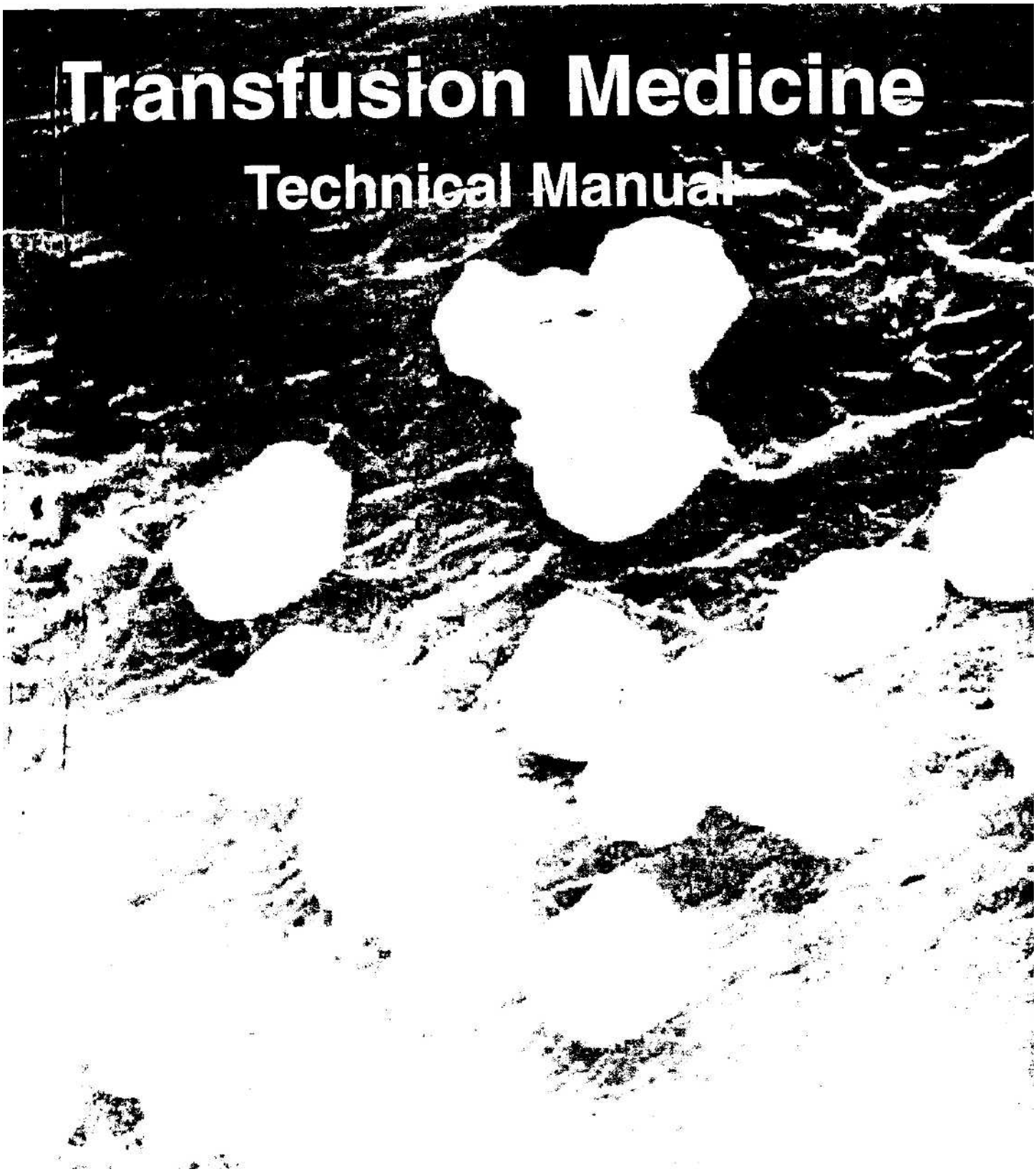
Transfusion Medicine

Technical Manual



Directorate General **of Health Services**
Ministry of Health & Family Welfare
Government **of India**

TRANSFUSION MEDICINE
TECHNICAL MANUAL

Second Edition 2003

Edited by
DR. R. K. SARAN

Sponsored by
WORLD HEALTH ORGANISATION

Liaisons
DRUGS CONTROLLER GENERAL (INDIA)
CENTRAL DRUGS STANDARD CONTROL ORGANISATION

DIRECTORATE GENERAL OF HEALTH SERVICES

MINISTRY OF HEALTH AND FAMILY WELFARE
GOVERNMENT OF INDIA, NEW DELHI

First Edition 1991

Second Edition 2003

Designed and printed by **Mehta Offset Pvt. Ltd.** New Delhi-110028

Dr. S. P. AGARWAL
M.S. (Sing) M.Ch. (Nauro)
DIRECTOR GENERAL

fsRrfur -*T^T, ^^ fosft-i 10001
GOVERNMENT OF INDIA
DIRECTORATE GENERAL OF HEALTH SERVICES
NIRMAN BHAVAN, NEW DELHI-110001
TEL. NO. 23018438, 23019063
FAX NO. 91-1123017924

FOREWORD

Number of technical and scientific changes have occurred in the practice of transfusion medicine and blood bank technology since the first edition of this book published in 1991. For example, additional screening tests for exclusion of potentially infectious blood with hepatitis C virus have been introduced, in addition to the testing of human immunodeficiency and hepatitis B viruses, to make blood and its components more safe for transfusion. Screening of all donors for evidence of transfusion-transmitted infections and mechanism of self-deferral of potential donors who are at high risk of the infection, have been quite successful to control this route of transmission of infections. Although some risk for the patient remains however this has produced a major change in the way transfusion therapy is viewed and utilized in clinical practice.

Under the scheme for the development and modernization of blood banking and transfusion services in the country, short term orientation courses have been provided so as to develop a framed workforce conversant in elementary and basic practices in transfusion medicine. Considering the development made in the field of transfusion medicine, a need was felt for the publication of the second edition of this book, having recent advances in transfusion medicine and the standards and federal regulations for blood banks to make blood more safe and to maintain the uniformity in the functions of blood transfusion services throughout the country.

With the above background in view, a technical committee of experts in blood transfusion services consisting of Dr. R. K. Saran, Consultant, Blood Transfusion Services, Sir Ganga Ram Hospital, New Delhi, Dr. Rama Bhasin and Dr. Kabita Chatterjee, Department of Transfusion Medicine, A.I.I.M.S., New Delhi, Dr. Vimla Ramalingam, Secretary General, Indian Red Cross Society, New Delhi and Mr. R. Narayana Swamy, DDC (I), DGHS, New Delhi was formed, to prepare an advanced and updated version of the existing Transfusion Medicine Technical Manual.

In this second edition of Transfusion Medicine Technical Manual, prepared by the technical committee, recent advances in transfusion medicine have been incorporated. Besides, the contributions of the other experts Prof. N. K. Mehra, Head of the Dept. of Histocompatibility and Immunogenetics, A.I.I.M.S., New Delhi, Dr. Shriniwas, Sr. Microbiologist, Inderaprastha Apollo Hospital, New Delhi, formerly Professor and Head of the Dept. of Microbiology, AIIMS, New Delhi and Dr. S. K. Sood, Sr. Hematologist, Sir Ganga Ram Hospital, New Delhi, formerly Professor and Head of the Dept. of Pathology, University College of Medical Sciences, Guru Tegh Bahadur Hospital, Delhi have been very useful in preparing this book.

I hope that this second edition of Transfusion Medicine Technical Manual would be of immense help in improving and standardizing the blood transfusion services in the country and keep the personnel in this field abreast of the advances in the field of transfusion medicine.

Dr.S.P.Agarwal

PREFACE

There have been many changes in transfusion medicine and blood banking in last one decade. Quite a few have been incorporated in this second edition of Transfusion Medicine Technical Manual like leukocytes- depleted blood products, which is recently drawing attention, peripheral blood and cord blood stem cells to replenish suppressed bone marrow, pharmacological alternatives to transfusion, transfusion support in organ transplant, human recombinant growth factors to activate bone marrow and peripheral blood stem cells and advances in the technology of apheresis used in a large variety of clinical conditions.

More emphasis has been given on clinical aspect of transfusion medicine, however appropriate weightage on laboratory aspects is also given. The inclusion of both clinical and laboratory components have been tried for communication to physicians and laboratory workers.

Certain clinical situations that are particularly related to the blood banking are given in detail, including autoimmune hemolytic anaemia, transfusion-transmitted viruses, human leukocyte antigen (HLA).

A chapter on transfusion safety and federal regulations clarifies the required quality assurance and inspection procedure by regulating authorities.

This book has been prepared with the intention to provide basic blood banking theory, techniques, regulatory guidelines, related transfusion medicine, and advanced concepts to facilitate learning. It is designed to provide the medical technologists, blood bank specialists with a concise and thorough guide in transfusion practice.

The technical committee is thankful to those individuals whose behind the scenes contributions made this book possible. Special thanks to Dr. Veena Doda, Head of the Blood Transfusion Service, R.M.L. Hospital, New Delhi for giving valuable suggestions. We also appreciate the tolerance help of the publisher M/S Mehta Offset Pvt. Ltd.

Dr. R.K. Saran is grateful to Dr. S. K. Sama, Head oftheDept. of Gastroenterology and Chairman of the Board of Management, Sir Ganga Ram Hospital, New Delhi for his advice and providing facilities in preparing this book.

R.K. Saran
Rama Bhasin
Kabila Chatterjee
V. Ramalingam
R. Narayana Swamy

CONTRIBUTORS
(Technical Committee)

Dr. R. K. Saran
Consultant, Blood Transfusion Services
Sir Ganga Ram Hospital, New Delhi

Dr. Rama Bhasin
Head of the Dept. of Transfusion Medicines
C.N.C., A.I.I.M.S., New Delhi

Dr. Kabita Chatterjee
Dept. of Transfusion Medicines
A.I.I.M.S., New Delhi

Dr. Vimla Ramalingam
Secretary General,
Indian Red Cross Society, New Delhi

R. Narayana Swamy
Deputy Drugs Controller (India),
Directorate General Of Health Services, New Delhi

OTHER CONTRIBUTORS

Prof. N. K. Mehra
Head of the Dept. Histocompatibility & Immunogenetics
A.I.I.M.S., New Delhi
HLA System & Laboratory Techniques
(R. Jaini, G. Kaur, N. K. Mehra)

Dr. Shriniwas
Sr. Microbiologist, Inderaprastha Apollo Hospital, New Delhi
Formerly Prof. & Head of the Dept. Microbiology A.I.I.M.S., New Delhi
Blood *Transfusion Transmitted Diseases*

Dr. S. K. Sood
Sr. Consultant Hematologist, Sir Ganga Ram Hospital, New Delhi
Formerly Prof. & Head of the Dept. Pathology
University College of Medical Sciences
Guru Tegh Bahadur Hospital, Delhi
Auto Immune Hemolytic Anemia

CONTENTS

1
Organization of Blood Transfusion Services

Blood Transfusion Services (BTS) is the vital part of modern health care system without which efficient medical care is impossible. The aim of blood transfusion services should be to provide effective blood and blood products, which are as safe as possible, and adequate to meet patients' need.

A Blood Transfusion Service is a complex organization, requiring careful designing and management.

BASIC GUIDELINES FOR THE ORGANIZATION OF BTS

The development of blood transfusion service should be based upon the following recommendations of the International Society of Blood Transfusion and Immunohematology (ISBTI), and the World Health Organization (WHO),

- The development of blood transfusion service should base on voluntary and non-remunerated blood donations. Reliance on family/replacement donors should be phased out.

- The development and implementation of national strategy for the screening of all donated blood for transfusion-transmitted infections, using the most appropriate and effective assays to test for HIV 1 & 2, hepatitis B and C viruses. syphilis and malaria.

- To enact effective legislation governing the operation of blood transfusion service so that it meets the prescribed standards.
- Develop good manufacturing and laboratory practices in blood bank in order to protect the health of both blood donors and recipients of blood and its products.
- To provide safe and adequate blood and its components to meet patients need. An adequate service includes availability of at least whole blood, red blood cells, platelets, fresh frozen plasma (FFP), cryoprecipitate (Factor **VIII)** and plasma.
- The maintenance of a register of voluntary non-remunerated blood donors.
- To establish a voluntary blood donation unit within the blood transfusion service, with an officer responsible for the blood donation programme and designated as donor recruitment officer.
- The training of staff responsible for donor education, motivation, recruitment and selection.
- The training of staff of blood transfusion service for safe blood collection procedures, including donor selection and donor care.
- To train laboratory technical staff in all respect of blood screening, blood grouping, compatibility testing, components preparation and the issue of blood and its products for transfusion.
- To develop good laboratory practice, including the use of standard operative procedures, ; in all respect of blood screening, blood collection and processin.
- Monitoring and evaluation of clinical use of blood.
- To promote cooperation between the blood transfusion services, health services and hospitals, educational institutes, religious, social and industrial organizations, mass media **and the** general public.

- To ensure adequate and separate budget for blood transfusion services.
- Blood Transfusion Service in a country may be centralized, regionalized, hospital based or some combination of them. Once a system has been established in a region it is difficult to change. The size, history, culture, political structure, level of economic development and administrative control effect the evolution of blood transfusion services. So in a developing country of big size the development of centralized or regionalized blood transfusion services at the level of metropolitan and big cities, states and/or district levels is more practical.
- Estimation of donor requirement is essential for the development of blood transfusion services. Estimates of need may be based on fixed percentage (5 % recommended by WHO) of the population. But this assumption ignores the disparity between the size of population and the number of hospital beds in an area. Estimate of blood needs on the number of hospital acute beds is more realistic. The figure may vary from 5-15 units per bed per year. The lower ratios apply to hospitals where blood is needed in the management of bleeding as a complication of pregnancy or trauma or simple surgery. The higher ratios apply to hospitals with more specialized facilities like oncology, open-heart surgery,

renal dialysis/transplant or replacement therapy in thalasseamia, hemophilia, leukemia, and other blood disorders.

DONORS RECRUITMENT STRATEGIES

Donor's recruitment is critical to the success of supply of safe and adequate blood and its products to meet patients need.

Donors Recruitment Strategies Are:

- Pure voluntary-based recruitment.
- Social persuasion-based recruitment.
- Remunerated-based donations.

The first two types of strategies result in voluntary blood donation, because they do not rely on monitory remuneration and the information of their health provided by these donors can be trusted. Remunerated based blood donation is done by blood sellers, who conceal the facts about their health and diseases which they may carry, about their last donation and their identity. Their blood is not safe.

Pure Voluntary Recruitment strategy:

It depends on the internally generated sense of altruism or community responsibility. A voluntary donor donates the blood on his/her own free will without distinction of caste, creed, religion, color and status of recipient and does not expect any monitory benefit from the collecting facilities or other sources at the time of donation or in future.

However, most donors do not donate blood as often as they can but small incentives like pins, badges and plaques given to donors who have donated blood to a specified numbers express appreciation to regular donors and enhance their feelings of altruism and encourage more frequent donations.

Social Persuasion-based Recruitment Strategy:

It is associated with persuasion and pressure of friends and colleagues, heads of religious organizations and political leaders to donate blood. Such donations are often associated with outdoor (mobile) blood donation drives at the work place like colleges, schools, factories, offices, or at some other place where a group of individuals collect as a social, political or religious unit. In many cases, group or organizer promises the recruiter a minimum number of donations of blood units and the social pressure on individual member of the group is quite effective to encourage donations and to meet the promised target.

In second form of social persuasion-based strategy are replacement donors also who donate blood for specific patients, usually close friends or relatives without any monitory reward. These donors may feel an expression of their caring and concern for the recipients. The replacement donors can be easily motivated and persuaded to become regular voluntary donors. There is often undue pressure (coercion) upon the family/friends of the patients, which force them to pay professional paid donors to serve as family surrogates and donate blood resulting in unsafe supply of blood. Reliance on replacement donations may be phased out.

Remunerated-based Donations:

Remunerated-based donations are done by blood sellers (paid professional donors) who are poor people and give blood for money. It is recognized all over the world that their blood is mostly of poor quality and may carry infection of many diseases like hepatitis B virus (HBV), human immune deficiency virus (HBV), hepatitis C virus (HCV) and syphillis etc. It is not safe blood and may be hazardous to the recipients. This system survives due to the inefficiency of voluntary blood transfusion service and apathy of the general public. It should not be encouraged and stopped.

MOTIVATION AND PROPAGANDA

Effective and wide propaganda is imperative for the recruitment of voluntary blood donors. The following enumerated available means and methods of communication for voluntary blood donation should be followed

Oral Communication

This is the most effective method of recruiting donors. Talk on need of blood, shortage of blood, ease of donation and myth about blood donation, possibly illustrated by films is very effective. The speakers/recruiters must have the persuasive power to appeal to the humanitarian feelings of the audience. Time should be available in the end of talk for the audience to ask questions and to give precise answers.

Personalized Communication

Personalized communication is achieved through circulars from professional and religious associations, clubs newsletters, and school magazines or by direct mail.

Printed Communication

Brochures, posters and informative leaflets are valuable forms of communication. Material must catch the' eye', and be easy to understand.

Publicity materials e.g. posters, television advertisements, jingles, cartoons etc. should be prepared by professionals. Greeting cards on Birthday, Marriage Anniversaries, New Year's Day or other auspicious days carrying motivational slogans for voluntary donations are also effective.

National or Local Mass Media

The awareness of the need of blood and voluntary blood donation and urgent appeal can be made by the announcements on radio, television and through news papers, magazines and other influential reading materials.

Educational Institutions

Education among the young is useful to remove superstition and myth connected with blood donation. It is important to introduce the subject of blood donation into schools as part of science and civic studies. The young are potential donors.

Recruiters

The importance of the contribution made by recruitment officers, who may be social workers or often blood donors themselves, should not be under estimated in recruiting donors. Their enthusiasm and empathy with new donors is invaluable.

Recruiters are the conduits to the general public and they should have all information about voluntary blood donation, scientific and technical advances relating to transfusion medicine, so that they can give precise answers to donors. There should be meetings for the recruiters at which they can express and exchange views about their work.

Motivating relatives and friends of patients

Hospital staff specially clinicians can actively contribute in motivating the relatives and friends of patients, who had or will need blood transfusion, to donate blood. Patients' relatives and friends who have donated blood can be easily motivated to become regular voluntary blood donors.

DONORS RETENTION STRATEGIES

Blood Collection Facilities

Public image building and the retention of donors are more successful if static and mobile blood collection centers are attractive and all facilities for the comfort and convenience to donors are available. The donation must be made a pleasant and rewarding experience for the donors.

Static collection centers

There should be attractive reception area and adequate seating arrangement at the collection center. The likely waiting time should be indicated to donors. Donors must be comfortable during the blood donation.

Mobile collection venues

Mobile collection venues are very convenient for many blood donors, and also attract people to donate first time on impulse. About 80% of voluntary blood donation (excluding replacement donations) is received in mobile (out door) blood donation camps and they have some advantages.

- Camps are organized at convenient locations for the donors e.g. shopping centers, sports stadium, banks, factories, colleges, schools, offices or any open public place.
- It has a very congenial atmosphere and provides a festive look.
- Blood donors and organizers can interact with each others and the public.
- It attracts the people who want to help others or do some social service.

ORGANIZATION OF OUT-DOOR BLOOD DONATION CAMPS:

- Blood donor organizer identifies a 'key' person amongst the target group who can act as chief donor recruiter within that group.
- In consultation with the contact 'key' person, a talk on a date convenient to the organizer may be arranged, if desired by the organizer.
- The date, time and venue of blood donation camp convenient to the organizer are fixed.
- Informative posters, brochures etc. are given to the contact person to display at the venue of blood donation camp.

- Blood collection team should reach at the venue of out door blood donation camp in time and should stay till the organizer wants.

Dealings with donors

It is of prime importance to dispel donor's fear. If a new donor is convinced by personal experience that blood donation is painless and harmless, he or she will usually return to give blood again. These are effective to motivate public to be regular blood donors.

Staff

The staff personnel should be courteous interested, cheerful and friendly, as well as professional and efficient. If the donors encounter ill-tempered or in-civil staff and badly run, disorganized, or dirty collection centers, they are unlikely to return to donate blood again.

Incentives

Incentives like pins, badges and plaques for specified number of donations help in repeat donations. Other incentives or awards, simple and attractive of minimal commercial value, are useful in retaining donors.

Post donation strategies

Donors are bled skillfully, treated well, given the light refreshment and donors cards. The donors should be thanked for the contribution and encouraged to donate again. Simple letters thanking donors/organizers for their donations are important.

Special ceremonies

Annual award ceremonies should be held to acknowledge and congratulate the people who have donated blood many times or assisted in promoting the voluntary donations. These occasions should be widely publicized and prominent citizens should be invited to address the donors and organizations/institutions for their valuable and outstanding service to the community. Donors, recruiters, institutions and organizations should be given cups, trophies and shields for their contributions in voluntary blood donations.

Greetings

Greetings on special occasions like Birthday, Marriage Anniversary, New Years Day etc. to voluntary donors increase repeat donations.

CONCLUSIONS

There is no short cuts to effective recruitment programming. The only way to achieve good results is to approach the task systematically and professionally. The work of voluntary blood donor recruitment is essentially progressive and active. The challenge is ever present.

2
Donors Selection and Blood Collection

The first and most important step, in ensuring that blood and its products for transfusion do not have any pathogenic virus/acteria, is the proper selection of blood donors. It should be done carefully. The donor should be in good health in order to avoid any untoward effect to the donor or the recipient.

Blood collected from voluntary donors and relations/friends of patients without any coercion on them is safe.

DONOR SCREENING

The donor screening process has four major aspects:

(1) Registration, consent of the donor, and demographic information. Demographic information should be complete and correct so that the donor can be informed of any laboratory testing abnormality or he/she may be called for future donation.

(2) Medical history

(3) Limited physical examination

(4) Simple laboratory tests

The salient demographic information include:

1. Donor's full name
2. Father/husband's name
3. Date of birth/Age
4. Gender-Male/Female
6. Residential and official addresses with phone numbers

INFORMATION PROVIDED TO THE DONORS REGARDING HIV/AIDS

All donors must be given educational materials informing them of high-risk activities for HIV transmission, of the clinical signs and symptoms of HIV infection and AIDS and of the importance of refraining from blood donation if they have been engaged in these activities or experienced signs and symptoms.

High Risk Group Donors for HIV Transmission are

1. Male homosexual or bisexual
2. Promiscuous men or women
3. Male or female sex entertainers
4. I. V. Drugs abusers
5. Individuals who have had sexual intercourse with any one in any of these groups.
6. Blood sellers (Professional Paid Donors)

Symptoms of AIDS Related Complex (ARC):

Within 6 months any of the following:

1. Unexpected weight loss
2. Night sweats
3. Unexplained fever above 99°C for more than 10 days
4. Swollen lymph nodes for more than 1 month
5. Persistent diarrhea
6. Persistent cough with shortness of breath
7. Blue or purple spots typical of Kaposi's sarcoma on, under the skin, or on mucus membrance.
8. Persistent white patches or unusual blemishes in the mouth

Medical History

The donor's medical history is evaluated. The donor is accepted by a suitably qualified person trained to follow prescribed guidelines for the selection of blood donors. This person works

under the instructions of a physician of the blood bank. A donor with any abnormal condition is referred to the physician of blood bank, who takes the final decision on whether blood should be collected from such a donor or not. In doubtful cases the donor should be deferred.

BLOOD DONORS QUESTIONNAIRE:

- Are you at present in good health?
- When did you east last?
- Are you taking any medicine?
- Have you been vaccinated or immunized recently?
- Have you ever suffered an epileptic fits, convulsions or mental disorder?
- Have you ever had jaundice or hepatitis?
- Have you ever been positive for HBV or HCV ?
- Have you been in contact with a person suffering from jaundice (hepatitis) during the past 6 months?
- Do you know about AIDS?
- Have you (if male) had sex with another man?
- Have you had unsafe sex with an individual at increased risk for AIDS?
- Have you lost significant weight in last 6 months?
- Have you ever been positive for HIV?

Occupational hazards:

Air Crews, drivers of long-distance heavy-duty vehicles and construction workers on high buildings are advised not to give blood within 12 hours of going on duty.

Respiratory Infections

• Cold, flu, cough, sore throat or acute sinusitis	Defer until all symptoms subside and temperature normal
• Chronic sinusitis	No deferral unless using antibiotics
• Asthmatic attack	1 week after last attack if chest is clear
• Asthmatics on steroids	Defer

Pregnancy and Abortion

• Pregnant or recently delivered	Defer for 6 months after delivery
• Abortion	Defer 6 months after abortion
• Breast feeding	After baby weaned (defer till baby is on breast feeding)

Surgical Procedures:

• Major surgery	6 month after recovery
• Minor surgery	3 months after recovery
• Open heart surgery- including By-pass surgery	Permanently defer ..
• Cancer surgery	Permanently defer
• Localized skin cancer that was removed	6 months after removal
• Tooth extraction or dental manipulation	Defer for 3 days
• Dental surgery under anaesthesia	Defer for 1 month

Heart Diseases

• Has any active symptom (Chest pain, shortness of breath, swelling of feet)	Permanently defer
• Restricted activity	Permanently defer
• Cardiac medication (digitalis.nitroglycerine)	Permanently defer
• High blood pressure controlled with medicine	Acceptable if B.P. normal

Cardio-Vascular Diseases

• Myocardial infraction	Permanently defer
• Coronary artery disease	Permanently defer
• Angina pectoris	Permanently defer
• Rheumatic heart disease with residual damage	Permanently defer

Seizures	
Fainting, Convulsions & Epilepsy	Defer, if not taking medicine or free from seizures for >2 years can be accepted after evaluation.
Endorcranial Disorders	Permanently defer

Infectious Diseases

Donors should be free from infectious diseases known to be transmissible by blood, so far as can be determined by usual examination and history.

Viral Hepatitis

• Has had hepatitis (jaundice) other than Hepatitis A,	Permanently defer
Positive test for Hepatitis B (HBsAg), Hepatitis C (HCV)	Permanently defer
• Exposure to hepatitis by tattoos, acupuncture or body piercing	Defer for 12 months
• Worked in renal dialysis	Defer for 12 months
• Received transfusion of blood and its components	Defer for 12 months
• Close contact with individual suffering with hepatitis	Defer for 12 months

Jaundice

Has ever had jaundice associated with:

• Newborn	No deferral
• Rh disease	No deferral
• Gall stone	No deferral
• Mononucleosis	No deferral

HIV Infection / AIDS

High risk group donors for HIV infection Anti-HIV positive donor Donors having symptoms of AIDS	Permanently defer

MALARIA:

Travelers who have been in an area considered endemic for malaria may be accepted as regular donors one year after returning from the endemic area irrespective of the receipt of antimalarial prophylaxis, provided they have been free from unexplained febrile illness. Immigrants, refugees, or citizens coming from a country endemic for malaria may be accepted as blood donors three years after departure from endemic area, if they have been asymptomatic in the interim.

In a region endemic for malaria, it is not practicable to reject donors who have history of malaria infection or who have taken antimalarial drug(s). Patients may be given anti-malarial drugs.

History of malaria in endemic area but duly treated and free from any symptoms	Accepted 3 months after treatment

Syphilis

Genital sore or generalized skin rashes	Defer for 12 month after rashes disappear & completion of therapy

Tuberculosis Defer for 5 years after cessation of symptoms and treatment

Fever

Had prolonged fever or Rheumatic fever	Defer till fully recovered and off medication

Kidney diseases

- Acute infection of kidney (pylonephritis) or acute infection of bladder (cystitis) — Defer for 6 months after cessation of treatment and symptoms free
- Chronic kidney diseases/failure — Permanently defer

Digestive system

- Stomach ulcer with symptoms or with recurrent bleeding — Permanently defer
- Chronic liver diseases with impaired organ — Permanently defer

Vaccination and Inoculation

1. **No waiting period before donation if symptoms free**
   Inoculation with toxoid or a killed viral/bacterial vaccine

Tetanus	Influenza
Diphtheria	Pertusis
Typhoid	Polio (salk vaccine, injection)
Paratyphoid	Rabies as prophylactic
Cholera	Plague
Prophylactic Hepatitis B	

2. **Two-weeks deferral from time of vaccination.**
   Smallpox (two weeks after scab falls off)
   Polio oral (sabin vaccine, oral)
   Measles (rubeola)
   Mumps
   Yellow fever

3. **Four-weeks deferral from time of vaccination**
   Anti-tetanus serum
   Anti-venom serum
   Anti-diphtheria serum
   Anti-gas gangrene serum
   Rubella (German measles)

4. **Twelve-months deferral from time of vaccination**
   Anti-rabic vaccination as a result of animal bite
   HBIG (hepatitis B immune globulin)
   Gamma globulin

MEDICATION

If a donor is taking some medicine it may not be in his/her own interest to donate blood and may also effect the patient who would receive the blood.

Medicines

	Accepted/Deferred
• Oral contraceptives	
• Analgesics	Accepted
• Vitamins	Accepted
• Mild sedatives and transquillisers	Accepted Accepted
• Salicylates(aspirin) taken in last three days	Not accepted if blood be used for preparing platelets
• Isotretinoin (accutane) used for acne	Defer for 1 month after the last dose
• Finasteride (e.g. Proscar) used to treat benign prostate hyperplasia	Defer for I month after the last dose
• Oral anti-diabetic drugs with no vascular complication	Acceptable
• . Diabetics on insulin	Defer while taking the drug
• Antibiotics (oral)	Defer for 3 days & till symptoms free
• Antibiotics (injection)	Defer for 4 days & till symptoms free/after the last injection
• Cortisone	Defer for 7 days after the last dose
• Medicine to treat hypercholesterolemia	Accepted

Donors taking following medicines are permanently rejected :

Anti-arrhythmics	Immunosuppressive
Anticonvulsions	Pituitary growth harmones of human origin
Anticoagulants	Sedatives or tranquilisers in high doses
Antithyroid drugs	Vasodilators
Cytotoxic drugs	Etretinate (e.g.Tegison) to treat psoriasis. It is teratogenic.
Digitalis	Vasodilators
Dilantin	Drugs for Parkinson’s Disease

Other Conditions Requiring Permanent Deferral

Abnormal bleeding tendency or blood coagulation disorder like hemophilia
Severe allergic disorder

Donors with relatively Minor Cell Abnormalities
Thalassemia Trait
It is associated with little or no reduction in red cell life and Hb concentration is usually normal. Persons with thalassemia trait may be accepted as donors, provided they have normal Hb concentration and normal reticulocyte count.

Polycythemia Vera
Blood from people suffering from the polycythemia vera is not used for transfusion as it is a precancerous condition and its recipient sometimes develops leukaemia or other malignant cells, though it is rare.

Glucose-6-Phosphate Dehydrogenase (G-6-PD) deficiency
The use of donor blood with this abnormality imposes a small but definite risk to the recipient. Although the survival of G-6-PD deficient red cells is almost normal for about 80-90 days., but if the recipient ingests drugs like phenacetin, sulphonamides, vitamin K, primaquine etc. there may be rapid destruction of the G-6-PD deficient cells.

On storage, the viability of red cells from G-6-PD deficient donor is less than that of normal red cells.

PHYSICAL EXAMINATION:

General Appearance:
A prospective donor should be in good health.

Age: 18-60 years

Weight:
A donor weighing 45 kg can give 350 ml blood (8-9 ml/kg body weight) in addition to the pilot samples for processing. Those weighing 60 kg or more may give 450 ml blood as well as pilot samples.

Blood Pressure:
The systolic blood pressure should be between 100 and 180 mm of Hg and the diastolic pressure between 50 to 100 mm of Hg.

Pulse:
The pulse should be between 80 to 100 beats per minute and regular.

Temperature:
Oral temperature should not exceed 37.5°C.

Donation Interval
The interval between the donation of a unit of blood should be at least 12 weeks except in unusal circumstances. Whole blood donation must be deferred for atleast 72 hours after plasma- or platelet-apheresis.

Donor Skin:
The skin at the venipuncture site should be free of any lesion or scar of needle pricks indicative of addiction to narcotics or frequent blood donation as in the case of blood sellers.

Systemic Examination:
Clinically heart, lungs and abdomen should be normal. Liver and spleen should not be palpable.

LABORATORY TESTS:

Hemoglobin (or Hematocrit):
Hemoglobin (Hb) or Haematocrit (Hct) should be determined each time the donor present himself/ herself. The Hb should not be less than 12.5 g/dl (or 38% Hct) The Hb may be measured by the following methods:

(a) Specific gravity method using copper sulphate solution of specific gravity 1.053. (See chapter "Preparation of Solutions and Methods")
(b) Sahlis method
(c) Cyanmethaemoglobin method using spectrophotometer or photoelectric colorimeter. (See chapter "Preparation of Solutions and Methods")
(d) Hemo-Cue method (See chapter "Preparation of Solutions and Methods")

The copper sulphate specific gravity test is generally used as a Hb screening procedure. It is simple, quick and inexpensive test.

ABO and Rh (D) Blood Grouping:
Donor is screened for ABO ad Rh (D) group by slide/tile or tube method.
Hb g/dl and ABO and Rh(D) group is recorded on the donor form.

COLLECTION OF BLOOD:

Donation Premises
It should be attractive, well lighted, clean and air-conditioned so that donors feel comfortable and relaxed.

Out-door blood collection

Out-door blood collection sites can present special problems. An individual trained in safety principles should make an advance visit to the collection site to ensure that hazards are minimized. All personnel in out-door blood collection camps should be trained to recognize unsafe conditions and understand infection control policies and procedures. The responsibility for site safety should be assigned to a senior-level employee.

Hand washing access is essential at all collection sites. Carpeted or difficult-to-clean surfaces can be protected with a clean suitable overlay. Portable screens are helpful to protect and maintain safe work areas. Refreshment service areas should be separated from areas of blood collection and storage. Blood-contaminated waste must be packaged and returned to a central location for disposal using thermal (autoclave, incinerator) or chemical disinfectant in accordance with local regulations for medical wastes.

Personnel
Personnel should be interested, friendly as well as professional and well-trained. Blood is drawn from the donor by a qualified physician or under his/her supervision by assistants trained in the procedure. A physician should be present on the premises when blood is being collected.

Equipment and Materials

a) Blood Containers

Blood containers are polyvinyl chloride (PVC) plastic bags which are closed system of single, double or triple bags for collection of 350 ml or 450 ml blood. The anticoagulant solutions citrate-phosphate-dextrose (CPD) or citrate-phosphate-dextrose-adenine (CPDA-1) are used in the volume of 49 ml for 350 ml or 63 ml for 450 ml of blood (14 ml CPD or CPDA-1 for 100 ml blood). Currently CPDA-1 anticoagulant solution is mostly used. It should be sterile, pyrogen free.

(b) Sphygmomanometer, automatic mixing of blood and weighing of blood bag machine (Blood Collection Monitor-Scale/Mixer like Combimix-X3, Baxter; Biomixer-321, Lungberg & Kogel AB etc.)

(c) Plastic clips, stripper, di-electric tube sealer or aluminium clips, sealer, cutter.

(d) Sterile cotton swabs and band-aids/bandages

(e) Methylated spirit, tincture of iodine, providone-iodine solution (1 %) & alcohol

(f) Emergency drugs and other articles: Aromatic spirit of ammonia; Intravenous crystalloid normal saline (sodium chloride 0.9%); Injections: Dexamethasone sodium phosphate; Calcium gluconate; Mephantine; Perinorm; Oxygen cylinder with regulator and mask; papers bags to breath; tissue papers; towels; basins.

Identification
Blood bag and pilot tube(s) are identified by a donation number at the time of collection of blood, so that it can be traced back to the donor. The dates of collection and expiry, and ABO, Rh (D) group are written on the label of bag for blood collection.

Volume of blood
A donor weighing 45 kg can give 350 ml (369g) of blood and those weighing 60 kg or more can give 450 ml (474 g) of blood and blood sample in pilot tube (8-9 ml of blood per kg body weight or 12% of blood volume)

Blood Volume : Anticoagulant Solution.
Volume of blood collected shall be proportionate to the volume of anticoagulant with ± 10% i.e. 350-385 ml blood in the bag for 350 ml of blood and 450-495 ml blood in the bag for 450 ml of blood. If less blood is to be collected. the amount of the anticoagulant must be reduced proportionately.

14 ml of CPD or CPDA-1 solution is used for 100 ml of blood. If less amount of blood is to be drawn two factors must be calculated:

(1) How much blood can be safely drawn from the donor.

(2) How much anticoagulant solution is needed to preserve the blood.
The calculations when less amount of blood is to be drawn in a bag for 450 ml blood:

a) First, the amount of blood to be drawn is determined by the donor's weight using the following formula:
(Donor's weight in Kg 55) x 450 = amount (ml) to be drawn

b) Next, the correct amount of anticoagulant required is determined:
(Amount of blood to be drawn 100) x 14 = amount (ml) of anticoagulant required.

c) The amount of anticoagulant to be removed is calculated:
63 ml-amount of anticoagulant needed = amount of anticoagulant to remove from bag.

For example, if whole blood is to be drawn from a donor who weighs 48 kg,
the calculation would be:

(48 55) x 450 = 392.72 say 390 ml of blood to be drawn
(390 100) x 14 = 54.6 ml anticoagulant needed
63-54.6 ml = 8.4ml of anticoagulant to be removed from the bag for proper storage of blood.

METHOD OF PHLEBOTOMY:

1. The phlebotomist should wash his/her hands with soap and water and should wear sterile gloves.

2. Inspect the bag for leakage or any other defect. The anticoagulant solution must be clear. Check the donor name, donation number on the form and the bag. Donation number on the bag and form should be same. Place the bag on a balance and ensure that it is below the level of the arm.

3. Choose the site of venipuncture in the anticubital area of the arm.Apply blood pressure cuff, inflate to 50-60 mm of Hg and select a prominent and firm vein in the area that is free of any skin lesion/needle marks. Asking the donor to close the fist usually helps in bringing the vein into prominence.

4. Release the blood pressure cuff and thoroughly clean the proposed site of venipuncture with an antiseptic lotion in the following way:

   (a) Clean 4-5 cm area starting at the site of venipuncture and moving outwards in a concentric spiral way with methylated spirit/alcohol.

   (b) Apply 10% providone-iodine solution (betadine) or tincture of iodine in the same way as spirit. Allow it to dry.

   (c) Clean with methylated spirit or alcohol allow the solution to dry.

   (d) The cleaned area is ready for venipuncture and should not be touched.

5. Inflate blood pressure cuff to maintain pressure 50-60 mm of Hg. Ask the donor to close the fist. Uncover the sterile needle and perform venipuncture immediately, using aseptic procedure.

6. Ask the donor to open and close hand or to squeeze a rubber ball.

7. The donor should be under constant observation throughout the phlebotomy and should never be left unattended.

8. Mix the blood and anticoagulant gently and periodically during collection of blood. Mixing can be done by hand or blood collection monitor -Scale/Mixer.

9. The flow of blood should be uninterrupted and constant, otherwise, it will not be suitable for preparation of platelet concentrate, fresh frozen plasma or cryoprecipitate.

10. Monitor the volume of blood being drawn using a balance or blood collection monitor-Scale/Mixer. One ml of blood weighs 1.05 g. Thus, 350 ml of blood weighs 367 g. and 450 ml weighs 472 g. The initial weight of the bag and the anticoagulant solution should be taken into account while measuring the total weight.

11. As soon as the required amount of blood is collected, clamp the tubing of the bag with plastic clip. Deflate the cuff or release the tourniquet. Place the sterile swab at the venipuncture site, apply light pressure and withdraw the needle.

12. Remove blood pressure cuff or tourniquet.

13. Ask the donor to put the fingers of the other hand on the swab at the venipuncture site and to raise the arm.

14. Take the bag to the processing table.

15. Loosen the artery forceps/plastic clip and apply light pressure on the bag to transfer 5-6 ml of blood in the pilot tube(s) with same number as on blood bag.
16. Seal the tube with di-electric tube sealer and separate the needle.

17. Strip blood bag tubing, starting at seal, pushing blood into bag. Do it quickly, to avoid allowing the blood to clot in the tubing. Invert bag several times to mix blood thoroughly; then allow tubing to refill with anticoagulated blood from the bag. Repeat the process a second time.

18. Seal the tubing attached to the bag into segments (2-3) with di-electrictube sealer.

19. Keep the blood bag at 2-6°C in the refrigerator immediately after collection. If platelets are to be harvested, blood bag should be kept at 20-24°C until platelets are separated. Platelets should be separated within 6-8 hours after the collection of the whole blood.

20. The donor should remain on the bleeding couch for 8-10 minutes under the observation of the staff. Then the donor is allowed to sit up and go for refreshment.

21. Check the arm and apply band-aid after bleeding stops.

22. Light refreshment like biscuits and tea/coffee/soft drink are given to donors.

23. The donor should be given donation card and thanked for the contribution and encouraged to donate again.

ADVERSE DONOR REACTION

Although the blood donation process is usually safe and uncomplicated, occasionally donors experience adverse reactions during or after donation; but they are usually harmless. Personnel in the phlebotomy area must be trained and prepared to respond quickly to those reactions. In general, if adverse reactions occur:

1) Remove/deflate the tourniquet and withdraw the needle from the donor's vein at the first sign of a reaction. Put the sterile swab at the venipumcture site and apply pressure with thumb.

(2) Call for assistance from other personnel or the medical director, if needed.

(3) If possible, remove the donor to an area where he/she can be attended to in privacy.

Donor adverse reactions are:

Syncope (fainting or vasovagal syndrome):

The symptoms may include sweating, weakness, dizziness, pallor, loss of consciousness, convulsions, and involuntary passage of urine and feces. The skin is usually cold, blood pressure falls and the pulse becomes thready.

Management

[a] Place the donor on his/her back and raise the legs above the level of the donor's head.
[b] Loosen tight clothings.
[c] Ensure adequate air-way.
[d] Administer inhalation of aromatic spirit of ammonia.
[e] Apply cold compresses to donor's head.
[f] Check the blood pressure, pulse and respiration until donor recover.

Tetany (Twitching or Muscular spasm)

Anxiety and deep breathing may cause the excited donor to loose excess of carbon dioxide, which may cause tetany characterized by twitching or muscular spasm due to hyperventilation.

Management
The donor is asked to breath into a paper bag which brings prompt relief.
Don't give oxygen.

Nausea **and vomiting.**

Management:

a) | Make the donor as comfortable as possible.
b[Ask the donor to breath slowly and deeply.

c) Turn the donor's head to a side to avoid aspiration of vomits.

d) If donor vomits, provide suitable receptacle and towel or cleansing tissues.

Hematoma Management

a) Deflate the blood pressure cuff. Ask the donor to open the fist and withdraw the needle from the vein.

b) Place 3 or 4 sterile gauze pieces or cotton swabs over the haematoma and apply digital pressure for 7-10 min with the donors arm held above the heart level.

c) Apply ice to the area for 5 min. if desired.

Convulsions
True convulsions are rare

Management

In case they occur:
[a] Prevent the donor from injuring himself/herself
[b] Place tongue blade between the teeth to prevent him/her from biting the tongue
[c] Ensure adequate air way.

Cardiac problems

Serious cardiac problems are extremely rare in the blood donor. If the donor is in cardiac arrest, begin cardiopulmonary resuscitation and continue until medical aid arrives.
The nature and treatment of all adverse reactions should be noted in donor's record.

Instructions to Donors (after donation)
1. Drink more fluids than usual in the next 4 hours. Do not remain hungry.
2. Do not smoke for half an hour.
3. Do not take alcoholic drinks for at least 6 hours.
4. If there is bleeding from phlebotomy site, raise the arm and apply pressure.
5. If there is feeling of faintness or dizziness, either lie down or sit with head between knees. If symptoms persist, ask for help, return to the blood bank or consult a doctor.
6. Remove the bandage/band-aid after 5-6 hours.

PROCESSING OF DONOR BLOOD

The general considerations in processing donor blood are:
1. Number of donation on the blood bag. processing pilot tube and donor record should be rechecked prior to processing.

2. ABO group is determined by testing the red cells with anti-A, anti-B and anti-AB sera and testing the serum with known A, B and O cells. Test with anti-AB serum is optional if monoclonal anti-A and anti-B are used. Blood must not be released for use until the discrepancies, if any, arc not resolved.

3. Rh group must be tested for Rh(D) only with anti-D serum. Rh(D) negative units must be tested for weak Rh(D) i.e. D^u by anti-human globulin (AHG) serum. Units those are Rh(D) negative and D^u positive must be labeled Rh(D) positive and those which are Rh(D) negative and D^{11} negative must be labeled Rh(D) negative. Routine testing for other antigens of Rh system is not recommended.

4. Donor blood should be tested for unexpected antibodies by saline, albumin/enzyme and anti-human globulin tests with screening cell panel, or with pooled 3 fresh O group red cells. See chapter on 'Screening and Identification of Antibodies'

5. Tests for blood transmissible diseases:

(a) Hepatitis B surface antigen and anti-HCV by ELISA method.
(b) Test for anti-HIV 1 and 2 by ELISA to eliminate the risk of transmission of HIV infection.
(c) Test for syphilis - Floculation test, Veneral Disease Research Laboratory (VDRL) assay or Rapid Plasma Regin (RPR) assay.
(d) Test for malarial parasites.

- Blood may be tested for malarial parasites by blood smear but it is difficult to find parasites in blood film in short time especially if the number of parasites is less than 100 per µl of blood. .
- Test for malarial antibody based on indirect fluorescent antibody test or ELISA.
- Malarial antigen test.

Tests using monoclonal antibodies for detection of malarial antigens are available but they are costly.
As there is no appropriate test which can be done easily in screening blood donor for malaria, it has been suggested that anti- malarial drugs may be given to the recipients of blood in highly endemic areas.

Preservation and Storage Of Blood

The first anticoagulant preservative was introduced by Rous and Turner in 1916. It consisted of a citrate-glucose solution in which blood from rabbits was stored for two weeks, which prevented anemia when transfused in other rabbit who had suffered from blood loss. Rous Turner's solution was used for storage of human blood during the First World War (Mollison 1987).

The next important development occurred in 1943 during the Second World War when acidified citrate dextrose (ACD) solution was introduced for clinical use by Loutit and Mollison.

In 1957 Gibson et al developed an improved preservative of citrate-phosphate-dextrose (CPD), which was less acidic than ACD and maintained 2,3-diphosphoglycerate (2,3-DPG) level better than in ACD solution. CPD eventually replaced ACD and became commonly used preservative for storage of blood/red cells in liquid form. Shelf-life of blood stored in CPD at 2-4 °C was 21 day.

In 1978 citrate-phosphate-dextrose with adenine (CPDA-1) preservative was developed. The addition of adenine improved the synthesis of adenosine triphosphate (ATP) in the stored blood, which prolongs the storage of blood/red cells at 2-4 °C to 35 days.

APPROVED PRESERVATIVE SOLUTIONS

Table. 3.1 Compositions **of** Preservatives/Anticoagulants

	ACD	**CPD**	**CP2D**	**CPDA-1**
Trisodium citrate (g)	22.0	26.30	26.35	26.35
Citric acid (g)	8.0	3.27	3.27	3.27
Dextrose (g)	24.5	25.50	51.10	31.90
Monobasic sodium phosphate (g)	-	2.22	2.22	2.22
Adenine (g)	-	-	-	0.27
Distilled water (ml)	1000	1000	1000	1000
Preservative (ml) / 100ml blood	15	14	14	14
Preservative (ml) / 450 ml blood	67.5	63	63	63
Initial pH of preservative	5.0	5.6	5.6	5.6
On first day pH of blood in bag	7.0	7.2	7.4	7.3
Storage time (days) at 2-6 °C	21	21	21	35

Action of ingredients of anticoagulant solution

Glucose _ supports ATP generation by glycolytic pathways.
Adenine _ synthesizes ATP, increases level of ATP, extends the shelf-life of red cells to 42 days.
Citrate _ prevents coagulation by chelating calcium.
Sodium di-phosphate preventsfall in pH

RED CELLS PRESERVATION

The goal of blood preservation is to provide viable and functional blood components for patients requiring blood transfusion. More than 70% of red cells should remain viable in circulation 24 hours after transfusion of stored blood in CPDA-1 for 35 days. The blood is stored at 2-6 °C to maintain the optimal viability.

The loss of red cells viability is correlated with the "lesion of storage" due to various biochemical changes:

Decrease in pH
Build up of lactic acid
Decrease in glucose consumption
Decrease in ATP level
Low 2,3-DPG levels

Decrease in pH

When blood is stored at 2-6 °C, glycosis is reduced but does not stop. Giycosis results in the production of lactate, with subsequent decrease in pH. Whole blood collected in CPD has a pH 7.20 on day 0 and 6.84 on day 21. Preservative solutions provide buffering capability to minimize pH changes and optimize the storage period.

Loss of Adenosine Triphosphate (ATP)

ATP is associated with the red cells viability. Loss of ATP causes increase in cellular rigidity and decrease in red cell membrane integrity and deformability. A decrease in ATP allows the leak of Na^+ and K^+ through red cell membrane at levels exceeding those normally seen in vivo. The ATP level in CPDA-1 red cells at day 35 is 45 % (±12) of the initial level.

Decline of *2,* 3-Dipnosphoglycerate (2,3-DPG)

A fall in pH in the stored blood results in a decrease in red cell 2,3-DPG level, which results in increase in hemoglobin-oxygen affinity. DPG-depleted red cells have impaired capacity to deliver oxygen to the tissues. The degree of reduction of 2,3-DPG levels depends upon the preservative solution used. ACD solution has lower pH than that of CPD solution. Thus 2,3-DPG falls within the first few days in ACD where as blood stored in CPD/CPDA-1 maintains adequate levels of 2,3-DPG for 10-14 days.

The pathological effects of the transfusion of red cells with low 2,3-DPG levels and increased affinity with oxygen include increase in cardiac output and a decrease in mixed venous PO_2 tension. 2,3-DPG levels in transfused blood are important in certain clinical conditions. Myocardial functions improve following transfusion of blood with high 2,3-DPG levels during cardiovascular surgery. In patients with shock the transfusion of DPG-depleted red cells makes a significant difference in recovery.

After transfusion, the red cells continue to synthesize 2,3-DPG and levels return to expected normal values within 24 hours. The acid-base status of the recipient, phosphorous metabolism and degree of metabolism influence the rate of restoration of 2,3-DPG.

Adenine

Simon (1962) showed that in CPD solution supplemented with 17 mg (0.25 mM) adenine per 63 ml of anticoagulant and 25% more dextrose, the survival of red cells 24 hours after transfusion of blood stored for 35 days was 80 ± 6.5 %. Adenine synthesizes ATP and its level is 56.4 ± 15.9% of the initial level in the stored blood for five weeks.

Adenine nephrotoxicity due to its unmetabolized product, 2,8-dioxyadenine, is negligible at a level of 15mg/kg body weight. This amount is present in 30 units of fresh CPD adenine (0.5 mM/unit) blood or in 60 units each having 0.25 mM adenine The quantity of adenine is less if red cells are used.

Changes in$Na^+$and K^+ levels

During refrigerated storage, Na^+ and K^+ leak through the red cell membrane rapidly. The cells lose K^+ and gain Na^+, however, the K^+ loss is greater than the Na^+ gain during storage.

Temperature

The lower temperature keeps the rate of glycolysis at lower limit and minimizes the proliferation of bacteria that might have entered the blood unit during venipuncture or from atmosphere. The rate of diffusion of electrolytes (Na* and K^+) across the cell membrane is also less at lower temperature.

l

Table 3.2 Biochemical Changes in Stored Blood

		CPD		**CPDA-1**		
Characteristics	Whole Blood	**Whole Blood**	**Whole Blood**	**Whole** Blood	**Red** Cells	Red Cells
Days of storage	0	**21**	**0**	35	0	35
% Viable cells (24 hours after transfusion)	100	**80**	100	79	100	71
pH (measured at 37 °C)	7.20	6.84	7.55	6.98	7.60	6.71
ATP (% of initial value)	100	86 (±16)	100	56.0	100 (±12)	45
2,3-DPG (% of initial value)	100	44	100	<10	100	<10
Plasma K^+ (mmol/L)	3.9	21	5.10	27.30	4.20	78.5
Plasma Na^+ (mmol/L)		156	169	155.0	-	111.0
Plasma Hb(mg/L)	17	191	78	461	82	658.0

Addtive Solutions

Traditional preservatives were put into use when whole blood was the major product. With the advent of component therapy use of red cells increased. This resulted in several problems in preservation. In preparing red cell concentrates 40% adenine and glucose present in CPDA-1 solution is removed with plasma and there is decrease in viability of the red cells, particularly in the last two weeks of storage. Red cell concentrates relatively void of plasma are more viscous and difficult to infuse in emergency situations. To overcome this problem red cell concentrates are prepared with hematocrits of less than 80%. This allows adequate plasma to remain for red cells nourishment and to improve flow properties. This results in lower plasma yields, affecting fresh frozen plasma and cryoprecipitate production.

The use of additive solutions allows recovery of maximum amount of plasma and preparation of red cells unit with a final hematocrit of about 60%. This new blood collection system has a primary bag containing a standard anticoagulant (CPD) and a satellite bag containing an additive solution. Blood is collected in the primary bag containing anticoagulant solution. After the plasma is removed from the whole blood into another empty satellite bag, the additive solution is added to the red cells, thus providing nutrients to red cells for improved viability. The red cells can be stored for six weeks at 2-6°C. The additive solution should be added to red cells within 72 hours since phlebotomy.

Three additive solutions are available (1) Adsol (AS-1) [Baxter Laboratories], (2) Nutricel (AS-3) [Medsep Corporation, formerly Cutter Biologicals] and Optisol (AS-5) [Terumo Corporation |.

Table 3.3 Composition of Additive Solutions

	As-1	AS-3	**AS-5**
Sodium Citrate	-	588 mg	-
Monobasic sodium phosphate	-	276 mg	-
Citric acid	-	42 mg	-
Dextrose	2.20 g	l.l0 g	900 mg
Adenine	27 mg	30 mg	30 mg
Mannitol	750 mg		525 mg
Sodium chloride	900 mg	410 mg	877 mg
Volume	100 ml	100 ml	100 ml
Primary bag anticoagulant	CPD	CP2D	CPD

One major benefit of the additive system is increase in the level of ATP, and red cells viability is enhanced, extending the shelf-life of the red cells to 42 days. This facilitates better inventory control of blood as well as wider use in pre-deposit autologous donations. More than 80% red cells survive in circulation 24 hours after transfusion of blood stored for 42 days. The additive solutions do not increase 2,3-DPG levels. Additive solution having mannitol are not routinely used for exchange or neonatal transfusion. There is no restriction on the use of additive solutions in any other type of transfusion recipients. In addition, the use of additive solutions allows extraction of more plasma/platelet rich plasma for optimal production of platelets, factor VIII yields and fresh frozen plasma.

Table 3.4 Biochemical Changes in Stored Additive Red Cells

		As-3 !	AS-5
Days of Storage	42	42	42
% of viable cells (24 hours after transfusion)	76(64-83)	83±10	80.0
pH (measured at 37°C)	6.6	6.5	6.5
2,3, DPG (% of initial level)	<5.0	<10.0	<5.0
ATP (% of initial level)	60.0	58.0	68.5
Plasma K^+(mmol/L)	50	N/A	45.6
Plasma Na^+(mmol/L)	117	121	N/A
% of hemolysis	0.5	0.7	0.6

Heparin

Heparin prevents coagulation by inactivating the prophylactic activity of thrombin after complexing with AT 111 and thrombin.

1000 IU of heparin is equal to 10 mg heparin but IU and mg are not strictly interchangeable because commercial preparations of heparin vary in composition.

Dose of heparin for anticoagulation is 0.5-2.0 IU/ml. of blood e.g. approximately 500 IU of heparin for 500 ml of blood.

l

Heparinized blood should be used with in 24 hours. Earlier heparinized blood was used in open heart surgery but now usually it is not used as extracorporeal pumps are now usually primed with crystalloids and not with blood.

The effect of heparin can be neutralized with protamine sulphate. 1 mg of protamine sulphate neutralizes 1 mg of heparin e.g. to neutralize 5000 units of heparin (50 mg), 5 ml of 1 % solution of protamine sulphate will be needed.

Rejuvenate Solutions

Rejuvenate solutions having phosphate, inosine, glucose, pyruvate and adenine increase the levels of 2,3-DPG and ATP in stored red cells. These solutions can be added at any time between 3 days post collection and 3 days after expiration of red cells. The solution is added directly to the red cells, mixed and incubated at 37 °C for one hour.

The rejuvenated red cells are either washed with saline (2 Litres of unbuffered 0.9% NaCl) and can be kept at 2-6°C, however, it should be transfused with in 24 hours after washing or they are glycerolized for keeping red cells in frozen state to improve the quality of red cells.

Red blood cells rejuvenation solution, 50 ml sterile vial (Rejuvesol, Cytosol Laboratories, Braintree, MA) is commercially available. The rejuvenation process is expensive and time consuming and is rarely used.

Plastic Bags

Many other factors may limit the viability of transfused red cells. One of the factors is also the plastic material used for the bags. The plastic material should be sufficiently permeable to CO2 in order to maintain higher pH during storage. Currently the blood is stored in plastic bags made of polyvinyl chloride (PVC) with plasticizer, di-(2-ethylhexyl) phthalate (DEHP). It is known that DEHP leaches from plastic into plasma and cell membrane during storage and may be harmful to the patient. The accumulation of excessive amount of acid due to glycosis even at low storage temperature is also a major problem in liquid preservation of red cells. So there is need to develop improved plastic blood bags as well as preservative solutions.

RED CELL FREEZING

Smith in 1950 reported that glycerol could prevent freezing injury in human red cells and that red cells, mixed with glycerol could be frozen without damage.

Effect of Freezing

It is believed that freezing damages red cells due to the intracellular ice formation and probably to some extent due to hypertonicity. If glycerol (cryoprotective agent) is added to the cells they can be frozen and thawed without damage (Polge et al. 1949). The effect of the glycerol is probably due to the fact that it limits ice formation and provides liquid phase in which salts are distributed; as cooling proceeds excessive hypertonicity is also avoided (Lovelock, 1953). Glycerol which permeates red cells fairly rapidly during freezing is most effective in protecting the human red cells.

Frozen red cells are primarily used for autologous transfusion and the storage of rare group blood. For freezing red cells a cryoprotective agent is added to red cells that are less than 6 days old. Glycerol is used most commonly and is added to the red cells slowly with vigorous shaking so that glycerol permeates into the red cells. The cells are rapidly frozen and stored in a freezer. The freezing and storage temperature depends on the concentration of glycerol. Two concentrations are used to freeze red cells, a high concentration glycerol |40% weight in volume (w/v) and a low

concentration glycerol [20% weight in volume (w/v)] in the final concentration of cryopreservative. Most blood banks use the high glycerol technique.

Table 3.5 ADVANTAGES OF HIGH CONCENTRATION GLYCEROL TECNIQUE OVER LOW CONCENTRATION GLYCEROL TEHNIQUE

Advantages	High Glycerol	Low Glycerol
Initial freezing temperature	-80 °C	-196 °C
Need to control freezing	No	Yes
Type of freezer	Mechanical	Liquid nitrogen
Maximum storage temperature	-65 °C	-120 °C
Shipping requirement	Dry ice	Liquid nitrogen

Frozen cells are deglycerolized before transfusion. Removal of glycerol is achieved by systematically replacing the cryoprotectant with decreasing concentrations of saline. The cells are washed with 12% saline, followed by 1.6% saline, with a final wash with 0.2% dextrose in normal saline. The shelf life of thawed red cells stored at 2-6 °C is 24 hours. Commercially available Cell Washing System manufactured by several companies can be used.

Generally cells are glycerolized and frozen with in 6 days of collection of blood in CPD or CPDA-1. Red cells stored in additive solutions can be frozen up to 42 days.

The frozen red cells can be stored for 10 years. The outdating period of the thawed red cells stored at 2-6°C is 24 hours.

Method of freezing and preservation of red cells in frozen state:

1 Glycerolized red cells having final concentration of 40% W/V of glycerol can be frozen at -80°C over a period of 30 min using mechanical refrigeration, then can be preserved at -60 to -65°C for 10 years.

2 Glycerolized red cells having final concentration of 20% W/V of glycerol can be frozen at - 196°C using liquid nitrogen for 2-3 minutes and can be preserved in the gas phase of liquid nitrogen at -120°C for 3 years.

High glycerol solution (40% W/V concentration). The glycerolizing solution consists of 6.2 M glycerol solution that contains 57 gm% glycerol, 1.6 gm% Na lactate, 0.03 gm% KCl and a total of 25 mEq/1 of monobasic and disodium phosphates to produce a pH of approximately 6.8 (Meryman et al. 1972)

Prior of glycerolization, whole blood in CPD solution, fresh or stored at 4°C for no more than 3-4 days, is centrifuged at 3000x g for 7 min; supernatant plasma is expressed into satellite bag and used for preparation of components or freezed. Appropriate volume of 6.2 M glycerol solution is added in two stages e.g. 300 ml when the weight of the packed red cells is 150-230 g. First 100 ml of glycerolizing solution is added to the cells in the collecting bag with vigorous shaking. After at least two minutes of equilibration, the remainder glycerol solution and the partially glycerolized cells are transferred to a 850 ml polyolefin bag (Hebia blood bag). The bag is centrifuged, the supernatant is expressed and the red cells are frozen at -80°C using a deep freezer. They can then be stored at -60 to -65‘‘C.

Low Glycerol Solution (20% W/V cencentration)

The glycerolizing solution consists of 35.0 gm% glycerol, 2.88% mannitol, and 0.65g sodium chloride.

Whole blood in CPD is centrifuged at 3000x g for seven minutes. Plasma is taken off and freezed. Low glycerol solution equal in volume to the red cells (e.g. 250 ml of solution for 250 ml of red cells) is added with vigorous shaking. The glycerolized red blood cells are transferred to a polyolefin plastic bag and kept in aluminium container which is then placed upright in a bath of liquid nitrogen at -196°C and then stored at -120°C in liquid nitrogen vapour.

Thawing and Deglycerolizing

Frozen red cells are thawed in a water bath at 37°C for about 10 min. Glycerol must be properly removed from the thawed cell to avoid haemolysis in vivo and/or in vitro. The intracellular environment of glycerolized cells is hypertonic relative to plasma and the first solution used for deglycerolization must be also somewhat hypertonic. This allows the glycerol to begin diffusing out of the red cells while the intracellular environment remains hypertonic. Subsequently followed by washing with solution progressively less hypertonic and finally with isotonic electrolyte solution containing glucose. Glycerol contents must be reduced to 1-2% otherwise they will haemolyse on contact with plasma.

The 40% W/V glycerolized red cells are diluted with 150 ml of 12g% sodium chloride buffered to about pH 7.2 with 0.15% disodium phosphate and allowed to equilibrate for 5 minutes. Then wash with one to two litres of 1.6g% sodium chloride solution buffered to pH 7.2 with 0.03g% of disodium phosphate and finally with one litre of 0.9g% sodium chloride solution containing 0.2g% glucose buffered with 0.065g% disodium phosphate to a pH of about 6.8 (isotonic glucose solution). The washed cells are finally suspended in isotonic glucose solution and ready for transfusion. The shelf-life of the processed unit is 24h.

The 20% W/V glycerolized red cells are diluted with 500 ml of 3.2g% sodium chloride solution buffered to about pH 7.2 and then washed with 1-2 litres of 0.9g% sodium chloride solution containing 0.2g% glucose buffered with 0.065g% disodium phosphate to pH 6.8. The washed cells are finally suspended in isotonic glucose saline and ready for transfusion. Shelf-life of the processed unit is 24 h.

The washing can be carried out either by continuous flow washing in the Haemonetic Blood Processor or by continuous flow washing in Fenwal Elutramatic system or serial centrifugation in the IBM blood processor. Protocols for each instrument should be followed as advised by the manufacturers.

Clinical consideration

1. Deglycerolized red cells are comparable in volume and Hct to standard unit of red cells.
2. Deglycerolized red cells consist of red cells in electrolyte solution. Virtually all plasma, anti-coagulants and most of the leucocytes and platelets have been removed.
3. In vivo survival and functions of red cells are comparable to fresh drawn liquid stored red cells because 2,3-DPG and oxygen dissociation curves are normal.

Indications for the use of frozen red cells

1 Freezing of rare blood groups enables long-term storage and supply on a regional and national basis.
2 Storage of blood for patients with antibodies against high frequency antigens.
3 Storage of blood for autotransfusion, specially in patients with rare blood group.
4 Prevention of non-haemolytic febrile transfusion reaction in patients sensitized to leucocytes, platelets or plasma protein.
5 Prevention of sensitization against HLA antigens in potential recipients of tissue transplants.

FREEZING AND THAWING OF RED CELLS (REAGENT CELLS)
USING HIGH GLYCEROL CONCENTRATION SOLUTIONS

Solutions required

1. *5% trisodium citrate ($Na_3C6H_50_7$)*
   I. weigh 100 g of trisodium citrate
   II. place in 2 Litre volumetric flask
   III. half fill with distilled water and mix until crystals are dissolved.
   IV. make up to 2 litre with distilled water and mix
2. *12% glycerol solution*
   Dissolve 120 ml analytical grade glycerol in 880 ml of 5% trisodium citrate solution

3. *60% glycerol solution*
Dissolve 600 ml analytical grade glycerol in 400 ml of 5% trisodium citrate solution

Freezing Procedure :

1 Blood is collected in CPDA-1 double pack. The pack is centrifuged at 3000 X g for 7 min and the plasma is removed.
2 12% glycerol solution equal to half the volume of the packed cells is added to the packed cells, mixed and allowed to equilibrate at RT for 10 min.
3 60% glycerol solution equal to half the volume of the packed cells is added to the pack and mixed and allowed to equilibrate for 10 min.
4 Dispense into labeled 10 ml sterile plastic/glass tubes (15X100 mm)
5 Freeze at -40° C, keep it in lowest part of freezer and switch freezer to rapid freeze or place in top of liquid nitrogen tank, i.e. vapour phase for 5-10 min.
Note: Tubes should be placed in a metal rack for faster freezing.
6 When frozen, tubes may be stored at -20° C.

Procedure for thawing and deglycerolization:

1 Thaw red cells in water bath at 37° C.
2 Prepare 25 cm strips of dialysing tubing by soaking in 0.9% saline. Seal one end by folding.
3 Transfer thawed red cells to dialysing tubing. Seal both ends by folding and clamp with paper clip.
4 Suspend tubing by means of paper clip supported by an applicator stick in a large beaker having *0.99c* saline (approximately 2 L).
Note: Cells in 4 dialysing tubings can be immersed in the same beaker for dialysing.

5 Dialyze for at least 1 -2 h at RT or overnight at 4° C.
6 Transfer cells from tubing to labelled tubes, spin and wash cells with 0.9% saline until supernatant is clear.
7. Add an equal volume of AB serum to packed cells.
8. Stopper lubes and store at 4"C.

PLATELETPRESERVATION

The preservation of viable and functional platelets depends on the following factors:

Temperature

Platelets should be stored at 22-24° C (controlled temperature) with continuous gentle agitation in platelet incubator and agitator.

pH
pH should be above 6.0.

Plastic Bag

Maintenance of pH and function of platelets depend on the permeability of the storage bag to oxygen and carbon dioxide. Platelets stored in bags made of polyvinyl chloride (PVC) with plasticizer di-(2-ethylhexyl) phthalate (DEHP) have shelf life of 3 days. New plastic bags made of polyolefin with no plasticizer (Baxter's PL 732) and thin walled PVC with tri-(2-ethylhexyl) trimellate plasticizer (TOTM) [Baxter's PL 1240 and Cutter CLX] maintain pH and functions up to about 7 days. However it is recommended to store platelets in new bags for 5 days only from the date of collection of blood

The pooled platelets can be stored for 4 hours at 22-24° C before they are transfused.

FRESH FROZEN PLASMA (FFP)
Shelf life of FFP is 12 months at - 18°C or lower. After thaw FFP can be stored at 2-6° C for 12 hours before transfusion. If FFP can not be used with in 1 year or thawed plasma is not used within 12 hours it is re-designated as single donor plasma which can be stored farther for 4 years at -18° C or lower.

SINGLE DONOR PLASMA
Shelf-life of Single donor plasma is 5 years at -18° C or lower. Thawed plasma can be stored at 2-6 °C for 5 days before transfusion

CRYOPRECIPITATE (Factor VIII)
Croprecipitate can be stored for 12 months at -18° C or lower. Thawed Cryoprecipitate can be stored for 6 hours at 2-6° C and pooled cryoprecipitate kept at 2-6° C should be used within 4 hours

GRANULOCYTES

The shelf life of granulocytes is 24 hours at 22-24$^{(1}$C. They do not need agitation. Post transfusion recovery of granulocytes in circulation and migration into inflammatory loci is better if transfused with in 8 hours of storage than that if granulocytes stored for 24 hours.

SHIPPING OF BLOOD PRODUCTS

The temperature of blood and blood products should be monitored during shipment from one place to another. Freshly drawn blood, which will not be used for preparing platelets, should be transported at the temperature between 2-10°C. Units from which platelets are to be made must be transported at temperature 20-24° C.

Transporting Red Cells/Whole Blood

Transporting red cells or whole blood from one facility to another requires that the temperature of the blood during shipment be kept between 2-10°C. Sturdy, well insulated carriers with some type of refrigerant are used. Wet ice is a good refrigerant for red cell shipment. Ice should be put at the top inside the container so that the cool moves down through the shipping box. In climates that are extremely warm or where the shipment journey will be lengthy, ice may be placed at the bottom also of the container. Ice should not come in direct contact with blood at any time because it can cause hemolysis of the red cells or blood.

Transporting red cells/whole blood with in the hospital

The blood and red cells should be transported within the hospital in insulated carrier or in cold insulated boxes if the ambient temperature is more than 25° C. Instructions should be given to keep in refrigerator if there is possibility that blood/red cells will not be transfused immediately.

Shipping of Platelets

Shipping of stored platelets to the transfusion facility should use a well-insulated container, with no ice, to maintain the temperature between 20-24° C.

Shipping of Frozen Components

Fresh frozen plasma and cryoprecipitate must be shipped at -18° C or below. Dry ice is used to maintain the frozen state. Dry ice should be kept at the bottom and at the top inside the well-insulated container. These frozen products are fragile, insulate the product by dry packing material or plastic air bubbles to check breakage during shipment.

General Requirements for Storage of Blood Products

The anticoagulant/preservative solutions are not the sole variables in maintaining the viability and stability of the blood and its products. Physical storage conditions best suited for products maximum survival are also important.

All storage equipment, whether a refrigerator, a freezer or a platelet environmental chamber should have the following facilities:

- Refrigerators for storing blood/red cells should have a fan for circulating air to ensure proper temperature 2-4° C in all compartments.
- Refrigerators, freezers and platelets incubators should have a system to monitor and record the temperature continuously. Temperature recording charts should be changed regularly. They should also have alarm systems with audible signals. These facilities should have battery back up.
- Separate areas should be reserved for storing untested, tested and quarantined products. No food should be stored in the refrigerators and freezers.

Table 3.6 Blood Component Storage Summary

Product	Description	Storage °C	Expiration
Whole Blood Red Cells	All components of donor blood plus anticoagulant CPD, CPDA-1 whole blood with 200-250 plasma removed; final volume- 300 ml, Hct < 80% AS : with most plasma removed And 100 ml additive soln. added; Final volume - 350 ml Hct 55-65%	2-6 ml 2-6	CPD — 21 days CPDA-1 - 35 days Closed system - see whole blood Open system - 24 hr. AS additive - 42 days
Red Cells - Leucocytes Reduced	R.C. modified by centrifugation removing buffy coat or filtration to remove >70% leucocytes while retaining >70% of original R.C.	2-6	Closed system - see whole blood/Red cells Open system - 24hr.
Red Cells Washed	R.C. washed with normal saline;	2-6	24 hr
Red Cells Frozen-Deglycerol	R.C. frozen with glycerol,	Frozen at - 65 (high glycerol) - 120 (low glycerol) Deglycerolized-at 2-6	10 yr 3 yr 24 hr
Platelets	>5.5 x $l0^{10}$ in 40-70 ml plasma	20 - 24 with agitation	3 or 5 days depending on storage bag 4 hr after pooling
Platelets, pheresis	>3.0x 10" in about 200 ml plasma	20 - 24 with agitation	5 days
Granulocytes	About 1 x $l0^{10}$ in 200-250 ml plasma	20 - 24 without agitation	24 hrs
FFP	200-250 ml with anticoagulant	Frozen -18 or below Thawed 2-6	l yr 12 hr
Plasma	200-250 ml with anticoagulant Liquid plasma	Frozen -18 or below Thawed 2-6	5yr 5 days after whole blood expiration
Cryoprecipitated AHF	80-120 units Factor Vlll 40-70% von Willebrand factor	Frozen < -18 Thawed 2-6 After pooling	l yr 6 hr 4 hr

INSPECTION OF BLOOD

Blood must be inspected prior to transferring the units from one facility to another or issuing for patient use. The blood should be inspected to check for possible bacterial contamination, which may produce abnormal colour in the red cells or plasma. Units are inspected for hemolysis, visible clots, or purple, brown and red plasma. Abnormal units should not be issued and the cause should

be investigated. Plasma with a green hue need not to be rejected because this is caused by exposure of bilirubin pigments to the light.
Prior to issue plasma in both the main bag and segments should be visually inspected for hemolysis or discolouration.
Yercinia enterocolitica, a bacterium, can grow at 4°C, the PO of the unit decreases and the blood is hemolyzed. This metabolic activity in the unit is not duplicated in the sealed segments. When the segments of the contaminated units are cultured, they are found to be sterile, while the blood from the unit may grow yercinia entrocolitica. This causes change of colour in the unit but not the colour of the segment.
In case of hemolysed blood the colour of blood both in bag and tubing is changed to purple.

4
Principles of Immunohematology

The Concepts of immunology are derived from the study of resistance to infections. Contribution to immunology comes from both the basic sciences (biochemistry, genetics, and hematology) and the study of clinical entities (allergy, infectious diseases, organs transplant, immune deficiency diseases and oncology).

Role of Immune System

The immune system plays two important roles in the human body. First it provides the immune mechanism which protects the body against external foreign substances, and second, it plays important role in the identification and destruction of abnormal cells. These abnormal cells may be malignant cells, cells infected with micro-organism or cells coated with antibodies.

COMPONENTS OF THE IMMUNE SYSTEM

Cells and Tissues of the immune system

Human immune system consists of a number of organs and cells which recognize non-self antigens accurately and specifically. Pluripotential cells, whose descendants, the lymphopoietic stem cells and hematopoietic stem cells are located within bone marrow, fetal liver, and yolk sac of the fetus, give rise to both inflammatory and immune cell lines. Off-springs of these stem cells, differentiate into red blood cells, platelets, leukocytes and phagocytic cells.

PLURIPOTENTIAL STEM CELLS

Hematopoietic stem cells	Lymphopoietic stem cells
Erythroid precursor Platelet precursor Granulocyte/monocyte precursor	B-cells precursor T-cells precursor
Erythrocyte progenitor Megakeryocyte progenitor Granulocyte progenitor Monocyte progenitor	B-cell progenitor T-cell progenitor
Red cells Platelet Neutrophil Monocyte	Bcell Tcell
Eosinophil Macrophase	Null cell
Basophil	NKcell

Immune system cells are found in blood, thymus, spleen, liver, lymph nodes and tissues.

Monocyte- Macrophase Cell System

Bone marrow stem cell's produce blood *monocytes, which circulate to sites of inflammation or* migrate to various tissues. Monocytes in tissue differentiate into tissue macrophages, and are found throughout the body but specially prominent in the liver, spleen and lymph nodes.

Different types of receptors and histocompatibility antigens are present on the surface of macrophages and they are important in blood banking.

An important cell surface receptor on macrophages is the receptor for the Fc portion of immunoglobulin. Cells coated with antibody can attach to macrophages when the Fc portion of immunoglobulin comes in contact with the Fc receptor on macrophage. In this way many kinds of abnormal cells - microbial planted, autologous or transfused cells may be removed from the circulation by macrophages.

Tissue macrophages also possess receptor for C3b component of complement. Cells or micro-organisms to which complement is attached can be removed by phagocytic macrophages. Some blood group antibodies are capable of complement activation, resulting in complement coated red cells. These cells are susceptible to C3b receptor on macrophages and they are removed by the macrophages.

A second group of cell surface protein found on macrophages are those of major histocompatibility. Macrophages express both class 1 (HLA -A, -B, -C) and class 11 (HLA -D, -DR, -DQ, -DC) antigens. Class 11 antigens which are important for transplantation immunity. The macrophages participate in phagocytosis, inflamation, and immunity.

LYMPHOCYTES

Lymhocytes differentiate into T-cells, B-cells and Null cells. They may be similar in appearance but they can be distinguished by the presence of distinctive cell markers.

T Lymphocytes (T-Cells)

T lymphocytes originate from lymphopoietic stem cells in the bone marrow. Precursor cells leave bone marrow and travel to thymus gland where they devel P and are released into the circulation as mature T-cells. About 75 to 80in the blood are T-cells. T-cells

circulate from the blood to lymphatic organs and return to blood stream via lymphatic ducts. The different kinds of T-cells are named according to their respective function: helper T-cells, suppression T-cells, cytotoxic T-cells and others.

Many proteins on T-cells are identified by monoclonal antibodies, and have been classified as clusters of differentiation (CD) antigens. Out of these antigens, the CD4 and CD8 are significant because their detection and identification is useful in some clinical conditions. CD4 cells enhance and promote the action of other immune cells and are called helper T-cells; CD8 cells have suppressive or cytotoxic effects and are called suppressor T-cells.

CD4 markers recognize antigen together with maximum histocompatibility complex (MHC) class 11 molecules. CD8 markers interact with MHC class 1 molecules.

The helper T-cells : suppressor T-cells ratio in healthy and immune-competent individual is about 2:1. In acquired immunodeficiency syndrome (AIDS), the virus destroys CD4 cells, and their number in the body decline and there is a decrease in CD4 : CD8 ratio.
T-cells participate in the regularization of humoral (antibody) response and cellular response.

B Lymphocytes (B-Cells)

B lymphocytes derive from lymphopoietic stem cells and develop in bone marrow in humans. Like T-cells, B-cells circulate from the blood to lymphatic organs and return to blood stream via lymphatic ducts. About 15% of lymphocytes are B-cells.

B-cells are the most important cells of the humoral immune system. B-cells are precursors of antibody producing plasma cells. B-cells, like other immune cells, exist in a resting form and an activated form. If B-cells are triggered they undergo a process called clonal expansion producing one kind of antibody and the proliferation of that particular cell line. However, in most cases, the trigger for B-cell antibody production also requires the presence of helper T-cells. These helper T- cells secrete chemicals (lymphokines), known as B-cell growth factors, that bind to specific B-cell receptors and induce clonal expansion and antibody production.

Many surface proteins have been recognized on B-cells and are grouped into three categories: cell surface markers, major histocompatibility antigens (MHC clas 11), surface immunoglobulin and receptors. Each B-cell expresses only one type of surface immunoglobulin and when triggered produce only one type of antibody. Therefore, the surface immunoglbulin on B-cell serves as the receptor for the particular antigen to which that B-cell reacts.

The majority of activated B-cells differentiate into IgM-secreting plasma cells. Upon continued stimulation, B-cells frequently change the expression of its heavy chain, known as isotype switching. This usually involves an IgM-to-IgG switch. This also results in the generation of memory B-cells with IgG surface immunoglobulin and the secondary antibody response results in the production of IgG antibody.

Null Cells

Some lymphocytes that do not carry T or B cells markers are found in blood and are called natural killer NK cells. These cells have the capacity to lyse or destroy a wide variety of infected cells and tumor cells without any apparent antigenic stimulus.

IMMUNE RESPONSE

Immune response may be:

a) Innate immune response (non-specific)
b) Adaptive immune response (specific)

Innate Immunity

Innate or natural immunity is the primary line of defense. First line of this defense mechanism is always present since birth and protect the individual from foreign invaders and does not change on repeated exposures to the same antigen, so it is considered as non-specific. This mechanism consists of physical and biochemical barriers that prevent the entry of pathogens into the body.

The exterior of the human body (skin) and mucous membranes are impermeable to most infections. Biochemical defenses that inhibit or destroy bacterial growth are lactic acid and fatty acid in sweat, sebaceous secretions. Many body secretions like tears, nasal secretions, and saliva have lysozyme (serum protein enzyme) has the capability to destroy bacterial cell wall. Biochemical defenses are also present in other parts of the body.

The innate system's second line of defense is internal. If an organism penetrates epithelial surface, leucocytes and phagocyte cells recognize foreign or non-self organisms they bind them, internalize them and destroy them.

Adaptive Immunity

Adaptive immunity has the ability of specificity, recognition, memory and specific reactivity. The ability to recognize 'self and 'non-self is established in fetal life but the immune response develop later after initial contact with the foreign substance. The acquired immunity is specific and the antibody recognizes to the antigen which initialized its production. The acquired immunity has memory, the ability to remember the antigen and to improve the immune defense process upon successive exposure.

Non-self or foreign material initiates adaptive changes that result in either production of antibodies called humoral response or cell mediated response

Cellular Immunity

Cell mediated immunity or cellular immunity, is localized reaction to organism, usually intracellular pathogens, mediated by lymphocytes and phagocytes rather than by antibody production. T-cells and macrophages play major role in cell mediated immunity, either as a result of direct cytotoxicity or through the liberation of lymphokines. Cell mediated cytotoxicity is important in lysis of virus infected cells and rejection of allo-graft and tumor cells.

Other cytotoxic cells involved in cell mediated immune response are null cells (NK). These NK cells are able to attack the target cells and kill them.

Humoral Immunity

Humoral immunity is mediated by antibodies produced by Lymphocytes (B-cells). Antibody has the capability of reacting with the specific antigen responsible for its production.

Antibody production involves two type of cells-macrophages, B-cells and T-cells. Mostly antigens that stimulate this response are T-dependent antigens, however, some antigens, particularly polysaccharides, activate B-cells to produce antibody independent of T-cells. In general antibody produced by T-independent antigen is IgM..

Cytokins produced by activated T-cells are important for many phases of B-cells response, and are essential for isotype switching from IgM-to-IgG and for the generation of memory cells.

CYTOKINES

Cytokins are polypeptides that act as biological mediators of immune and tissue cells. Cytokins are divided into lymphokines, which are produced by Lymphocytes, monocyte and macrophages. Cytokines modulate the host response to antigens by regulating growth, mobility and differentiation of leucocytes.

A variety of lymphokins are secreted by a number of different types of cells:

Lymphokines	Activity
• Interleukin (IL-2)	Stimulate T-cell proliferation
• Interferon-y	Enhances macrophages cytotoxic activity
• B-cell growth factor (IL-9)	Stimulates cell division
• B-cell differentiation factor (IL-6)	Stimulates B-cell differentiation into plasma cells
• Interleukin (IL-3)	Stimulates T-cells proliferation
• Interleukin -4 (IL-4) differentiation	Promotes T-cell and B-cell growth and
• Colony stimulating factors (CSF)	Growth of various cell lines

Endogenous Pyrogens

Endogenous pyrogens are low molecular weight proteins which are liberated by various leucocytes particularly monocytes, granulocytes and tissue macrophages, Three endogenous pyrogens have been recognized: interleukin-1 (IL-1), interferon (INFS) and tumour necrosis factor (TNFS). All these factors directly stimulate the synthesis of prostaglandin in the hypothalamus, the effect is to raise the level at which body temperature is set. Presumably, in the febrile reactions due to leucocyte antibodies in the recipient, IL-1 is released from donor leucocytes and causes fever.

PRIMARY AND SECONDARY IMMUNE RESPONSES

The immune responses, that occur when immunocompetent individual encounters a non-self antigen for the first time are primary immune responses. In addition to immune responses, the primary immune response generates memory cells. These memory cells contribute to the immune response on second or subsequent exposure to the same antigen - the secondary or anamnestic immune response

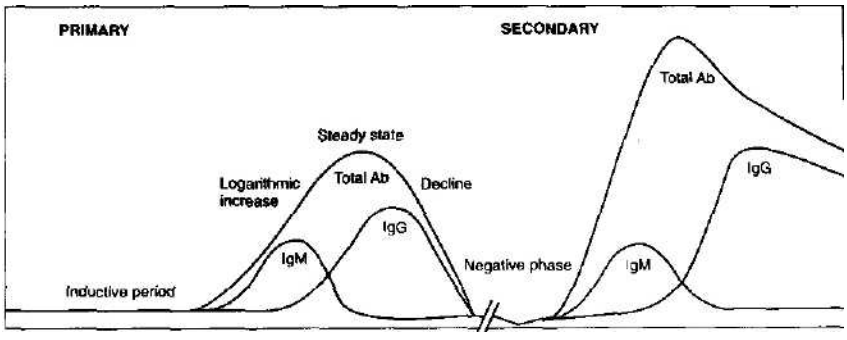


Fig. 4.1 Primary & Secondary Antibody responses

Detectable antibody production occurs after a few days to a few months following an antigen first encounter. During this latency period, T-cells and B-cells response is occurring and memory cells are formed. The secondary response becomes apparent more rapidly, with increased antibody level detectable in few hours.

Antibody class: Two classes of antibodies are made in the primary immune response. The earliest antibodies are IgM. With in 2-3 weeks IgM antibodies level decline and IgG antibodies are formed. This isotype switching from IgM-to-IgG antibody production is caused by T-cell lymhokines and DNA mutations in dividing B-cells.

In the secondary immune response, the memory cell responds to subsequent antigenic stimulation, forming mostly IgG antibodies. IgM antibodies, also formed at the beginning of the response, rapidly decline as they are replaced by igG antibdies (see Fig. 4.1)

The secondary response antibodies have a higher avidity for antigen, are produced by lower doses of antigen than antibodies formed during the primary response, and are usually formed quickly in 1-2 days.

HYBRIDOMAS

The monoclonal antibody technique devised by Kohler and Milsten has proved useful in producing high-titer and specific antibodies. Laboratory animals, usually mice are immunized for the production of monoclonal antibody. After suitable immune response, mouse spleen cells containing antibody-secreting lymphocytes are fused to neoplastic plasma cells of infinite reproductive capacity from a mouse (i.e. myeloma cells). The resulting hybridomas are screened for antibody with the required specificity and affinity. The antibody-secreting clones may then be propagated in tissue culture or by inoculation into mice, in which case the antibody is collected as ascites. The clonal line produces a single antibody, there is no need for absorption or to remove heterospecific antibodies. All antibody molecules produced by a clone of hybridoma cells are identical in terms of antibody structure and antigen specificity. Once one antibody-secreting clone of cells has been established, antibody with same specificity and reaction characteristics will be available indefinitely.

The blood banker's role is predominantly concerned with tests aimed at detecting blood group antigens and antibodies, their actions and their interaction with another system, the component system. As this role has expanded, especially in bone marrow and stem cells transplantation, a basic knowledge of the overall immune response is necessary

ANTIGENS

An antigen is a substance, usually a complex protein or polysaccharide in nature, which when introduced parentally into an individual whose tissues do not possess that particular substance, is capable of instituting the production of antibody specific to itself.

ESSENTIALS OF ANTIGENS

The substances should have sufficiently high molecular weight (more than 40,000-50,000 M.W) to act as antigens. Substances below 40,000 MV are called haptens. They generally fail to act as antigens and produce no antibody unless coupled to a carrier protein of a size approximately more than 10,000 M.W.

On the surface of red cells are minute glycoproteins and glycolipids, which are under genetic control. These substances are of sufficiently high molecular weight to act as antigens and are known as blood group antigens. The antigens on the red cells may be on, below or protruding from the surface of the red cell membrane. Therefore, in some instances, IgG molecules are capable of causing the agglutination of red cells if the corresponding antigen is on or protruding from the cell membrane but may not agglutinate when corresponding antigen is buried within the cell membrane.

The specificity of the blood group antigens has been shown to be determined by the sequential addition of sugar residues to a common precursor substance as a result of gene action. The precursor substance, which is located on or within red cell membrane, is composed of four molecules of three different sugars, viz. D-galactose (2 molecules), galactosamine (lmolecule) and N-acetyl glucosamine (one molecule). There are two types of precursor substances known as Type 1 and Type 2 which differ in terminal linkage (See Figs. 5.2,5.3 and 5.4 in chapter on ABO system). The action of the gene causes the production of an enzyme which in turn causes the addition of another sugar to the basic precursor substance, which determines the specificity of the antigen. For details see the ABO Blood Group System (Chapter5).

The number of antigen sites on the red cells varies according to specificity. Thus there are approximately lmillion ABO antigen sites and 25,000 Rh (D) antigen sites on red cells. Red cells antigens are present not only on red cells but some have also been detected on leucocytes, platelets, in body fluids, saliva, milk, seminal fluids, plasma (Lewis antigens) and in most tissues of the body. Antigens of some specificities are confined wholly on the red cell membrane (e.g. Rh antigens).

The antigens on the red cells are not always constant throughout life, Some antigens are poorly developed at birth (e.g. 1, Lewis). Some can be altered in certain disease states e.g. Acquired B antigen in A, individuals usually resulting from the action of a gram-negative organism. A or B antigens may be weakened in patients with leukaemia. Changes in blood group antigens have also been noted during pregnancy (e.g. Lewis).

Blood Groups Genetics

The blood group antigens are the products of specific genes. Each gene occupies a specific position (locus) on the DNA of the chromosome. The particular locus may be occupied by one or more different forms of the gene, and these alternatives are known as alleles. When these allelic genes on a pair of chromosomes are identical (i.e. the individual have inherited gene of same characteristic from both the parents), the individual is said to be homozygous for that factor or characteristic, when the alleles are different, the individual is said to be heterozygous. An individual who is homozygous for a particular gene will have antigen product of the respective gene in double dose (homozygous) and an individual who is heterozygous for a gene will have antigen product of respective genes in single dose (heterozygous). An antibody may react strongly with red cells of an individual who is homozygous for the corresponding antigen but react weakly or not at all with red cells of an individual who is heterozygous for an antigen.

A gene may be a dominant gene; this is always expressed as product antigen regardless of whether it occurs in homozygous or heterozygous state (e.g. antigens A, B, M and N). A gene may be recessive gene; this can produce product antigen only when it is in homozygous state (e.g. A_2). If both the inherited genes are dominant, the products of both the genes are detectable and they

are called co-dominant, genes (e.g. M and N; A and B). Certain blood group genes appear to produce no product even when present in a homozygous state; these are known as amorphous or amorphic genes (e.g. O).

Genotype and Phenotype

The term genotype refers to the sum total of genes present on the chromosomes regardless of whether or not they produce detectable products. The genotype is determined through direct testing of gene products (antigens) and family study.

The terms phenotype refers to the detectable products (antigens) demonstrated through direct testing only.

The concept of genotype and phenotype is explained below

Phenotype	Genotype
A1	**A1A1**
	A1O
\	**A2A2**
	A2O
B	**BB**
	BO
0	**00**
K	**KK**
	Kk

ANTIBODIES (IMMUNOGLOBULIN)

Antibodies belong to a group of proteins known as immunoglobulins (Igs) in the plasma/serum. These Igs make up approximately 20% of total serum/plasma proteins. There are five different forms of blood group antibodies (IgM, IgG, IgA, IgD and IgE).

All antibodies have two main features in common.

1. The basic structure of the molecule
2. The function of the molecule i.e. the ability to combine specifically with a corresponding antigen.

Blood group antibodies IgM, IgG or IgA are significant in blood banking and they are dealt in detail.

Production of antibodies

The introduction of red cell antigen into the circulation of an individual (lacking that antigen) may stimulate the production of a corresponding antibody. This may occur as a result of blood transfusion therapy or feto-maternal blood group incompatibility in pregnancy. These are called

incomplete or acquired antibodies (IgG). Mostly immune antibodies are IgG that reacts best at 37°C and require the use of anti-human globulin serum for detection. Common immune antibodies react with Rh, Kell, Duffy, Kidd and Ss blood group system.

Certain antibodies occur without known antigenic stimulus. These are known as complete or natural antibodies (lgM, lgA). Theoretically, these antibodies are produced in response to the substances in the environment that genetically are identical with or similar to red cells antigens. The common occurrence of naturally occuring antibodies suggests that their antigens are widely found in nature - that is in animals, bacteria and pollen. Mostly they are IgM cold agglutinins, which react best at room temperature or below, and activate complement components. Common naturally occurring antibodies react with the ABO, Hh, Ii, lewis, MN and P blood group systems. Individuals do not generally produce antibodies when red cells possess the corresponding antigen.

The structure of the immunoglobulin molecules. (Fig 4.2)

The basic immunoglobulin (1 g) unit consists of two identical polypeptide heavy (H) chains each having higher molecular weight (55,000-75,000 MW) and two identical polypeptide light (L) chains each having lower molecular weight (22,500 MW).

The immunoglobulins are named according to the structure of heavy (H) chains. The five types of heavy chains are designated as alpha (lgA), delta (igD), epsilon (lgE), gamma (IgG) and mu (1 gM). Only two types of light (L) chains Kappa and Lambda are found in all classes of immunoglobulins and two light chains of any Ig are identical being either Kappa or Lambda.

Heavy (H) polypeptide chains are joined by covalent (disulphide) and non-covalent bonds to each other and to a pair identical light (L) chain, The co-valent are mainly in the hinge region.

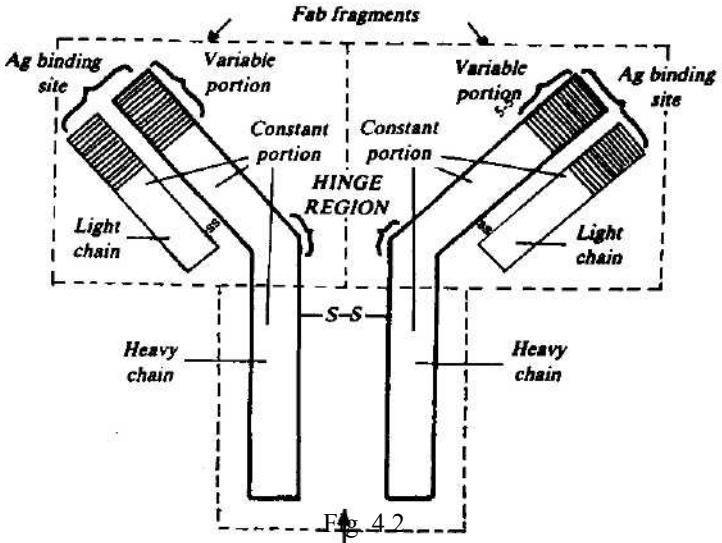


Fig. 4.2
The basic structure of Immuno globulin (Ig)

Basic chemical structure of antibodyMolecule

Each light chain contains 220 amino acids and each heavy chain has 440 amino acids. The first 110-120 amino acids of both heavy and light chains have a variable sequence and form variable region, which is considered to determine the antibody specificity. The rest of the light and heavy chains represent regions with constant amino acid sequence.

The heavy chain has a coiled hinge region that gives the molecule flexibility and it is in this region that enzymes or albumin act.

Antigen binding sites are believed to be located at the end of heavy and light chains in the variable region of the molecule. Biological activities other than antigen binding reactions are the responsibility of the constant portion of the heavy chains.

Functions of various parts of lg molecules.

Enzyme papain splits the immunoglobulin at the hinge region into three fragments. One crystallizable (Fc) fragment and two identical (Fb) fragments. The Fc fragment is composed of two halves of two heavy chains bound by co-valent bonds and it is responsible for complement fixation, and placental transfer.

Each Fb fragment is composed of one light chain and half of one heavy chain bound by covalent disulphide bonds. Antigen binding sites are believed to be located at the end of heavy and light chains in the variable region of the Ig molecule.

IgG Immunoglobulin

The IgG molecule consists of one basic structural unit known as a monomer, possessing two heavy chains (gamma chains) and two light (kappa or lambda). See Fig-4.3. Free IgG is usually Y-shaped but it is capable of changing its shape to combine with antigen or for other functions i.e. complement fixation. The change in shape is facilitated at the hinge region, where the chains uncoil, allowing considerable flexibility. IgG immuno-globulin can transfer from mother to fetus causing hemolytic disease of newborn.

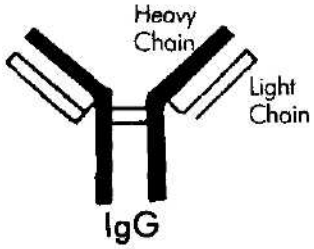


Fig. 4.3
IgG molecule

IgG immunoglobulins are antibodies to Rh, kell, Duffy, kidd and other systems antigens. These antibodies are detected by serological tests based on their characteristics, such as reactivity at 37° C, complement activation, and indirect globulin test.

IgG constitutes about 80% of the immunoglobulin in serum/plasma and is also present in extravascular fluid.

There are four sub-classes of IgG known on the basis of structural and morphological differences. These are IgG 1, IgG2, IgG3 and IgG4. IgG 1 and IgG3 activate the complement strongly but IgG2 activates weakly and IgG4 not at all. IgG in plasma have half-life of 23 days. More details are beyond the scope of this book.

IgM Immunoglobulin

The 1 gM molecule is made up of five basic structural units in a circular arrangement known as pentamer. It has additional disulphide bonds linking the Fc portions of alternate heavy chains so as to produce a molecule with central circular portion and five radiating arms (like a five-legged spider). See Fig-4.4. The typical pentamer IgM molecule has 10 antigen-antibody sites, however, owing to steric restriction, only 5 are functional at any time. In spite of this limitation, IgM are potent agglutinating antibodies and activate compliments. IgM is more effective than IgG in activating complement.

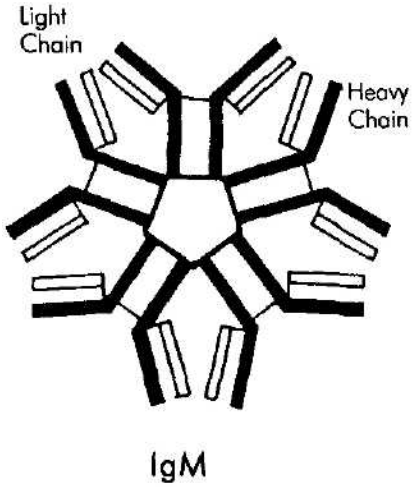


Fig. 4.4

IgM normally constitutes 10% of the immunoglobulin in serum/plasma, very few of these molecules diffuse into interstitial fluid. IgM in plasma have half-life of 5 days. IgM, natural antibodies, are in ABO system and in other groups like Lewis, Ii, P and MNS.

IgM does not cross the human placenta.

If both IgM and IgG antibodies are present in any serum, IgM may interfere with detection of clinically significant IgG antibodies by masking their activity. IgM can be dissociated through the cleavage of covalent bonds interconnecting the sub-units by the use of 2-mercaptoethanol (2-ME) or dithiothreitol (DTT). This treatment destroys both hemolyzing and agglutinating activities of IgM antibodies and IgG antibodies can be identified in a mixture of IgM and IgG antibodies.

Immunoglobulin (IgA)

IgA is usually present as monomer in plasma IgA is the only Ig present in epithelial secretions. In secretions IgA occurs as a dimmer, two IgA units are linked lo small glycoprotein known as secretory piece or T-components, which makes IgA more resistant to proteolytic enzyme. (See Fig-4.5). The presence of IgA in secretions is believed to be due to local production rather than transportation from plasma.

IgA does not fix complement and is not transported across the placenta. Although IgA has no bactericidal effect, it is thought to play an important role in localization of certain infectious agents.

IgA immunoglobulin is important in immunohematology. Approximately one-third of anti-A and anti-B are IgA class (two-third are IgM and occasionally IgG). Sometimes anti-IgA are formed following transfusion of plasma products in patients who have no IgA. Transfusion of even few milliliters of blood or its products having IgA may cause anaphylactic reaction in patients having anti-IgA.

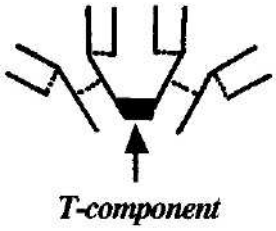


Fig. 4.5
Secretory IgA

Immunoglobulin (IgE)

Immunoglobulins IgE are normally monomoric and are found in a very low concentration in serum or plasma and has the shortest half-life span. IgE antbodies in patient blood may react with the foreign protein (allergen) in transfused blood or plasma products and histamine is released which causes allergic reactions or allergen in patient's blood and IgE antibody in transfused plasma may also react and causes allergic reaction.

No blood group antibodies have been reported to belong to this class.

Main Properties of immunoglobulins in serum

Characteristics	**IgG**	igM	**IgA**
Structure Spider	Monomer Y-shaped 2 units in Dimer	Pentamer Five legged Spider	Monomer Yshaped 2 units in Dimer
Molecular dimension	12.5x3nm	35x4.7nm	12.5 x 3 nm
Heavy chains	Gamma (y)	Mu (μ)	Alpha (a)
Light chains	Kappa or Lambda	Kappa or Lambda	Kappa or Lambda

Main Properties of immunoglobulins in serum (Continued)

Mol.weight ■ . \	150,000	900,000	160,000 330,000 (in dimer)
Sedimentation Coefficient	7S	19S	7S 105 S (in dimer)
Cone, in serum (mg/l00ml)	800-1680	50-190	140-420
Crosses placenta	Yes	No	No
Serological behaviour	Incomplete	Complete	Complete
Temperature of reaction	37°C.	20°C.	37°C
Serological behaviour After heating at 56°C for 3 h	Unaffected	Reduced	Unaffected
Complement fixation	Yes	Yes	No
Usual antigenic stimulus	Transfusion or pregnancy	Often naturally occurring	-
External secretions	No	No	Yes
Effect of 2-ME and DTT	Not affected	Inactivated	Partially inactivated
Valency (antigen binding sites)	Bivalent	Multivalent	-

Antibodies in the new born infants

Under normal circumstances the human fetus does not begin to synthesize antibodies to any significant degree until after birth.

IgM is the only type of immunoglobulin made by the fetus before and at the time of birth. Since IgM is not transferred across the placenta, all IgM antibodies in cord serum are of fetal origin. In general, IgM concentration in cord serum is between 5-10% of that found in adult serum. Several IgM antibodies can be found in cord serum, viz. Anti-I, anti-A and anti-B. The concentration of IgM starts to rise within 2-3 days after birth, reaches to 50% of that of the adult level in 2-3 months and 100% in about 9 months after birth.

At birth, the small amount of IgG in the cord serum is mostly maternal, that is the IgG derived from the mother by placental transfer.

IgA cannot be detected in the cord serum. By the age of 2 years, the amount of IgA in the serum reaches to about 20% of that of the adult level.

COMPLEMENT SYSTEM

The complement system proteins are the major component of the humoral innate immune response. The function of the complement system is the lysis of the cells through interaction with antibodies, mediation of phagocytosis through opsonization, and control of inflammation.

The complement system consists of a group of serum proteins that circulate in an inactive pro-enzyme state and are activated in a very precise biochemical sequence. The proteins in

complement system are given either numerical or letter designations. In many cases activation of the proteins is associated with cleavage of protein component. The larger fragment produced by the protein cleavage is responsible for continuation of the complement activation sequence. The smaller fragment often has the function of promoting the inflamatory response. These proteins can be activated by two main pathways using specific and non-specific immune mechanism that convert the inactive proenzymes into active enzymes. These two ways are:

1) *The Classic Pathway:-* Erythocytes antigen-antibody complexes activate the complement proteins C1-C9 by specific immune response.
2) *The Alternate Pathway:-* The complement proteins are activated by innate, non-specific immune reactions with polysaccharide and lipopolysaccharides found on the surface of many micro-organism, tumor cells and aggregates of IgA and IgG4 that do not activate complement component C1.

Sequence of Reactions of the Classic Pathway

This pathway is mostly responsible for the lysis of antibody sensitized red cells. Activation of the classic complement pathway is initiated by interaction of antigen with complement fixing antibody. Not all immunoglobulns (Ig) activate the classic pathway. IgM and IgG subclasses IgGl, IgG2 and IgG3 are the immunoglobulins that bind and activate CI. CI is a complex protein compound of three subunits, Clq, Clr, and Cls, which are held together in the presence of calcium. The nature of antigen-antibody-C1 interaction is not clear, but it is known that C1 binds to the Fc fragment of antibody in the antigen-antibody complex by a non-covalent bond.

The binding of CI to antibody leads to activation of CI. This activation causes cleavage of CI into Clq, Clr, and Cls. The activated antigen-antibody-C 1 complex now is capable of interacting with the next complement component C4 in sequence. C4 is cleaved by C1, acting as an enzyme, and two cleavage fragments C4 and C4b are formed. C4a floats free in the surrounding medium, and has anaphylatoxic activity. The activated C4b molecules on the cell surface attacks the proenzyme molecules C2. This reaction involves cleavage of the component C2 into a small free-floating C2b fragment and has no biological activity. The larger C2 a fragment attaches to the cell surface leading to the formation of activated C4b2a complex attached to the cell surface. After this Clqrs and immunoglobulin are no longer needed, if the antibody is dissociated, the process can still continue. The activated C4b2a complex is called C3 convertase and cleave C3 into two fragments C3a and C3b. C3a is released into surrounding medium and has anaphylatoxins activity. C3b is bound to the erythrocyte membrane and form activated C4b2a3b complex.

The C14b2a3b can bind and cleave C5 into two fragments, C5a and C5b. C5a is released into the surrounding medium and has anaphylatoxins activity. Activated C5b attaches to the cell membrane and is the nucleus for the formation of membrane attach complex.

Membrane attach complex-Membrane bound C5b binds C6, and *C7* by adsorption. This complex attaches to the cell membrane and binds C8, and C9 molecules. In the end terminal membrane attack complex C5b6789 is formed which lysis the cell.

Classic Pathway

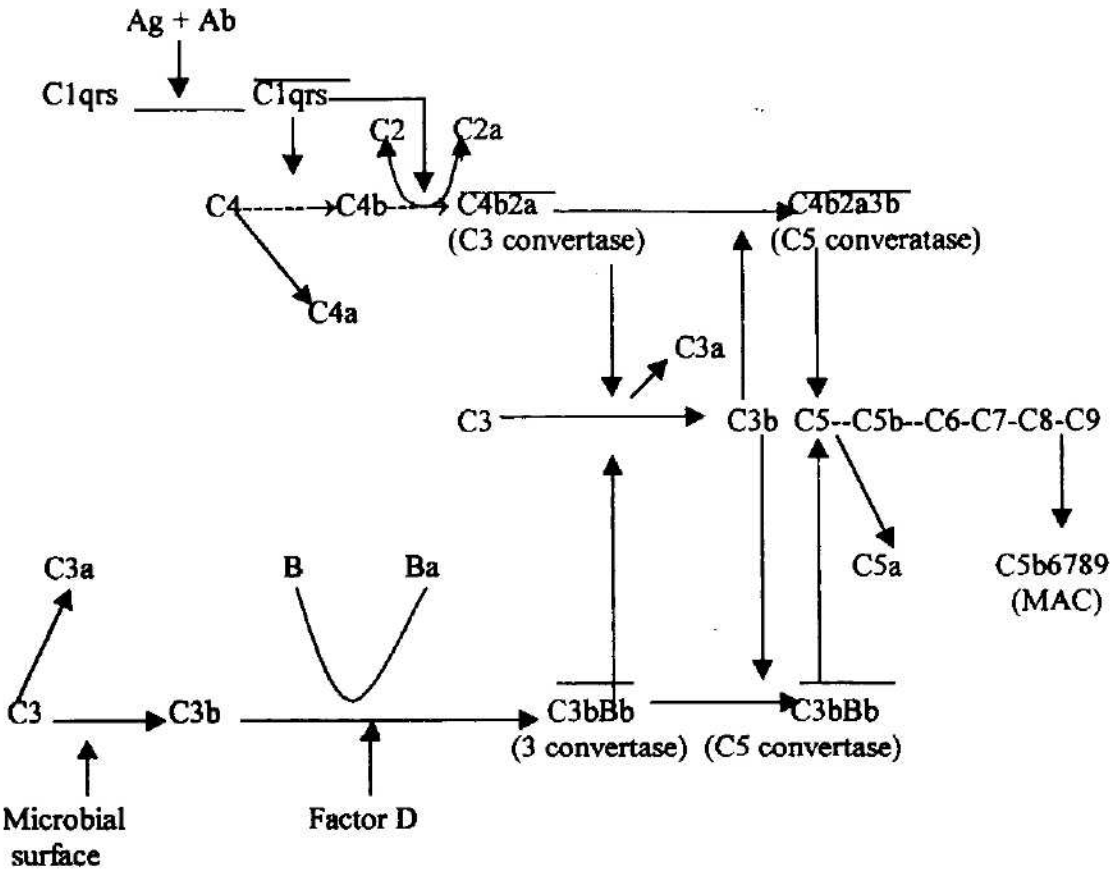


Fig. 4.6

Alternative Pathway

Figure- 4.6 Schematic diagram illustrating the sequential activation of the complement system via the classic and alternative pathways. The activated complement complex is indicated by the bar above the letters and numbers. The classic pathway utilizes C1, C4, C2, the alternative pathway utilizes factors **D, B** and C3.

Alternative Complement Pathway

The alternative pathway does not utilize the classic complement components, C1, C4 and C2 and also does not have an absolute requirement of antibody. Four proteins participate in the alternative pathway: factor B, factor D, properdinCfactor P), and C3. which are analogous to the components of classic complement pathway and which function to form a C3 cleaving enzyme. Factor D is analogous to CI of the classic pathway. Factor B is analogous to C2 and is important in the cleavage of C3. Small amount of C3b is generated continuously owing to the spontaneous hydrolytic cleavage of C3 molecules. C3b molecules bound to the surface of the target cells. C3b in combination with factors B and D forms an enzyme C3bBb which is capable of cleaving C3. This activated enzyme C3bBb interact with C3 of the classic pathway and form C3Bb3b which activate *C5* as in classic pathway and complement cascade continues. See Fig. 4.6.

8
The Antiglobulin Test

Antiglobulin serum (Coombs'Serum) was discovered by Coombs etal in 1945. The antiglobulin test can be used to detect red cells sensitized with IgG alloantibodies, IgG autoantibodies or complement components. Sensitization of red cells can occur in vivo or vitro. The use of AHG serum to detect sensitization of red cells in vitro is a two stage technique known as indirect antiglobulin test (IAT). The sensitization of red cells in vivo is detected by one stage technique the direct antiglobulin test (DAT).

ANTIBODIES REQUIRED IN ANTIHUMAN GLOBULIN (AHG) SERUM

Anti-human globulin reagent (AHG) is prepared by immunizing animals, usually rabbit, with whole human serum or with specific fractions of human globulins.

Whole human serum is used for the preparation of polyspecific (broad spectrum) sera which contains antiglobulin antibodies (IgG) and anti-complement antibodies like C3b, C3d, the break down product of C3; and may also contain C4d, break down product of C4.

Mouse monoclonal anti-IgG have been evaluated for use in AHG reagents and it has been

demonstrated that they have no advantage over anti-IgG reagent prepared in rabbits. However monoclonal break down products anti-C3b, anti-C3d and C4d of C3 and C4 complements are useful in the production of AHG reagent. It has been observed that monoclonal anti-C3b and anti-C3d in appropriate concentrations, blended with rabbit anti-IgG produce better AHG serum. For prepartion of monoclonal AHG sera, see Hybridoma in the chapter 4 of Principles of Immunohematology.

Anti-IgG

AHG serum must contain antibody activity to non-agglutinating blood group antibodies. Majority of these are IgG. Rarely non-agglutinating IgM may be found, they fix complement on red cell membrane and may be detected by anti-complement activity of polyspecific AHG. So presence of anti-IgM is not needed in AHG.

IgA antibodies have never been found without the presence of IgG and/or IgM antibodies of the same specificity. Thus the presence of anti-IgA in the reagent is also not required. Thus anti-IgG is the only essential antiglobulin antibody in AHG reagent.

Anti-Complement

Some antibodies fix complement components C3b and C3d to the red cells membrane after complexing of the antibody with its corresponding antigens. These membrane bound complement components can be detected by the anti-complement activity in AHG.

An antiglobulin serum for the use in the direct antiglobulin test (DAT) contains anti-IgG, anti-C3d, and possibly anti-C4d. An antiglobulin serum for the use in the indirect antiglobulin test (IAT) contains anti-C3b and C3d antibodies.

Antiglobulin reagent to be used for both DAT and IAT should contain anti-IgG, anti-C3b, anti-C3d and may contain anti-C4d antibodies.

PRINCIPLE OF ANTIGLOBULIN TEST

Washed red cells coated with IgG and / or complement components C3b or C3d will show agglutination with broad spectrum AHG serum. The coating (sensitization) of red cells can occur in vivo or in vitro following incubation at 37°C with serum containing antibody.

The incomplete antibodies (IgG) attach to red cell membrane by the Fab portion of the immunoglobulin molecule (IgG). The IgG molecules attached to the red cells are unable to bridge the gap between sensitized red cells which are separated from each other by the negative charge on their surface and the sensitized red cells do not agglutinate. When AHG serum is added to the washed sensitized cells, the Fab portion of the AHG molecule (anti-IgG) reacts with the Fc portions of two adjacent IgG molecules attached to red cells thereby bridge the gap between sensitized red cells and cause agglutination (Fig. 8.1)

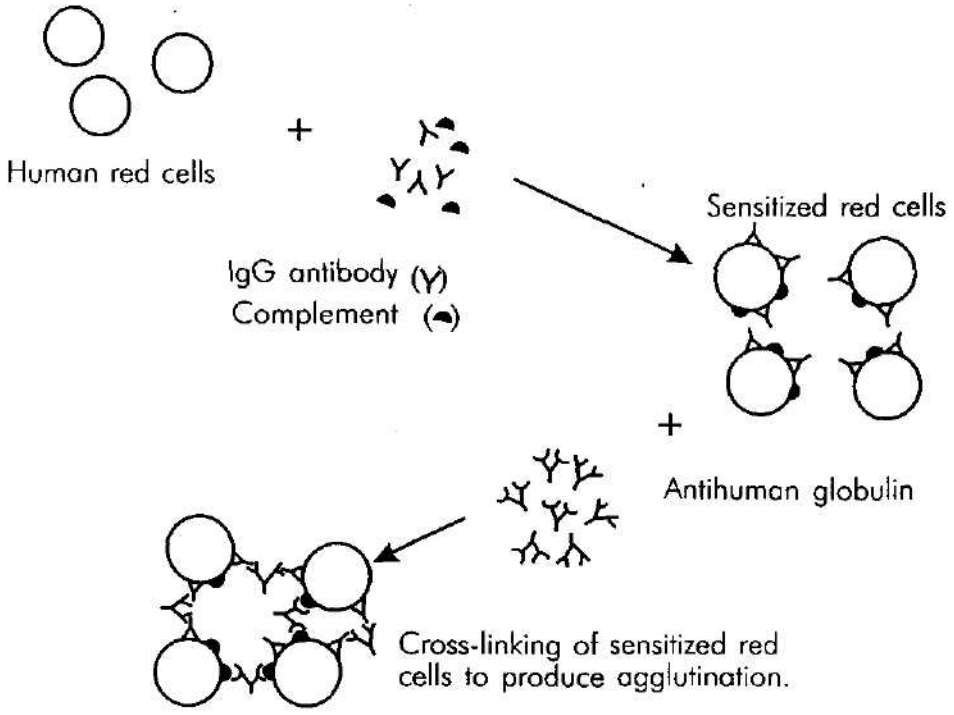


Figure 8.1

Agglutination of the sensitized *red* cells by anti-human

ANTI HUMAN GLOBULIN REAGENTS

Reagents	**Contents**
Polyspecific	
Rabbit Polyclonal	Contains anti-IgG, anti-C3b and anti-C3d [may contain other anti-compliment antibody(s) e.g. C4dj.
Rabbit+murine monoclonal	Contains rabbit polyclonal anti-IgG and blend murine monoclonal anti-C3b and anti-C3d.
Murine monoclonal	Contains murine monoclonal anti-IgG, anti-C3b and ant-C3d.
Monospecific Anti-IgG	
Rabbit Polyclonal	Contains anti-IgG with no anti-complement activity
Monoclonal IgG	Contains monoclonal anti-IgG only
Anti-Complement	
Rabbit polyclonal	Contains antibodies against designated Anti- C3d and anti-C3b compliment components, no anti-IgG
Murine monoclonal anti-C3d	Contains antibodies against C3d
Murine anti-C3b and C3d	Contains antibodies against C3b & C3d

Control Cells for AHG Tests

Positive Control Cells	IgG sensitized O Rh (D) positive cells
Negative Control Cells	Unsensitized O Rh (D) positive cells Sensitized O Rh(D) negative cells

The positive and negative controls are put along with the test.

The best control for the DAT and I AT is the addition of IgG sensitized O Rh (D) positive cells to any AHG test that is non-reactive. If there is agglutination the result is valid.

PREPARATION OF O RH (D) POSITIVE SENSITIZED RED CELLS

Reagents required:

1. Human polyspecific anti-D or Human IgG + Monoclonal IgM or Monoclonal IgG + IgM anti-D Serum
2. O Rh(D) positive cells

Procedure:

1. Select a dilution of polyspecfic anti-D serum that coats the O Rh (D) positive washed red cells at 37 °C in vitro but that does not agglutinate them. One has to determine by experience to what extent the anti-D serum should be diluted to give sensitized cells (no agglutination).
2. Add 3-5% washed red cells suspension of O Rh (D) positive cells equal to the volume of diluted anti-D serum.
3. Mix, incubate at 37 °C for 30-45 minutes.
4. Look for agglutination. If there is agglutination, the procedure is repeated by taking more diluted anti-D serum.
5. If there is no agglutination, wash the cells three times with a large volume of normal saline manually or by automatic cell washer. Make a 5% suspension of sensitized cells in saline.
6. Add 2 drops of polyspecific AHG serum to 1 drop of the 3-5% sensitized and washed red cells.
7. Mix, spin immediately at 1000 rpm for 1 minutes.
8. Cells should show +2 agglutination, if there is no agglutination the whole procedure is repeated by taking less diluted anti-D serum.

DIRECT ANTIGLOBULIN TEST (DAT)

The direct antiglobulin test (DAT) detects sensitized red cells with IgG and/or complement components C3b and C3d in vivo.

In vivo coating of red cells with IgG and/or complement may occur in:

(1) Hemolytic disease of newborn (HDN)
(2) Autoimmune hemolytic ancmias (AIHA)
(3) Drug induced hemolytic ancmias (AIHA)
(4) Hemolytic transfusion reaction (HTR)

Procedure of DAT

Blood Sample - It should be as fresh as possible not more than 24 hours old, otherwise, the sample should be taken in EDTA (1.5 mg EDTA for I ml of blood) to protect the uptake of complement.

1. Take 2-3 drops of blood to be tested in a clean labeled tube.
2. Wash the red cells 3-4 times in a large volume of saline to remove free globulin molecules. Remove all supernatant after each wash. Completely decant the final supernatant wash.
3. Add 2 drops of polyspecific AHG serum in 1 drop of sensitized washed red cells or in 1 drop of 3-5 % suspension of sensitized cells immediately.
4. Mix, Centrifuge at 1000 rpm for 1 minutes immediately.
5. Gently shake the tube to dislodge the cell button and see for agglutination, use optical aid if needed. Record the result.
6. Add I drop of IgG coated red cells to a negative test. Mix, centrifuge at 1000 rpm for 1 min. Immediately look for agglutination. If a negative result (no agglutination) is obtained the test result is invalid and whole test should be repeated. If agglutination is obtained, the result is valid.

Characterization of autoimmune hemolytic anemias requires the detection of IgG or C3d or both. The detection of C3d on red cells membrane is important in the investigation of warm and cold autoimmune hemolytic anemias. Many cases of warm autoimmune hemolytic anemias, including drag-induced hemolytic anemia are associated with IgG or C3d or both. While in cold autoimmune hemolytic anemia, C3d may be the only globulin detectable on red cells. In the investigation of autoimmune hemolytic anemias, initialy a DCT is performed with polyspecific AHG serum subsequently testing with monospecific AHG sera anti-IgG and Anti-C3d is performed to identify coating globulin. In the investigation of HDN testing complement components is not necessary as the newborn cells are sensitized with maternal IgG.

Table-2 Patterns of Reactivity in Autoimmune Hemolytic Anemia

Anti-IgG	Anti-C3d	type of AIHA
+	+	WAIHA (67%)
+	-	WAIHA(20%)
-	+	CHD, PCH, WAIHA(13%)

WAIHA - warm autoimmune hemolytic anemia, CHD - cold hemagglutinin disease, PCH - Paropxysmal cold hemoglobinuria.

INDIRECT ANTIGLOBULIN TEST (IAT)

Indications

The IAT is done to determine the presence of sensitization of red cells with IgG and/or complement in vitro in the following conditions.

1. Compatibility testing.
2. Screening and detection of unexpected antibodies in serum.
3. Determination of red cells phenotype K, Le^a, Fy^a Fy^b, Jk^a, Jk^b and sub-group of Rh etc by using known sera.

Procedure:

I. Place 2-3 drops of the test serum in a tube. Serum should be fresh for detecting complement components and complement binding antibodies, otherwise, fresh AB serum should be added to it.

2. Add 1 drop of 3-5% suspension of washed O Rh (D) positive red cells to the serum in the tube,
3. Mix and incubate at 37°C for 30-40 minutes.
4. Centrifuge at 1000 rpm for 1 minutes.
5. Examine for hemolysis and/or agglutination. Use optical aid if necessary. Agglutination at this stage indicates the presence of saline (complete) antibodies.
6. If no agglutination is seen, wash cells 3-4 times in large volume of saline. Decant supernatant in each wash as completely as possible.
7. Add 2 drops of AHG serum to the cells.
8. Mix and centrifuge at 1000 rpm for 1 minutes immediately.
9. Gently shake the tube to dislodge the button and examine for agglutination, using optical aid. Record the result.
10. Add 1 drop of IgG coated red cells to any test that is negative. Mix and centrifuge at 1000 rpm for 1 minutes. Look for agglutination. If there is no agglutination, the test result is invalid and the whole test is repeated. If agglutination is obtained the result is valid.

I1. Auto control should be kept with IAT.

For Detecting Antigens LEWIS, Kell, Duffy, Kidd etc.

1. Take known reagent serum corresponding to the antigen as in step (1) instead of test serum.
2. Add 1 drop of 3-5% suspension of washed test cells as in step (2) instead of ORh(D) positive cells.
3. Follow all steps (3-10) as in IAT.

BOVINE ALBUMIN(22%)-IAT

One Stage Method - Additive method

Procedure:

1. Two drops of albumin 22.5% are added in step (2) of saline-IAT
2. Mix and incubate for 20-30 minutes at 37°C
3. Proceed further as in saline-IAT procedure.

ENZYME-IAT

Procedure

Mostly one stage method with papain cystein solution is used in Enzyme-IAT

1. Add I drop of papain cystein solution in step (2) of saline-IAT
2. Incubate for 20-30 minutes at 37°C
3. Proceed further as in saline -IAT procedure.

LOW IONIC STRENGTH SOLUTION (LISS) - IAT

Procedure

1. Red cells are washed two times in normal saline and once in LISS. Make 2% cell suspension in LISS
2. Take equal volume of serum and 2% cell suspension in LISS
3. Incubate at 37°C foe 10-15 minutes
4. Proceed further as in step 4 in saline-IAT

For preparation of papain cystein and low ionic strength solution (LISS) see chapter 21 on Preparation of Solutions and Special Methods.

FACTORS EFFECTING THE SENSITIVITY OF IAT

1. Ratio of Serum to Cells

Increasing the ratio of serum to red cells increases the degree of antibody coating on red cells thereby the sensitivity of the test is enhanced. A commonly used ratio is 2 drops of serum to 1 drop of 3-5% red cells suspension. Equal volume of serum and 2-3% suspension of red cells in LISS should be used.

2. Suspending Medium

The sensitivity of IAT can be increased with the addition of 22% bovine albumin or enzyme solution or LISS. Albumin and enzymes allows antibody coated cells to come closer with each other and agglutination is better. Low Ionic Strength Solution (LISS) enhances antibody uptake and decrease in incubation time.

Sensitivity of IAT is increased if albumin is incorporated in the reaction medium. The reaction mixture consists of 2 drop of serum, 2 drops of 22% bovine albumin and 1 drop of 3-5% red cells. It shows the same sensitivity at 30 minutes of incubation as a 60 minutes saline test. The use of albumin does not seem to provide any advantage over LISS technique and does add to the cost of the test.

Low Ionic Strength solutions enhance antibody uptake and the incubation time is decreased to 10-15 minutes. The reaction mixture consists of 2 drops of serum and 2 drops of 3% cell suspension in LISS. If serum to cell ratio is increased the ionic strength of the mixture is increased, leading to a decrease in the sensitivity.
For more details see chapter 21 on Preparation of Solutions and Special Methods .

3. Temperature

The rate of reaction of the majority of IgG antibodies and complement activation is optimal at 37°C. So this is the usual incubation temperature for IAT.

4. Incubation Time

The usual incubation time for cells suspended in saline is 30-60 minutes in IAT. Most of the clinical significant antibodies can be detected after 30 minutes incubation. If LISS technique is being used incubation time is 10-15 minutes.

5. Washing of Cells

Red cells must be saline-washed at least 3 times before adding AHG reagent. Washing of cells removes free unbound serum globulin. Inadequate and improper washing may result in false negative reaction because of neutralization of the AHG serum by residual unbound globulin.

Washing should be uninterrupted and performed in as short time as possible to minimize the elution of low-affinity antibodies. The cell button should be completely re-suspended in adequate saline. All saline should be discarded after the final wash as residual saline may dilute the AHG serum and decrease the sensitivity of the test. Washing can be done by automated cell washer.

6. Addition of Antihunan Globulin Reagent

AHG reagent should be added to red cells immediately after washing to minimize the chance of elution of antibodies from the cells and subsequently neutralizing the AHG serum.

SOURCES OF ERRORS

Sources of False-Negative Results in AHG Tests:

1. Inadequate or improper washing of cells
2. AHG serum may be non-reactive due to improper storage
3. AHG serum not added
4. Inadequate incubation conditions in IAT
5. Cell suspension either too weak or strong
6. Under centrifugation
7. Poor result reading technique.

Sources of False-Positive Results in AHG Tests:

1. Improper blood specimen (refrigerated or clotted) may cause complement attachment to red cells in vitro
2. Bacterial contamination of cells
3. Agglutinated cells in saline used for IAT
4. Saline stored in glass bottle may contain colloidal silica
5. Saline stored in plastic container for long period may have decrease pH
6. Dirty glassware
7. Over centrifugation
8. Contaminating antibodies in AHG serum.

GEL AND GLASS MICROBEADS METHOD

Gel and Glass Microbeads Methods see chapter on Preparation of Solutions and Special Methods.

Screening and Identification of Antibodies

The purpose of antibody screening is to detect red cells antibodies other than expected anti-A and anti-B. These are called 'unexpected' antibodies. Only 0.3 to 2.0% of general population have unexpected antibodies and the incidence is higher in women due to the pregnancy. These unexpected antibodies are usually allo (directed against an RBC antigen that is lacking on the antibody producer's RBCs), but may be auto (directed against an antigen on the antibody producer's RBCs). In addition, these unexpected allo- and auto-antibodies may be further subclassified by their temperature of reactivity into cold or warm reactive. The unexpected alloantibodies are usually red cells immune and formed by exposure to red cells due to blood transfusion or pregnancy.

Unexpected antibodies may be suspected/detected during:

Unexpected antibody is usually suspected during routine serological tests:

1. In ABO cell and serum grouping.
2. In antibody screening
3. Positive auto-control
4. Positive DAT
5. In major cross-matching

Effects of clinically significant unexpected antibodies:

1. Shortens the survival of transfused red cells
2. Hemolytic transfusion reaction (HTR)
3. Hemolytic disease of new born (HDN)

Proper detection and identification of antibodies is important for:

1. Selection of appropriate blood for transfusion
2. In investigation of hemolytic anaemias
3. In investigation of HDN
4. Transfusion reaction
5. Serological changes in antenatal period

Panel of red cell for antibody screening

Antibody screening tests involve testing patients' and donors' sera against 2-3 reagent RBCs called screening cells. Group O red cells are used so that natural expected anti-A and anti-B may not interfere. Two specially selected group O R_1R_1 and O R_2R_2 red cells are used. These cells must carry the most commonly encountered and clinically significant antigens of Rh, Kell. Duffy, Kidd, MNSs, P and Lewis (and Lutheran if possible) blood group systems. Many antibodies can be detected more easily if the antigens are homozygous on the red cells, particularly of common groups.

Table 9.1 Typical Panel of Screening Cells

DonorGeno	Rh	MNSs	P	Kell	Duffy	Lewis	Kidd	lutheran
No. type	C c D E e	M N S s	PI	K k	FY^a FY^b	E^a I^cb	JK^a JK^a	LU^a LU^b
1 R_1R_1	+ - + - +	+ +-+	+	+ +	+ -	- +	- +	- +
2 R_2R_2	- + ++ -	+ - +-	+	- +	+ +	+ -	+ -	- +

To maintain the optimum strength of the antigen, the two red cells ORJRJ and OR^, should be used separately (in conjunction with each other) and are not pooled. This will increase the chance of detecting the weak antibodies.

Cell panels are commercially available in developed countries, however, they can also be prepared by the individual laboratory. The institution that prefer to prepare their own panel, should determine the full phenotype of individuals on staff and bled them regularly, or freeze red cells of large donations from these individuals in glycerol. For freezing of red cells in glycerol see chapter on 'Preservation and Storage of Blood.'.

If panel of screening cells is not available fresh pooled cells of 2-3 group O blood may be used. The second course is much less desirable than the first and should be taken if panel of screening cells is not available.

METHODS FOR SCREENING OF ANTIBODIES

The techniques commonly used are:

1. Saline test at room temperature for IgM
2. Albumin method for IgG
3. Enzyme method - papain cysteine, or enzyme pretreated cells for IgG
4. IAT for IgG and anti-complement antibodies

For the details of techniques 1,2,3 see chapter on 'Preparation of Solutions and Methods' and for IAT see chapter on ' Antihuman Globulin Test.'
On screening if antibody is detected it should be identified.

IDENTIFICATION OF ANTIBODIES

Before beginning the antibodies identification, it is useful to review:

1. Medical history - diagnosis
2. History of transfusion
3. History of pregnancy
4. Drug therapy (including Rh immunoglobulin)
5. Review of the results of previous testing

Repeat Preliminary Tests

1. ABO cell and serum grouping
2. If patient is group A, perform A subtyping using anti-A, (lectin)
3. Perform Rh(D) typing
4. DAT for IgG and anti-complement antibodies

Specimen Requirements

Either serum or plasma may be used for antibody identification but mostly serum is used. Plasma is not suitable for detection of complement activation. A 10 ml of whole blood in plain tube usually contain sufficient serum for identification of antibodies.

When autologous red cells are studied, the use of cells anticoagulated with EDTA avoids problems associated with the in-vitro uptake of complement components by red cells.

Selection of Panel of cells for identification of antibodies

These panel cells are commercially available, but they can be prepared in the individual laboratory. They are usually 8-10 group O cells from different donors. These cells must carryD, C, E, c, e, M, N, S, s, Fy^a, Fy^b; Jk^a, Jk^b; K, k; Lu^a. lu^b; Le^a, Le^b; P_1 antigens, to identifyclinicallysignificant alloantibodies. There must be sufficient number of cells that lack, and sufficient red cells samples that carry, mo,st of the reagents.

Table 9.3 typical panel of Cells for Detection of Antibodies

	Rh	MNS	P	Lu	Le	K	Fy	Jk
No. Cell	**C c D Ee C^w**	**M N S s**	**P1**	**Lu^a Lu^b**	**Le^a Le^b**	**K K Kp^a Kp^b**	**Fy^a Fy^b**	**Jk^a Jk^b**
1 R_1 R_1	+ -+ - + -	- + - +	-	- +	- +	+ +- +	- +	- +
2 R_1R_1	+ - + - + +	+ - + +	+	-+	- +	- + - +	+ +	+ +
3 R_2 R_2	- + + + - -	+ + + -	+	- +	- -	- + - +	+ -	+ +
4 Ror	- + + - + -	- + + +	+	- +	- +	- + - +	+ +	+ -
5 r' r	+ + - - -	+ - - +	**w**	- +	- -	- + - +	- +	+ -
6 r"r	+ + - -	- + + +	-	+ +	- +	+ + - +	+ +	+ +
7 r"r	- + - + + -	+ + + +	+	-+	+ -	+ - - +	+ +	+ -
8 rr	- + - - + -	+ + + + +	+	- +	- -	- + - +	+ -	- +
9 rr	- + - - + -	+ + - +	-	- +	+ -	- + + +	+ +	+ -
10 rr	- + - - + -	+ + - +	**w**	- +	- +		- +	

Autologous Control

It is important to know how a serum reacts with autologous red cells. This helps to determine whether allo-antibody, autoantibody, or both are present. Serum that reacts only with the reagent cells usually contains only alloantibody, whereas reactivity with both reagent and autologous red cells suggests the presence of autoantibody, and allo-antibodies*. If autologous control is positive, DAT should be performed. In case DTA is positive, elution technique is performed, and adsorption studies may be necessary to establish if it is not masking alloantibodies.

Cord Red Cells

Two group O cord cells one Rh(D) positive and one Rh(D) negative may also be included. Cord cells have i antigen and I antigen is absent.

Methods of Antibody Identification

The serum should be tested against panel of cells (8-10), own cells (autocontrol) and two cord cells. Three techniques are commonly used:

1. Saline test at room temperature for IgM
2. Enzyme (one stage technique) or albumin (additive technique) for IgG
3. I AT for IgG and anti-complement antibodies

For the details of the techniques 1 and 2 see chapter on Preparation of Solutions and Methods and for 3 see chapter on Antihuman Globulin Test.

Interpretation of Results

Panel results will include a range of positive and negative reactions, and they help to reach a final conclusion. First non-reactive panel of cells is examined and the possibility of antibodies corresponding to the antigens present on cells may be excluded and the corresponding antigens may be crossed out. Then the cells reactive with the serum are evaluated. Conclusive antibody(ies) identified are confirmed by the absence of the antigens present on the red cells of the test sample.

Example 1

		Rh				MNS;							P	Lu	Le							py		Jk		Patient's Serum Panel Cells
No	Cell	C	c	D	E	e	Cw	M	N	S	8	PI	Lu^a	Lu^b		Le^a	Le^b	K	k	Kp^a	Kp^b	Fya	Fyb	Jk'	Jk^b	JCT PAP/ SAL
1	R_1R_1	+	-	+	-	-	-	-	+	-	+	-		-	+	-	+	+	+	-	+	-		+	-	+3	- -
2	R_1R_1		-	+	+	-	+	+	-	+	+	+		-	+	-	+	-	+	+		+	+	+	+	+4	+4
3	R_2R_2	-	+	+	+	-	-	+	+	+	-	+		-	+	-	-	-	+	+		+		-	+	+4	- +4
4	Ror	-	+	+	+	-	-	-	+	+	+	+		-	+	-	+	-	+	+		+		+	-	+4	- +4
5	r'r	+	+	-	+	-	-	+	-		+	w		-	+	-	-	-	+	+		-		+	-		-
6	r'r	+	+	-	+		-		+		+			+	+		+		+	+		+		+	+	-	-
7	r'r	-	+	-	+	+	-	+	+	+	+	+		-	+	+	+	-	+	+	-	+	+	+	+	+3 +3	+1
8	r r	-	+	-	-	+	-	+	+	+	+	+ +		+	+	+	-	+		+		+	+	+		-	-
9	rr	-	+	-	+		-	+	+		+	-		+		+	-	-	+	+		+		+	-	-	-
10	IT	-	+	-	+		-	+	+	-	+	w		+		-	+	-	+	+ +		-		+	-	-	-
Auto		-	+	-	+																					-	-
Cord	1																									-	-
Cord	2																										

Cell No. 5 eliminates C, c, e, M, s, P, Lu^b, k, Kp^b, Fy^b,Jk^a, leaving D, E, C^w, N, S, Lu^a, Le^a, Le^b, K, Kp^a, Fy^a, and Jk^b.

Cell no. 6 eliminates N, Lu^a, Le^b, Fy^a, leaving D, E, C^w, S, Le^a, K, Kp^a, JK^b.

Cell No. 7 eliminates E, S, Le^a, K, JK^b leaving D, C^w, and Kp^a.

Cell No. 10 eliminates Kp^a leaving D, C^w.

Anti-C^w can be eliminated on statical basis and only anti-D may be present.

Example 2

Cell No. 10 eliminates Kp^a leaving D, C^W
Anti-C^w can be eliminated on statical basis and only ant-D may present.

Example 2

		Rh				MNS						P	Lu^a				Lu^b	Leb	K			Fy		Jk		Patient's Serum Panel Cells		
No.	Cell	C	c	D	E	c	Cw	M	N	S	s	PI	Lu^a	Lu^b	Le^a	Le^b	K	k	Kpa	Kp	Fya	Fy	Jk'	Jka	ICT	PAP	AL
1		+	-	+	-	+	-	-	+	-	+	-	-	+		+	+	+	+		-	+	-	+	+3	-	-
2	Rw,R,	+	-	+	-	+	+	+	-	+	+	+	-	+		+	-	+	+		+	+	+	+	-	-	-
3	R1R1	-	+	+	+	-	-	+	+	+	-	+	-	+	-		-	+	+		+	-	+	+	-	+4	+2
4	Ror	-	+	+	+	+	-	-	+	+	+	+	-	+	+	+	-	+	+		+	+	+	-	-	-	-
5	r'r	+	+	-	+	+	-	+	-	-	+	w	+	+	-	-	-	+	+		-	+	+	-	-	-	-
6	r'r	+	+	-	+	+	-	-	+	-	+	-	+	+	+	+	-	+	+		+	+	+	-	-	-	-
7	r"r	-	+	-	+	+	-	+	+	+	+	+	+	+	+	-	+	+	+		+	+	+	+	+3	+3	+1
8	rr	-	+	-		+	-	-	+	+	+	+	+	+	-	+	+	-	+		+	-	+	-	+4	+4	-
9	rr	-	+	-	-	+	-	-	+	+	+	-	+	+	+	+	-	+	+		+	+	-	+	-	-	-
10	rr	-	+	-	-	+	-	+	+	-	+	w	-	+	-	+	-	+	+		-	+	+	-	-	-	-
Auto	-	+	+	+		+											-								-	-	-
Cord	1																								-	-	-
Cord	2																										

Cell No. 2 eliminates C, D, e C^w, M, S, s, P_1, Lu^b, Le^b, k, Kp^b, Fy^a, Fy^b, Jk^a Lk^b and leaving c, E, N, Lu^a, Le^a,K, Kp^a.
Cell No. 4 eliminates c, N, leaving E, Lu^a, Le^a, K, and Kp^a.
Cell No. 6 eliminates Lu^a leaving E, Le^a, K, Kp^a
Cell No. 9 eliminates Le^a, leaving E, K, and Kp^a
Cell No. 10 eliminates Kp^a leaving E and K
So the antibodies in the serum are E and K

As further proof, the patient must be typed for the antigens to which the antibodies have been detected. The patient
should be negative for antigens to the corresponding detected antibodies.

Pretranfusion Compatibility Testing

The purpose of pretranfusion testing is to select blood components that will have acceptable survival when transfused and will not cause harm to the recepient. Compatibility (pre transfusion) testing is done to ensure safe transfusion therapy.

Compatibility Testing Involves

- Identification of the recipient's blood sample.
- Checking of recipient's previous records in case there is history of blood transfusion, if possible-
- ABO and Rh (D) grouping of the recipient.
- Antibody screening of recipient's serum for commonly encountered antibodies and their identification, if needed.
- Selection of ABO and Rh (D) compatible donor blood free from any irregular antibody and infectious diseases that may be transmitted through its transfusion.
- Cross-matching of the recipient's serum against the donor's cell to confirm donor-recipients compatibility.
- Proper labeling of donor blood before issue.

Identification of the Recipient's Blood Sample

The steps involved in identification of recipient's blood sample are as follows:

- The correct recipient's blood sample should be taken in a clean and dry test tube labeled before venipuncture. It is the responsibility of patient's physician to collect this blood sample.
- The blood bank laboratory staff should ensure that the blood sample is appropriately labeled and information such as patient's name, admission/registration number, ward and bed number and date of collection are same on the blood sample as on the request form.
- Unlabeled or hemolysed samples should never be accepted.
- The request form for blood should give details such as the patient's full name , hospital admission/ registration number, age, sex, ward and bed number, clinical diagnosis and relevant serological history (i.e. in case of previous transfusion or pregnancies).
- A new blood sample is required if the earlier transfusion was given more than three days back, in order to detect antibody antibodies which may have been produced recently.

Checking Recipient's Previous Records if Possible

If the patient has a history of blood transfusion, his /her previous transfusion records must be checked (if possible) for:

- ABO and Rh(D) group
- unexpected antibody /antibodies
- any problem in cross-matching
- any transfusion reaction

ABO and Rh (D) Grouping of Recipient

ABO Grouping

Red cells of the patient must be tested with anti-A, anti-B, anti-AB and serum is tested with A, B and O red cells. For techniques and interpretation see chapter on ABO Blood Group System.,

Any descrepancy in the ABO tests should be resolved before giving blood. If the problem cannot be resolved and the patient needs blood, group 'O' red cells may be issued after a cross-match.

Rh(D) Grouping

The patient's red cells are tested for Rh (D) antigen with anti-D. Routine testing for other Rh antigens is not recommended . It is not necessary to test recipient's red cells for weak D (D^u), because no harm results if weak D (D^u) individual is given Rh (D) negative blood. See chapter on Rh system.

Antibody Screening in Recipient's Blood

- The patient's serum is screened for commonly encountered irregular antibodies with OR_1 R_1 and OR_2R_2 cells of screening panel which have a double dose of the corresponding antigen (homozygous) because they react better with the antibodies of certain blood group systems notably Rh. Kell, Duffy and Kidd. If cells of screening panel are not available three pooled group 'O' cells can be used for antibody screening. The antibodies screening tests are carried out using the saline technique at room temperature and albumin/enzyme

and indirect antihuman globulin methods at 37°C. For details see chapter on Screening and Identification of Antibodies.

- If antibodies screening tests show that some antibody/antibodies is/are present, they are detected by a cell panel having 8 or 10 group O cells with as many antigens as possible. If antibody (ies) is (are) identified, select the blood whose red cells lack the corresponding antigen. For details see chapter on Screening and Identification of Antibodies.
- If it is not possible to determine the specificity of an irregular antibody either because of the lack of a cell panel or because there is an emergency requirement for transfusion, the patient's serum should be cross-matched with several units of the same ABO and Rh (D) type as the patient to select blood compatible with the unidentified antibody/antibodies.

Selection of Blood

Donor's blood should be already processed for ABO and Rh (D) group, screened for irregular antibody /antibodies and for transfusion transmitted disease like HIV 1 &2, HBs Ag, HCV, treponema pallidem and formalaria.

For selection of safe blood, the criteria followed are:

ABO System

ABO blood group system is still the most dangerous because all recipients have anti-A and/or anti-B antibodies in their serum (except those of AB group) and incompatiblity in the ABO group between patient and donor blood will cause severe hemolytic transfusion reaction.

Rh System

The Rh (D) antigen is very immunogenic and it is important to avoid immunization of Rh negative patients, particularly child bearing female patients, because of the likelihood of Rh haemolytic disease of new born in subsequent Rh (D) positive babies.

Other Blood Group Systems

Atypical antibody(ies) active at 37°C of other systems is(are) serologically significant. In case the patient has atypical antibody(ies) active at 37°C, the survival of donor red cells may not be normal or the patient may have hemolytic transfusion reactions.

Choice of Blood in ABO System

- Same ABO group as that of the patient is preferred.
- If anti-A, in A., or A_2 B group patient is active at 30°C and above or strongly active at room temperature the group A_2 blood is selected for transfusion; otherwise anti-A, in A_2 or A_2B group patient reactive at R.T. has no clinical significance.
- Very rarely antibody anti HI (O) may be present in A_1 group patient, which is not inhibited by H substance and is active at temperatures above 30° C. In such case A, group blood is selected for transfusion.
- Bombay blood group (Oh) patient should be given Oh blood only.
- A, individual with acquired B like antigen are given A_1 blood.
- If same ABO group type blood is not available, transfuse packed red cells of different ABO group provided they are compatible. See table 10.1

Choice of alternate blood groups Table 10.1

Patient's Blood Group	Alternative Blood Group (Red Cells)	
	First Choice	Second
A	O	None
A_2 without anti-A_1 or with anti-A_1 reacting at RT or	O	None
A_2 with anti-A, reacting	O	None
$A_1$with anti-HI(O)	None	None
B	0	None
AB	A or B	O
A_2B without anti-A_1 or with anti-A, reacting at RT or	A or B	0
A_2B with anti-A, reacting at	A_2 or B	o
A_1Bwithanti-HI(O)	None	None

- In AB group patients, if AB group blood is not available, group A blood is preferable to group B blood as in A group blood anti-B is weaker than anti-A in group B. It is not advisable to change from Group A to Group B blood or vice versa when more than one unit are given as a continuous transfusion. If the patient has received more than one unit of any of these two groups and more blood is needed, it is best to use group O blood (free of hemolysins) or red cells.
- If more than one unit of alternate blood group transfusion has been given. The decision to change back to group specific blood should be based on the presence or absence of anti-A or anti-B in the subsequent sample of the patient. If freshly drawn patient's blood sample is compatible with group specific blood, the group specific blood can be used.

Choice of Blood in Rh System

The blood of the same Rh (D) group should be used as that of the patient. When Rh (D) negative blood is not available, Rh (D) positive blood may be given to Rh (D) negative patient rather than with hold blood from a patient whose need is critical:

When Rh(D) negative is not available, criteria or priorities are:

1 Patient who already have Rh antibody(ies), blood lacking the corresponding Rh antigen(s) is selected.

2 Rh (D)negati ve women who have not passed child bearing age, to avoid transfusion reaction in future and HDN in subsequent Rh (D) positive baby, Rh (D) negative blood should be given.

3 Infant suffering from HDN are given transfusion of Rh (D) negative blood.

4 In Rh(D) negative male or Rh(D) negative female after child bearing age: Rh(D) positive may be given rather than with hold for a patient whose need is critical, provided.

(i) Anti-Rh (D) antibodies, have never been detected in recipient's serum and no antibody(ies) is found in test serum on screening with red cells panel by AHG and enzyme techniques.

(ii) Blood is compatible

WeakD(D^u)

- D^u donor blood is taken as Rh (D) positive blood.
- The weak D (D^u) recipient is treated as a Rh (D) negative individual, though transfusion of Rh (D) positive blood in D^u positive patient involves very little risk of immunization to Rh (D).

Choice of Blood in Other Group System

- If irregular antibody(ies) of other systems in patient's serum are active at 37°C, they must be taken into account as they may cause hemolytic transfusion reaction or the survival of donor red cells may not be optimum. In such cases appropriate blood lacking the corresponding antigen is selected for transfusion, e.g. K negative blood for patient with anti-K; and Fy^a negative blood for those with anti-Fy^a
- If it is not possible to identify the specificity for an irregular antibody either because of the lack of a cell panel or because there is an emergency transfusion requirement, the patient's serum should be cross matched with several units of the same ABO and Rh (D) type blood as that of the patient in order to select the compatible units of blood.
- In some cases when it is not possible to find compatible units of blood, the patient's relatives, especially siblings; may be compatible.
- If the patient is known to have irregular antibody(ies), autologous transfusion may be considered in case it is practicable.

SELECTION OF BLOOD IN SPECIAL CONDITIONS

Hamolytic Disease of New Born (HDN)

ABOHDN

In ABO HDN, group O red cells of the same Rh type as that of baby should be used.

RhHDN

- In Rh HDN, Rh (D) negative blood of the same ABO group as that of baby is used if it is the same as that of the mother or if it is compatible with mother's blood.
- When baby's ABO group is not compatible with mother's ABO group or if donor's unit is prepared before delivery, it should be O Rh (D) negative and it should be free from hemolysins, anti-A and anti-B.
- In case if exchange transfusion is required more than once, subsequent blood should be of the same ABO and Rh type as that used during the first transfusion.

- When O group blood is given to an infant who is group A, B or AB, red-cell concentrates should be used. It is a good practice to use packed cells resuspended in one-third volume of a fresh AB plasma (or A plasma or B plasma as appropriate).
- Blood should be as fresh as possible and should not be more than five days old, to avoid high level of plasma potassium in the transfused blood and to ensure long survival of transfused red cell in infant.

COMPATIBILITY TESTING (CROSS MATCH)

Compatibility tests are done to ensure that particular unit of blood may be safely transfused to a patient. Normally group specific blood, ABO and Rh (D), as that of the patient is selected. However in certain situation, because of the non-availability of group specific blood, group O blood (red cells) is selected for A or B patient and A or B blood for an AB patient as explained earlier.

Compatibility testing (cross match) includes

- Major cross match - Recipient's serum is cross matched with donor 's red cells for IgM and IgG antibodies compatibility.
- Minor cross match—Donor's serum is cross matched with recipient's red cells for IgM and IgG antibodies compatibility. If donor's serum has been screened for irregular antibodies with OR_1R_1, and OR_2 R_2 cells of antibody screening panel and found negative, minor cross-match can be avoided.
- If incompatibility is not detected in cross matching then, it is likely that the donor blood transfused into patient will survive normally.
- Finding of incompatibility indicates that transfusion of such blood is potentially dangerous and further steps should be taken to identify the antibody.

Compatibility Tests

Major Compatibility Tests

It is done both for IgM and IgG antibodies

Requirement:

1. Recipient's serum.
2. Donor's red cells taken from the tube attached to the bag.

Saline technique

Saline technique is designed to detect compatibility of IgM antibody(ies) in patient's serum against antigens on donor's red cells.

Method

1. Label 1 tube for each donor sample to be tested.
2. Put 2 drop of patient's serum in labeled tube.
3. Add 1 drop of 2-4% saline suspended red cells of donor

4. Mix and incubate for 5-10 min. (spin method) or incubate for 30-60 min (sedimentation method) at RT.
5. Centrifuge at 1000 rpm for 1 min. in spin method (after 5-10 min. incubation); centrifugation is optional in sedimentation method.
6. Read the result, observe for hemolysis and agglulination.
7. Negative result should be confirmed under microscope.

Interpretation

Agglutination or hemolysis indicates a positive result (incompatible)

Note: In emergency spin technique is acceptable.

Saline technique is inadequate as a complete compatibility test because it is inadequate to detect clinically significant IgG antibodies.

Compatibility Test for IgG Antibody(ies)

Anti -Human Globulin Test (IAT)

Indirect anti human globulin test (IAT) is the most important and widely used serological procedure in modern blood banking to test the IgG compatibility between recipient's serum and donor's cells. The majority of incomplete antibodies are IgG and are detected by AHG test.

Method:

1. Put 2 drops of patient's serum in a labeled tube.
2. Add 1 drop of 2-4 % saline suspended red cells of donor.
3. Incubate for 30-60 min at 37° C
4. Centrifuge at 1000 rpm for 1 min, check for hemolysis/agglutination
5. If there is no hemolysis/agglutination, wash the cells three times with normal saline.
6. Perform IAT test

   - Add 2 drops of polyspecific AHG serum to washed cells
   - Centrifuge at 1000 rpm for 1 minute
   - See for agglutination
7. Add IgG coated red cells to negative AHG test.
8. Centrifuge and check for agglutination - if there is no agglutination test is invalid.

Interpretation

Hemeolysis or agglutination at any stage indicates incompatibility.

Note: Cross-match can be done by two tubes technique for IgM amd IgG separately as described above or by one tubes in which donor' cell and the patient's serum after step 5 in saline technique is incubated at 37°C for 20-30 minutes and then do IAT.

In major-cross for IgG antibodies albumin or enzyme or LISS can be used with IAT to increase sensitivity. For techniques see chapter on Antiglobulin Test.

Compatibility Testing in Emergencies

- On the request of the clinician blood can be issued in emergency after ABO and Rh(D) typing followed by cross match by immediate spin tube technique for IgM compatibility.
- In extreme cases, where there is no time to take sample and to test it, O, Rh(D) negative blood, preferably red cells, may be given on the request of the clinician.
- Donor's unit that has not been tested or partially tested for compatibility for IgM and IgG antibodies against patient's serum, should be clearly labeled 'Uncrossed-match Blood'.
- It is advisable to complete the routine cross-matching both for IgM and IgG compatibility, after issue of blood with incomplete or without compatibility test.

Minor-Cross Match

- If donor's serum has not been screened for irregular antibodies with OR_1R_1 and OR_2 cells of screening panel, and found negative, it is advisable to do minor cross matching.
- In minor cross- match donor's serum is cross-matched with recipient's red cells for both IgM and IgG antibodies compatibility.

Method

Method is same as that of major cross-match except in minor cross-match donor's serum is tested against patient's cells both for IgM and IgG antibdies compatibility.

MASSIVE TRANSFUSION

- When massive transfusions are given, that is when the number of units transfused in a 24 hours period exceeds the recipient' s blood volume, compatibility testing may be reduced to checking the ABO and Rh (D) types of the transfused units. If the patient has known allo-antibody reactive at 37° C, the blood should be negative for the relevant antigen, as much as possible,
- After the emergency has been dealt with, if antibodies are detected in pre-transfusion sample, and in case further transfusion is necessary compatible blood is selected.
- Donor's units that have not been tested or have been partially tested against the patient's serum, should be clearly labeled "uncross matched blood".

For details see Massive Transfusion in chapter on Tansfusion Practice in Clinical Medicine.

PRE - TRANSFUSION TESTING OF NEONATES

(In less than 4 months old infant)

Procedure:

- Group ABO and Rh (D) group of infant cells should be determined by cell grouping only.
- Direct AHG test on baby's cells is performed.
- Maternal serum should be screened for any irregular antibody, if mother's blood sample is available.

- If the antibody screening and DAT is negative in mother's blood, and no evidence of HDN, cross -match blood of the same ABO and Rh (D) type as that of the infant using mother's serum (if ABO compatible and sample is available) or baby's serum. This is to exclude the possibility that incomplete antibody from the mother may be in baby's serum in the absence of the antigen on the baby's red cells to. that antibody.
- If the antibody screening is positive, the direct AHG is positive or HDN is present; the donor's blood must be cross- matched against maternal serum. The neonate's serum can also be used (if mother's blood sample is not available),
- When group O blood needs to be given to an infant who is group A and / or B, then red cell concentrates of units with low titre anti-A or anti-B should be used. It is a good practice to use packed cells resuspended in one third volume of AB plasma (or A plasma or B plasma as appropriate).

Issue of Blood / Components for Transfusion.

1. Cross-match report should be sent along with the blood to be issued.

The cross -match report form must include:

- Donor's blood identification number
- Donor ABO and Rh (D) groups
- Collection & expiry date of blood product.
- Patient's name and identification number etc.
- Patient's ABO and Rh (D) group
- Interpretation of cross- matching tests
- Date and time of issue, and
- Identification of the person performing the tests

2. Label or tag is securely attached to be unit of blood it should contain:

- Donor's blood identification number
- Donor's ABO and Rh (D) group
- Donor's blood non-reactive to HIV & HCV antibodies, HB_sAg, VDRL
- Patient' s name and identification number
- Patient's ABO and Rh (D) groups
- Interpretation of cross-matching test
- Collection and expirry date of blood product

3. At the time of issue of blood observe the following:

- Recheck the identification of the patient and donor blood
- Check the blood expiry date to avoid issuing out dated blood
- Inspect the unit to make certain that is does not have abnormal color or appearance

CROSS MATCH RECORD

Patient's Name .....Admission No…….....

PATIENT'S GROUP

Cell Grouping					Serum Grouping			Group	
An	An	An	An				C	AB	Rh (D)

• _____ Antibody screening in patient's serum: In saline _____ in Alb/Enz/AHGS_____ with OR_1R_1, OR_2 R_2 cells or 3 pooled O group cells for IgM & IgG.

• DAT in patient's cell if indicated .

• Autologus control

CROSS-MATCH

Donati	Bl	IgM Antibodies		IgG Antibodies (IAT)		Compatible	
		Maio	Minor	Major	Mi	Yes	No
			-				

Remarks (if any)_____

Signature of Technologist

Note : A positive result (agglutination and /or Hemolysis) indicates incompatibility.

11
Adverse effects of Blood Transfusion

Transfusion of blood and its product is, ordinarily, a safe and effective way of correcting hematological defects but adverse effects do occur during or after transfusion and they are commonly called blood transfusion reactions. These untoward effects vary from being relatively mild to lethal and some of them can be prevented while others cannot.

The time between the suspicion of a transfusion reaction and the investigation and the initiation of a appropriate treatment should be as short as possible. The transfusionist nurses/clinicians in the wards, the medical officers and the technicians in the blood banks must have the knowledge about the transfusion reactions and its management.

Categories of Transfusion Reactions

Reaction	**Acute (onset within < 24 hours)**	**Delayed (onsec within days or months)**
Immune-mediated	Haemolytic Febrile nonhemolytic	Hemolytic Alloimmunization
	Allergic	‘ Post-transfusion purpura
	Anaphylactic	TR- Graft-vs-host disease
	TR-acute lung injury	Immunomodulation

*Categories of Transfusion Reactions (Continued):*Non-Immune-Mediated

Bacterial contamination	TA-
Circulatory over load	Hepatits B & C
Physical or chemical -	HIV1&2
damage to RBCs	Syphilis
Hyperkalemia	Malaria
	Iron over

Immediate Immune - Hemolytic Transfusion Reactions (IHTRs)

IHTRs occur very soon after the transfusion of immunologocal incompatible whole blood or red blood cells to a recipient. The commonly red cells antibodies e.g. anti-A, anti-B, anti-Kell, anti Jk^a and Fy^b cause immediate hemolytic transfusion reactions. These antibodies bind complement on the surface of red cells, causing lysis of red cells. Immune mediated hemolytic transfusion reactions can destroy red cells by one of two mechanisms: (1) intravascular hemolysis or (2) extravascular hemolysis. In both the initial event is binding of patient antibody to the antigen on the surface of transfused incompatible red cells forming antigen-antibody complex.

In intravascular hemolysis Ag-Ab complex activate the complement which hemolysesred cells and hemoglobin and stroma of red cells are liberated in the circulation.

In extravascular immune-mediated hemolytic transfusion reactions Ag-Ab complex causes incomplete or no activation of complement. So RBCs lysis does not occur in intravascular circulation and there is no release of hemoglobin and red cells stroma.

Causes of Immediate Immune-Hemolytic Transfusion Reactions (IHTRs)

1. Clerical Errors

(i) Inadequate or incorrect labeling of blood bag, recipient's blood sample.

(ii) Confusion in the identity of the patient at the time of collection of sample or at the time of transfusion

(iii) Improper identification of patient's blood sample by blood bank technician

(iv) Wrong blood issued

2. Technical Error

(i) Error in blood grouping and cross-matching.

(ii) Incompatability not detected in cross-matching due to improper method.

(iii) Weak antibodies are not detected by routine tests.

(iv) Destruction of recipients red cells by donor antibodies.

It may occur when donor blood has antibody(ies) against antigen(s) in recipients. It may not be serious as donor antibody(ies) is diluted in patient's blood. It mostly occurs in the transfusion of group O blood to group A, group B or group AB recipients. This usually results from the indiscriminate use of group O whole blood, which has not been screened to ascertain the anti-A and anti-B titer and hemolysins.

(v) Incorrect interpretation of the test results.

The interaction of antibody with antigen on a red cell membrane can initiate a sequence of neuroendocrine responses, complement activation, coagulation effects and cytokine effect, that result in the clinical manifestation of an acute HTR.

Immediate-Immune Haemolytic Transfusion Reactions.

Signs and symptoms

Sign and symptoms that may occur with impending or established transfusion reactions include:

- Fever > 1 °C or 2° C, with or without chills
- Shaking chills(rigors), with or without fever
- Pain at infusion site, or in chest or back (flanks)
- Blood pressure changes, usually hypotension
- Respiratory distress, including dyspnea, tachypnea or hypoxemia
- Oozing from intravenous line site
- In anesthesized patient during surgery there is diffuse oozing from surgical site, hypotesion, hemoglobinuria, pink or red urine may be seen when the patient has in-dwelling urinary catheter
- Hemoglobinuria
- Oliguria
- Anuria
- Shock

Immediate non-immune mediated hemolytic reactions (IHTRs)

Signs and symptoms

Non-immune mediated hemolytic reactions cause destruction of red cells and produce signs and symptoms like those immune-mediated hemolytic transfusion reactions. They must be considered in the differential diagnosis of hemolytic transfusion reactions. Immediate non-immune hemolytic transfusion reactions may be due to:

- Blood infected with bacteria
- Thermal exposure of transfused red cells
- Mechanically hemolysed red cells
- Chemically effected red cells
- Hyperkalemia

Bacterial contaminated blood :

Bacterial contamination of refrigerated blood is rare now because blood is collected in disposable plastic bags. Transfusion reactions due to bacterial contamination are commonly caused by endotoxins produced by bacteria capable of growing in cold temperature such as Pseudomonos species. Escherichia coli and Y.enterocolitica.

They are commonly attributed to platelet transfusion because platelets are stored at $20 - 24^0$ C.

Causes:

- Contamination can occur at the time of phlebotomy and it is therefore important to clean the site of venipuncture very carefully and all aseptic precautions should be taken in phlebotomy.

The mechanically hemolysed unit:

Causes

- Roller pump like used in cardiac surgery
- Infusion under pressure through small bore needles
- Blood pumps

Thermally hemolysed unit

Causes

- Blood cooled to -3°C or warmed to > 42° C may be hemolysed.

Chemically hemolysed unit

Causes

- Hypotonic saline or 5% glucose may hemolyse blood
- Concomitant administration of blood and drugs through a common administration set or incorporation of drugs into blood bag.

Hyperkalemia in massive transfusion

After massive transfusion, a life-threatening load of potassium may be released into the plasma and causes signs of hyperkalemia particularly arryhthmias.

Signs and symptoms of contaminated products or hemolysed units :

They appear rapidly during transfusion or with in 30 minutes after transfusion. Clinically this type of reaction is termed warm and characterized by:

- Dryness and flushing of skin
- Sever Hypotension
- Fever and chills
- Muscular pain
- Vomiting
- Abdominal cramps
- Hemoglbinuria
- Shock
- Renal failure
- DIC

Preventive measures :

At the time of issue of blood check evidence of contamination which include gross hemolysis or change in colour of blood, particularly in comparision to the color of the attached segmented tubing or the presence of particular matter or clots.

In case of hemolysis of red cells there may be change of color (purple) both in the bag and the attached tubing segments.

In case of bacterial contamination there may be change in the color of blood in the bag and the color of blood in the tubing of the bag is not changed and there may be clots in the blood.

Management of Immediate Immune & Non-immune HTRs

The treatment of HTRs depends on the amount of incompatible/hemolysed/contaminated blood transfused, the specificity of the offending antibody, and the clinical severity of the reaction.

1. Always stop the transfusion and disconnect the entire infusion set from the needle/ catheter.
2. Using a new infusion set, keep the intravenous (IV) line open with a drip of normal saline.
3. Check the label on the blood bag, cross-matching report and identify the patient to confirm that the patient received the correct unit of blood or component..
4. The patient's physician should be informed immediately.
5. Notify the blood bank and describe the signs and symptoms.
6. A post-transfusion fresh blood sample (10 ml of blood in plain test tube and 2 ml in EDTA), taken from another vein, should be sent to the blood bank along with blood bag and transfusion set and transfusion reactions report.
7. First voided urine should be sent for to the laboratory for the analysis of free hemoglobin

Laboratory Investigations

1. Check identity of patient, donor blood and all relevant papers to ensure that there was no clerical error.
2. Compare the color of plasma of patient's pre and post-transfusion blood specimens.
   (i) Pink red discoloration in post-transfusion sample indicates the presence of free hemoglobin due to the destruction of red cells.
   (ii) Yellow or brown discoloration in patient's samples drawn 4-10 hours after the transfusion indicates increased bilirubin.
3. Check the color of bag -
   (i) purple color and clots in blood bag (no change in color in the segments of tubing of the bag) may be due to bacterial contamination
   (ii) Change in color both in the bag and the tubing of the bag may due to hemolysed blood.
4. Repeat ABO, Rh(D), testing in the patient's pre- and post- transfusion blood samples, and blood from the bag or from a segment of the tubes still attached to the unit to check any error in ABO and Rh(D)
5. Perform direct antiglobulin test on patient's blood.
   (i) If DCT is negative, the red cells are not coated with IgG antibodies and there is no incompatibility.
   (ii) If antibody coated donor incompatible cells are not immediately destroyed, the DCT on the post- reaction blood sample will be positive.

(iii) If the patients blood sample is drawn several hours after the suspected reaction, the antibody coated donor red cells are destroyed and the DCT will be negative.

(iv) In non-immunological reaction DCT will be negative.

6. Bacteriological smear and culture of donor's blood is done to rule out bacterial contamination.

Interpretation of Laboratory findings

1. If nothing abnormal is found in the above findings, it indicates that there has not been an acute hemolytic reaction.
2. If any finding is positive or doubtful or the patient's clinical conditions strongly suggest a hemolytic reaction, the following investigations are warranted:
   (i) Repeat the cross-match, testing both pre and post transfusion sample of the patient against the sample of blood from the bag or a segment of tube still attached to the unit by a saline, enzyme/albumin and indirect antiglobulin tests techniques.
   (ii) Repeat antibody screening in patient's pre- and post- transfusion samples and in blood from the bag or segment of the tube still attached to the unit with OR_1R_1, and OR_2 R_2 screening cells by saline, enzyme and IAT.
   (iii) If irregular antibody is detected on screening.
      (a) Identify antibody (ies) by panel of cells by saline, enzyme and IAT. Auto-control and two cord cells (one ORh(D) positive, one ORh(D) negative) are also included; [See chapter 9 on Screening and Identification of anti body(ies)]
      (b) Identify the antigen(s) on donor's red cells corresponding to the implicated antibody(ies).

Optional Tests for Clinical Evaluation of Patients:

1. Serum non-Conjugated bilirubin is tested in pre and post transfusion blood samples of the patients. Peak levels of bilirubin occur 5 to 7 hours after transfusion and disappear with in 24 hours if kidney function is normal.
2. Measure Haptoglobin in pre and post transfusion blood samples of the recipient. If recipients blood sample is taken after 1 -2 days, its value is diminished in post transfusion blood sample. Visible hemoglobinemia results only when haptoglobin reserves are depleted, so there is no point in measuring haptoglobin level. Haptoglobin can rapidly regenerate when depleted, so if measurements are made several days after hemolytic reaction, normal levels may already been restored.
3. Methemalbumin test is done in pre and post-transfusion samples of recipients by Schumm's test.
4. Estimation of free hemoglobin is done in post-reaction blood sample. If sample is taken after some time the plasma may be clear of hemoglobin and it may not be visible. Normal plasma hemoglobin is 10-40 mg/litre

In a patient with transfusion-associated hemoiysis for which both immune and nonimmune causes have been eliminated, the possibility might be considered that the patient or donor has an intrinsic red cells defect.

Treatment

If the patient develops a severe reaction with hypotension, shock, and renal dysfunction intensive clinical management is required even before the cause of reaction is investigated.

Therapy for hypotension

0.9% Normal saline intra-venously, to hydrate kidneys

Low-dose dopamine < 5 mg/kg/min. produce selective renal vasodilution, other vasopressive drugs are avoided

Therapy of renal failure

Treatment should be to prevent systemic hypotension and the maintenance of renal cortical blood flow. Low to-moderate doses of furosemide (20 - 100 mg, intravenously) or mannitol are given to improve renal cortical perfusion and redistribution of renal blood flow, producing diuresis when hydration is adequate. In adult, urine output of 100 ml/hour for 24 hours should be maintained.

Therapy of hyperkalemia

After massive hemolysis life-threatening load of potassium may be released into the plasma. It is important to monitor plasma potassium level and must be treated promptly. Hyperkalemia is one of the indication for dialysis after a massive hemolytic transfusion reaction.

Therapy of Disseminated lntravascular Coagulation: See chapter 16 on Transfution Practice in clinical Medicine.

Therapy for bacterial contamination

Management

As the reactions are potentially fatal, they must be recognised and treated at once if bacterial contaminated transfusion is suspected. One should not wait for confirmatory laboratory tests.

Start aggressive broad-spectrum antibiotic therapy and supportive care, corticosteroids are often given empirically. The patient's intravascular volume should be maintained with crystalloid solution and possible vasopressor drugs such as dopamine.

Prevention

There should be written procedure for all aspects of procuring, issuing, and administering transfusion. All staff should be trained in proper use of equipment, intravenous solutions, and drugs used during the administration of blood and blood component. Equipment must be properly maintained and records kept of how and when items are used. Medications that can be given intravenously must never be injected into blood bags, and care must be exercised in selection and use of intravenous access devices.

1. Febrile Nonhemolytic Transfusion Reactions (FNHTR)

It is often described as in increase of 1°C or more temperature, above the patient's base line temperature, during or within 24 hours of transfusion without any other medical explanation. Febrile reaction are often accompanied by chills and rigors.

These are self-limiting and are usually seen in multi-transfused patients or multiparous women who have an antibody directed against donor leukocytes. Such antibody-antigen reaction can activate complement and stimulate cytokines production, which results in the release of endogenous pyrogens. In some cases, cytokines accumulating in stored blood may directly activate endogenous pyrogens.

Signs and symptoms

Generally symptoms are mild and benign

- Temperature I°C or more with or without rigor
- Hypotension is rare.

Sometimes symptoms may be severe and include

- Hypotension
- Cyanosis
- Tachycardia
- Tachypnea
- Dyspnea
- Cough
- leukopenia

Management

Once acute hemolysis is ruled out, supportive care includes :

- Antipyretics e.g. paracetamol, aspirin (aspirin may be avoided in thrombocytopenic patient).
- Antihistamine intramuscularly, (e.g. chlorpheniramine)
- If a second reaction does occur, leukocyte-reduced blood components should be given.
- Routine use of prophylactic antipyretics is not recommended because they may mask symptoms of acute hemolysis.

Allergic Reaction

- These are attributed to foreign protein (allergen) in donor plasma that react with immunoglobulin IgE in the patient attached to mast cells and basophils.
- The donor plasma having reagins (IgE) combine with allergens in the patient plasma.

The antibody antigen reaction initiate histamine release which causes hives, itching and rearly laryngeal edema.

Signs and symptoms

Mostly allergic reactions are mild and not life threatening. The common signs and symptoms are:

- Local erythema (redness)
- Pruritis
- Hives (urticaria)
- Fever may or may not be present

Management

The patient should be monitored carefully because urticaria can be the only sign of a most serious allergic reaction.

- The patient should be treated with an antihistamine to ease discomfort. If the only symptoms is skin rash or hives, and if the symptoms resolve with 30 minutes of the treatment the transfusion may be restarted.
- Patients who have had two or more allergic reactions may benefit from oral or parental antihistamine prophylaxis one hour before transfusion and at the start of transfusion.
- Transfusing saline washed RBCs may help patients with frequent severe allergic reations.
- Corticosteroids are indicated only in severe repetitive cases.

Anaphylactic Reactions

Anaphylactic and anaphylactoid reactions are due to immediate hypersensitivity of immune system. They may begin after infusion of only a few ml of plasma or plasma-containing blood products. They are usually mild at first but can progress to loss of conciousness, shock and in rare cases death.

Sysmptoms may involve one or several systems

- respiratpory tract (cough, bronchopasm, dyspnea)
- gastrointestinal tract (nausea, vomiting, diarrhoea)
- circulatory system (arrhythmias,hypotension,syncope)
- skin (generalized flushing, utricaria)

Generalized reactions may not begin immediately; some develop as long as an hour after transfusion is completed.Good transfusion practice calls for close observation during the first quarter hour of infusion and less intensive afterwards but nonetheless continuing surveillance is kept throughtout and after the transfusion.

Causes

Anaphylactic and anaphylactoid reactions are generally caused in patients who are congenitally IgA deficient and have developed anti-IgA antibodies by the sensitization from transfusion or pregnancy.

IgA deficiency is the most common cogenital immune deficiency, affecting one in 700-800 persons, of whom as many as 30% have circulating anti-IgA antibodies. Anaphylatic transfusion reactions, however, are quite rare.

Management

- Stop transfusion. Keep intravenous line open with normal saline
- Inject epinephrine (adrenaline) subcutaneousIy/I.M. (usually about 0.5 ml of 1:1000 solution)
- Inject antihistaminics (may also be given parentally).
- If hypoxia develops, give oxygen by nasal catheter or mask. Endotracheal intubation may be necessary.
- For those rare patients with a history of an anaphylactic reaction to blood/plasma and who have congenital IgA deficiency and have anti-IgA antibodies, blood component depicted of plasma should be used.

- For RBC transfusion, this can be accomplished by using saline-washed or frozen-thawed RBCs.
- If plasma is needed, it should be from a known IgA deficient donor.

Transfusion-related Acute Lung Injury: (TRALI)

Also known as non-cardiogenic pulmonary edema, this unusual, life threatening complication is associated with altered permeability of the pulmonary capillary bed from activation of complement, histamine-mediated events, or prostaglandins, which leads to fliuds accumulation, inadequate oxygenation, and reduced cardiac return. The reaction is also attributed to anti-leukocyte antibodies in donor or patient plasma that react with leukocyte in the pulmonary microvascular system, resulting in leucocyte emboli aggregating in the lungs capllaries. Other suggested cause includes cytokines in the donor units.

Manifestation

Transfusion-related acute lung injury (TRALI) is manifested by an acute onset of respiratory distress, dyspnea, cyanosis, fever, and chill. An X-ray of chest will show bilateral pulmonary infilterates, but no other signs of left heart failure are seen. Potentially fatal hypoxia may occur and persist for 24-28 hours.

Treatment

- The corner stone of therapy is effective supportive care for respiratory insufficiency. Oxygen therapy and sometimes ventilatory assitance i.e.intubation may be required
- Intravenous steroids are used imparically, but their effectiveness have not been proven.
- If TRALI is caused by patient anti-leucocyte antibodies the leucocyte-poor components should be used.

Usually pulmonary sufficiency returnes within 10 - 12 hours.

Donor who have been implicated in a case of TRALI and who possesses potent leuko-agglutinins can trigger reactions in other patients and should be deferred from donating blood.

Circulatory overload

Patients who are very young or very old, and have underlying congestive heart failure or have chronic normovolumic anaemia and an expanded blood volume are at greatest risk from circulatory overload. When too much blood is transfused too quickly, these patients cannot tolerate the volume increase and consequently develop congestive heart failure and acute pulmonary edema, manifested by

- Cyanosis
- Dyspnea
- Tachycardia
- Peripheral edema
- Hypertension
- Congestive heart failure

Management

When transfusion -induced heart failure is suspected :

- The transfusion may be stopped or continued very slowly
- Intravenous diuretics
- Patient may be put in upright position
- Oxygen is given by mask in order to facilitate breathing
- If symptoms are not relieved or if pulmonary edema persists, phlebotomy may be done.
- Hypertension may need tratment

Prevention

Transfusion associated congestive heart failure can be prevented by identifying susceptible patients and take preventive measures like :

- Transfuse red blood cells rather than whole blood or give small aliquots of concentrated products over a long period.
- Infusion rate should be I ml/ Kg body weight/ hour, transfusion should over within 4 hours.
- Intravenous diuretics may be given before transfusion

DELAYED HAEMOLYTIC REACTIONS (DHTRs)

There are two types of delayed haemolytic transfusion reactions.

Primary Alloimmunization

This reaction occurs several weeks after transfusion and is mostly mild. It is the result of the primary allo-immunization due to the incompatibility of Rh, Kell, Duffy, Kidd, and other systems. It may be due to blood transfusion, tissue transplantation or pregnancy.

The antibodies are formed many weeks after transfusion the signs and symptoms may be mild:

- Mild fever
- Fall in hemoglobin level
- Irregular antibody (ies) may be present
- DAT may be positive

Anamnestic or secondary Response

It is due to the transfusion of incompatible red cells in a patients who is already immunized by previous transfusion. It is also due to the incompatibilty of Rh, Kell, Duffy, Kidd and other blood group systems. Few days after transfusion, the antibody(ies) concentration increases and they destroy red cells causing delayed hemolytic reactions.

There is no reaction at the time of transfusion .Afterwards there may be.

- Fall in hemoglobin;
- Rise in bilirubin and mild jaundice occur 5-7 days after the transfusion
- Renal failure is very rare.

Management

- Transfusion with compatible blood if required.
- It is impossible to prevent all DHTRs. When RBCs antibodies are identified by the blood bank, physicians should inform their patients & counsel them to provide this information when hospitalized elsewhere.
- A transfusion alert card should be carried by the patient.

PLATELET INCOMPATABILITY

A rare complication of blood transfusion, usually involving platelet concentrate, is thrombocytopenic purpura as a result of anamnestic production of platelet antibodies. Post-transfusion purpura occurs in cases who are already immunised by earlier transfusions of platelets. Post-transfusion thrombocytopenic purpura is a rare event occuring usually in multiparous females. Some patients develop a platelet specific allo-antibody anti-Pl^{A1}. The antibody destroys not only the transfused $P1^{A1}$ positive platelets but also causes non-specific destruction of the patients $P1^{A1}$ negative platelets

Antibodies to platelet in multi-transfused patients may cause reaction such as: chills, fever and dyspnea etc. similar to the leukocyte incompatability.

- Febrile reactions

  Chils

  Fevr

  Dyspea
- The thrombocytopenia is usually severe and produces generalised purpura about a week after the blood transfusion.

Management

The thrombocytepenia is usually self-limiting and platelet transfusion is usually not beneficial. Treatment includes:

- Corticosteroids
- Plasma exchange (plasmapheresis)
- Intravenous immunoglobulin.

GRAFT-VERSUS - HOST DISEASE (GVHD)

Transfusion associated graft-versus-host disease is a complication of blood component therapy or bone marrow transplantation. GVH disease occurs if donor-functional lymphocytes engraft and multiply in a severly immunodeficient recipient. These engrafted donor cells react against the foreign tissue of the host (recipient).

GVHD is a rare complication and the patients at risk are :

- Lymphopenic patients
- Bone marrow supressed cases
- Fetus receiving intrauterine transfusions
- New bom infants receiving exchange transfusions
- Individuals with congenital immunodeficiency syndrome

- Patients with hematologic and oncologic disorders
- Patients receiving blood components from blood relatives

The fatality rate with TA-GVHD is about 84% with a median survival of 21 days post transfusion. Death is usually caused by infection or hemmorhage secondary to bone marrow aplasia.

The sysmptoms of GVH disease may include :

- Fever
- Skin rashes
- Hepatitis
- Diarrhoea
- Bone marrow suppresion
- Infections.

Management:

- Pre-transfusion irradiation of all blood components containing lymphocytes will prevent GVH diseases.The functions of red cells, granulocytes and platelets are not affected by such irradiation.
- Corticosteroids
- Cyclosporine
- Methotrexate etc.

The clinical efficacy of these drugs have not been proved adequate in TA-GVHD.

Immunomodulatory Effects of Transfusion

Transfusion has been known to modulate immune responses since observations of improved renal allograft survival in transfused patients in the 1970's. Transfusion is thuoght to have other, less benificial effects in other clinical settings, including increase rates of solid tumor recurrence and increased rates of postoperative bacterial infections. These effects are controversial but suggest that the relationship between transfusion and the immune system is more complex than previously considered.

No specific mechanism has been definitly proved for post transfusion immunodulation (immunosuppression)

No specific therapy regimen is available. Transfusion of appropriate blood component(s) is advised to have beneficial effects.

NON-IMMUNE DELAYED COMPLICATIONS

HAEMOSIDEROSIS (IRON OVERLOAD)

Every unit of red cells contains approximately 200 mgm of iron. Chronically transfused patients specially those with hemoglobinopathies like beta-thalassemia major, congenital hemolytic anemias, or aplastic anemia have progressive accumulation of iron and no physiological means of excreting it. Storage occurs initially in reticulo endothelial sites, but when these are saturated there is deposition in parenchymal cells. Iron deposition interferes with the functions of the heart, liver and endocrine glands. Hepatic failure and cardiac toxicity cause most of the morbidity

Treatment is directed at removing iron without reducing the patient's circulatory haemoglobin. Metered subcutaneous infusion of desferrioxamine, an iron chelating agent is valuable for reducing body iron stores in these patients.

Desferrioxamine is usually prescribed in a daily dose of 20-40 mg/Kg body weight, administered subcutaneously in 8-12 hours (usually over night) by a portable infusion pump.

Transfusions of neocytes (young red cells) have been effective in longer survival of neocytes, causing longer transfusion interval, and lower amount of iron accumulation.

TRANSFUSION TRANSMITTED DISEASES

A major risk of blood transfusion is transmission of infectious diseases, few of which have clinical importance. The blood should be screened for transfusion-transmissible diseases like HIV 1 & 2, Hepatitis B & C, syphilis and malaria to ensure safe blood transfusion.

Mean Time Between Infection and Seroconversions :

Infection	Mean Time	Range
HIV	22 days	6 to 38 days
HCV	98 days	56 to 189 days
HBsAg	56 days	24 to 128 days
HILV	51 days	37 to 72 davs

Treponema pallidum:

- Incubation period in transfusion syphilis varies from 4 weeks to 4.5 weeks.
- In citrated blood stored for more than 72 hours at 2-6°C, spirochetes are unlikely to survive.

Malaria

Incubation period for transmission of malaria depends on the number and strains of plasmodia transfused.

Strains-of Plasmodia	Incubation Time
• P. Falciparum & P. Vivus	Between 1 week to 1 month
• P. Malaria	May be many months

For detail see chapter 12, on 'Transfusion-Transmitted Diseases and their Screening'.

TRANSFUSION REACTION INVESTIGATIONS RECORD

Patient's Name _____ __Age _____ Sex _____
Hospital _____ Adm. / Reg. No. _____
Bag No. _____ Date & Time of Supply _____
Receipt of reaction report with bag and blood sample of the patient on date _____Time____
Approx. remaining product in bag No._____
Any clerical error, Yes/No _____
Colour of blood in bag _____ in tube attached to bag_____

Colour of Patient's blood sample	Pre-transfusion	Post-transfusion
Haemolysis		
Icterus		

Regrouping

Blood Samples	Cell grouping/typing				Serum grouping		ABOGr.	Rh(D)
	A	-B	-AB	-D	A Cells	B Cells		
Pre-transfusion								
Post-transfusion								
Donor-bag/tube								

DAT : Pre-transfusion sample _____ Post-transfusion sample _____
Re-cross matching

Donors blood with					For For IgM	
	Major	Minor	Major		Minor	
	Saline		Alb/Enz	IAT	Alb/Enz	IAT
Pre-transfusion						
Post-transfusion						

Red Cell Antibody Screening

Pre-transtrans. Sample	Cells	RT	37°	AHG	z/Alb	Post-trans. Sample	Cells	RT	37°	AHG	nz/Alb
	Pooled O Cells 1						PooledO cells1*				
	Pooled O cells 2**						Pooled O cells 2**				
	Auto						Auto				

* OR_1R_1 cells; **OR_2R_2 cells

Irregular antibody detected on screening _____ Yes/No _____
Identification of antibody, If antibody found on screening (if possible) _____
#Post transfusion patient's blood sample Free Hb _____
Total Bilirubin _____Direct Bilirubin _____Indirect Bilirubin_____
Urine sample void after HTR for free Hb _____
Donor's Blood Smear (Pathogenic Organism) _____ . _____
Donor's Blood Culture _____
Interpretation _____

Counter Checked Sr. Tech. Officer
(jfGencraly done in Pathology Lab) **Signature of Technician with date**

Blood Transfusion Transmitted Diseases

Introduction

Florence Nightingale, more than 100 years ago said “No stronger condemnation of any hospital or ward could be pronounced than the single fact that zymotic (infectious) disease has originated in it, or that such a disease has attacked other patients than those brought in with them”.

It should, therefore, be obligatory on those who are involved in transfusion of blood to a patient for saving his life, that the blood transfusion does, no harm to the patient. Nothing could be worst than the fact that in an attempt to save life, blood & blood products having transmissible infectious agents have been given. Many of these infectious agents may cause death or prolonged illness. Hence it is necessary to understand the organisms which could be transmitted through blood transfusion and means by which this could be prevented.

Infectious agents

There are four main groups of micro-organisms known to cause infections namely viruses, bacteria, protozoa and fungi

Only first three groups of microbes - viruses, bacteria and protozoa - have been reported to be transmitted by blood transfusion. Individuals with fungal infections are usually too sick to be accepted as blood donors. Viruses are most commonly transmitted by transfusion.

Recently, a new form of infectious agent - the prion - has been identified. At this time, there is no evidence to suggest that they could be transmitted by blood transfusion.

Viruses

Viruses are the simplest forms of life. They infect all forms of life, they lack certain components needed to live and their growth hence depend on the host cell that they infect to provide these missing components .

Following are some of the viruses which are known to be transmitted through blood:

1. Human immunodeficiency virus (HIV)
2. Hepatitis B virus
3. Hepatitis C virus
4. Hepatitis A virus
5. Hepatitis G virus
6. Non - A, Non - B Hepatitis
7. Epstein Barr Virus
8. Cytomegalo virus (CMV)
9. Human T Lymphocytic virus (HTLV - 1 & HTLV - 2)

Some viruses have the property of latency. This is the ability of a virus to join its own nucleic acid with the nucleic acid of the host cell without taking complete control of the host cell as a virus would normally do. Latency usually occurs after an active infection when the individual has recovered and immunity is building up. The viral nucleic acid exists in an inactive form that does not seem to harm the host cell. When the host cell divides, the cell nucleic acid is copied, together with the viral nucleic acid. In this way, the viral nucleic acid becomes part of the cell nucleic acid and is copied every time the cell divides.

Latency is usually indefinite and without any harmful effects on the host cell. However, at any time, the latent nucleic acid could become active and take over the cell functions, resulting in an active infection. This is called a reactivation of infection (recrudescence), which is caused by the reactivation of virus already present in the individual.

TRANSMISSION OF INFECTIOUS AGENTS BY BLOOD TRANSFUSION.

In order to be transmitted by blood transfusion, an infectious agent must be present in the donated blood. Each blood transfusion service or blood bank or laboratory should, therefore, screen for evidence of the microbes that are known to cause infections with this route of transmission .

There are three basic conditions that will determine whether an infectious agent is likely to be transmitted by transfusion:

1. The agent must be capable of using the blood stream as a route of entry in host.
2. The infected donor must be essentially free of any noticeable signs and symptoms of disease, otherwise, they would have been identified during donor screening and the donor would have been excluded or deferred.
3. The agent must exist naturally for a period of time, either free in the plasma or present in a cellular component in the blood stream of an infected donor.

Any infectious agent meeting all these conditions can be transmitted by blood transfusion. However, whether transmission actually occurs or not depends on a number of other factors, particularly on the immune status of the patient and the amount of infectious agent transfused.

It is known that the transmission of certain infectious agents through blood transfusion can, and do occur, and it can be an important route of infection, however, the key point to remember here is that there are a number of ways by which risk can be reduced.

Strategies to reduce the risk of transfusion- transmitted infections.

- The careful selection of donors to ensure that, as far as possible, blood is not collected from people who are likely to be carriers of infectious agents. Safe blood donation depends on, building a panel of regular, voluntary, non - remunerated donors as the first step in ensuring a safe and adequate supply of blood. In our country where most of the blood is collected from family/replacement donors, the risk of transfusion transmissible infection is higher.
- The direct screening of the blood for evidence of the presence of infectious agents or markers produced by them..
- The removal of specific components of blood thought to harbor infectious agents, for example, by the filtration of blood to remove white blood cells.
- The physical/ chemical inactivation of any contaminating agent that may be present: for example, heat treatment of 5 % albumin during production.

Not all infectious agents can be detected directly in donated blood. Blood is often screened for evidence of previous infection by looking for the presence of specific antibodies raised against the infectious agent.

Clearly, it is only by understanding what markers of infection are produced by the infectious agent that screening for the correct marker can be introduced.

INFECTIOUS AGENTS TRANSMISSIBLE THROUGH BLOOD:

Any viral agents can be transmitted by blood, however, important amongst them are HIV, HB V, HCV, CMV, and HTLV-I & HTLV-2 (Table 12 -1)

TRANSFUSION MEDIATED INFECTIONS (VIRAL) (Table 12-1.)

Infection Agents	**Illness**
HIV-1	AIDS
HIV-2	Illness similar to AIDS
HBV	Viral Hepatitis B
HCV	Cirrhosis Liver - Late Carcinoma Liver - Sequelace
Cytomegalovirus	Hepatitis, Lymphadenopathy, rashes, Intrauterine infection of newborns.
HTLV-1, HTLV-II	Adult Cell Leukemia

HUMAN IMMUNODEFICIENCY VIRUS (HIV)

HIV causes AIDS. This syndrome was recognized in 1981, well before the discovery of the causative virus. Wider implications of the immune disorder were noted when, in 1982, AIDS was

reported in three hemophiliacs and in a 17-month-old infant whose multiple transfusions at birth included a unit of platelets from a donor who subsequently developed AIDS. Within a few years, over 50% of hemophiliacs receiving clotting factor developed HIV-1 infection.

HIV was first isolated from the cells of an infected patient in 1983 (HIV-1). The virus was subsequently identified as the causative agent of AIDS. In 1986 a second type of HIV, HIV-2, was identified in certain areas of West Africa. HIV-2 appears to cause the same diseases as HIV-1, but may be less pathogenic. It is morphologically similar to HIV-1. The two types can be distinguished by the presence of proteins and glyco-proteins specific to them. Although cross-reactivity occurs between the core protein of both viruses, the envelope proteins are different.

Components of the HIV Virus (Table 12-2.)

	Bands Observed		
Gene	**HIV1**	**HIV2**	**Proteins**
Gag	pl8,p24,pl5	pl6,p26,p55	Core
Pol	p31		Endonuclease
	p55, p65	p68	Reverse transcriptase
Env	gp41	gp36	Transmembrane proteins
	gpl20,gpl60	gpl40,gpl25	Envelope

p= Protein, gp=Glyco protein,
Number indicates molecules

Cross-reactivity

Cross-reactivity occurs when an antibody recognizes not only its own antigen but also other antigens that have certain similarities. In the case of HIV, this means that an individual infected with HTV-1 would produce antibodies that recognize both core and envelope proteins of HIV-1 and core proteins of HIV-2. Similarly, an individual infected with HIV-2 would produce antibodies that recognize both core and envelope proteins of HIV-2 and core proteins of HIV-1.

THE STRUCTURE OF HIV

There are two types of nucleic acid :

- ribonucleic acid (RNA)
- deoxyribonucleic acid (DNA)

DNA is usually double-stranded. It is the genetic material passed to daughter cells when a cell divides. It is DNA that is responsible for the transmission of hereditary characteristics from parents to children.

The nucleic acid in the HIV virus is RNA. There is no DNA present. Instead, the virus uses the machinery of the human cells that it enters to convert its RNA to DNA so that the virus can replicate or integrate itself in the cell's DNA.

The viral RNA is condensed in a cylindrical core together with two closely-associated structural proteins and an important enzyme called RNA dependent DNA polymerase. This is

more commonly known as reverse transcriptase. This enzyme is found in all retroviruses as it is needed to copy the viral RNA into DNA.

The way that viral and other such proteins are described is based on their molecular weight (measured in daltons) and on whether they are proteins or glycoproteins. The two proteins associated with the RNA of HIV are 7000 daltons (7kDa) and 9000 daltons (9kDa). These are abbreviated to p7 and p9 respectively. The reverse transcriptase enzyme is a 66kDa protein, which is abbreviated to p66. Glycoproteins are similarly abbreviated to "gp".

The core is totally enclosed in a cone-shaped shell of p24 protein. This is called the major core protein and appears to be the same in both HIV-1 and HIV-2. The whole unit is called the viral capsid. (Fig 12.1)

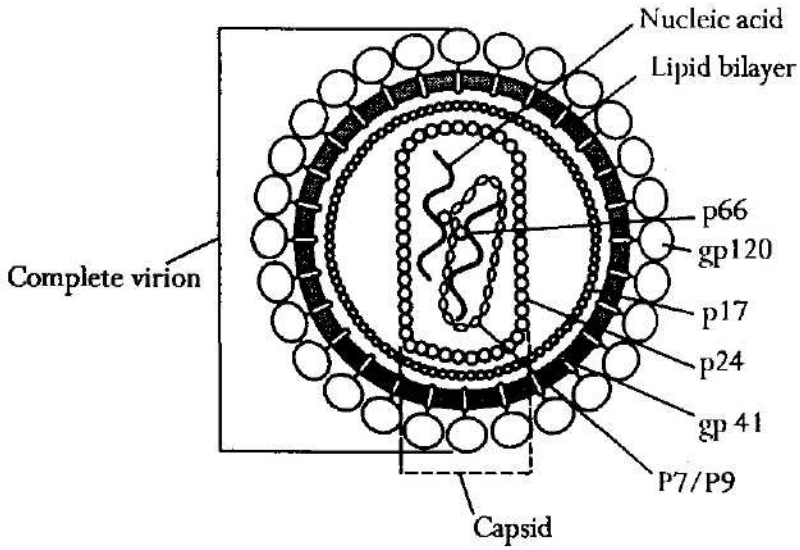


Fig. 12.1

The Structure of HIV

The capsid itself is covered by two layers. The first of these is a shell of pl7 matrix protein to which proteins that project from the surface of the virus particle are attached. This is covered by a lipid bilayer. Projecting through the lipid are many transmembrane proteins. These proteins, gp41, are attached to the p17 matrix and themselves attach the gpl20 envelope proteins. These appear as small projections on the surface of the virus particle. It is the structure of these small projection and their attaching proteins that appears to be the major difference between HIV-1 and HIV-2. The corresponding HFV-2 proteins are gpl 10/130 and gp 36 respectively. Antibodies to these two specific sets of proteins do not cross-react.

The entire virus particle is called the virion. This is the infectious particle that is secreted and transmitted between individuals. The complete virion is 100 -120 pm in diameter.

All retrovineses contain three main structrual genes the gag gene which codes for the core proteins, pol (polymerase) and env (envelope) gene which codes for the envelope glycoproteins.

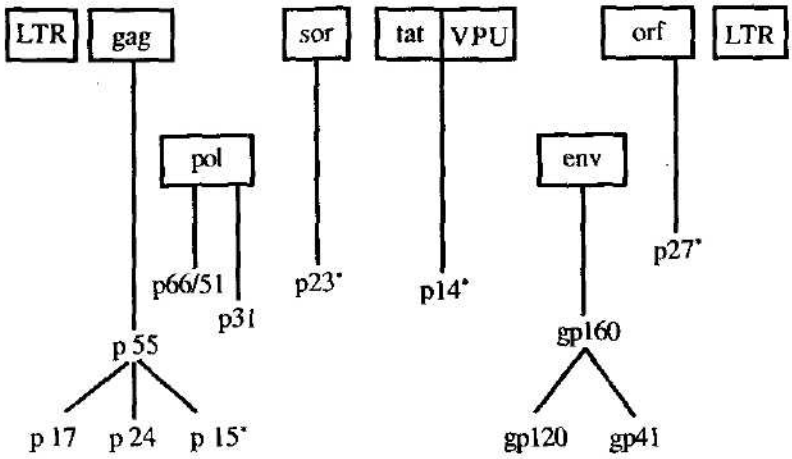


Fig. 12.2

Genetic Structure of HIV includes the nine genes, but their are three main genes - gag, pol and env and they are antigenic, others genes serve to regulate the expression of these virion genes. Gene products marked with asterisk are not usuallay visible on a Western Blot.

ENTRY OF HIV INTO CELLS

HIV enters susceptible cells by binding to a receptor - a protein called CD4 - on the cell surface. CD4 is found on the surface of a number of different cells within the immune system:

- the T cells that help to stimulate the immune response
- the T helper cells (Th cells)
- the macrophages which engulf virus particles in many parts of the body.

Following fusion of the virion to the cell membrane, the uncovered capsid passes into the cytoplasm of the cell. Within the cytoplasm, the RNA is copied to double-stranded DNA by the reverse transcriptase enzyme present in the capsid using raw materials from within the cytoplasm. The DNA then passes into the nucleus of the cell and integrates into the cellular DNA. Once in the cell, the DNA remains latent.

The final stage of infection occurs when the virus starts to replicate. Large quantities of infectious virus (virions) are produced. As the virus emerges (buds) from the cell, it is packaged in the cell membrane to produce the complete virus particles. These virions are then released and can infect other cells.

THE CLINICAL PRESENTATION OF HIV INFECTION AND AIDS

Initially it was thought that infection with HIV only produces AIDS. It then became clear that HIV infection could lead to a number of different conditions of varying severity, although it usually (and finally) results in AIDS.

HIV have a predilection for infection and destruction of CD or helper T cells, thereby producing severe immundeficiency and associated diseases.

In individuals suffering from AIDS, the main cause of illness is the occurrence of secondary infectious diseases, the opportunistic infections. These opportunistic infections are caused by infectious agents which do not produce disease in normal individual.

Following are common infections associated with HIV/AIDS :

- Pneumonia caused by Pneumocystis carinnii
- Tuberculosis produced by Mycobacterium tuberculosis
- Mycobacteriosis caused by Mycobacterium avium/intracellulais
- Chronic cryptosporidiosis
- Toxoplasmosis
- Viral infection, such as cytomegalovirus

Secondary cancers such as Kaposi's sarcoma and non-Hodgkins' lymphoma are other conditions sometimes found in AIDS patients. These cancers are usually aggressive and do not respond very well to standard chemotherapy. Kaposi's sarcoma, as originally described, was a benign malignancy found in elderly men which has no adverse affect on the individual.However, the Kaposi's sarcoma found mainly in AIDS patients in Africa is a fast-growing, and usually fatal malignancy.

In many parts of the world, patients with AIDS Related Complex (ARC) or AIDS present simply with severe diarrhoea. The presence of opportunistic infections or secondary cancers is only determined following clinical and laboratory investigation.

LABORATORY DIAGNOSIS OF HIV INFECTION

Shortly after exposure, the core protein, p24, has been found in some individuals. Within few weeks antibodies to both envelope (gp41) and core(p24) proteins appear in almost all infected individuals. During the early phase of infection a non-specific acute "viral illness" may occur. Once antibodies appears, they increase in titer even though the host is asymptomatic. During this phase of infection viral cultures of isolated lymphocytes demonstrate the presence of virus. As infection progress, changes in the ratio of T-lymphocytes with specific surface markers, CD4 (helper) to CD8 (suppressor) cell, are observed. The ratio of CD4 to CD8 in healthy and immune competent individual is about 2:1. In acquired immune deficiency syndrome (AIDS), the virus destroys CD4 cells and their number in the body decline and there is decrease in CD4:CD8 ratio. HIV-positive persons with fewer than 200 CD4+ T cells per u.1 are considered as having AIDS in the absence of symptoms and /or opportunistic infection.

Before HIV was identified, AIDS was diagnosed by its clinical appearance. Any laboratory tests that were used were surrogate tests. All of these initial tests measured the results of HIV infection rather than specifically detecting either viral antigen or antibody against the virus.

The production of specific tests for HTV helped to understand both HIV infection and AIDS. The finding of anti-HIV antibodies in patients with previously unexplained immunodeficiency is diagnostic of AIDS.

The presence of anti-HIV in an asymptomatic individual means that the individual has been exposed to the virus. It is accepted that, in almost all cases, the virus will be present in the individual. Seroconversion in sequential samples means that the infection has been recent.

Some research papers report that *antibodies are present as early as 14 days after infection, while others indicate that they may not be present until 28 days or more after infection.* The antibodies produced are directed against both core and envelope proteins. The most important antibodies are specifically anti-p24 (core) and anti-gp41 (envelope). Although antibodies to other virion proteins are produced, the presence of these two antibodies has been found to provide the

best confirmation of infection. It has also been found to be the best means of monitoring the progress of infection.

Following infection and prior to the production of antibodies, there is 'window period' of varying length during which the infection establishes. During this period when no antibodies is detected, viral antigen (p24, gp41) could be detected. *The length of time that antigen can be detected is very short, often no more than 1-2 weeks.* Although detection of HIV antigen would theoretically provide evidence of infection at an earlier stage, there are very few commercial antigen assays and they are also not very sensitive. However, in some instance where antigen assays have been used, HIV antigen has been detected at a stage when anti-HIV antibodies had not appeared or just appeared. At present, therefore, it appears that HIV antigen assays may have limited value in blood transfusion service. However, it is possible that their value may grow with an increasing prevalence of HIV in the donor population.

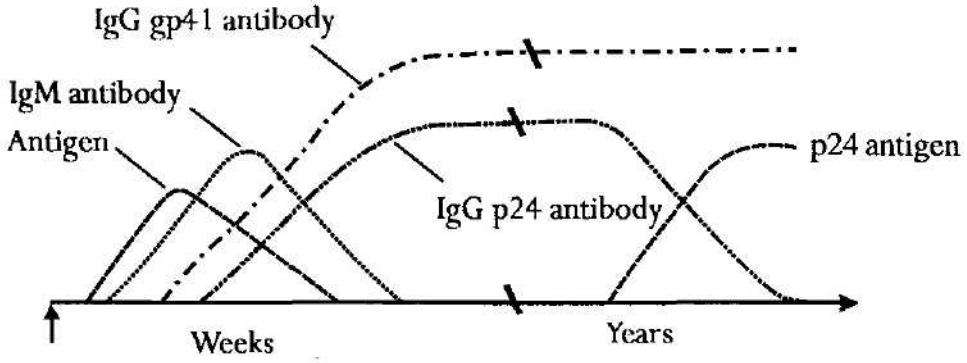


Fig. 12.3
Serological events following HIV infection

Figure 12.3 shows the events following infection by HIV. Soon after antigen has appeared, antibody emerges corresponding with a decrease in free antigen. The levels of antibodies to p-24 and gp-41 peak and remain constant throughout the stages of persistent asymptomatic infection. It is not clear whether p24 antigen production stops completely. Using detergent treatment of serum samples, p24 antigen complexed to anti-p24 antobody has been detected in some patients previously considered to possess only anti-p24 antibody. It is possible that p24 antigen production never ceases completely, but that the circulating antigen - antibody complexes are formed, hence the antigen could not be detected.

As ARC develops, level of anti-p24 antibodies falls and detectable p24 antigen appears. At this stage, anti-gp41 antibodies and p24 antigen are detectable. This situation is the prelude to the development of full-blown AIDS.

It may be noted that viremia is maximum during window period and after ARC and AIDS develops.

TRANSMISSION OF HIV INFECTION

The modes of transmission of HIV infection are now well-established. Whilst virus can be isolated from many body secretions, infection is transmitted in only a limited number of ways.

There are three principal modes of transmission of HIV infection :

1. Unprotected penetrative sexual contact with an infected person, either between men or between man and woman.
2. Inoculation of infected blood, either by blood transfusion or as the result of the use of contaminated needles, syringes or knives used, for example, in injecting drug, ritual scarification or tattooing.
3. From an infected mother to her child, in the uterus, during birth or by breast feeding.

Blood transfusion can be a significant route of infection. The efficiency of the transmission of HIV through blood transfusion is estimated to be more than 90%. WHO reports that the viral dose in HIV transmission through blood is so large that one HIV-positive transfusion leads to death, on an average, after two years in children and after three to five years in adults. Nevertheless, the extent to which blood transfusion is an actual route of transmission depends on the prevalence of infected individuals in the population and on the effectiveness of the screening program used. In a population with a low prevalence of infected individuals and a good screening program, transmission by blood transfusion may be extremely rare. In a population with a high prevalence of infected individuals and with a poor or non-existent screening program, transmission by blood transfusion is likely to be relatively common and would be an important route of infection in the population.

Blood transfusion, therefore, can spread HIV infection very widely if blood is not systematically screened.

Transmission of infection by blood transfusion

Transmission of infection by blood transfusion, or the infusion of blood products, can also be avoided. The first approach to the prevention of transmission by transfusion is the selection of donors who are at low risk for transfusion-transmissible infections. Remember that a safe donor makes a safer donation. The following are important points to remember while selecting a donor:

- identifying low-risk donor groups
- avoiding unsuitable blood donors
- recruiting voluntary non-remunerated blood donors
- promotion of self-exclusion by individuals at risk through an effective donor education program
- predonation counselling, including an assessment of risk factors and an opportunity for self-exclusion or confidential unit exclusion
- a brief medical history, including possible signs and symptoms related to transfusion-transmissible infections
- a basic health check, including a brief examination of the arm for needle marks
- promoting regular voluntary non-remunerated blood donation

Self-exclusion is probably the most effective approach in preventing transmission, but is dependent on the, education of potential donors about risk behaviour. It is particularly important to encourage self-exclusion by people such as prostitutes, homosexual and bisexual men, injecting drug users, those who have any unprotected sexual contact other than with a regular partner, and the sexual contacts of any of these people.

Finally, screening tests for HIV infection are needed to enable infected donors to be excluded and the donated blood to be discarded.

Testing for HIV Viral Markers

The detection of anti-HIV is the most suitable approach for identifying HIV-infected blood donations. The three main kinds of screening assays to detect anti-HIV available are:

- Enzyme Linked ImmunoSorbent Assays (ELISA/EIA)
- Particle agglutination assays
- Specialized rapid assays

While selecting assays for testing anti-HIV following features may be taken into account:

- High specificity
- High sensitivity
- Simplicity of test
- Incubation time
- Cost

Enzyme Linked ImmunoSorbent Assay (ELISA)

It is the most common used assay and is based on the use of immobilized viral antigen which captures anti-HIV antibodies present in the test sample.

Manufacturers often use the terms first generation, second generation and third generation assay.

- First generation assays are purified or unpurified native virus or virus infected cells lysates prepared from cell culture.

- Second generation assays use recombinant antigen produced by cloning fragments of viral nucleic acid into yeast, growing large amount of the engineered yeast in bulk culture and purifying the viral proteins produced.

- Third generation assays are synthetic viral polypeptides artificially produced by chemical synthesis.

Assays are based on the same principles but differ in the way the viral antigen is immobilized:

- On the sides of wells of a polystyrene microplate

- On small polystyrene beads. This method needs specialized equipment.

There are three types of ELISA:

(1) Antiglobulin type ELISA:
(2) Viral antibody present in test sample is bound to immobilized viral antigen and is detected by enzyme labeled anti-human antibody.
(3) Competitive ELISA:
(4) It is widely used assay, in which antibody present in test sample competes with enzyme-linked specific antibody for binding sites on immobilized antigen.
(5) Sandwich ELISA:
(6) This is highly specific type of ELISA in which viral antibody in test sample is bound to immobilized antigen and then detected by free enzyme-labeled viral antigen.

Antiglobulin Type ELISA Method (or EIA)

It is solid phase enzyme immunoassay utilizing polystyrene wells of microplates or beads coated with HIV specific proteins representing HIV core and envelope antigens. See figure 12-4.

(1) Serum or diluted serum is added to the wells coated with HIV specific proteins (p24 & gp 41). Positive and negative controls are added to a number of wells on each plate run.

(2) They are incubated for the defined period of time and at the correct temperature.

(3) During the incubation, any specific antibody present in the test serum binds to the viral antigen.

(4) At the end of incubation, the wells are washed at least three times with washing fluid to remove unbound serum and to prepare them for the next stage, (manual washing is done using multi-channel washer to fill and then empty the wells with wash fluid or by mechanical washing using an automated plate washer).

(5) After final wash, the wash fluid is removed. It is very important that well are as much dry as possible. The plate can be turned upside down and gently tapped dry on some absorbent tissue if the wells are still wet.

(6) Conjugate solution is added to all wells and they are incubated at the defined period of time and at correct temperature.
Conjugate solution contains anti-human globulin antibody which has been chemically linked to an enzyme usually horse radish peroxidase or alkaline phosphate. Conjugate binds to only human antibodies that are bound to the antigen immobilized on the wells. Conjugate does not bound in those wells that did not contain anti-HIV sera bound to antigen.

(7) At the end of incubation, the wells are again washed three times to remove excess, unbound conjugate and are prepared for the next stage of the assay as described earlier in step 4&5.

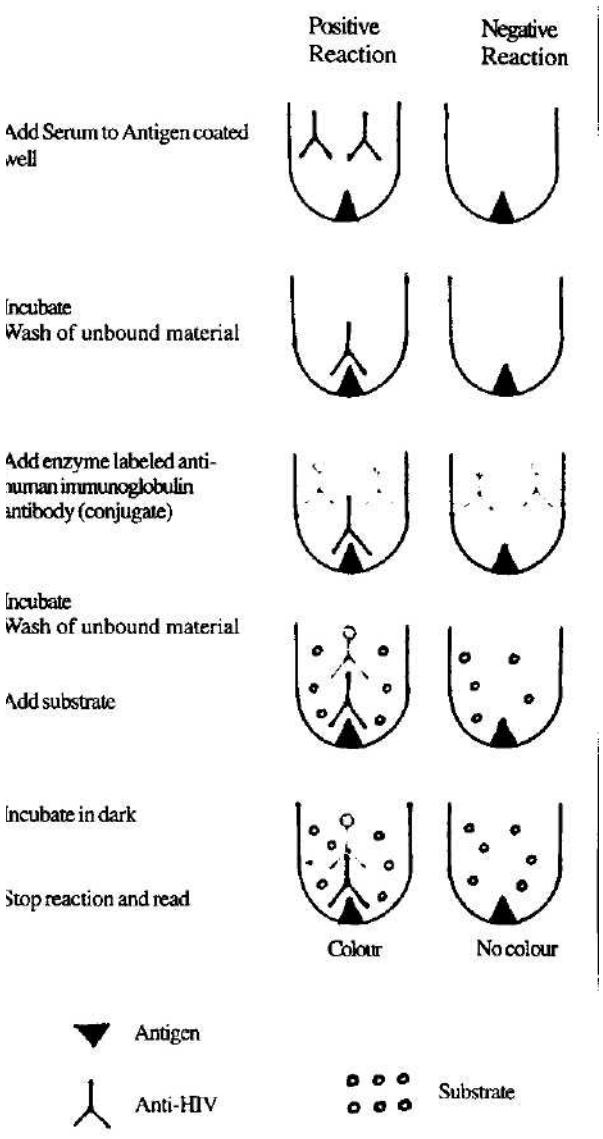


Fig 12-4.
Antiglobulin Type ELISA Method

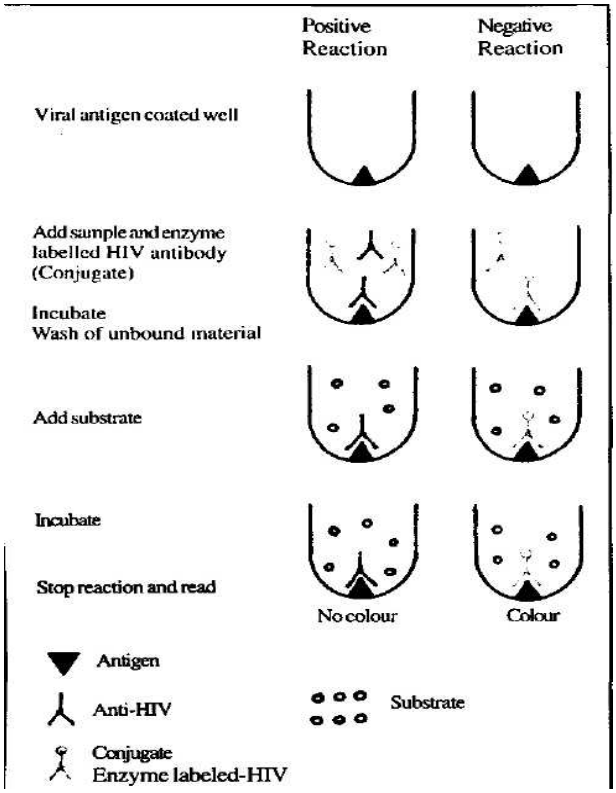


Fig 12-5.
Competitive ELISA Method

(8)Substrate solution is added immediately to all wells and incubated in dark for the defined period of time at correct temperature.

When the substrate solution is added, color develops in the wells containing bound conjugate due the activation of substrate by enzyme. Wells having no bound conjugate do not change the color of the substrate. Thus reactive wells having anti-HIV positive sera are colored, and the wells containing no anti-HIV sera are colorless. The controls show appropriate color changes.

(9) At the end of incubation, diluted acid (1N H_2SO_4) solution is added to all wells to stop reaction. The acid inactivates the enzyme and fixes the color. The intensity of color change is directly proportional to the antibody concentrate present in the samples/controls. The color change can be read visually.

(10) The optical densities (OD values) of the solutions in the microwells are measured by ELISA reader at the specific wave length after determining the cut-off value and the results are determined.

Competitive ELISA Method

The principles of the competitive Elisa are the same as that of antiglobulin assay but it differs slightly in the way in which anti-HIV is detected.

- The conjugate and the test sera are added at the same time in wells and incubated together.
- The conjugate is enzyme-labeled anti-HIV antibody in competitive ELISA, rather, than labeled non-specific antibody as in antiglobulin-type ELISA.
- The conjugated anti-HIV competes with anti-HIV in test sera for the antigen binding site. A test smaple containing anti-HIV will block the binding of conjugate to antigen while sample not containing anti-HIV will allow binding of the conjugate to antigen.

Method: (See figure 12-5)

1. Undiluted sera and conjugate are added in wells at the same time. Positive and negative controls are also put.
2. The wells are incubated for the defined period and at correct temperature. During this

period anti-HIV present in the test serum competes with the conjugated anti-HIV for the binding sites on the viral antigen.

3. At end of the incubation period, the wells are washed with washing fluid to remove excess sera and conjugate and prepared for the next step as described in the antiglobulin-type assay.
4. Substrate solution is immediately added to all wells and incubated in dark for the defined period and at correct temperature.
5. At the end of the incubation period, dilute acid (1 N H_2SO_4) is added to all wells to stop the reaction.
6. An intense color signifies a non-reactive sample, while lack of color signifies a reactive specimen.
7. The optical densities (OD values) of the solution in the microwells are read by the ELISA reader after determining the cut-off value, and the results are determined.

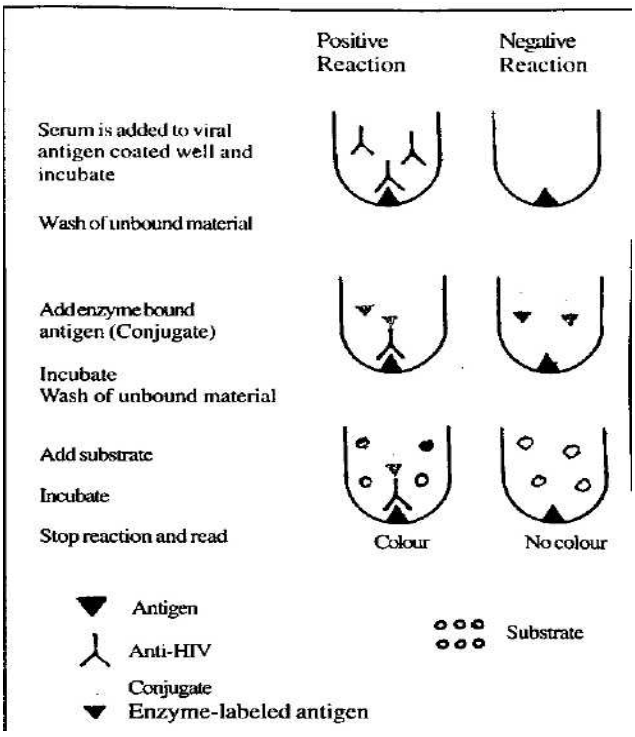


Fig 12-6
Sandwich ELISA Method

Sandwich ELISA Method:
The basic principle of the sandwich ELISA is again the same as that of antiglobulin-type ELISA, but it differs in the way in which the anti-HIV is detected.

- Antigen, usually synthetic peptides are attached to the surface of wells in microplates.
- The conjugate is enzyme-labeled synthetic antigen in sandwich ELISA, rather than enzyme-linked anti-human immunglobulin in the antiglobulin-type assay.
- During the incubation period, the conjugated antigen binds anti-HIV antibody bound to the antigen immobilized on the microwells.
- A sandwich is built of antigen-antibody-antigen.
- At the end of the incubation period, dilute acid (1 H_2SO_4) is added to all wells to stop the reaction.
- An intense color signifies a reactive sample having HIV, while lack of color signifies a non-reactive specimen having no HIV.
- The optical densities (OD values) of the solution in the microwells are read by the ELISA reader after determining the cut-off value, and the results are determined.

Method: (See figure 12-6)

1. Sera or diluted sera are added to antigen bound wells. The positive and negative controls

are also put. The wells are incubated for the defined period of time and at the correct temperature. During this period anti-HIV present in the sera bind to the antigen.

2. At the end of incubation period, the wells are washed with washing solution to remove the excess sera and the wells are prepared for the next step as described earlier.
3. The conjugate is added to the wells. The wells are incubated for the defined period of time and at the correct temperature. During this period, the conjugate binds to antibody bound to the immobilized antigen. A sandwich is built up of antigen-antibody-antigen.
4. At the end of incubation period, the wells are washed with washing fluid to remove unbound conjugate and the well are prepared for the next step, as described earlier.
5. Substrate solution is immediately added to all wells and they are incubated at the defined period of time and at correct temperature.
6. At the end of incubation period, diluted acid (1 N H_2SO_4) is added to all wells to stop the reaction.
7. Color develops in the wells having sera containing anti-HIV and no color will develops in the wells having sera with no anti-HIV.
8. The optical densities (OD values) of the solutions are read by the ELISA reader after calculating cut-off value, and the results are recorded.

Precautions in ELISA

- Hemolysed, lipemic or contaminated sample sera should not be used.
- Correct volumes of test samples, conjugate and substrate are added.
- Instructions given by the manufacturers of kits should be strictly followed.

Particle Agglutination Assays

Particle agglutination assay detects the presence of anti-HIV by the agglutination of particles coated with HIV antigens. Particles are made of gelatin or latex. The assay is performed in microplates.

Method:

1. HIV antigen is immobilized on particles made out of gelatin or latex.
2. Test samples and controls are diluted in microwells with test diluent which is provided with assays.

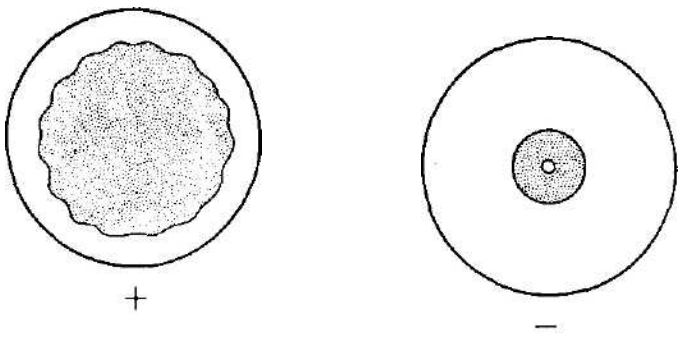


Fig 12.7
Particle agglutination assays

3. The HIV coated particles are added to the diluted samples and controls. Then they are incubated, usually at room temperature (20-24°C) for the defined period. During incubation, the particles are agglutinated by anti-HIV present in the serum.
4. At the end of the incubation period, the result of tests can be read with naked eyes. If gelatin particles are used, they appear in bluish color. If latex particles are used, they appear white in color which can be seen against a black background.
5. A reactive result appears as an even mat of agglutinated particles across the bottom of wells. A non-reactive result appears as a button or ring of non-agglutinated particles, that settle in the center of well. See figure 12-7.

Advantages of particle agglutination assay:

- Expensive equipment are not needed.
- Do not have different stages of reactions.
- Do not need washing equipment.
- Results can be read visually

Disadvantages of particle agglutination assay:

- Subjective error in weak reaction
- Test serum having non-specific agglutinins, may agglutinate both sensitized and nonsensitized particles.

Specialized Rapid Spot Test

It detects anti-HTV. It is rapid and simple and based on the ELISA/EIA technique. The HIV antigen, usually recombinant or synthetic peptide, is immobilized on either porous or semi-porous membrane usually set in a well, in a plastic cassette containing absorbent pad. Most of the specialized rapid assays are in the form of a kit having every thing required for the test.

Method;

1. The test sample and buffer solution (provided with the kit) are put on the porous membrane and allowed to soak in. Pre-dilution of the test sample may be required. This is achieved by the addition of drops of diluent and sample in a suitable vial. The diluted sample is then directly put on the porous membrane.
2. It is incubated for 10-15 minutes. During this period the sample passes through the membrane and if it contain anti-HIV antibodies, it will bind to the HIV antigen on the membrane.
3. The membrane is rinsed with the buffer solution to remove unbound antibodies.
4. Then conjugate is added. The composition of the conjugate varies between assays. Some assays use an enzyme conjugated anti-human immunoglobulin as in antiglobulin-type ELISA. When such conjugate is used, a further wash step and addition of chromogen is required to visualize the results. Some assays use protein A labeled with colloidal gold as the conjugate. It will bind to anti-HIV present and gives a red /purple color.
5. The final result is read visually and compared with the expected results described by the manufacturer.
6. Although control is not required, it is good practice to set up a negative control and a weak positive control.

Note: The instructions provided by the manufacturers of screening kits should be followed strictly for reliable results.

Merits of Specialized Rapid Test

- Very sensitive
- Has the advantage of speed and sensitivity
- Useful in small blood banks.
- Useful in emergency
- Results can be read visually
- No calculation is required

Demerits of Specialized Rapid Method

- Costly
- False positive results due to particles in the test samples (Ghost Dot)
- Results can not be preserved.

Confirmatory/Supplementary Assay

Western Blot is highly specific confirmatory/supplementary test and at one time it was considered as 'gold standard'. The technique is useful in determining with which viral components the antibodies react. It is relatively specialized and expensive test and is not appropriate for screening blood donations. It is no longer recommended as a routine confirmatory test.

Western Blot or Immunoblot

This is enzyme-linked immunoelectro-transfer blot (immunoblot) technique and is used to detect human anti-HIV. It is the confirmatory test of choice. Detergent disrupted purified HIV virions are separated into various proteins (antigens) according to their relative molecular weight by electrophoresis on a polyacrylamide slab gel in the presence of sodium dodecylsulfate (SDS). The separated HIV proteins are transferred to nitrocellulose membrane. They retain their relative positions achieved on separation. The antigen impregnated nitrocellulose membrane is then cut into strips, each strip having the full range of viral proteins which were separated in the gel (figure 12.8)

Method:

- Individual nitrocellulose strips are incubated with serum or plasma specimens in wells of incubation tray. The non-reactive and weakly reactive controls are included with each run, regardless of the number of test specimens.
- The strong reactive control is used to establish criteria of reactivity of bands and is included with the first run for each kit and is not included in subsequent run unless the strip is misplaced. Figure 12.9 shows HlV-specific bands for the strongly reactive control.
- During incubation, if HIV antibodies are present in the specimen, they will bind with viral antigens bound to the nitrocellulose strip.
- At the end of incubation the wells of plastic tray are aspirated and the strips are washed to remove unbound material.
- Anti-HIV bound to HIV antigens on strips are detected by antihuman immunoglobulin antibody to which biotin has been attached. The binding of this tracer antibody to the

human immunoglobulin is detected by the addition of an enzyme-avidin conjugate followed by the application of substrate. This substrate changes color in the presence of enzyme and permanently stains the strips.

Location or position at which antibodies in specimen attach to viral antigen on strips indicate whether antibody is specific for viral antigen or not.

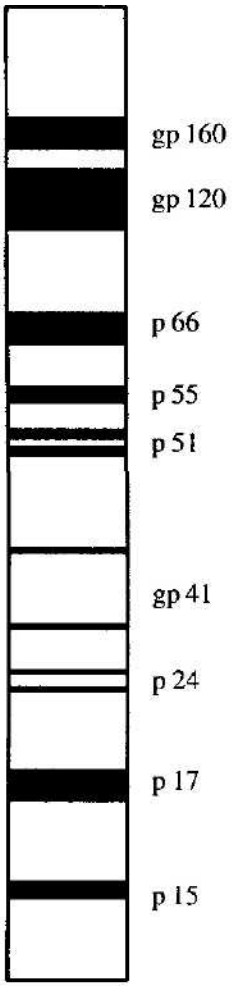


Fig. 12.8
HIV-1 antigens on nitrocellulose strip

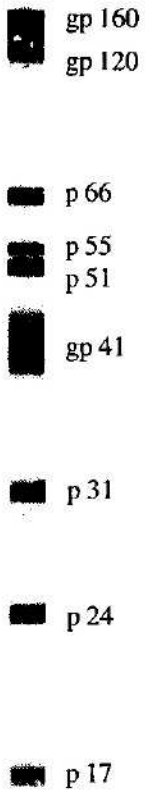


Fig. 12.9
H1V-1 Western blot pattern of reactive control

Interpretation of results
The presence or absence of anti-HIV in a specimen is determined by comparison of each nitrocellulose strip of test specimen with the strips used for non-reactive and weakly reactive controls tested with the run, and the strip used for strongly reactive control tested once with the kit. Figure 12-9, indicates the bands on the strip used with the strongly positive control.

- A donor is regarded positive for anti-HIV if bands to the gag protein p24; pol protein p 31 and env glycoprotein gp 41 and gpl 60/120 are present.
- Recently less stringent but equally specific criterion are emerging, requiring the presence of antibodies to at least one of the gag proteins (p 17, p 24, and p 55), one of the pol proteins (p 31, p 51, p 66) and one of the env glcoproteins (gp 41, gp 120, and gp 160).
- Other bands that do not meet the criteria for positive results are evaluated as "indetermined" and are reinvestigated at a later date after 3 to 6 months.
- If there is diagnostic information suggestive of HIV infection, the blot pattern may be interpreted more liberally, and bands representing only two structural genes are required for a positive interpretation.
- A blot pattern without lines is interpreted as "negative"
- One of the difficult patterns to interpret is the finding of the antibodies reactive to one of the gag proteins. This pattern is found during seroconversion in persons exposed to HIV infection and develop a full immune response later. This pattern may also be found among individuals who have no risk for HIV infection and they do not develop full immune response to other HIV gene products afterwards.

Recommendations for HIV 1 & 2 testing
Sera of all blood donations are tested for anti-HIV 1&2 by ELISA/EIA. Sera non-reactive on the first screening are considered as HIV negative and are recommended for transfusion while a serum reactive on the first screening is considered HIV positive and is not used for transfusion and is discarded.

Confirmatory tests are important to determine how to counsel a healthy blood donor and whether or not may be notified.

The general scheme is given below:

ELISA Screening

Reactive

Repeat ELISA in duplicate

If one or both postive

If both negative, HIV negative

Non-reactive, donation is safe for transfusion

Western Blot is done

Positive
Counsel or Notify (if required by law)

Negative
Revaluate after 3 months (notify-optional)

In the selection of HIV antibody tests for use, the first ELISA kit should have the highest sensitivity, where as for repeat tests ELISA kits should have higher specificity than the first.
WHO has recomended anti-HIV 1 & 2 test with ELISA or Rapid spot assay.

HIV Antigen Detection Assays

These are sensitive and specific tests targeting viral antigens or nucleic acids and their availability has led to proposals for using these assays in screening of donated blood to detect HIV infected blood earlier than with current antibody assays.

The detection of HIV antigen has very limited value in blood transfusion service, however, it may be useful in the following conditions:

- Detection of HIV infection during window period before seroconversion.
- Confirmation of HIV infection in infants born to HIV infected mothers.
- May help to resolve the indeterminate Western Blot test results.
- To monitor HIV infected patients on antiviral therapy.

Methods for HIV antigen testing.

- ELISA for testing HIV p24 antigen
- Polymerase Chain Reaction (PCR.)
- 'Viral isolation

Screening for HIV Antigen (ELISA):

Screening for HIV antigen (usually p 24) is performed using sandwich ELISA. The difference between HIV-antigen and anti-HIV antibody screening is that HIV-antigen test uses a sandwich of antibody-antigen-antibody, unlike HIV antibody screening which comprises a sandwich of antigen-antibody-antigen.

- Antibody (usually mono-clonal) is bound to the surface of the micro wells.
- Test serum and positive and negative controls are added to the microwells and incubated. At the end of incubation period, the excess serum is washed off.
- Conjugate is added and incubated. The conjugate is an enzyme-labeled specific antibody (usually monoclonal).
- During the incubation, the conjugate binds HIV-antigen bound to the anti-HIV immobilized on the microwell. A sandwich is formed of antibody-antigen-antibody.
- The excess conjugate is washed away and substrate (chromogen) is added in the same way as in the antiglobulin assay and incubated.

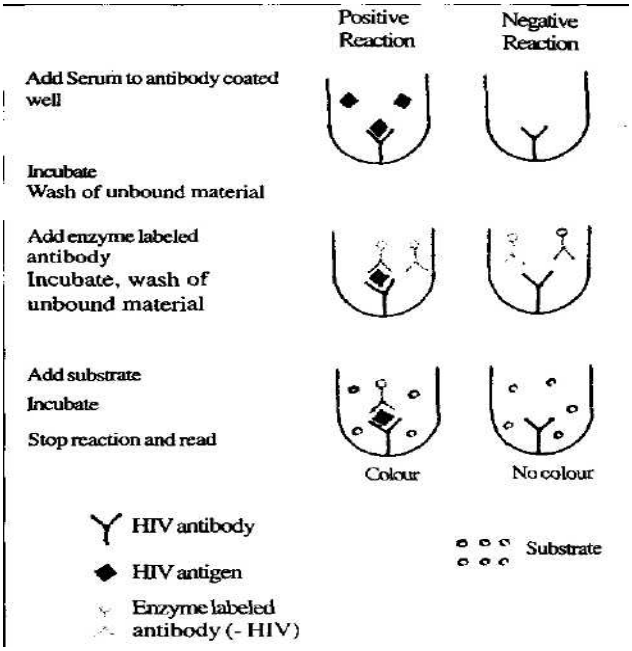


Fig 12.10 HIV Antigen ELISA

- At the end of the incubation period, diluted acid (1 H_2SO4) is added to all wells to stop the reaction.
- An intense color signifies a reactive sample having HIV, while lack of color signifies a non reactive specimen having no HIV.
- The optical densites (OD values) of the solution in the microwells are read by the ELISA reader after determining the cut-off value, and the results are determined (Fig. 12.10).

Polymerase Chain Reaction (PCR) for HIV

PCR is the most sensitive assay for the detection of HIV infection. PCR detects HIV infection before tests for antigen or antibody by other methods and thus further shortens window period (time between infection and sero conversion). This test depends on the amplification of HIV integrated in the DNA of infected cells. Polymerase chain reaction systems require several cycles with a thermostable polymerase at carefully controlled temperatures and need specific primers present at templates for the amplified DNA. The detection of the amplification product is by a labeled probe in an immunoblot assay. This method requires very careful control to ensure that positive reactions are specific.

The routine PCR tests may have only a small impact on blood safety with regard to HIV, however the potential for closure of the window period for HIV infection is significant. The implementation of routine PCR testing of donors' blood will require development of automated systems that will eliminate the time consuming steps necessary to extract DNA and to prepare samples.

TRANSFUSION ASSOCIATED HEPATITIS

At least four viruses have been associated post-transfusion hepatitis:

1. Hepatitis A virus (HAV)
2. Hepatitis B virus (HBV)
3. Hepatitis C virus (HCV)
4. Hepatitis D virus (HDV)

Several other viruses causing hepatitis have been reported. Hepatitis E virus has been reported to cause epidemic hepatitis associated with contaminated water. The majority of transfusion associated hepatitis are due to HBV or HCV.

Hepatitis A Virus

Infection is usually a result of viral spread by contaminated food or water due to fecal-oral mode of transmission. HAV is present in blood for a short period and there is no chronic carrier state in humans. Incubation period is 15-40 days.

Transfusion-transmitted HAV infection has occurred, but it is very rare. An outbreak of HAV infection has been reported in recipients of solvent detergent-treated coagulation factor.

Infection by transfusion require that donor has viremia and the recipient be susceptible to virus. Viremia, if it occurs, is usually berief and at the time of illness, when no one will donate blood. Antibody to HAV regularly appears after infection and thus large number of the population has immunity. The rarity of HAV infection after transfusion does not want routine testing of donors.

Hepatitis B Virus

Hepatitis B virus is in the family of hepadnaviridae. In 1945, it was fond in humans by Blumberg and coworkers in the serum of an Australian aborigine, which they called Australia antigen.

In human the virus is 42 nm in diameter consisting of a 28 nm core with double stranded circular DNA and DNA polymerase. The core is surrounded by a coat protein HBsAg, which also occurs free in the serum as 22 nm spheres and 22 nm to 200 nm filaments. The virion contains further two proteins; hepatitis Be antigen (HBeAg) and core antigen (HBcAg). Both these antigens (proteins) are associated with the capsid core of the virion (Fig. 12-11).

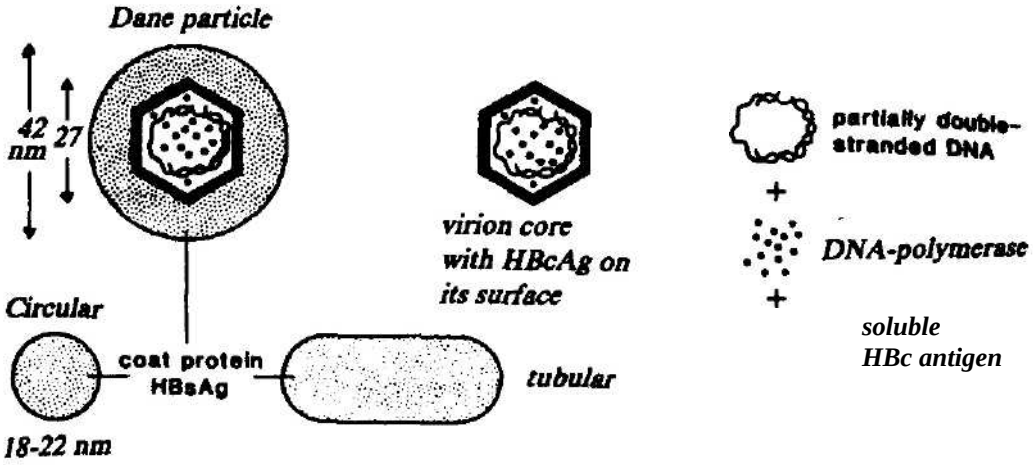


Figure 12.11 Diagramatic representation of HBV

Transmission of HBV infection:

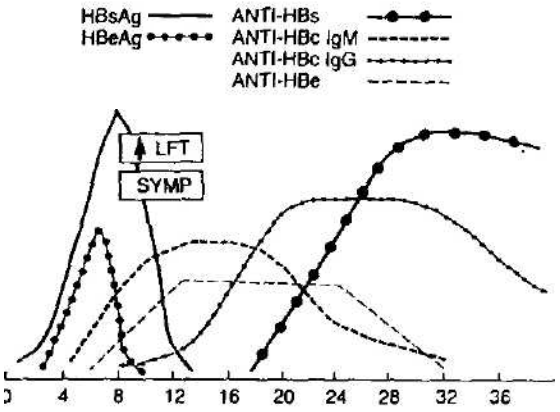
The transmission of HBV is mainly by parental route which involves direct contact with body fluid. The most common route of infection are:

- Contact with infected blood, either by exposure of wounds to infected blood or to contaminated needles and syringes used in injecting drugs, tattooing, ear piercing, or acupuncture.
- Sexual contact
- Neonatal or perinatal transmission
- Transfusion of infected blood or blood products.

Serological Findings (Figure 12-12)

The incubation period of HBV infection is about 30 to 150 days during which the patient has no sign and symptom but the virus may be detected.

- When an individual is infected by HBV several of antigens and antibodies can be detected by serologic tests. Usually the first marker of HBV to appear is HBsAg. It remains detectable from a few days to several months. This marker is also found in some (5% - 10%) infected persons who become chronic carriers of HBV.
- Anti-HBs becomes detectable after HBsAg disappear which indicates recovery from acute illness. Sometimes appearance of anti-HBs is delayed for weeks and months after HBsAg becomes undetectable. During this period, called 'core antibody window', anti-HBc may be the only detectable marker of recent HBV infection.



Weeks after exposure
Figure 12.12 Markers in HBV infection

- Shortly after infection and before clinical signs and symptoms or biochemical changes in liver functions occur, two other markers - HBeAg and anti-HBc are detectable in the serum of infected person.
- Initially the anti-HBc is IgM, however as the infection progress IgG anti-HBc appears and the later persists in persons who recover from the infection.
- HBeAg usually disappears when the patient enters the convalescent phase.
- In others with chronic infection (persistence of HBs Ag for longer than 6 months), HBeAg is cleared, and anti-HBe is found.
- In persons who do not develop immunity to HBV, HBsAg, HBeAg, and anti-HBc can be present.
- Individuals, who recover from infection, will have anti-HBs and/or anti-HBc in their sera.

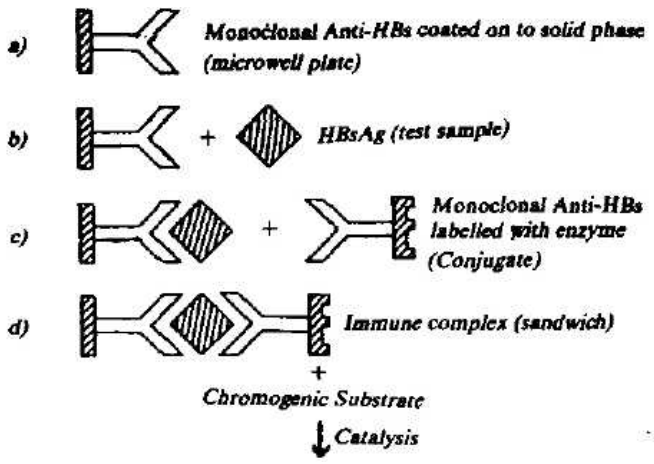
Serologic markers of Hepatitis B infection and their diagnostic significance.

Marker	Significance
HBsAg	Active infection, acute or chronic
Anti-HBs	Clinical recovery, infection resolved, immunity develop
Anti HBc (IgM)	Early acute infection
Anti- HBc (IgG)	Active or past infection (carrier state)
HBeAg	Acute or serious chronic infection
Anti- HBe	Resolution of acute infection, may signal late sequelae

Screening Test for HBV

A variety of serological markers appear following the infection with HBV, and one of these is HBsAg. This antigen appears before biological evidence of liver disease or jaundice. This persists throughout the acute phase of the disease and declines during convalescence.

Procedures for the detection of HBsAg have evolved from the relative insensitive agar gel diffusion method to the sensitive and reliable techniques of radioimmunoassay and enzyme immunoassay. Both are third generation tests and are equally sensitive. ELISA procedure has wide application in the detection of HBsAg and anti-HBs antibodies.



Colour Development
(slopped by adding H_2SO_4)

Figure 12.13 Diagrammatic sequence for detection of HBsAg by ELISA.

Enzyme Immunoassay (ELISA/EIA)

It is based on a one step 'sandwich' principle. It involves the use of solid support (wells of microplate or beads) coated with unlabeled anti-HBs antobody. Number of commercial kits are available and the recommended procedure by the manufacturers should be followed. The general steps of the technique of the test in microplate are given below.

See figure 12-13.

1. Put appropriate volume of the test sample and equal volume of positive and negative controls in the wells of microplate coated with anti-HBs.
2. Incubate for the correct period of time at defined temperature.
3. At the end of incubation period, the wells are washed to remove excess of serum and dry the wells as much as possible before the next stage of the assay.
4. Then add appropriate volume of conjugate (enzyme-linked anti-HBs anti-body) to each well. Enzyme label is usually horseradish peroxidase.
5. Incubate for the correct period of time at defined temperature.
6. After incubation, the wells are washed and are prepared for the next step.
7. Add appropriate volume of chromogen substrate into wells.
8. Incubate the plate in dark for correct period of time, development of colour suggests the presence of HBsAg, and no or low color suggests absence of HBsAg.
9. The results can be read visually or by ELISA reader.

Radio Immuno Assay (RIA)

The RIA method is similar to ELISA. It is also solid phase sandwich test. Plastic beads, tubes or microtiter wells are coated with anti-HBs antibody. The general principles and the steps of technique are described below: (Fig. 12-14.)

1. In the first stage HBsAg in the test serum is bound to the solid phase antibody. After incubation unbound extraneous proteins and tluid in the test is removed by aspiration and washing.
2. In the second stage radio-active iodine (1^{125})-linked anti-HBs antibody is added. After incubation, the unbound iodine-linked antibody is removed by aspiration and washing.

3. Measure the amount of bound iodine-linked anti-HBs with gamma counter. Calculate the mean of the negative controls.

A positive result is usually any value more than 2 to 2.5 times of the mean count of negative control.
False positive reactions for HBsAg may occur with EIA or RIA. The results may be confirmed by repeat or confirmatory neutralization test.

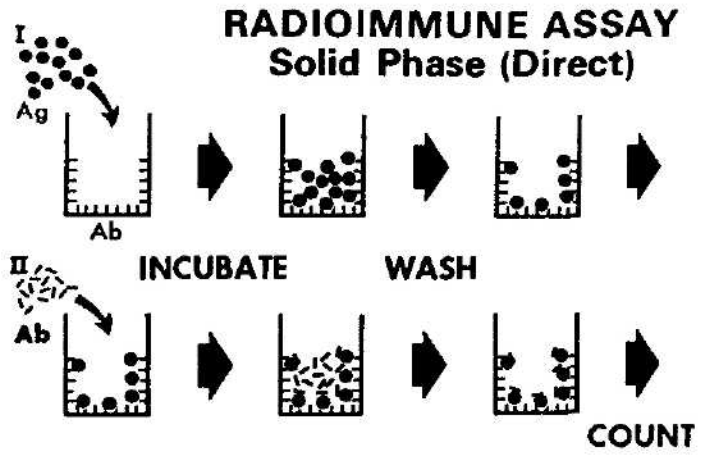


Figure 12.14: Diagrammatic sequence for detection of HBsAg by RIA.

Safety measures for personnel working in the laboratory.

- Eating, drinking and smoking are prohibited in the laboratory.
- Mouth pipetting is forbidden.
- Personnel should wear disposable gloves when handling specimen.
- Hands should be washed before leaving laboratory.
- Laboratory staff should bear laboratory coats to protect their clothes.
- HBsAg positive material and disposable used in the test should be put in leak proof containers. They should be labeled as infectious and autoclaved before disposing them or incinerated.
- Working table should be cleaned after test. Remove spills with swabs soaked in 1.0 per cent hypochlorite solution immediately.
- In case of accidental innoculation of HBsAg positive blood or secretion in the individuals, prophylactic injection of HBIG should be given.

The types HCV, HDV, HEV and HGV have been identified, from amongst the Non - A, Non-B (NANB) hepatitis virus group, however, there are still some types of Hepatitis which do not show antibodies to any of these known types and hence the term Non-A, Non-B (NANB) is still in use. Search for yet more and new hepatotropic viruses is continuing.

HEPATITIS C (HCV)
Hepatitis C Virus (HCV) is the most common cause of post transfusion Non-A, Non-B hepatitis all over the world. The prevalence of HCV antibodies in blood donors in

developed countries ranges from 0.4 - 2%. However in Egypt it is as high as 14 %. The prevalence of HCV in Indian blood donors has not been studies extensively but as per some available reports the prevalence of anti HCV varies form 0.6% to 5.2% in voluntary/ replacement donors.

75% - 80% of HCV infection is reported to progress to chronic infection of which 10% - 20% may progress to cirrhosis and hepatocellular carcinoma.

The average incubation period is 6 - 7 weeks, it may be as less as 2 weeks or as much as 26 weeks, the acute illness (jaundice) is mild.

HCV shares relationship with flaviviruses. Structure of Hepatitis C virus (HCV) has been recently identified by cloning techniques. The virus is an enveloped single standed RNA virus, 50-60 mm in diameter with a genome containing approximately 9500 bases coding for approximately 3000 aminoacids. The genome consists of a highly conserved 5' noncoding region (NCR), generally used for PCR amplification and have regulatory functions. Downsteam to the noncoding region are the regions coding for the structural elements, including the core or nucleocapsid (C) and the envelope (El, E2/NSI), it is presently unclear whether NS1 is apart of the envelope region or the first nonstructural gene. The 5' end of E2/NSI contains a hypervariable region (HV) that mutates rapidly and probably plays a key role in the virus ability to escape neutralization. Next follows a series of non-structural genes (NS2-NS5) with enzymatic on membrane-binding functions (Figure 12.15).

The initial clone discovered was from the NS 4 region, the derived protein was designated 5-1-1. This was expanded to form the cl00-3 antigen, the basis of the first generation anti-HCV EIA assay.

Second generation EIA assay added the c22 core antigen and c33c antigen from the NS3 region. These antigens increased the sensitivity of the second generation assay by about 20% over the first generation assay.

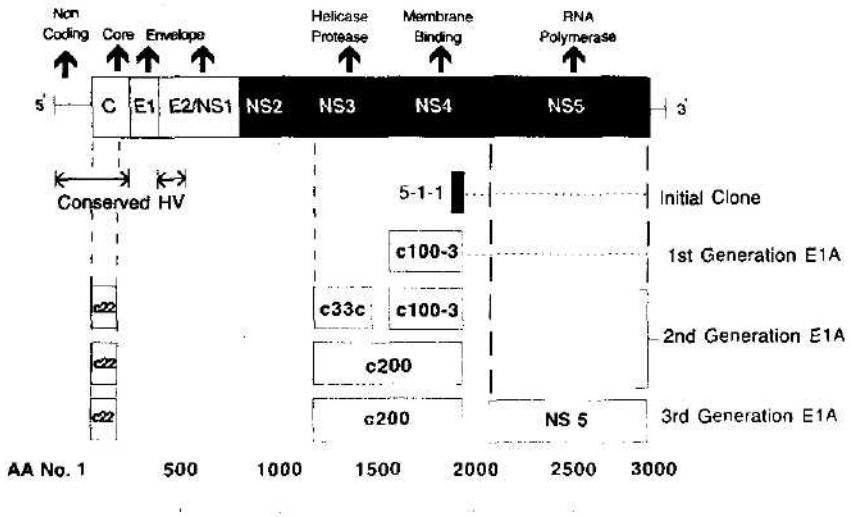


Figure 12.15: Proposed genome of HCV: functional equivalents and major antigens used in antibody detection assay.

Third generation EIA assay adds an NS5 protein and reconifigures some of the earlier antigens. Now it has synthetic peptides which offer the advantage of minimizing the incidence of non-specific reactions. The third generation EIA have only incremental benefits in the disease prevention.

Instructions given for the methods by the manufacturers of the kits should be strictly followed.

Because of the problem of non-specificity, it is important to confirm EIA reactivity with a supplemental assay, a recombinant immunoblot assay (RIBA) that displays the key epitopes in a linear form on a nitrocellulose strip. Now in RIBA the recombinant proteins c22-3 and cl00-3 are replaced with synthetic peptides representing only a portion of the respective coding regions and replaces 5-1-1 antigen with recombinant NS5.

Polymerase Chain Reaction (PCR) for HCV

Detection of HCV infection during early phase of infection is possible by the detection of HCV-RNA by various molecular biological techniques-nucleic acid amplification techniques.
PCR amplification can detect low level of HCV RNA in serum. Testing of HCV RNA is a reliable way of demonstrating that hepatitis C infection is present and is the most spefific test.Testing of HCV RNA by PCR is useful in:

- In imunodepressed patient.
- In patient who have organ transplant recently.
- In indetermined recombinant immunoblot assay.
- In acute infection as HCV viremia occurs well before the development of anti-HCV.

Certain rapid antibody detection techniques such as immunochromatic test (ITC) are also available.

Prior to the development of specific markers certain surrogate markers i.e. determination of serum alanine aminotransferase (ALT) and antibodies to hepatitis core antigen (anti-HBc) have been used to identify the high risk donors for transmission of NANB hepatitis (HCV). Although the surrogate markers played an important role in the prevention of hepatitis C prior to the introduction of HCV specific assays but presently the value of these tests remain inconclusive and controversial. In a study reported from India very little co-relation was found between the surrogate markers i.e. (anti-HBc & ALT) and anti - HCV reactive donors. It is too early to comment whether hepatitis C (HCV) is the only cause of parenteral NANB hepatitis as the current HCV antibodies assay fails to detect 100 % NANB hepatitis.

Community acquired or Sporadic NANB hepatitis.
Sporadic community or acquired NANB hepatitis due to hepatitis C virus (HCV) with no history of parenteral exposure has been identified.

HEPATITIS D (Delta virus)
It is also known as delta hepatitis. It is caused by defective RNA virus that is unable to produce its own protein coat and thus coats itself with HBV surface antigen. The delta virus is dependent on preexisting or concomitant HBV infection for propagation. Co-infection causes acute viral hepatitis which often becomes fulminant and is usually fatal, while superinfection causes acute hepatitis progressing to chronic active hepatitis and cirrhosis in 75 % of carriers.

Delta hepatitis has parenteral mode of transmission and usually follows HBV infection. Although tests for delta antigen and antibody exist but should not be used in blood donor screening as all infections with delta virus are positive for HBsAg. So routine testing for HBsAg will eliminate the risk of its transmission.

HEPATITIS G VIRUS (HGV)

Hepatitis G virus is an enveloped RNA virus and belongs to the flavivridae family. The virus was discovered in 1995. The virus genome is similar to that of HCV with which it shares a 25 % homology at the nucleotide level. The HGV appears less variable although variants have been described. HGV replicates in peripheral blood cells but its replication in the liver is still questionable. The mode of spread of HGV is almost same as HCV. The HGV can be detected in the serum by nucleic acid technology (PCR). Recently an immuno-assay has been developed to detect antibody to HGV.

The prevalence of HGV in blood donor population by doing antibody test in the developed countries ranges between 1 to 5% but the detection of antibody generally indicates recovery from an infection and probably immunity to the HGV agent.

CYTOMEGALOUS VIRUS (CMV) INFECTION

CMV is known to be carried in leukocytes, therefore blood components containing white blood cells are more likely to transmit CMV infection. Plasma-derived components and derivatives are not likely infectious for CMV. Transfusion-transmitted CMV can cause severe disease in seronegative premature baby of low birth weight and seronegative immunosuppressed patients e.g. bone marrow transplant recipients or patients with HIV infction.

Since more than 50-80% of adults in developed countries and more than 90% in underdeveloped countries have antibodies to CMV, so it is not practical or advisable to screen donors blood for CMV infection. However, blood transfusion services should have panel of CMV negative donors. In high risk group seronegative patients it is advisable to use CMV negative blood or use leukocytes depleted blood.

Screening tests for CMV

- Latex agglutination
- Enzyme immunoassay
- Complement fixation

The latex agglutination test is mostly used in blood transfusion centers because it is simple and rapid.

Human T- cell lymphotropic virus type I & II

HTLV I & II are retroviuses. Both are cell associated and are not present in plasma. HTLV-1 has been shown to be associated with adult T-cell leukemia-lymphoma and various neurological conditions originally called tropical spastic paraparesis but now often called HTLV-associated myelopathy (HAM). Incubation period for HTLV-1 infection is very long and 2-4% of sero-positive persons develop the disease.

The importance of HTLV-11 is not clear. It is usually associated with intravenous drug abuse and has been found in few cases of Hairy cell Leukemia.

HTLV-1 infection can be transmitted by blood, which has caused concern in those parts of the world where the virus is prevalent, notably in some countries in the Western Pacific and the

Caribbean basin. Surveys conducted in Europe and North America indicate that HTLA-1 infection is very rare.

Transmission

- It is transmitted with transfusion of cellular components of blood. After refrigerated storage for 10 days or more, red cells from an infected donor are far less likely to cause sero-conversion

- Transmission by sexual contact (predominantly male to female)

- Mother-to-child transmission through breast milk

Donor screening should be considered in those countries where HTLV-1 is endemic. Preliminary epidemiological studies are therefore necessary (WHO, 1992, Guidelines for the organization of a Blood Transfusion Service).

ELISA techniques are used for screening HTLV-1 antibodies, and Western blot, immunofluorescent, or radioimmune precipitation techniques are used for confirmatory testing. It is difficult to differentiate between HTLV-1 and HTLV-11 unless advanced techniques such as polymerase chain reaction are available.

BACTERIA

Bacteria consist of distinct cells which possess cell walls, and have a very simple structure and lack a true nucleus. Bacteria may have capsules which are often important in the immune response. Example of common bacteria which are known to be transmitted through blood is Treponema pallidum.

Syphilis
Etiological agent for syphilis is Treponema pallidum. Blood and its components may transmit syphilis. The incubation period for transfusion transmitted syphilis is 1 to 4 months, the blood recipient exhibiting the signs and symptoms of secondary syphilis.
Transfusion-transmitted syphilis is not a major hazard of modern transfusion therapy, its chief reasons are:

- The spirochetes do not survive in citrated blood stored at 4°C for 72 hours.
- Now the blood is mostly collected from the voluntary donors and sexually promiscuous persons are excluded from blood donation.
- Most patients who need transfusion of blood or its components receive antibiotics therapy because of their clinical condition.
- Widespread use of penicillin and other antibiotics to treat syphilis and its carrier might account for the rarity of infected cases.

Role for syphilis in prevention of posttransfusion syphilis:

Spirochetemia usually is common in the early stages during the invasion of lymphnodes. The screening tests used for blood donors often are negative in early syphilis when spirochetes could be transmitted by blood transfusion. Only 25% of patients with primary syphilis have a reactive serological test for syphilis (STS). and the rest do not become positive until the 4"' week after the

onset of primary syphilis. By the time the person develops a positive STS, the spirochetemia has typically cleared.

In addition, a high proportion of healthy people have biological false positive reactions even they do not have circulating spirochetes. It may be due to viral infections, immunization, lupus erythematosus and dysproteinemias.

The prevention of spirochete transmission is not well accomplished by STS test. Anyhow it is considered safe to test blood donation for syphilis due to the following reasons:

- There is possibility of transmitting syphilis.
- Demand of fresher blood for exchange transfusion, and platelets.
- Screening for spirochetes helps to exclude donors who are in high risk group for HIV and HBV infections.
- Cost for STS is low.

Tests

The serological tests are of two types:

- Non-specific tests
- Specific test

Non-specific test:
The most commonly used test in blood donor screening are non-specific tests because they are simple, rapid and economical, They are:

- Venereal Disease Research Laboratory (VDRL) test
- Rapid plasma reagin (RPR) test

Specific tests:
These tests are mainly confirmatory tests and have lengthy procedures. They are not suitable for routine screening of donors blood. Tests are:

- Treponema pallidum hemagglutination test (TPHA)
- Fluorescent treponemal antibody absorption test (FTA-ABS)
- Treponema pallidum immobilization test (TPI)
- Enzyme Immuno Assay

PROTOZOA
Protozoa are unicellular organisms. They have a well-defined cellular structure with a clear nucleus and other organelles. A typical cell is enclosed by a cytoplasmic membrane. This may be covered in an outer cytoplasmic layer. Proteins present in the membranes are important in immune response.

Examples of common protozoal infections, which can be transmitted due to blood transfusion are Plasmodium species.

Malaria

Malaria is caused by intra-erythrocytic protozoan parasites. Plasmodium vivax, P. falciparum, P. ovale or P.malariac. The usual mode of transmission is via the bite of anopheles mosquito.

Blood infected with malarial parasites can also transmit the infection. Transfusion-transmitted malaria may become evident a week to several months following the transfusion of infected blood. The incubation period of plasmodium vivax and P. falciparum is shortest e.g. one week to one month, while that of P. malariae is the longest e.g. many months.

Malarial parasites survives for at least one week at 4°C in whole blood and red cells concentrates, and at least for one week at room temperature in platelets.

Screening tests for the malaria

(1) Microscopic examination of thick and thin blood smear.
It is difficult to find parasites in blood film in short time especially if the density of parasites is less than 100 per microlitre of blood. However it is best practical method for testing malarial parasites

(2) Tests for antibody to malarial parasites are:

- Indirect fluorescent antibody test (IFA)
- ELISA

(3) Immuno-diagnostic testing, including EIA methodology

(4) Nucleic acid probe methodology, including polymerase chain reaction (PCR).

None of the above methods except microscopic examination of blood smear is practical and possible.

Since there is no appropriate method for screening malarial parasites in blood donation, it has been suggested that chemoprophylaxis therapy for malaria should be given to all recipients of blood in highly endimic areas.

VIRAL INACTIVATION

With the emergence of HIV/AIDS there is great demand for guaranteed safe blood and blood products. Today there is rigorous screening of blood to prevent transmission of blood borne infection, the transfusion of blood products have already achieved high level of safety. The "window period" viraemia can be further reduced by screening the donated blood for nucleic acid testing methods. Thus further reducing the risk and increasing the already high cost of testing.

METHODS OF VIRAL CLEARANCE

Several methods of removing or inactivating viruses that may be present in plasma pools from the source or recovered plasma donations are being tried. These steps could be divided into two broad categories: removal, or partitioning, is the physical separation of the viruses or viral particles from the blood and blood products; and inactivation, which destroys the virus so that the remaining viral fragments lack the structure and components needed to infect an individual receiving the blood or blood product.

Removal processes include filtration, affinity chromatography, ion-exchange chromatography, and polyethylene glycol fractionation. Inactivation of viruses has been attempted by heating.

Further, some processes, such as ethanol fractionation results in both removal and inactivation of viruses. Finally, a number of new methods are under development, such as irradiation, photoinactivation and treatment with a variety of chemicals. Several of these novel techniques

have been incorporated into the production of investigational products. In order to be effective, viral inactivation techniques must destroy at least one viral element essential to replication. Photosensitizing techniques use light - activated dyes that are irradiated, causing the dyes to convert to molecules that can alter DNA or membrane lipoproteins. Heat treatment denatures viral proteins and nucleic acids, rendering viruses incapable of replication. Irradiation processes may destroy viral nucleic acids by inducing breaks and linkages. Solvent detergent techniques destroy the viral envelop on lipid - enveloped viruses.

Ultra Violet(UV) inactivation was one of the method thought to be useful for this purpose, as most viruses are quite sensitive to UV, however, current expert opinion is that viral inactivation sufficient for the purpose is not feasible without intolerable level of damage to the different components of the blood.

Photochemical inactivation of viruses in platelet concentrates by use of novel Psoralen and long - wave length U V light, significantly reduces the viral load but cannot be relied on for total removal. This process is based on photochemical reaction of novel Psoralen (S - 59) with nucleic acid, upon illumination with long wave length UV light (UVA, 320 - 400 nm).

Recently (Nov. 2000) Gambro BCT announced inactivation of pathogens in blood products like packed Red Blood Cells by Riboflavin and UV light. Riboflavin can inactivate pathogens in all the three major blood components that is Red Blood Cells, platelets and fresh frozen plasma.

Hemolytic Disease of the New Born (HDN)

Introduction

Hemolytic disease of newborn and the foetus is due to the premature destruction of the red blood cells of the foetus in uterus and neonates in early neo-natal period after delivery. The red cells destruction is brought about most commonly by IgG antibodies produced by the mother due to the blood group incompatibility between mother and foetus. The mother can be stimulated to form IgG antibodies by previous pregnancy or transfusion, and in small number during the pregnancy itself.

Less common causes of the hemolytic disease of new born are due to red cells abnormalities such as in thalassemia, deficiency of glycolytic enzymes and hereditary spherocytosis, or neonatal infections.

HDN can also be due to prematurity and physiological jaundice due to insufficient production of glucuronyl transferases.

Blood transfusion service is mostly concerned with the HDN caused by maternal antibodies and it will be discussed in detail.

The most common cause of HDN sufficiently severe to require treatment is incompatibility of Rh system and in 95% of these cases the D antigen is responsible. Rarely c. C and E antigens have been implicated.

HDN due to ABO incompatibility is usually mild.

Other antigens like Kelt. Kiddand Duffv have also been rarely involved.

Disease Mechanism
Hemolytic disease of the new born and foetus is caused by the destruction of the red cells of the foetus or new born by IgG antibodies produced by the mother. Only antibodies of the immunoglbulin IgG class actively transfer across the placenta, other classes as IgA and IgM do not. In HDN, the mother antibodies act against the antigens on the fetal red cells that are inherited from the father

Pathology (fig. 13.1)
The three most important features of the pathology of HDN are:

1. Anemia due to the destruction of the infant's red cells.
2. Hyperbiliribinemia resulting from the catabolism of the hemoglobin released from the destroyed red cells.
3. Changes in the tissues.

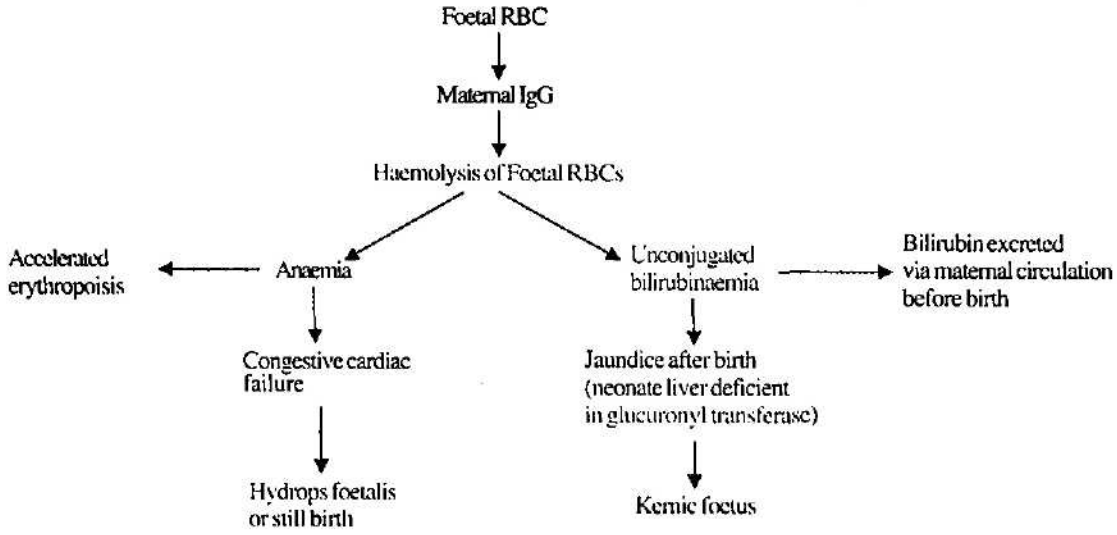


Fig. 13.1 Diagrammatic sequence of events in HDN

Anemia
Destruction of foetal red cells and the resulting anemia stimulate the foetal bone marrow t produce red cells at an accelerated rate, even to the point that immature red cells (erythroblasts are released into the circulation. The term erythroblastosis fetalis is used on this finding. When the bone marrow fails to produce enough red cells to keep up with the rate of red cells destruction erythropoiesis outside the bone marrow is increased in the spleen and liver. The liver and splee are enlarged, resulting in portal hypertension and hepatocellular damage.

Severe anemia along with hypoalbuminemia cause cardiac failure with generalized edem effusions, and ascites known as hydrops foetalis.

The process of red cells destruction goes on in neonate. as long as the maternal antibodies remain in the infant's circulation for several days to weeks after delivery.

Hyperbilirubinemia
The continuing destruction of the red cells of the foetus and infant releases large quantity bilirubin for disposal during both intrauterine and neonatal period. This bilirubin is indirect bilirubin

This indirect bilirubin crosses the placenta and conjugates in the maternal liver to direct bilirubin, The conjugated bilirubin is excreted by the mother. The levels of total bilirubin may be elevated in the amniotic fluid. At birth the infants are anemic but may not be jaundiced.

However after birth, the infant is unable to conjugate bilirubin efficiently especially in premature infants owing to the lack of glucuronyl transferase to convert indirect to direct bilirubin, and jaundice develops (icterus gravis neonatorum). When level of bilirubin exceeds to the binding capacity of the albumin, the unconjugated bilirubin can reach to levels toxic (more than 18 g/dl) to the infant's brain. If untreated, it can cause kernicterus or permanent damage to brain.

Tissue Changes

The destruction of red cells in the spleen and increased erythropoietic activity in liver and spleen causes enlargement of the liver and spleen in HDN.

RH (D) HAEMOLYTIC DISEASE OF THE NEW BORN

Anti-Rh(D) IgG antibodies are the prime cause of severe HDN. Iso-immunization of Rh(D) negative mother may result from Rh(D) positive pregnancy or transfusion of Rh(D) positive blood.

Factors influencing the production of Rh-antibodies are:

1. Size of Foeto maternal haemorrhage / leak

The amount of foetal red cells that cross the placenta into maternal circulation in the third trimester of pregnancy is usually small and insufficient to cause antibody production. In addition, elevated steroid levels and other factors, associated with pregnancy may suppress the mother's primary response. Usually the first Rh incompatible foetus is unaffected.

At delivery, a transplacental haemorrhage is not uncommon. The amount of foetal blood entering the maternal circulation varies from less than 1 ml to 10 ml or more. The Rh(D) positive foetal red cells stimulate the production of anti-Rh(D) in about 5-9% of these Rh(D) negative mothers. The antibody appears in the mother's blood within 6 months of delivery and 7-12% of the mothers become sensitized but do not have demonstrable levels of antibodies until they have secondary stimulus during a subsequent pregnancy.

When pregnancy with a second Rh(D) positive foetus occurs, the foetal red cells crossing the placenta from about the 24^{th} week of gestation either stimulate the sensitized mother to produce antibody or stimulate the existing antibody to a higher titer. This is secondary response and therefore, only a small amount of red cells are capable of producing antibody sufficient to cause HDN. The anti-Rh (D) formed is IgG, thus it is capable of crossing the placenta into foetal circulation, where it combines with foetal Rh(D) positive red cells, leading to their destruction.

2. Parity

Incidence of sensitized

1 Para	II Para	III Para	IV Para	V Para & above	Average
-	1:50	1:29	1:20	1:9	1:33

About 70% cases are confronted with HDN in II and III pregnancy. Usually first pregnancy is unaffected.

3. Effect of ABO incompatibility between mother and the foetus:

ABO incompatibility between mother and foetus provides some protection to the foetus from Rh-HDN. It is believed that the existing maternal ABO allo- antibodies destroy the Rh(D) positive foetal red cells immediately as they enter the maternal circulation before they can sensitize the mother. Once the mother becomes immunized to Rh antigen, this protective effect is lost. (See table 13.1).

Table: 13.1: ABO group of mother and baby which are incompatible.

Mother	Baby
O	A or B (baby can not be AB if mother is O group)
A	BorAB
B	AorAB

4. Zygosity of father

If the father is heterozygous for the implicated antibody there is 50% chance of the child being effected by Rh-HDN. It is not possible to determine the genotype of the father for Rh (D) antigen with centainty unless the father has a living D- negative child. However, for C and E antigens true genotype can be determined.

5. History of Blood Transfusion

A Rh(D) negative mother who has been immunized by Rh(D) positive blood transfusion has a risk of delivering a baby with Rh-HDN even in the first pregnancy. The risk of immunization is greater with Rh(D) positive blood transfusion than with Rh(D) positive pregnancy in a woman since the antigens load is much greater in transfusion.

5. Medical termination of Pregnancy (MTP) or Miscarriage can also immunize the Rh-negative mother.

Antenatal Assessment of Rh-HDN

The objective of the antenatal assessment is to identify women with a high risk of having their babies effected with HDN so that they can be investigated during pregnancy to estimate the degree of involvement for proper management.

Investigation should be started at approximately the 12^{th} week of pregnancy or at the first obstetric visit.

(I) Detailed obstetric history

Women with bad obstetric history (i.e. hydrops foetalis, stillbirth or HDN) or evidence of Rh immunization should be closely followed up.

(II) History of Blood Transfusion

Rh positive blood transfusion may immunize an Rh negative woman; its significance has been discussed earlier.

(III) Grouping
ABO and Rh grouping including D^u grouping

(IV) Antibody Screening
The sera of both Rh (D) positive and Rh (D) negative women should be screened for red cells antibodies on the first visit since some IgG antibodies (i.e. Kell, Duffy, Kidd, Ss) that can cause HDN may be found in Rh (D) positive women. A sampling regime is given below:

Stage of Pregnancy	Samples to be collected from
Early pregnancy	All cases
28^{th} week	Rh (D) negative mother
$34\text{-}36^{th}$ week	All cases

Screening test for IgG antibody (ies) should be done with at least two individual O group screening cell panel (see chapter 9 on Screening and Identification of Antibodies) by indirect antiglobulin test, using the polyspecific antiglobulin serum and the two-stage enzyme technique . The latter has greater sensitivity for detection of Rh antibodies.

(V) If antibody (ies) is /are detected at any stage then the following be done:

(a) Repeat titer every month : Severity of the disease is generally, though not always, correlated with the titer of the antibodies. A titer of 1:32 or above and a rising titer at repeated testing is significant and is an indication for aminocentesis. For the technique of titeration of antibody see chapter on Special methods.
(b) Identify the antibody (ies) with 8-10 cells panel (see chapter 9)
(c) Carry out Rh phenotype to confirm the implicated antibody.

(vi) Father's Probable Rh genotype
The father's red cells are tested with anti-D,-C,-E,-c and -e to determine Rh phenotype. From these data, the probable genotype can be estimated. Whether the father is homozygous or heterozygous for D cannot be determined with certainty unless he has a Rh(D) negative living child. However, for C and E antigens, the true genotype can be determined.If the father is heterozygous there is a 50% chance of the child being unaffected with Rh-HDN.

(vii) Antibody Dependant Cell-medicated Cytotoxicity (ADCC) Test
The result of ADCC correlates well with the severity of HDN. A high ADCC indicates serious HDN while if the result remains low until the end of the pregnancy, the infant is mildly affected or is unaffected (for more information the reader is referred to large text books).

(viii) Aminocentesis
Amniotic fluid is almost colourless but in case the infant has a severe hemolytic process it is bright yellow due to bilirubin. Amniotic bilirubin concentration can be estimated by spectrophotometric examination and it is extremely valuable in predicting the degree of the hemolytic process in pregnancy and severity of HDN. The main indications of amniocentesis are:

(a) The liter of maternal IgG antibodies by IAT is 1:32 or above or a rising titer on repeated testing by about 28 weeks of gestation;
(b) Previous obstetric history of still birth, hydrop foetalis or neonatal death in Rh(D) negative mothers.

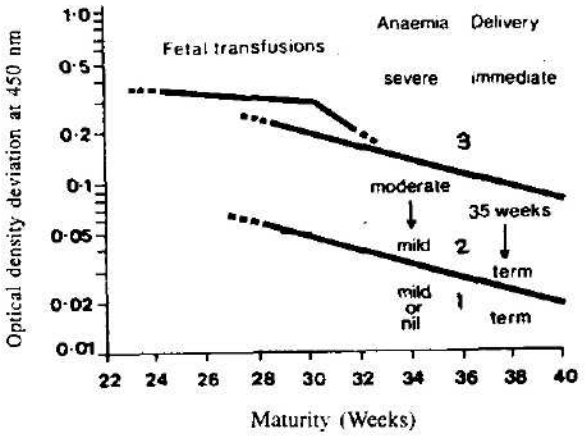
Amniocentesis is usually done between the 28^{lh} -32nd week of pregnancy. It can also be done earlier. Amniocentesis can be repeated after 2 weeks. Two samples of amniotic fluid are helpful to verify the results and establish whether optical density is constant, increasing or decreasing. The optical density of amniotic fluid (at 450-460 nm) in normal pregnancy is about 0.05 at the 30^{lh} week, falling to about 0.02 at term. The fall is probably due to the decrease in albumin concentration in the amniotic fluid. The fall is also present in infants having HDN.

Optical density at 450 nm varies according to the period of gestation. If the optical density at 450nm is in the lower zone, the baby will be unaffected or it may have mild disease.

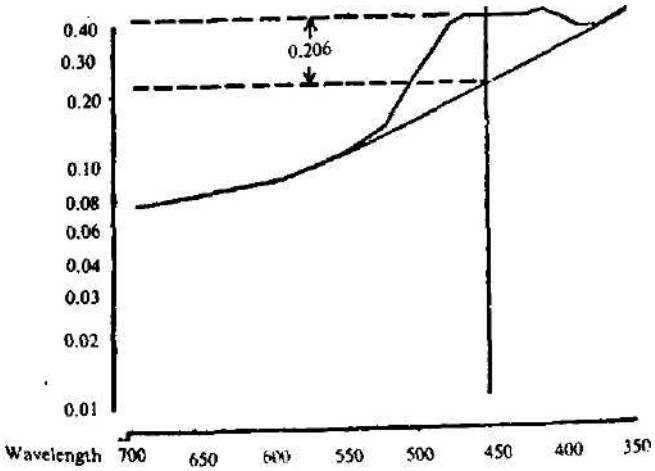
A middle-zone pattern suggests that the baby will probably be moderately effected, and one should be prepared for an exchange transfusion after delivery.

If the optical density is in the top zone prior to 32 weeks gestation, intrauterine transfusion should be done.

Termination of pregnancy can be done at 34 weeks or earlier depending on the studies of foetus maturity (see Fig 13.2)



Curves showing three zones indicating the approximate severity of HDN from O.D. reading of amniotic fluid



Spectrophotomerric- pattern of the amniotic fluid in a ease of <w> affected erthyroblastic baby

Fig. 13.3

Precautions:

1. The first amniocentesis is usually not done before 26 weeks, as foetus is too small for any kind of intervention.
2. The amniocentesis should not be performed without a clear cut indication because of complications like infection and enhancement of maternal immunization.
3. Samples should be protected,from light, which oxidizes bilirubin pigments.
4. Samples should be free from muconium and foetal maternal blood, since they increase the optical density

.

Procedure
Amniocentesis is done under ultrasound to find a placental free window for inserting the needle through abdominal wall and uterus into uterine cavity. It is generally done at 28"[1] to 32[nd] week of pregnancy; it can also be done earlier.

The aspirated amniotic fluid is analyzed spectrophotometrically, and the optical density (OD) is measured at different wavelength over a range of 400-600 nm. In the normal, a smooth curve is obtained while in HDN a peak at 450-460 nm suggests increase in bilirubin levels of amniotic fluids.(fig 13.3)

Foetal Maturity
A determination of lecithin/ sphingomyelin (L/S) ratio in amniotic fluid may be valuable in predicting the maturity of foetal lungs and thus the ability of the foetus to survive after early delivery. An L/S ratio of less than 2.0/1.0 indicates the lung immaturity and intra-uterine transfusion may be required.

The foetal condition can be evaluated by ultrasound scanning. This has replaced aminography.

INVESTIGATION OF NEW BORN
Investigations of cord blood samples from Rh-negative mother play an important role in the management of HDN. A sample of venous blood taken from the infant after birth (even 30 min) is far less valuable than cord blood samples in assessing the severity of HDN.

Cord blood samples should be obtained from the maternal side of the umblical cord after it is divided, by inserting a needle attached to a syringe into the umbilical vein. One samples should be taken into anti-coagulant, EDTA (lmg EDTA in lml). It is used for ABO and Rh grouping, antiglobulin test and for measuring hemoglobin value. The second sample is taken in a dry plain tube; and serum is separated which is used for the determination of bilirubin and for screening the antibodies.

Investigations on newborn include:
1. ABO grouping
2. Rh(D) grouping including D^u
3. Direct-antiglobulin test (DAT)
4. Screening and identification of antibodies in cord's serum (elute if necessary)
5. Hemoglobin estimation on cord blood
6. Cord blood bilirubin estimation
7. Hematological parameters on cord blood. Number of nucleated cells and reticulocyte count is increased. Anyhow they are not specific for HDN.

ABO Grouping
ABO grouping of the new born is done only by cells grouping because alloantibodies in the cord blood are of maternal origin. Red cells should be washed 4-5 times to avoid false-positive results due to Wharton's jelly.

Rh Grouping
Accurate Rh grouping may be difficult if red cells are heavily coated with IgG antibodies. Either false-positive or false-negative results may occurs.

False Rh(D) positive results may occurs if;

(i) Cells are contaminated with Wharton's jelly
(ii) Rh (D) negative cells are heavily coated with antibody (ies) other than anti-Rh(D)
(iii) Reagent anti-D serum may contain potentiator of agglutination that may cause non-specific reaction.

The problem can be solved by

- using cells washed 4-5 times in saline
- gentle elution of antibody followed by repeat Rh(D) grouping.
- using reagents diluent as control. If there is agglutination in the control tube, saline or chemically modified anti-D serum can be used to determine the Rh(D) group.

False-negative or weak-positive results : Sometimes false negative or weak positive results occur with saline anti-D when the new born is Rh positive and the red cells are fully coated with maternal anti-D that no D sites are available to react with the reagent serum. This should be suspected when the immunized mother is Rh negative and the baby's cells give a strong positive DAT. Maternal anti-D can be removed from these cells by heat elution (see chapter on Special Methods) and Rh test is repeated.

Direct Antiglobulin Test (DAT)
DAT usually gives strong positive results in HDN due to anti-D or antibodies to other red cells antigens; reactions are much weaker or negative in HDN due to ABO. If the DAT is positive and the maternal serum has a negative antibody screening test, suspicion falls on ABO-HDN or HDN due to antibody against a low incidence antigen not present on screening cells panel.

Identification of antibody in cord blood
The maternal Rh antibody may or may not be found in the infant's serum, however, identification of antibody can also be performed in the elute of cord cells.

Cord Hemoglobin Value
Cord blood hemoglobin correlates well with the clinical severity of HDN. The cord hemoglobin level (Tablel3.2) serves as a guide to assess the severity of HDN. Normal cord blood Hb is 18.6-19.6g/dl)

Table 13.2 Showing cord Hb. Levels and severity of HDN

Hb level (g/dl)	Severity of HDN
Over 14	Uneffected
11-14	mild to moderate
8-11	moderate to severe
below 8	severe

Cord Blood Bilirubin
(Normal cord bilirubin is 0.7-3.1 mg/dl)
There is also a correlation between cord blood bilirubin concentration and severity of HDN but it is less close than the cord hemoglobin. The cord blood bilirubin concentration is valuable to assess the severity of the disease when Hb value is within normal limits. Cord blood bilirubin above 4mg/dl is usually suggestive of very severe disease. Peak bilirubin levels usually reached by the third or fourth day of life.

ANTENATAL MANAGEMENT OF Rh IMMUNIZATION
If after taking into account the mother's previous obstetric history, the levels of anti -D and amniocentesis findings, if the obstetrician is convinced that the foetus might not survive a full-term pregnancy, the following options are available at present:

1. Intrauterine transfusion: Intrauterine transfusion can be
   (a) Intraperitoneal
   (b) Intravascular

(a) Intraperitoneal Transfusion (IPT): The procedure is preformed under ultrasound localization and monitoring after about 24-26 weeks of gestation and it is usually repeated after every two to three weeks until delivery at 34-35 weeks when infant is mature enough.

The packed red blood cells of group O Rh (D) negative are given and they should be less than five days old, leucocyte depleted and irradiated (preferably) and compatible with mother's blood. The volume of red cells for transfusion is calculated by using the formula: (gestation age in weeks - 20) xl0ml. Leucocyte poor red cells that have been irradiated will reduce the possibility of the mother developing white cells or platelet antibodies, and even graft versus host disease in the foetus.
(b) Intravascular transfusion (IVT): Direct intravascular transfusion of compatible irradiated, leucocyte poor red cells via the umblical cord vein under foetescopic or ultra sound monitoring is a better procedure than IPT in infants who need transfusion before-25 weeks of gestation and are severely anemic.

Red cells suspended in Adsol or SAGM should not be used for transfusion to the fetus in uterus.

2. Premature induction of labour : The majority of high-risk pregnacies are terminated selectively after 34 weeks of gestation to lower the incidence of still-births and to reduce severity of HDN in live born infants. The decision to induce premature labour depends upon analysis of amniotic fluid, prior obstetric history, antibody titer and foetal ultrasound studies for maturity. Premature induction of labour at 30-32 weeks is possible with improved medical and nursing care.

3. Plasmapheresis : Intensive plasmapheresis has been effective in reducing antibody level in maternal serum and enhancing chances of foetal survival. Optimum volume of plasma to be plasmapherized has still not been determined. Promising results have been obtained in some cases with exchange of small volume of 250ml to 600 ml plasma every week beginning at 10-20 weeks of pregnancy till the baby is delivered at 34-35 weeks gestation. The safest replacement fluid is 4-5% human albumin solution and small supplement of frozen fresh plasma (from Rh negative donors) is necessary to maintain normal IgG levels, coagulation factors, etc.

There is no consensus about the therapeutic value of plasmapheresis in Rh-immunized pregnant women and this remains an experimental approach of unproven value.

POST - NATAL TREATMENT OF INFANT
About 40% of infants born with DAT positive, require no treatment while others, if untreated, die within a few hours due to cardiac failure or become deeply jaundiced and may develop kernicterus

at any stage after the age of about 36 hours. It is desirable to select at the earliest, cases of HDN requiring treatment.

Blood Transfusion
Infants with mild HDN (Less than one week old) who exhibit only anemia may be transfused with red cells concentrate to correct the anemia. Occasionally a partial exchange transfusion may be necessary.

Exchange Transfusion
In infants with moderate to severe HDN, an exchange transfusion is performed with the following objectives:

(a) Correction of anemia by providing compatible red cells with adequate O_2 carrying capacity.
(b) Removal of unconjugated bilirubin.
(c) Removal of baby's antibody coated red cells.
(d) Removal of the offending antibody (ies)

Indication for exchange transfusion
There is little agreement on criteria for exchange transfusion. However, cord blood haemoglobin, bilirubin and birth weight are the major determinants of exchange transfusion. All new borns at risk, must be monitored closely by bilirubin determination at 4-8 hours intervals, with the objective of keeping the serum bilirubin level below 20 mg/dl in full term infants and 15 mg/dl in premature infants.See table 13.3.

Table 13.3: Parameter of exchange transfusion in Rh-HDN

Parameter	Observe	Consider exchange	Do exchange when
At Birth			
Cord blood			
Hemoglobin	>14g/dl	12-14 g/dl	<12 mg/dl
Serum Bilirubin	<4 mg/dl	4-5 mg/dl	>5 mg/dl
After Birth			
Capillary blood	>12g/di	<12g/dl	<12g/dl and falling in
hemoglobin			first 24 h
Serum billirubin	<18 mg/dl	18-20 mg/dl	20 mg/dl in first 48 h*

* If the bilirubin level rises at a speed indicating that it will reach 20mg/dl before the child is 48 hrs of age.

Selection of Blood for Exchange Transfusion in Rh-HDN

1. Blood should be as fresh as possible (48 h old) but it should not be more than 5 days old

for exchange transfusion to ensure long survival of red cells in the infant and to avoid high level of plasma potassium in the transfused blood.

2. Rh(D) negative blood of the same ABO group as that of the baby's is used if ABO group of the baby is the same as that of the mother or is compatible with mother's blood.

Table 13.4 : Selection of ABO group blood for exchange transfusion in Rh -HDN

Baby's blood group	Mother's Blood Group	Blood selected for exchange transfusion
A	A	Aor O
	B	O
	AB	A or O
	O	O
B	A	O
	B	B or O
	AB	B or O
	O	O
	O	O
O	A	A or O
AB	B	B or O
	AB	AB,A,B,O
	O	O
	A	O
O	B	O
	AB	O
	O	O

3. When baby's ABO group is not compatible with mother's ABO group or if donor's unit is prepared before delivery, it should be O Rh(D) negative blood and it should be free form hemolysins anti-A and anti-B.

4. In case exchange transfusion is required more than once, subsequent blood should be of the same ABO and Rh type as that of the first time.

5. If the mother's antibody is reactive against a high frequency antigen and no compatible blood is available:

(a) The mother's siblings can be tested for compatible blood.

(b) A unit of blood can be collected from the mother, if the obstetrician agrees that it is safe. Mother's red cells are constituted in AB plasma.

(c) If no compatible blood is available and the clinical situation is urgent, exchange transfusion with incompatible blood (for the high frequency antigen) is preferred to no transfusion at all as it helps to remove antibody (ies) and bilirubin. Withholding blood may result in death of the infant.

6. In areas where sickle call anemia is prevalent, the donor blood should be screened for sickle cell trait before the blood is used for exchange transfusion.

Volume and hematocrit of blood for exchange transfusion
Ideally an exchange transfusion equal to twice the newborn infant's blood volume is recommended. The blood volume of a full term newborn is approximately 85 ml/kg. Exchange transfusion with partially concentrated red cells having hematrocrit of 55-65% is preferable to whole blood. Some clinicians like to exchange relatively large volume of blood, e.g 200 ml/kg in 60-90 min.

Special consideration in compatibility testing for exchange transfusion

1. If ABO group of mother and infant are compatible, it is convenient to obtain the serum from mother than from the infant for compatible test.

2. If mother's blood is not available or the ABO group of mother is not known or the ABO group of mother and infant are incompatible, baby's serum or the elute from cord cells is used for cross- matching.

3. Blood should be cross-matched against mother's serum by IAT and enzyme method.

Procedure
Exchange transfusion is usually accomplished through a single vascular access (umblical vein). A three-way-stop-cock joins the unit of blood, the baby and extension tube that leads to a graduated discard container. A maximum of 5 ml/kg is used for each draw and infusion .The donor blood should be properly mixed during exchange; the exchange is completed in about 90 min.

An in-line blood warmer, if possible, and a standard blood filter should be incorporated in the administrative set. The following considerations may apply to exchange transfusion in new born:

1. Oxygenation and acid - base balance should be monitored; it is often necessary to increase the inspired oxygen concentration during the procedure.

2. If the post-exchange platelet count is less than 25000/mm^3, it may be necessary to administer platelets.

3. Hypoglycemia may occur as a rebound phenomenon after exchange or a phenomenon secondary to the increase in insulin in plasma that may accompany severe hemolytic disease. Infusion of 10% glucose solution after exchange transfusion may be needed.

4. 1 ml of 10% calcium gluconate after infusion of 100 ml of blood may be given to avoid citrate toxicity.

PREVENTION OF HDN
The usual dose of 250-300 (ig of hyperimmune anti-D immunoglobulin (RhIg) is sufficient to counteract the effect of 15 ml of Rh(D) - positive red cells which correspond to 30 ml of foetal blood.

If more number of foetal cells are detected in the materanal circualtion by Kleiharu Betke Acid Elution test, the dose must be increased proportionately.

1. RhIg (300 (µg) is given intramuscularly within 72 hrs to Rh(D) negative women who have
   - (i) Delivered an Rh(D) - positive infant and have not developed anti-D in the serum. It is not useful once immunization has occurred,
   - (ii) Undergone abortion or medical termination of pregnancy
   - (iii) Undergone aminocentesis: or
   - (iv) Have had antipartum haemorrhage

2. It has been advocated that the risk of immunization is further lowered if 300 ug RhIg is given antipartum at 28 weeks gestation and a second dose of 300 ug RhIg is repeated within 72 h of delivery.

ABO HEMOLYTIC DISEASE OF NEW BORN

ABO incompatibility is the most common form of blood group incompatibility between mother and foetus. However, it usually results in a mild form of HDN. ABO-HDN occurs mostly in group O mother with A or B foetus and is due to maternal IgG anti-A or anti-B. Prior immunization with A or B cells is not essential for formation of IgG anti-A and anti-B, hence ABO-HDN can occur in any pregnancy including the first. ABO-HDN is usually mild because

(i) A and B antigens are not fully developed in the infant's RBC;
(ii) Tissue A and B antigens of the infant neutralise the anti-A and anti-B of mother, thereby decreasing the number of antibody molecules which can attach to the RBC (Mollison)

The following criteria are used for the diagnosis of HDN (Bhatia, 1977)

1. Exclude the presence of antibodies to other blood group antigens by checking maternal serum with a panel of reagent cells.
2. The mother is generally of Group O with infant of group A or B though on rare occasions heterospecific pregnancy in A or B group mother may result in ABO-HDN.
3. Anti-A and or Anti-B titer higher than 1:128 and presence of hemolysins in the mother may further support the diagnosis.
4. Since incomplete (IgG) anti-A and anti-B titer upto 32 are normally found in Indian O group mothers (Gupte et.al 1972) a higher titre is a diagnostic features.
5. Direct coombs' test is often negative or occasionally weak positive.
6. Hematological changes like increased fragility and spherocytosis in the jaundiced infant with above features further support the diagnosis of ABO-HDN

Investigation in ABO-HDN

Tests in the mother

The following tests are carried out:

1. ABO and Rh(D) grouping.
2. Estimation of IgG anti-A and anti-B in maternal serum. Titer above 32 is considered significant.
3. Antibody-dependent cell-medicated cytotoxicity (ADCC) test in relation to severity. A good correlation has been observed between the results of ADCC using mother's serum, and the severity of HDN (Mollison 1987)

Tests in the Infant

The following tests are carried out:

1. ABO and Rh(D) grouping.
2. Direct antiglobulin tesst (DAT) using polyspecific and monospecific AHG serum (IgG; C3d). DAT is usually negative or weak positive.

3. Serum bilirubin levels are moderately increased and can be controlled by phototherapy but, very often may need exchange transfusion.
4. Hemoglobin level may be slightly decreased in cord blood in moderately severe ABO-HDN.
5. Hematological parameters:
   (a) Reticulocytosis 5%
   (b) Increased osmotic fragility
   (c) Spherocytes in blood smear

Management Of ABO- HDN
Severe anemia is very uncommon and therapy, when necessary, is related to the control of hyperbilirubinemia.

1. Phototherapy
2. Blood Transfusion: Infant with mild ABO-HDN who exhibit only anemia may be transfused with red cells concentrate .The blood should be as fresh as possible but it should not be more than 5 days old in any case.
3. Exchange Transfusion with whole blood should be carried out when the bilibrubin level threatens to exceed 20 mg/dl. Group O blood of the infants's Rh type should be used. Blood should have low titre of anti- A and anti-B and should also be free from haemolysins. When O group blood is given to an infant who is group A, B or AB, the red cells concentrates should be used. It is a good practice to use packed cells resuspended to one-third volume of fresh AB plasma (or A plasma or B plasma as appropriate)

Volume and Hct of blood for.exchange transfusion is same as that for Rh HDN.

Estimation of IgG anti-A and anti-B
Naturally occurring IgM anti-A or anti-B complicate the accurate determination of IgG anti-A or anti-B. Estimation of IgG anti-A or anti-B is done after partial neutralization or inactivation of IgM anti-A or anti-B by the following methods:

1. Partial neutralization of IgM anti-A or anti- B by blood group substance A or B (Witebsky's test)
2. Inactivation of IgM antibody by thiol reagents,
   (i) 2- Mercaptoethanol (2-ME)
   (ii) Dithiothereitol (DTT)

These reagents cleave interchain disulphide bond of IgM antibodies and destroy their acitivity. They have no effect on IgG antibodies.

Witebsky's partial neutralization test to determine IgG anti-A and anti-B

Reagents required are as follows;
Reagent saliva with A substance and B substance.
Reagent cells: 2-5% saline suspensions of A and B cells.
Patient's (Test) serum

Methods:
Witebsky's Partial Neutralization Test

1. Determine the saline (IgM) anti-A and anti-B titre of mother's serum, (see chapter 21 on Preparation of Solution and Methods)

2. On the basis of saline antibody titre, add an appropriate amount of A and B group specific saliva to neutralize and-A and anti-B respectively. Amount of saliva generally needed for partial neutralization is as follows:

IgM Titre in serum	Volume of serum	Volume of saliva
Upto 32	2 vol.	1 vol.
64-128	1 vol.	1 vol.
256-512	1 vol.	2 vol.
512 or more	1 vol.	3 vol.

Preparation of saliva is described in chapter on special methods

3. The mixture is kept at room temperature for 20 min and tested for saline anti-A and anti-B . If the reaction is weak or +1, proceed for incomplete (IgG) antibody titer.
   If the neutralized mixture gives +2 to +3 reactions, add more group specific substance saliva and repeat the test till it gives weak or +1 reactions.
   If the neutralized mixture gives negative reactions, neutralization procedure is repeated using lesser amount of substance (saliva)
4. Make two-fold serial dilution of the partially neutralized serum into two rows A or B. In row A do the titration of saline (IgM) anti-A or anti-B. In row B do the titration of incomplete (IgG) anti-A or anti-B antibody using albumin or enzyme or indirect AHG test technique.
   A typical example of incomplete anti-A in a case of HDN due to anti-A is given below:

Anti-A titre of ABO-HDN tested before and after neutralization of the serum with group substance (witebsky's Test)

Titre	Before-Neutralization		After-Neutralization	
	Saline	Incomplete	Saline	Incomplete
Neat	H	H	+2	+4
1:2	H	H	+	+4
1:4	H	H	+	+4
1:8	+4	+4	-	+4
1:16	+4	+4	-	+3
1:32	+4	+4	-	+2
1:64	+3	+4	-	+2
1:128	+3	+3	-	+
1:256	+3	+3	-	+
1:512	+2	+2	-	-
1:1024	+2	+2	-	
1:2048	+	+2	-	-
1:4096	+	+	-	-
1:8192	-	+/-	-	-

Aderson's Dextron -AB serum mixture can also be used in doing titer of IgG anti-A and anti-B (Gupte et.al. 1972)

Aderson's Dextron-AB serum Composition:

Neutral AB serum	25 ml
NaCl	1.3g ,
Dextran (Mol.wt. 100,000 to 150,000)	3.0g

Make up the volume to 100ml with distilled water.

Method; Add 1 volume each of Aderson's Dextran AB serum mixture and 2-5% cells suspensiosn of reagents A or B cells to each dilution. Incubate for 1 hour before reading results.

INACTIVATION OF IgM ANTIBODIES BY

2. MERCAPTOETHANOL (2ME)

Reagents required are as follows:

1. Phosphate Buffer pH 7.4
   (a) M/15 Na_2HPO_4 - dissolve 9.47 g anhydrous Na_2HPO_4 in 1000 ml distilled water.
   (b) M/15 KH_2PO_4- dissolve 9.08 g crystalline KH_2PO_4 in 1000 ml distilled water.
   (c) To make phosphate buffer pH 7.4 - Mix 80.8 ml of Na_2HPO_4 solution (a) with 19.2 ml of KH_2PO_4 solution (b) at room temperature.
2. 0.2 M 2- mercapatoethanol, prepared by adding 100 ml of phosphate buffer pH 7.4 to 1.56 ml 2- mercapatoethanol. This solution should not be stored for more than one month at 4°C.
3. Phosphate buffered saline pH 7.4
   (a) 0.15 M NaH_2PO_4 . 2 H_2O - dissolve 23.4 g of $NaH_2PO_4$2 H_2O - in 1000 ml of distilled water.
   (b) 0.15 M NaH_2PO_4-dissolve 21.3 g NaH_2PO_4-in 1000 ml of distilled water.
   (c) Make phosphate buffer iso-osmotic pH 7.4 - by mixing 18 ml. NaH_2 PO_4. $2H_2O$ Solution with 82 ml Na_2HPO_4 solution.
   (d) To make phosphate buffer saline - mix equal volume of iso - osmotic phosphate buffer pH 7.4 and 9g/L NaCl.
4. 0.2 M Iodoacetamide, prepared by dissolving 0.37 g iodoacetamide in 100 ml of phosphate buffered saline pH 7.4

Method

1. Mix 1 ml of 2- ME solution with 1 ml of test serum.
2. Make a control consisting of equal volume of isotonic PBS pH 7.4 and test serum.
3. Incubate 2- ME serum mixture and PBS serum mixture at 37°c for 15min.
4. Add 1 ml iodoacetamide to the test serum and control and keep it over night (14-16h) at 4°C or dialyze mixtures overnight (16 h) against PBS at 4°C. For small volume use 1 cm wide cellophane tubing.
5. Make two fold serial dilution of the test mixture and control mixture in isotonic saline in duplicate.Titer them with appropriate cells for IgM anti-A and /or anti-B by saline technique and for IgG anti-A and or / anti-B by albumin or enzyme or indirect AHG test technique.

Interpretation

1. If no reactivity is noted in the control, it indicates dilution of the anti body and invalidates the results.
2. Equal reactivity both in the test mixture and control mixture indicates that only IgG

antibodies are present.

3. If no reactivity is seen in the test mixture and the reactivity is seen in the control mixture, this indicates only IgM antibody present.
4. A four fold (two-tube difference) or greater reduction of acitivity when compared to the control tube indicates the presence of both IgM and IgG antibodies.

Note: 2-ME has noxious odour and should therefore, be used under a hood. Most recent investigations show 2 -ME is more effective than dithiothreitol. The addition of iodoaceetamide is necessary to prevent false - positive results.

Inactivation of IgM Antibodies by Dithiotheritol (DTI)

Reagents required are as follow;

1. 0.01M dithiothreitol is prepared by dissolving 0.154 g (DTT) in 100 ml of PBS pH 7.4. The DTT solution is stable at -20°C for upto 6 months. When stored in refrigerator at 4° C it deteriorates in less than one week.
2. Phosphate buffered solution pH 7.4.
3. 2 ml of serum to be tested.

Method

1. Dispense 1 ml serum into each of the two tubes.
2. To one tube, labeled control add 1 ml of PBS pH 7.4
3. To other tube, labeled test, add 1 ml of 0.01 M DTT
4. Mix and incubate at 37°C for 2h
5. Make two-fold serial dilution of the test mixture and control mixture in isotonic saline in duplicate. Titer them with appropriate cells for IgM anti-A or anti-B by saline technique and for IgG anti-A or anti-B by albumin or enzyme or indirect AHG test technique.

Interpretation

Effect of DTT on Blood group antibodies

	DILUTION					INFERENCE
Test Samples	1:2	1:4	1:8	1:62	1:32	
Serum + DTT	+3	+2	+2	+ 1	0	IgG
Serum + PBS	+3	+2	+2	+ 1	0	
Serum + DTT	0	0	0	0		IgM
Serum + PBS	+3	+2	+2	+ 1	0	
					0	
Serum + DTT	+2	+ 1	0	0	0	IgM, IgG
Serum + PBS	+3	+2	+2	+ t	0	

1. If no reactivity is noted in the control, it indicates dilution of the antibody and invalidates the results.
2. Equal reactivity both in the test and control mixture, indicates that only IgG antibodies are present.
3. If no reactivity is seen in the test mixture and the reactivity is seen in the control mixture, this indicates only IgM antibodies are present.
4. A four fold (two-tube difference) or greater reduction of acitivity when compared to the controlled tube after partial neutralization indicates the presence of both IgM and IgG antibodies.

Note: DTT is not as odorous as 2 -ME and provides a more rapid test. However, it may not be as sensitive as 2- ME

14

Blood Components Preparation and Their Uses

These days effective blood transfusion therapy depends upon on the availability of different blood components. These components, used separately or in combinations, can meet most patients transfusion need and keeping the risk of transfusion to a minimum.
A blood donor donates the product known as whole blood, from which components are prepared. The ability to separate various components from whole blood is desirable for the following reasons:

1. Separation of blood into components allows optimal survival of each constituents. For example after 24 hours storage of whole blood at 2-6°C, it has few viable platelets and levels of labile Factors V and VIII decrease, while after separation platelets can be stored for 5 days at 22°C and Factors V and VIII can be stored as FFP for 1 year at- 30°C or below.

2. Component preparation allows transfusing only specific blood component that the patient requires.

3. Transfusion of only the specific constituent of the blood needed avoids the use of unnecessary component, which could be contraindicated in a patient. For example, because of the risk of hypervolemia, an elderly anemic patient in congestive heart failure may not easily tolerate the transfusion of two units of whole blood, while the same patient can be transfused two units of red blood cells easily.

4. By using blood components, several patients can be treated with the blood from one donor, giving optimal use of every unit of donated blood.

5. Use of blood components, supplements blood supply - adds to blood inventory.

Blood components such as red cells, platelets, and plasma are prepared from a single whole blood donations. Components have tightly regulated preparation and storage requirements. Blood group compatibility between the component and the patient is considered during product selection. Each component carries the same risk of hepatitis, human immunodeficiency virus (HIV) transmission as the original unit of whole blood.
In contrast, plasma derivatives (fractions) such as albumin, immune serum globulins, and concentrated coagulation factors etc. are prepared from large pools of donor plasma. They have more flexible storage requirements and are given without regard to ABO compatibility. Depending on the manufacturing process, derivatives may carry a decreased risk of hepatitis and HIV transmission as compared with components.

The blood plasma components given below can be prepared in blood bank by conventional methods (e.g. centrifugation, freezing and thawing) for therapeutic use. The specific gravity of red cells and granulocytes is very similar so the centrifugation is not efficient method of separation them. While plasma derivatives (fractions) are prepared by biochemical or other manufacturing process under pharmaceutical manufacturing conditions in a well equipped Plasma Fractionation Laboratory.

Blood Components (Cellular and Plasma) and Plasma Derivatives (Fractions):

Cellular components	**Plasma components**	**Plasma deruivatives**
Red cell concentrate	Fresh frozen plasma	Albumin 5% & 25%
Leucocytes-Reduced Red cells	Single donor plasma	Plasma Protien Fractions
Platelet Concentrate	Cryoprecipitate	Factor VIII concentrate
Leukocytes-Reduced Platelet Concentrate	Cryo-poor plasma	Immunoglobulins
Platelet Apheresis		Fibrinogen
Granulocytes, Apheresis		Other coagulation factors

Preparation of blood components is possible due to :

- Multiple Plastic packs system
- Refrigerated centrifuge
- Different specific gravity of cellular components

Red cells specific gravity	1.08 - 1.09
Platelet specific gravity	1.03 - 1.04
Plasma specific gravity	1.02 - 1.03

Due to different specific gravity of cellular components, they can be separated by centrifuging at different centrifugal force in g for different time.

Centrifugation for blood component preparation:

Refrigerated centrifuge rotor speed and duration of spin are critical in preparing components by centrifugation. Each centrifuge should be calibrated for optimum speeds and times of spin for the preparation of each component, Times given in this manual include only the time of acceleration and its speed, not the deceleration time.

The blood components are prepared by centrifuging at different relative centrifugal force in g at different time. Conversion of relative centrifugal force (RCF) to rpm depends upon the radius of centrifuge rotor. It can be calculated by:

1. Nomogram illustrated in figure (14.1)
2. By any one of the formulae -

   i) Relative centrifugal force in g = $28.38\ R\left(\frac{rpm}{1000}\right)^2$

   R = radius of centrifuge rotor in inches

   ii) Relative centrifugal force in g = $118 \times 10^{-7} \times r \times N^2$

   r =: radius of centrifuge rotor in cm

   N = speed of rotation (rpm)

Relative centrifugal force in g (rcf x g) for preparing components:

Components	**Spin (rcf) & Time**
Red cells	
Plasma	Heavy Spin
Platelet Concentrate	5000 x g for 5 minutes
Croprecipitate	
Platelet-Rich Plasma	Light Spin
	2000 x g for 3 minutes

Equipment

- Freezer - 40°C and - 70°C
- Blood bank refrigerator 2-6°C
- Refrigerated centrifuge with swing out head and oval cups
- Laminar flow
- Weighing scale
- Dielectric sealer or aluminium clips & sealer
- Stripper
- Water bath 37°C or Plasma Defroster (Microvave)
- Platelet reciprocator (platelet incubator with agitator)
- Cryoprecipitate bath 4°C
- Sterile connecting device (sterile docking device)

This device allows the tubing from two separate bags to be welded together without losing sterility of either. The diameter and the material of the tubings to be connected should be similar. This device is useful in making paediatric units from a primary bag that has no satellite bags.

Consumable items

- 450 ml double & triple bags CPDA bags
- 450 ml double & triple bags with CPD & additive solution (Adsol/SAG-M)
- Thin board boxes for storing plasma in freezer

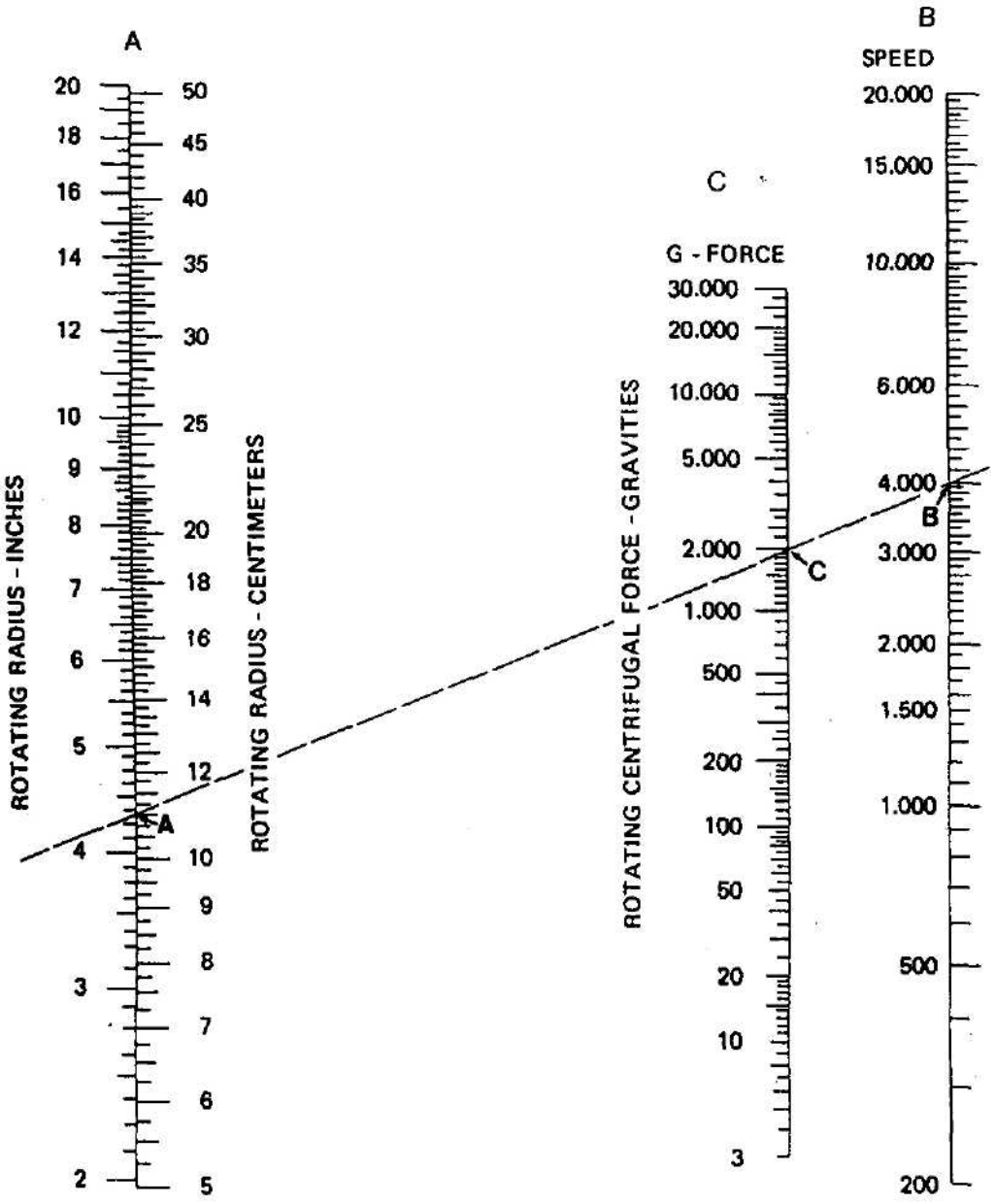


Fig. 14.1 Normogram for computing relative centrifugal force and speed.

Example:

To find the relative centrifugal force at a radial distance of 11 cm from the centre of rotation when operating the certrifuge at a speed of 4000 r/min, place a ruler on the chart connecting the 11 cm point on the Rotation Radius Scale (A) with the 4000 r/min point on the Speed Scale(B). Read the point at which the ruler interscts the Relative Centrifugal Force Scale(C) - in this case, 2000 x gravity.

Similary, if the desired relative centrifual force is known, the necessary speed for a given rotating radius may be determined by connecting the two known points and reading the intersection of the ruler with the Speed Scale.

- Plastic over raps for blood bags (preferably)
- Appropriate labels (sticker) and instructions for each component
- Optional
  Transfer packs
  Bag to bag connector

Precautions to be observed in preparing components:

In collection of blood (See chapter 2 on Collection of Blood)

- Proper selection of donor
- Clean and aseptic venipunture site to minimize bacterial contamination
- Clean venipunture with minimum tissue trauma and free flow of blood
- The flow of blood should be uninterrupted and continuous. If any unit takes more than 8 minutes to draw, it is not suitable for preparation of platelets concentrate, fresh frozen plasma or cryoprecipitate.
- A correct amount of blood proportionate to anticoagulant should be collected in primary bag that has satellite bags attached with integral tubings.
- Monitor the collection of blood with Automatic Mixer/Scale which is used for collecting the desired amount of blood and mixing the blood with the anticoagulant.
- If platelets are to be harvested the blood bag should be kept at room emperature 20-24°C until platelets are separated. Platelets should be separated within 6-8 hours from the time of collection of blood.
- Triple packs system with two attached bags makes it possible to make red cells, platelet concentrate and fresh frozen plasma. While quad packs system with three attached bags are used for preparing red cells, platelet concentrate, cryoprecipitate (factor VIII) and cryo- poor plasma. Double bags are used for making red cells, and plasma only.

In centrifugation

- Opposing cups with blood bag and satellite bags must be equal in weight otherwise excessive eccentric loads placed on rotor of centrifuge cause irregular wear and tear and eventual breakage.
- The bags should be so placed that its broad side faces the out side wall of the cup.
- Rubber discs should be used for balancing.
- Plastic over wears for bags may be used (optional).
- Correct speed of centrifugation and time must be maintained as they are the most critical factors in component preparation.
- Observe for any abnormal vibration till the required speed is attained, if there is any, stop the centrifuge and check the weight of the opposite cups with bags.

Centrifuges used for separation are calibrated to produce highest product yield in the shortest time at the lowest possible spin so as to cause the least trauma to each product and at the same me maintaining optimal temperature for component viability. In a unit of blood, the centrifuged

products settle in layers, starting from bottom: red blood cells, white blood cells, and platelet rich plasma. After separating platelet-rich plasma (PRP) from the red cells, the PRP is centrifuged at a heavy spin for a longer time. This time platelets settle to the bottom of the bag. The plasma is transferred into another satellite bag.

WHOLE BLOOD

Whole blood contains 450 ± 45 ml or 350 ± 35 ml of donor blood plus anticoagulant solution. The name of the anticoagulant is used with the name of the product e.g. CPDA-1 Whole Blood. Whole blood has a hematocrit of 30-40 per cent. Minimum 70% of transfused red cells should survive in the recipient's circulation 24 hours after transfusion. Stored blood has no functional platelets and no labile coagulation factors V and VIII.

PREPARATION OF RED BLOOD CELL CONCENTRATES (PACKED RED CELLS)

Red blood cells (packed red cells) are prepared by removing most of the plasma from a unit of whole blood. Red cells have higher specific gravity than plasma, the red cells settle in the lower portion of bag due to the gravitational settling (sedimentation) or centrifugation. The plasma is transferred into a satellite bag.

Red blood cells preparations are:

Sedimented red cells: They have a PCV of 60-70 per cent, 30 per cent of plasma and all original leukocytes and platelets.

Centrifuged red cells: They have a PCV of 70- 80 per cent, 15 per cent of plasma and all original leukocytes and platelets,

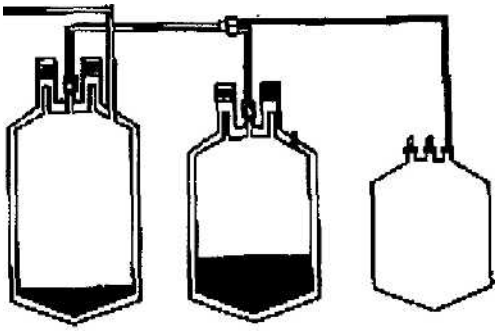
Red cells with additive (Adsol or SAG-M): They have PCV of 50-60 percent, minimum plasma and all Leukocytes and platelets.

Sedimentation:

The blood after collection is kept upright in refrigerator at 2-6°C, the red cells settle down and the clear supernatant plasma is transferred into a satellite bag.

Centrifugation:

1. Collect appropriate volume of donor blood in CPDA double or triple bag.
2. Store at 2-6^{o}C till processed.
3. Place bags in the buckets of refrigerated centrifuge and balance the opposite bags accurately.
4. Centrifuge at heavy spin (5000 x g) for 5 minutes at 2-6^{o}C.



1.Primary Container (CPD anticoagulant solution) 2.100 ml tion solution in 400ml container 3. Empty transfer pack

Tripa Blood packssystem, with additivesolution Adsol

(Fig. 14-2).

5. Express approximately 3/4 of the plasma into the satellite bag.
6. Double seal the tube between primary and satellite bags with plasma. Separate the satellite bag with plasma and keep at -30°C or below.
7. Keep the red cells at 2-6°C.

Red Cells in Additive Solution (Adsol, SAG-M)

1. Collect the appropriate volume of donor blood in primary bag of additive system, consisting of a primary bag containing anticoagulant solution CPD or CP2D attached with at least two satellite bags, one of which is empty and another contains 100 ml of additive solution e.g. Adsol or SAG-M. Fig. 14.2
2. Store at 2-6^{o}C till processed.
3. Centrifuge at heavy spin as above.
4. Remove most of the supernatant plasma in the empty satellite bag.
5. Add the additive solution to the red cells with in 72 hours of phlebotomy.
6. Keep the red cells at 2-4°C and plasma at -30°C or below.

Leukocyte-Reduced Blood Components

Leukocytes in blood components can cause:

- Non-hemolytic febrile transfusion reaction (NHFTR)
- Human leukocyte antigen (HLA) alloimmunizaion
- Transmission of leukotropic viruses (Cytomegalovirus(CMV), Epstein- Barr virus (EBV) and human T-cell lymphotropic virus type 1 (HTLV-1)
- Transfusion related Graft versus host disease
- Transfusion related acute lung injury (TRALI)
- Transfusion related immunosuppression

Reducing the leukocytes content to less than 5 x 10^{8} in one unit of RBCs prevents most of the non-hemolytic febrile transfusion reactions. For other complications such as preventing transmission of CM V or alloimmunization to HLA antigens, leukocytes content must be reduced to less than 5 x 10^{6}C in a unit of RBCs .

Leukocyte reduction has been used with considerable success to prevent FNHTR but at times post storage leuko-reduction is not much effective as cytokines generated by leukocytes during storage can cause FNHTR.

Cytokines are generated by leukocytes, even at 2-6°C but to a much greater extent at 20-24°C. Levels of interleukin one alpha plus beta (1L- la and IL-IB), interleukin 6(IL-6) and tumor necrosis factor alpha (TNF-a) rise sharply in units stored with leukocytes compared with similar units stored after leuko-reduction (prestorage leuko-redction). Cytokins levels rise in direct proportion to the number of leukocytes. Hence leuko-reduction before storage (pre-storage leuko-reduction) in blood bank is much better than the leuko-reduction after storage (post-storage leuko-reduction) at the bed side of the patient to eliminate FNHTR.

Donors lymphocytes engrafting in the recipient and reacting against host antigens may cause GVHD.

The reaction between leukocyte antigens and antibodies can result in leuko-agglutination, aggregates of white cells being trapped in the microcirculation of the lungs, causing pulmonary edema, that is transfusion related acute lung injury (TRALI). In most of the cases of TRALI.

leukocytes antibodies in previously sensitized transfusion recipient react with leukocytes in transfused blood or plasma, or reverse to it that is, leukocyte antibodies in blood or plasma react with the recipient's leukocytes forming leukocytes aggregates which are trapped in the microcirculation of lungs causing TRALI.

Approximate Residual Leukocytes in Cellular Blood Components

Fresh whole blood	10^9
Red blood cell concentrate	10^8.- 10^9
Buffy coat-depleted red cells	10^8
Red cells, leukocyte-reduced by filtration	$<10^7$
Washed red cell concentrate	10^7
Deglycerolized red	10^6- 10^7
Platelet concetrate	$<10^7$

Methods of the preparation of Leucocyte-Reduced Red cells

- Centrifugation and removing of buffy coat
- Filtration
- Washing of red cells with saline
- Freezing and thawing of red cells

Centrifugation and removing of buffy coat:
In the centrifugation method for leuokocyte reduction, the buffy coat layer between the red cells and the plasma which appear after centrifugation is drawn off along with plasma and some red cells into a satellite bag. A variation of this method is to spin the red cells in an inverted position so that red cells can be transferred into a satellite bag, leaving the buffy coat, some red cells and plasma behind in primary bag.

Method

1. The whole blood unit is centrifuged in an upright position at 5000 x g for 5 minutes at 4°C.
2. The supernatant plasma, buffy coat and some red cells are transferred into a satellite bag.
3. Double seal the tubing between the primary bag and the satellite bag, separate them.
4. Keep the red cells at 4°C.

This is one of the easiest and least expensive method and it can be done in close system, but it is the least efficient. It reduces the leukocytes level by 70-80% (less than 1 log) and sacrifices 20% of the red cells. It does not meet minimum standards for WBC reduction. Besides its hematocrit is more than > 80% which is difficult to transfuse unless some plasma is returned back or additive solution is added. It is also laborious method. Today this method has been replaced by other more efficient techniques.

Filtration :
Many types of filters are available today that can produce an acceptable leukocyte-reduced product depending on the requirement.

Microaggregate filters are polyester or plastic screen filters with a pore size of 20-40 micron., which trap most of the microaggregates composed of white cells, platelets, and fibrin threads that form in blood after 5-6 days of refrigerated storage. These microaggregates pass through standard blood filters and are trapped in micro-circulation of lung causing TRALI. The effectiveness of microaggregate filters of leuko-reduction is increased by cooling the unit at 4^0C for 3-4 hours after centrifugation and before filtration. Filtration of this type usually give a 1 -2 log reduction (90-92%) of leukocytes in the unit (< 5 x 10^8) and recover most of the red cells. Such LR-red cells reduce the incidence of NHFTRs only but does not achieve other goals of leuko-reduction.

Newer leukocyte-reducing filters (third generation) use selective absorption of leukocytes or leukocytes and platelets. They are made of polyester or cellulose acetate and will produce a 2 to 4 log (99-99.9%) reduction of the WBCs (< 5 x 10^6). There is very little loss of red cells and the process is quite easy. They prevent allommunization to HLA antigens, CMV transmission, and NHFTRs.

Leucoreduction can be done at three different point.

(1) Prestorage leuko-reduction
(2) After storage leuko-reduction in blood bank, before issue
(3) Bed side filtration

Prestorage leuko-reduction :

Leukocytes begin to disintegrate quickly when stored at 1-6°C. These white cells fragments may initiate an immune response to HLA antigens and carry viral activity. White cells in stored blood may also produce cytokines which may cause NHFTRs. In order to prevent these effects it may be desirable to remove white cells prior to storage. This can be done by using one of two technologies, the sterile tube connection device or the inline filter.

Using the sterile tube connecting device, a bedside LR-filter can be connected to a unit of blood prior to storage and to another sterile bag. The cells can be filtered from the primary bag through attached filter into the attached bag. This retains the original expiry date of the initial product and all red cells, platelets, and plasma.

The second technology is possible with specially designed blood collection bags system with Integral (in line) filters incorporated between primary collection bag and a satellite bag (fig, 14.3).

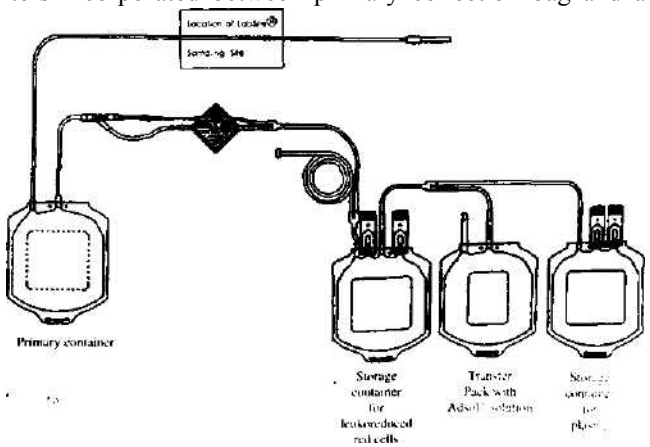


Fig. 14.3
Blood Pack with Integral Filter for Leukoreduction of Whole Blood

Within 8 hours after phlebotomy, the blood is passed through this filter into an attached collection bag. The filtered red cells in the bag are leukocyte reduced and retain the original expiry date.

Leukocytes removal with this system is 99-99.9 per cent (3 log), with > 90 percent red cells remaining.

Impact of Prestorage Leukoreduction

Results from Prestorage leukoreduction	Potential patient benefits
Cytokins poroduction is reduced or eliminated	* Decrease in febrile non-hemolytic transfusion reactions
White cells are removed before fragmentation	* Decrease alloimmunization * Reduce exposure to intracellular Viruses (e.g. CMV)
Tumor metastases are reduced in animals	* Prevent immunomodulation * Reduce tumor spread (Blajcman et al. Blood, 82 (suppl.392a, 1993)

After storage leukoredction:

The blood or red cells are filtered to reduce leukocytes in the blood bank before issue. In laboratory filtration of blood /red cell can be properly standardize and adapted to QC program. However red cells may have disintegrated leukocytes and cytokins.

Bedside filtration:

Bedside filtration commonly being practiced has been shown to be quite effective to prevent NHFTRs but they may also have disintegrated leukocytes and cytokines which may cause FNHTRs. They are not much effective for prevention of HLA alloimmunization.

Washing of Red Cells for leukocyte reduction:

Washing of red cells removes leukocytes, platelets and plasma, It can be done manually or using machine e.g. Haemonetic cell washing machine.

Manual Method

(1) Collect the blood in a double bag and store at 2-4°C, till it is processed.
(2) Centrifuge the bag at 5000 x g (heavy spin) for 5 minutes at 2-6°C.
(3) Separate the plasma along with buffy coat into a satellite bag/transfer bag.
(4) Double seal the tube between primary and satellite bags.
(5) Connect the bag with red cells to I.V. saline bottle with bag to bottle connector under laminar flow. Transfer about 250 ml saline in the red cell bag.
(6) Clamp the tube of bottle connector (transfer set) and seal the tube of the bag distal to the spike or needle of the transfer set by di-electric sealer. Remove the needle from the tube of the bag and cover the spike/needle with its plastic cover.

(7) Mix well red cells and saline. Centrifuge the bag at heavy spin (5000 x g for 5 minutes).

(8) Place the bag on the expresser and remove the saline in waste receptacle.

(9) Repeat the washing three times (steps 5 to 8).

(10) After the final wash add 60-70 ml saline in the red cells and mix.

(11) Seal the tubing close to the bag and separate the connector.

(12) Keep washed red cells at 2-6°C.

- Whole process is done under laminar flow.
- Shelf-life of the washed red cells is 24 hours.
- All aseptic precautions should be taken.

REMOVING OF LEUKOCYTES BY FREEZING AND DEGLYCEROROLIZATION.

See the chapter 3 on 'Preservation and Storage of Blood'.

PREPARTION OF PLATELET RICH PLASMA (PRP) AND PLATELET RICH CONCETRATE (PC)

Platelet concentrate can be prepared from:

1. Random donor platelet (prepared from 450 ml whole blood)
2. Single donor platelet prepared by apheresis.

Random Donor Platelet

It is prepared from 450 ml whole blood kept at room temperature (22-22°C) and within 6 to 8 hours of collection.

Procedure:

1. Collect 450 ml blood by a clean, single venipunctuer into 450 ml CPDA or Adsol / SAG-M triple bags system.
2. Keep the blood bag at room temperature (20-22°C) before preparing platelets, for not more than 6 hours. Do not chill at any time before or during platelet separation.
3. Keep the bags in the buckets of refrigerated centrifuge and balance them curately. Centrifuge the blood bags at 20-24°C at light spin for appropriate time (2000 x g for 3 minutes).
4. Separate 4/5 of the platelet rich plasma (PRP) into one satellite bag if CPDA triple bags systems is used. Double seal the tubing between the primary bag and the satellite bags. Separate the primary bag with red cells. One of the satellite bag contain PRP.
   If the Adsol /SAG -M triple bags system is used, transfere all PRP into empty satellite bag and add additive solution in to red cells. Double seal the tubing of the bag with red cells and separate it from the satellite bags. One of the satellite bags contains PRP. PRP may be used as such or processed further to prepare platelet Concentrate (PC).
5. Centrifuge the bag with PRP and another satellite bag at 20-40°C at 'heavy spin' for appropriate time e.g. 5000xg for 5 minutes.
6. Express supernatant platelet - poor plasma into another empty satellite bag.

7. Leave approximately 50 ml of plasma with the platelets and label it.
8. Leave platelet concentrate (PC) undisturbed at 20-22°C for 1 hour, then resuspend the platelet in plasma by gently mixing for l0 min.
9. Store platelet at 20-22°C under constant agitation in platelet incubator with agitator till used. The shelf life is 3-5 days depending on the type of plastic bags used (see chapter 3 on Preservation and Storage of Blood)

A unit of platelet concentrate prepared from 450 ml blood contains:

Plasma Volume	40-70 ml
Platelet Yield	5.5×10^{10}
WBC	$\geq 10^8$
RBC	Traces to 0.5 ml
pH	6.0 or more

Calculation of Platelet Yield

Number of platelet in whole blood = Platelet per mm^3 x 1000 x volume of whole blood (ml)
Number of platelet in PRP = Platelet per mm^3 x 1000 x volume of PRP (ml)
Number of platelet in P.C. = Platelet per mm^3 x 1000 x volume of P.C. (ml)

Calculation

$$\%\text{of platelets yield in PRP} = \frac{\text{Number of platelet in PRP X 100}}{\text{Number of platelet in whole blood}}$$

$$\%\text{ of Platelets yield in P.C} = \frac{\text{Number of platelet in PC X 100}}{\text{Number of platelet in PRP}}$$

Precaution and storage :

pH should never fall below 6. A decline in pH causes:

- Change in shape of platelets from disc to sphere.
- Pseudopod formation.
- Release of platelet granules.

The above changes are responsible for low recovery and poor survival of platelets in vivo.

Maintenance of pH and function of platelets on storage depends upon permeability of storage bag to oxygen and carbondioxide. Platelets are stored in bags made of standard plyvinylchloride (PVC) with Di-(2-ethylhexyle) phthalate (DEHP) plasticizer upto 72 hrs at room temperature (20-24°C). New plastic bags made of polyolefin with no plasticizer, or thin walled PVC with Tri -(2-ethylhexyl) trimellitate (TOTM) plasticizer maintain pH level and platelet function upto about 7 days but it is recommended to use them within 5 days from the date of collection of blood.

Agitation during storage helps the exchange of gases, maintenance of pH, and reduce formation of platelets aggregates. Agitator (flat bed) with 1" to 1.5" strokes at 70 cycles per minutes at 20-24°C, gives good results.

GRANULOCYTE CONCETRATE

Granulocyte concentrate can be prepared by:

1. Single donor unit
2. Leukapheresis by blood cells separator

As the specific gravity of red cells and granulocytes is very similar , the separation of granulocytes by centrifugation is not satisfactory. Leukapheresis is a better method.

Procedure: (Preparation of Granulocytes from single donor blood).

1. Collect 450 ml of donor blood in 450 ml of CPD A or Adsol SAGM triple packs system and keep at 20-24°C before separating the buffy coat, but in no case more than 6 hr after collection of blood.
2. Keep the bags in the buckets of refrigerated centrifuged and balance them accurately. Centrifuge the blood bags at 20-24°C at light spin for appropriate time e.g. 2000x g for 3 minutes.
3. Express the supernatant plasma into first satellite bag. Leave about 20 ml of plasma above the cellular layer (buffy coat) in the primary bag. Double seal the tubing between primary bag and satellite bag with plasma and separate it.
4. Express the 20 ml of plasma and the upper 20-25 ml of the cellular layer, rich in white cells, into another satellite pack. Double seal the tube and separate.

The yield of white cells by this method is about 1 x 10^9, of which about half i.e. 0.5-0.6 x 10^9 are granulocytes per 450 ml unit of blood. It is heavily contaminated with platelets and other leucocytes. Granulocytes can be stored at 20-24°C but they should be used as early as possible and not later than 24 hours from blood collection.

For details of leukapheresis see the chapter on ‘ Apheresis’.

FRESH FORZEN PLASAMA (FFP)

FFP is plasma obtained from a single donor either by normal donation or by plasmapheresis and rapidly frozen within 6-8 hours of being collected. It contains all coagulation factors and great care must be taken during collection of blood, freezing and thawing to preserve their activity.

Collection of blood

1. Blood should be collected by a clean, single venipuncture.
2. Row of blood should be rapid and constant.
3. Total time taken to collect 450 ml of blood should not be more than 8 minutes.

Procedure :

1. Collect appropriate volume of blood in 350-450 ml CPDA double bags systems or 450 ml SAGM/Adsol triple bags system.
2. Store at 4°C or in air -conditioned room till processed but not for more than 6-8 hours.
3. Place bags in the bucket of the refrigerated centrifuge, balance them accurately and centrifuge at heavy spin (5000 x g for 5 minutes) at 4°C.
4. Express approximately four fifth of the plasma into a satellite bag, if blood is collected in CPDA triple /double bag system. Double seal the tube between primary bag and the

satellite bag having plasma with metal clips or dielectrical sealer. Separate the satellite bag having plasma. If blood is collected in SAGM or Adsol triple bag system, express all plasma in satellite bag.

5. Label the plasma bag and is rapidly frozen. This should be done as soon as possible after collection, in any case within 6-8 hours. The complete freezing process should be as short as possible and preferably should not take more than one hour. Rapid freezing can be achieved by spreading the plasma in a thin layer (bags laid flat and not vertical) in freezer at - 70°C or placing the bags protected by a plastic over wrap at - 70°C in ethanol dry ice bath.
6. It has been shown that the most labile coagulation factors are preserved for one year if FFP is kept at -30° C or below. If FFP is not used within one year, it is redesignated as a Single Donor Plasma which can be kept further for 4 years at -30° C or below.
7. The formation of cryoprecipitates (containing factor VIIIC, fibrinogen and fibronectin) should be avoided during thawing as it would reduce the expected clotting properties. Thaw it in a plasma Defroster (Microwave) or place the bag in a plastic over wrap and put in a 37°C circulating water bath (the entry ports of the bag should remain above the water).

   The FFP should be administered as soon as possible after thawing, and in any event within 12 hours if kept at 2-6° C.

CRYO PRECIPITATE

Cryoprecipitate are precipitated proteins of plasma, rich in Factor VIII and fibrinogen, obtained from a single unit of fresh plasma (approximately 200 ml) by rapid freezing within 6 hours of collection. It is rich in factor VIII, von-Willebrand factor, fibrinogen (Factor XIII) and fibronectin. Several factors which improve the yield of factors VIII in cryoprecipitate are:

1. Clean, single venipuncture at the first attempt.
2. Rapid flow of blood, donation of blood (450 ml) obtained in less than 8-10 minutes should be used.
3. Adequate mixing of blood and anticoagulant.
4. Rapid freezing of plasma as soon as possible after collection in any case within 6-8 hours after collection as done for preparing FFP.
5. Rapid thaw at 4° C in circulating water bath.
6. Use of siphon technique which prevents thawed plasma remaining incontact with the cryoprecipitate.

Procedure

1. Prepare fresh frozen plasma (FFP), as described under FFP, for processing into cryoprecipitate.
2. Freeze the plasma at -70° C in freezer or in ethanol dry bath.
3. Thaw frozen plasma either at 4°C in a cold room (air thaw) or at 4°C in circulating water bath.

   - If FFP is thawed in a cold room, hang the bag in an inverted position with ports lower most and place the second satellite bag on a lower shelf. Observe the pack frequently to make sure the thawed plasma is flowing in to the satellite bag and not accumulating

in the primary bag. When 10-15 ml of plasma remain with cryoprecipitate seal the tubing and separate bags.

- If FFP is thawed in 4°C water bath, centrifuge the bag when the plasma is slushy at 5000 x g for 5 minutes at 4°C. Then supernatant cryo-poor plasma is siphoned out in the satellite bag, leaving 10-15 ml plasma with cryoprecipitate. Seal the tubing and separate the bags. Label bags.

4. Store the bag with croprecipitate at -30°C or lower and bag with Cryo-poor plasma in the second satellite bag is stored at -20°C or below. Storage and shelf life of croprecipitate : One year at -30°C or below.

Reconstituting cryoprecipitate

(Thawing and issue of Cryoprecipitate)

Reconstitute cryoprecipitate before issue by placing in an overwrap in a 30°C water bath until the cryoprecipitate has dissolved. Cryoprecipitate should be resuspended thoroughly by gentle kneading. After thawing pool the cryoprecipitate from all thawed bags into one bag under laminar flow by means of bag to bag connector. Wash the empty bags with 10 ml of normal saline to dissolve residual cryoprecipitate and add to pooled cryoprecipitate. Once thawed, cryoprecipitate should be kept at 2-6°C and administered with in 4 hours It should not be frozen.

One bag of cryoprecipitate contains on an average 80-120 units of factor VIII in 15-20 ml of plasma.

SINGLE DONOR PLASMA

Single donor plasma can be prepared by separating it from red cells any time upto 5 days after the expiration of the whole blood unit. When stored at -20°C or lower, single donor plasma may be kept upto 5 years.

A prolonged pre-separation storage period increases its contents of potassium and ammonia and at times free hemoglobin. Generally, it has no liable coagulation activity. Because of these disadvantages, plasma separated from out dated is avoided for infusion.

CRYOPRECIPITATE-POOR-PLASMA

It is a by-product of cryoprecipitate preparation. It lacks labile clotting factors V and VI11 and fibrinogen. It contains adequate levels of the stable clotting factors II, VII, IX and X. It is frozen and stored at -20°C or lower temperature for 5 years.

REPARATION OF LEUKO-REDUCED BLOOD COMPONENTS

(Semi-automated Method)

Leuko-reduction in blood component by removing the buffy coat from whole blood by manual procedure is laborious. This led to the need to automate the preparation of leuko-depleted components from whole blood to achieve consistency in the quality and reproducibility. M/s Baxter has developed Opti System with specially configured Optipacs to produce leuko-reduced blood components and to harvest leuko-reduced platelet concentrate from the buffy coat.

In newer technique called ‘top and bottom’ method, developed by M/S Baxter, whole blood is centrifuged at relatively high speed. After hard spin, the buffy coat contains both WBCs and

platelets. In fact, the law of physics dictate that not only the platelets at the top of bag will fall to the buffy coat but also that the platelets at the bottom of bags will rise to the buffy coat. So the buffy coat is good source of platelets also. The plasma is transferred into a satellite bag and red cells separate into the bag at bottom having SAGM additive solution. The primary bag, containing suspension of 10-20 ml red cells, buffy coat and some plasma, is centrifuged at soft spin and platelets suspended in plasma are expressed into empty satellite bag carefully and slowly.

OPTIPRESS (Fig 14.4)

This is an automatic extractor using two parallel pressure plates, one is stationary and other is pneumatically driven that simultaneously expel plasma in an empty satellite bag and red cells into another bag containing SAG-M an additive solution.

The system is designed to leave a specific volume of buffy coat with plasma in the original collection pack. A light transmitter and two photocells control the flow of red cells and plasma into separate bags by the means of two automated clamping devices in the optipress.

OPTIPACKS

The optipacs system consists of one 600ml bag made of PVC plasticized with DEPH having 63 ml CPD solution with two outlet at the bottom and one at the top. One of the bottom out let has a connecting tubing with needle for the phlebotomy, while the other is connected to a 400 ml bag made of PVC plasticized with DEPH containing 100 ml SAG-M additive solution. The top outlet

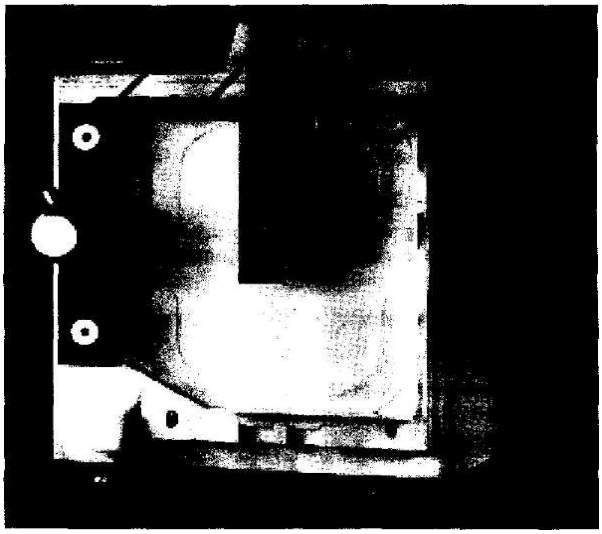


Fig. 14.4 OPTIPRESS

is connected via a V-piece tubing to two empty 400 ml bags made of PVC plasticized with TOTM, one for the collection of plasma and the other for the collection of platelets to store for 5 days.

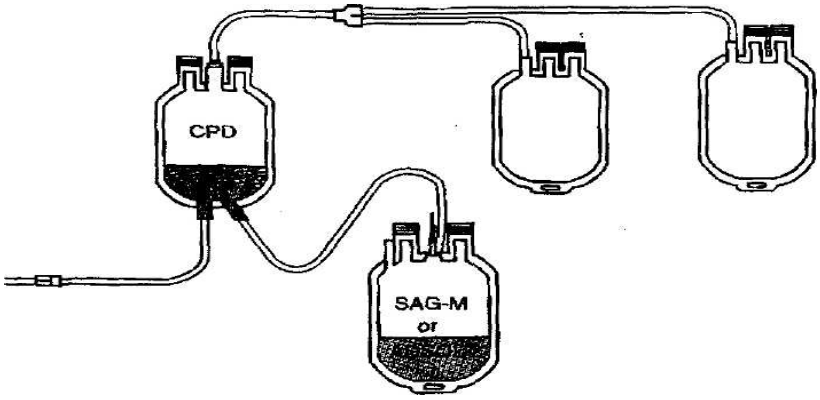


Fig. 14.5

Configuration of the OPTIAC quadruple blood pack unit

Preparation of LR-red cells Concentrate

Method:

1. 450 ± 10% blood is collected in the primary bag of optipac system having CPD anticoagulant. The anticoagulated blood is stored at room temperature (22°C) for about 4 hours before further processing.
2. The whole blood is centrifuged at heavy spin for 8 minutes.
3. The centrifuge unit is put in the optipress and a button is pressed to activate pressure plate.
4. Open the tube connection between the primary bag and plasma transfer pack to allow flow of plasma to one of the satellite bag. Then open the tube connection between primary bag and the bag with SAG-M to allow flow of blood.
5. When the process is complete both top and bottom tubings automatically clamp and the flow of red cells and plasma stop.
6. The bags containing red cells and plasma are sealed with di-electric sealer and separated.
7. Red cells in additive solution is kept at 2-6°C and plasma at -30°c or below.

Preparation of platelet concentrate from Buffy coat:

I. The primary bag with buffy coat and plasma with satellite bag is left hanging for about 2 hours at room temperature (22oC), then centrifuged at light spin at 22°C.

2. The supernatant plasma with platelets is slowly transferred into the empty bag using conventional extractor.

For more details see the instruction in the manual supplied with the equipment.

Conclusion

- The process is semi-automated.
- The consistency in the quality of the products and reproducibility is better.
- The whole process takes about 6-8 hours so the quality and shelf life of platelets is not effected and the levels of factors VIII and V in FFP do not drop.
- Red cell concentrates have on average 10^8 leukocytes e.g. one log leukocytes reduction, the incidence of FNHTR are significantly reduced in comparison to red cells in additive solution prepared without removing buffy coat. Further it provides good base for further leukoreduction in red cells having less than 5.5×10^6 using bed side leuko-reduction filters and even reduces the chances of HLA antigen sensitization.
- The yield of platelets in platelet concentrates prepared from buffy is on average $6\text{-}9 \times 10^{10}$ and leuko-reduction is about 90% which is significantly more than that prepared from PRP. Platelet concentrates have on average 10^7 leukocytes.
- Leukocytes are removed before storage, less amount of cytokines are formed, and the number of degenerated white cells is less in red cells and platelet concentrate. Incidence of FNHTRs is further reduced by their transfusion.
- Initial hard spin causes more yield of plasma leaving very little amount of plasma in red cells, this reduces the incidence of allergic and anaphylactic reactions after red cells transfusion. Besides, the yield of FFP is more and there by better yield of croprecipitate (factors V and VIII).

Disadvantages:

- There is loss of about 20% red cells.
- It is costlier.

THERAPEUTIC USES **OF BLOOD COMPONENTS**

WHOLE BLOOD

Functions of whole blood

- Red cells in whole blood carry O_2 to tissues
- Plasma in whole blood is blood volume expander
- Whole blood is a source of proteins with oncotic properties
- Is a source of non-labile coagulation factors

Indications for use :

Whole blood is indicated for those patients who have a symptomatic decrease in ***oxygen-carrying capacity*** combined with ***hypovolemia*** of sufficient degree associated with shock. Few patients fall into this category. The patients of trauma and for major surgery or exchange transfusion may be considered for whole blood transfusion.

Even in major trauma or surgical operation, a blood loss up to 30% can be corrected by the use of crytstalloid solutions alone. If the loss of blood is more than 30% and the patient is at the risk of hemorrhagic shock the transfusion of whole blood is the component of choice to restore blood volume and oxygen-carrying capacity. Even in most such cases, transfusing combination of

crystalloid and red cells or red cells and fresh-frozen plasma provides both volume expansion and oxygen-carrying capacity.

FRESH BLOOD

Whole blood or red blood cells concentrates less than 12-24 hours old from the time of collection are considered fresh. Processing of donor blood which includes screening for the markers of transfusion-transmitted diseases e.g. HIV, HBsAg, HCV, Treponema Pallidum etc., ABO and Rh typing and screening of antibodies, is rarely completed with in 24 hours. It is difficult to provide fresh blood ensuring the blood safety.

About 80-90% of platelets in a unit of blood become non-functional and about 30-40% of labile coagulation factors V and VIII are lost in 24 hours on storage at 2-6°C. Besides one or two units of fresh blood will not help a patient with a specific component deficiency.

Fresh blood could be justified earlier when no facilities for the separation of components were available and tests for the transfusion-transmitted disease were not mandatory. There is belief that it has some mystical humoral properties. But it is myth.

There are no valid indications for transfusion of fresh blood before completing all necessary tests. Before fresh blood is requisitioned, it is appropriate to establish diagnosis and to plan specific component (s) therapy.

Although justification for fresh blood is often difficult, it may be indented in a belief that fresh blood provides the greatest oxygen-carrying capacity because it has the maximum level of 2, 3 - di-phosphoglycerate (2, 3-DPG) and minimum amount of potassium as compared with older blood. Levels of 2, 3-DPG return to normal with in 12 to 24 hours after transfusion in recipients, so this does not present a problem for routine transfusion. 2, 3-DPG and potassium may be important in the premature neonates and other patients with impaired cardiac function, hemorrhagic shock, or sever pulmonary disease.

Newborns sometimes need fresher blood because they have high percentage of fetal hemoglobin, which does not release oxygen to the tissues as well as the adult hemoglobin. Their 2, 3-DPG levels may be severely decreased in conditions like respiratory distress syndrome, and the transfused blood represents a large proportion of their total blood volume. In such cases, blood less than 7 days old, with almost full levels of 2, 3 - DPG is of clinical significance. In exchange transfusion and in open heart surgery blood less than 7 days old is appropriate.

Concern about the high level of potassium and hyperkalemia caused by potassium in stored blood are rarely justified. Possible exceptions are patients who are persistently hypotensive, poorly perfused, and acidotic and who need large amount of blood.

Blood that is less than 7 days old after phlebotomy is more practical and can be readily available for few special patients.

A unit of whole blood of 350 ml blood will increase the hemoglobin by about 0.75 g/dl while a unit of 450 ml will increase Hb by about 1 g/dl in an adult patient of about 70 Kg body weight who is not bleeding. In paediatric patients the transfusion of 8 ml /Kg of red cells will increase the Hb approximately by 1 g/dl.

RED BLOOD CELLS (Packed Red Blood Cells)

Red cells are indicated for increasing red blood cells mass in symptomatic anemia who require increased oxygen-carrying capacity. In symptomatic anemia patients have pulse rate more than 100/minute, respiration rate more than 30 breaths/minute, dizziness, weakness, angina, and difficulty

in thinking. Indications of red cells transfusion are:

- In decreased bone marrow production conditions
  - Leukemia
  - Aplastic anemia
- In decreased red cells survival conditions
  - Hemolyic anemia
  - Thalassaemia
- In bleeding patients
  - Surgical bleeding
  - Traumatic bleeding

There is no set levels of hemoglobin that indicate a need of transfusion. However it is suggested that tiger values of hemoglobin of less than 6.0 g/dl in the absence of disease and between 8 and 10 g/dl with disease need transfusion of red cells.

Each unit of transfused red cells prepared from 450 ml of whole blood is expected to increase hemoglobin by about 1 g/dl (Hct 3 per cent) in a patient of 70 Kg body weight and who is not bleeding as with whole one unit of whole blood. The increase in hemoglobin and hematocrit is evident more quickly with transfusion of 1 unit of red cells than with 1 unit of whole blood.

Packed Red Cells

Reasons	**Indications**	
Surgery	• Patient need urgent operation and has Hb < 10g/dl • Anticipated surgical blood loss > 1000 ml	
Other acute blood loss	• Loss of blood	Replacement Fluid
	< 20% of blood vol.	None
	20-30% of blood vol.	Crystalloids/Colloids
	30-40% of blood vol.	RBCs & crystalloids
	> 40% of blood vol.	Whole blood or RBCs & Crystalloids

Others

- Anemia associated with incipient/established cardiac failure
- Hb value < 6 g/dl
- Patients approaching delivery and has Hb value < 7 g/dl
- In hereditary hemolytic anemias and beta thalassaemia major. Guidelines are more liberal

Advantages of transfusion of red cells

- Reduce risk of circulatory overload due to less volume of anticoagulant and plasma
- Lessens severity and incidence of allergic reactions
- ABO antibodies are reduced, red cells non-ABO identical to patient's group can be given, if compatible.
- Removed plasma can be used for preparing FFP and cryoprocipitate (factors VIII and V).

Contraindications of transfusion of red cells in:

- Well compensated anemic patients such as chronic renal failure.

- Nutritional anemia such as iron deficiency or pernicious anemia responsive to iron, folic acid, recombinant erythropoietin etc. unless patient shows signs of decompensation (need for increased oxygen-carrying capacity).
- To correct protein and coagulation factors deficiency
- Preoperative transfusion to raise Hb above 10 g/dl
- To enhance general well being, promote wound healing, prevent infection, expand blood volume when oxygen-carrying capacity is adequate.

LEUKOCYTES REDUCED RED BLOOD CELLS

Leukocytes-reduced red blood cells are indicated in the following conditions:

- Multitransfused patients like thalassaemic
- Leukemia
- Aplastic anemia
- Immunosupressed
- Immunodeficient
- Multiparous women
- Prevention of recurrent FNHTRs
- Prevention or delay of primary alloimmunization to HLA antigen
- Prevention of CMV transmission in at risk individual

Indications under investigation:

- Prevention of platelet refractoriness due to alloimmunization
- Prevention of recurrent FNHTRs to platelets
- Prevention of reactivation of latent CMV or HIV infection

Leuko-reduced red cells are not indicated

- To prevent transfusion related GVHD

WASHED RED CELLS

Washing of red cells removes 70 - 95 % of leukocytes and there is concomitant loss of 15 - 20 ml of red blood cells, but it is effective in removal of plasma proteins and microaggregates.

Indications

- Patients having recurrent attacks FNHTRs and urticarial reactions.
- Patients who have developed antibodies to plasma proteins.
- IgA deficient patient who has developed anti-IgA (incidence of IgA deficiency is 1 in 700 persons).
- Paraoxymal noctural hemglobiuria (PNH), sensitive to complement.

FROZEN/DEGLYCEROLIZED RED CELLS

Indications

- The provision of rare blood lacking common antigens for patients with corresponding antibodies.

- Autologous units of patients with multiple red cells alloantibodies stored for future surgery.
- IgA deficient patient with antibodies to IgA
- Its use to prevent FNHTRs has been supplanted by the use of LR-Red Cells.

PLATELETS

Functions of platelets :

- Formation of the primary hemosttic plug
- Maintain normal hemostasis

Platelet forms the hemostatic plug and provide the surface on which fibrin forms in a bleeding thrombocytopenic patient resulting in:

- Cessation of bleeding
- Correction of prolonged bleeding time
- Rise in platelet count

PRODUCTS AVAILABLE AND THEIR RELATIVE MERITS

Different platelet preparations

- Random donor platelet prepared from 450 ml blood
- Platelet - Apheresis (prepared by Cell Separator)

Relative Merits of Platelet-Apheresis and Random Donor Platelet

Platelet, Apheresis	**Random donor platelet prepared from whole blood**
• Average > 3 x 10^{11} platelets (equal to platelets obtained from 5 to 6 whole blood donations)	5.5 x 10^{10} platelets
• Plasma volume 200 ml	50-60 ml
• Leukocytes < 5.5 x 10^{6} Obviate the need of filtration	10^{8} in each unit filtration is required to reduce leukocytes
• Red cells - Traces	more
• pH - 6.0 or more	pH - 6.0 or more
• Exposes a patient to one donor	Exposes a patient to multiple donors
• Less exposure to infections	More exposure to infections
• Low risk to alloimmunization	Relatively more risk to alloimmunization
• HLA - or platelet- matched donor product can be prepared for the patients who have become refractory to platelets	Not possible
• Decreased risk of bacterial contamination and easy handlingas	More risk of bacterial contamination platelets are pooled
• Donation by apheresis requires great commitment	Routine donation, can be made from whole blood

- Sophisticated equipment required Not required
- Highly trained persons required Not much

Thrombocytopenia

The production of platelet in a healthy person is approximately 40,000 platelets / µl per day. This production rate balances platelet removal and sustains a normal platelet count as long as steady state conditions are maintained. However, if conditions altered to either less production or increase destruction of platelets, the equilibrium may be disturbed to produce thrombocytopenia.

Thrombocytopenia due to decreased rate of platelet production

- Leukaemia
- Chemotherapy
- Aplastic anemia
- Bone marrow transplantation
- Marrow infiltrative diseases (e.g.carcinoma, leukemia)
- Drug induced supression
- Radiation-induced hypoplasia

In these conditions endogenous and exogenous platelet survival is normal and platelet supportive therapy is useful.

Thrombocytopenia due to increased platelet destruction

■ **Immune platelet destruction or loss** :

- Idiopathic thrombocytopenic pupura (ITP)
- Alloimmune thrombocytopenia
  Neonatal alloimmune thrombocytopenia
  Post transfusion purpura
- Drug-induced thrombocytopenia (e.g. quinidine, heparin)
- Septicemia (severe infection due to gram negative organism)

■ **Non-immune platelet destruction or** loss

- Disseminated intravascular coagulation (DIC)
- Thrombotic thrombocytopemic purpura
- Hemolytic uremic syndrome

■ **Platelet sequestration**

- Hypersplenism
- Hypothermia

Endogenous and exogenous platelet survival is shortened. Benefit of platelet transfusion is low. Platelet therapy is given in life threatening bleeding at higher platelet count > 20 x 10^9/L. Dose is slightly higher, 1.50 units / 10 Kg of body weight.

Indications of **platelet transfusion when**

■ Platelet count is < 5000 / µl regardless of clinical condition

ABO compatibility between donor and recipient is of minor importance in platelet transfusion in most adults. Administration of ABO incompatible platelets is an acceptable transfusion practice. The possibility of Rh immunization by red cells contained in platelet concentrates should be considered in female patients. Rh (D) negative female patient of child bearing age should be given platelets from Rh (D) negative platelets, otherwise Rh(D) immune globulin Rhlg should be given in doses of 20 μg for each unit of platelet.

Risks associated with platelet transfusion :

Alloimmunization

When platelet-reactive and HLA-antibodies are induced by transfusion, platelets subsequently administered often fail to produce a therapeutic benefit. Alloimmunization to platelet antigens is common in patients who have received repeated platelet transfusions. However, an appreciable number of patients fail to form platelet-reactive antibodies for reasons that are not clear. Leukocytes present in routinely prepared platelet concentrates, provoke HLA-antibody formation more easily than platelets.

Platelet refractory state

Patient becomes refractory to platelet transfusion, if increment in platelet count one hour after transfusion is less than 20% of the expected increase in value on two occasions.

Platelet refractoriness may be due to:

- Immune-mediated platelet refractoriness
  Antibodies against HLA antigen
  Antibodies to platelet-specific antigen
- Non-immune based refractoriness
  Sepsis
  High fever
  DIC
  Hyperspleenism

Various techniques have been used to improve the effectiveness of platelets in alloimmunized patients.

- HLA- matched platelets from family members or unrelated persons.
- Platelets-Matched transfusion
- Platelet from single donor (prepared by apheresis)
- Leukocyte-reduced platelets

New approaches to this problem, still in experimental stage, are

- Treatment of platelets with ultraviolet light to abolish the immunogenicity of contaminating leukocytes.
- Transfusion of soluble, class 1 HLA antigens to induce active tolerance.
- Splenectomy
- Removal of antibodies by plasmapheresis
- High-dose intavenous immunoglobulin therapy

Infection

Infections transmitted by platelets transfusions are similar to those associated with other blood

products. An unusual but sometimes fatal, complication of platelet transfusion is infusion of bacteria that have proliferated in concentrates stored at 20 - 24°C.

Graft-Versus-Host Disease :- This is a rare complication of platelet transfusions that can be prevented by gamma irradiation of concentrates prior to transfusion to patients who have undergone bone marrow transplantation or have other form of immunodeficiency.

Adjunctive Therapies

Several agents have been used in addition to, or in place of platelets in the treatment of thrombocytopenia resulting from impaired production or platelet dysfunction. Epsilon-aminocaproic acid (EACA) and prednisone has been considered useful in these conditions by some clinicians, but there are no satisfactory studies that support their regular use. In uremia, bleeding responds in many cases to treatment with cryoprecipitate or desmopressin (DDAVP 0.3 µg/Kg), and platelet transfusion can be avoided.

Contraindications for Platelet Transfusions in:

1. Thrombotic thrombocytopenic purpura (TTP), Idiopathic thrombocytopemia purpura (ITP), Heparin-induced thrombocytopenia, unless life-threatening hemorrhage exists.
2. There is no role for prophylactic platelet transfusion in routine primary open heart surgery unless there is:
   a. Microvascular bleeding and platelet count 50,000/µl
   b. Microvascular bleeding (e.g. post-operative chest tube drainage greater than 500 ml within 6 hours) and non-diagnostic coagulation abnormality
   c. Microvascular bleeding and platelet function defect
3. Surgery or invasive procedures where platelet count is more than 50,000 /µl, prophylactic platelet transfusion is not indicated.

Conclusion

Indications of platelet transfusions can not be rigid and need to be individualized, depending upon the platelet count and clinical condition of the patient.

GRANULOCYTES

The role of granulocytes transfusion has decreased with

- The availability of new and better antibiotics
- The advent of recombinant granulopoietic growth factors e.g. granulocyte stimulating factor (rhG-CSF).
- Adverse effects of granulocytes transfusion

However the granulocyres trasfusion are still used at times with success in the following conditions:

- Septicaemia not responding to antibiotics. Infections in patients undergoing chemo / radio therapy for neoplastic diseases
- Neutrophil count less than 0.50 x 10^9/L (500 / mm^3), having gram-negative infection which fail to respond with antibiotics
- Temporary bone marrow depression for 1 - 2 weeks.

Granulocyte products available :

- Buffy coat
- Granulocytes, Apheresis

Buffy Coat prepared from 450 ml of blood contains :

- 0.60 x 10^9 granulocytes
- Contaminated with red cells, platelets, other leukocytes
- 15-20 ml plasma

Contents of Granulocytes, Apheresis

• Granulocytes	1 x 10^{10}
• Other leukocytes	1 x 10^8
• RBCs	5 - 10 ml
Plasma	200 - 400 ml
HES,if used	6-12%

Doses and Administration of Granulocytes

- In adult lxl0^{10} granulocytes daily e.g. 1 unit of granulocytes prepared by apheresis (equivalent to 18 - 20 units of buffy coat)
- Infected neonates need 0.5 - 0.6 x 10^9 granulocytes daily

Granulocytes transfusion should be discontinued when the patient becomes afebrile, or when granuolcyte count exceeds 1.0 x 10^9/L**.**

Contra-Indications for Granulocytes transfusion :

- Infections responsive to antibiotics

FRESH FROZEN PLASMA

Fresh frozen plasma (FFP) is plasma that is separated from whole blood and is frozen within 6-8 hours of collection. FFP contains plasma proteins and all coagulation factors, including the labile Factors V and VIII if stored at - 30°C or below.

Contents of 1 unit of FFP prepared from 450 ml Of whole blood

Plasma	175 - 230 ml
All coagulation Factors (including Factors V & VIII)	1 i.u. / ml of each factor
Fibrinogen	200 - 400 mgm

Indications of Fresh Frozen Plasma

- Actively bleeding and multiple coagulation factors deficiencies in
  - Liver diseases

- Disseminated intravascular coagulation (D1C)
- Coagulopathy in massive transfusion
- TTP
- When specific disorder cannot be or has not yet been identified

■ Familial Factor V deficiency
If concentrated Factor V is not available, FFP can be used as a source of Factor V.

■ Deficiency of Factors II, VII, IX,, and X
It is due to the absence of vitamin K or the use of drugs such as coumarin, like warfarin sodium and dicumarol that interfere with vitamin K metabolism. Administration of vitamin K is preferred to FFP in order to correct Vitamin K deficiency or coumarin over dose. Because several hours are required for vitamin K effectiveness, signs of hemorrhage may require transfusion of FFP in emergency.

■ Antithrombin III deficiency

■ Congenital or acquired coagulation factor deficiency

■ Use of FFP in conjunction with red cells has largely replaced the transfusion of fresh blood.

■ Cryoprecipitate-poor plasma contains 80% of the amount of Factor V in FFP and can be used as an alternative to FFP.

Dosage of FFP: About 10 ml / Kg of body weight. Post transfusion assessment of levels of aPTT, PT and fibonogen is done for monitoring the effect of FFP. FFP should be thawed at 30-37°C in circulating water bath.

Thawed plasma should be transfused as soon as possible, or within 12 hours, if stored at2-4°C.

Compatibility test before transfusion is not necessary. Plasma should be ABO compatible with the recipient blood, but not necessarily group specific. However plasma of the same ABO group as that of the recipient is preferred. The donor's plasma should not contain ABO antibodies that might interact with A or B antigen on the recipient's red blood cells.

Plasma ABO Compatibility Chart

Recipient's blodd group	**Plasma, Donor's blood group**
O	O,A,B,AB
A	A,AB
B	B,AB
AB	AB

Rh(D) positive plasma should not be given to Rh(D) negative women in the reproductive age group.

Contraindications for the use of fresh frozen plasma

- Blood volume expander
- Hypoproteinaemia
- Source of immunoglobulins
- When the prothrombin time is < 18 seconds

Solvent / Detergent Plasma

Plasma is treated with the solvent tri (n-butyl) phosphate (TNPB) and the detergent Triton X-100 to inactivate lipid-enveloped viruses such as hepatitis B and C and HIV. The solvent - detergent treatment has no effect on nonlipid-enveloped viruses, such as hepatitis A or parvovirus B19. The coagulation factors of solvent/detergent plasma are comparable to FFP. Indications for its use are the same as that of FFP.

Contraindications :

- Pregnant women, except at the time of delivery
- Neonates
- Chronic hemolytic anemia
- Sickle cell disease
- Patients under going treatment with bone marrow damaging chemotherapy or radiation
- Bone marrow transplant patients

Out date :12 hours at room temperature after thawing, do not refrigerate.
Doses and Administration is the same as that for FFP.

SINGLE DONOR and CRYOPRECIPITATE-POOR PLASMA

Plasma separated from one unit of whole blood on or before the fifth day of the expiration date is called as single donor plasma. Cryoprecipitate-poor plasma is a by product of cryoprecipitate preparation. Both these products lack the labile coagulation factors V and VIII, but contain stable clotting factors II, VII, IX, and X. Cryo-poor plasma lacks fibrinogen also.

Indications

- In deficiency of stable clotting factors (e.g. coagulopathies due to warfarin drugs)
- Burn

Doses : are the same as that of FFP.

CRYOPRECIPITATE

Description: Cryo is cryoprecipitated proteins derived from the fresh frozen plasma.

Each bag has approximately:

Plasma	10- 15 ml
Factor VIII	80 - 100 i.u.
Fibrinogen	150 - 250 mg
von-Willebrand Factor	40 - 70%
Fibronectin	55 mgm
Factor XIII	20 - 30% of the original

Indications :

- Hemophilia A
- von Willebrand's disease
- Congenital or acquired fibrinogen deficiency

- Acquired Factor VIII deficiency (e.g. DIC, massive transfusion)
- Factor XIII deficiency
- Source of Fibrin Glue used as topical hemostatic agent in surgical procedures and to remove fragmented renal calculi.

Factor VIII concentrate, 500 i.u. bottle, available as pharmaceutical product, is the product of choice for most of the hemophiliacs.

Doses and Administration of Factor VIII Concentrates

Croprecipitate may not be ABO identical however ABO compatible croprecipitate is preferred. The compatibility testing is not necessary, Rh type need not be considered when using this component.

In calculating the dose, it can be assumed that 1 i.u. of factor VIII / Kg body weight will increase the patient's factor by 2% (0.02 i.u./ml). Thus, if the patient's base line factor VIII level is 0.01 i.u./ml, a dose of 20 i.u./Kg body weight would be expected to raise the level to 40% (0.4 i.u./ml). The half-life of factor VIII is 10-12 hours.

If 20 units /Kg body weight are required, a person of 70 kg weight will require 1400 i.u. of factor VIII.

CRYO could be used to supply 1400 i.u. of factor VIII, but at 80 i.u. per bag this would require at least 17-18 bags of cryo.

The amount of factor VIII required can also be calculated as follows :

1. Weight (Kg) x 70 ml/Kg = blood volume (ml).
2. Blood volume (ml) x (1.0-Hct) = plasma volume(ml)
3. Units of factor VIII required = (desired factor VIII level in units/ml - Initial level of factor VIII/ml) x plasma volume in (ml)

Example:

A 70 Kg severe hemophiliac with hematocrit of 40% has initial factor VIII level of 2 i.u. per dl (0.02 i.u./ml, 2% activity). How many units of factor VIII concentrate should be given to raise his factor VIII level to 50 i.u. / dl ?

70 Kg x 70 ml/Kg	=	4900 ml (Blood volume)
4900 x (1.0 - 0.40)	=	2940 ml (Plasma volume)
2940 x (0.50 - 0.02)	=	1411 i.u. of Factor VIII required

This dose will increase the level to 50 i.u./dl.

Hemophilia A - Recommended Doses of Factor VIII

Type of bleeding	Doses (Factor VIII i.u/k	Frequency
• Acut hemarthosis		
Early	10	Seldom required
Late	20	20 i.u. every 12 hours
• Intramuscular hemorrhage	20-30	20 i.u. every 12 hours
Life - threatening situation	50	25-30 i.u. every 8-12 hours
Intracranial hemorrhage		
Major surgery		
Major trauma		
• Severe abdominal pain	20	20-25 i.u. every 12 hours
	20	20 i.u. every 12 hours
• Extraction of permanent teeth		often not necessary
	20	
• Tongue or mouth laceration		20 i. u. every 12 hours

The duration of treatment with factor VIII depends upon the type and location of hemorrhage. After major surgery, the factor VIII level should be maintained above 40-50 i.u./dl for at least 10 days.

Factor VIII assay should be done to serve as a guide to therapy. In emergencies the aPTT can be used as a rough guide to factor VIII activity.

In mild or moderate hemophilia A, Desmopression (DDAVP) 0.3 µg/Kg intravenous, is the treatment of choice.

Antifibrinolytic agents (aminocaproic acid and tranexamic acid) are useful in controlling bleeding in oral cavity. The recommended dose of amincaproic acid is 75 mg / Kg body weight every 4-6 hours, that of tranexamic acid is 25 mg / Kg body weight every 6-8 hours. Antifibrinolytic agents are given 1 day preoperative and continued for 7-10 day. The therapy of choice for sever hemophilia A is Factor VIII concentrate.

Pooling of cryoprecipitate :

Cryo is thawed at 30 - 37°C in circulating water bath and all bags are pooled in one bag by means of bag to bag connector under laminar flow. Wash each empty bag with 10 ml of sterile normal saline to dissolve residual cryoprecipitate and add to the pooled cryoprecipitate. It should be used preferably immediately after thawing and pooling.
Outdate : 6 hours after thaw: and 4 hours after pooling.

Blood Components and their uses (Summary)

Components	Composition	Approx. volume	Indications
Whole blood	RBC (approx. Hct 40%) WBC & some platelets; Plasma deficient in factors V, VIII	500 ml	Increase red cells and plasma volume
Red blood cells	RBC (approx. Hct 75%), WBC & some platelets, reduced plasma	250 ml	Increase red cells mass in symptomatic anemia
Red cells+Additive Solution	RBC (approx. Hct 60%) WBC & some platelets, reduced plasma, 100 ml of additive solution	330 ml	Increase in red cells mass in symptomatic anemia
Leukocytes-reduced RBCs	RBC > 85% of original volume WBC<5x 10^8 to < 5x 10^6 Few platelets & minimal Plasma	225 ml	Increase red cells mass, reduce FNHTR, WBC <5 x 10^6 decrease HLA immuni-zation & CMV transmission
Washed RBCs	RBC (approx. 75%) WBC <5 x 10^8 No plasma	180 ml	Increase red cells mass, reduce risk of allergic reaction to plasma proteins
RBCs frozen/ Deglycerolized	RBC (approx. Hct 75%) WBC < 5x10^6 no platelets & plasma	180 ml	Increased red cells mass, minimize febrile or allergic reactions; used for prolonged RBCs storage
Platelet, Concentrate (random donor)	Platelets 5.5xl0'°/unit few RBCs, WBC, plasma	50 ml	Bleeding due to thrombocytopenia or thromocytopathy
Plateletpheresis	Platelet > 3 x 1011/unit WBC < 5.5 x 10^6, plasma & minimal RBCs	300 ml	Same as platelet concentrate, sometimes HLA matched or platelet cross-matched platelets are prepared
Granulocytes, pheresis	Granulocytes > lx10^{10} Lymphocytes: some RBCs and platelets	220 ml	Used in selected cases with sepsis & sever neutropenia (500/µl)
Fresh frozen plasma	Plasma having all coagulation Factors	220 ml	Coagulation disorders,if PT > 18 sec, aPTT >60 seconds (> 1.5-1.8 times of controls)
Plasma	Plasma, stable clotting factors, no platelets	220 ml	Stable clotting factor deficiencies (II, VII, IX, X, XI)
Cryoprecpitated AHF (Factor VIII)	Factor VIII, von-Willebrand's factor XIII, fibrinogen	15 ml	Hemophilia A, von-Willebrand's disease, deficiency of fibrinogen & factor XIII

PHARMACOLOGICAL PRODUCTS AS ALTERNATIVES TO BLOOD PRODUCTS

Awareness of physicians regarding issues of blood / blood products safety, blood conservation, great emphasis has been placed on alternatives to allogenic blood products. These alternatives are pharmacological interventions few of them show promising result in reducing blood transfusion.

PHARMACOLOGICAL INTERVENTIONS ARE:

1. **Recombinant hematopoietic growth factors**
   Stimulate blood production, prepared by recombinant DNA technology
   - Recombinant human Erythropoietin (EPO)
   - Granulocyte colony-stimulating factor (G-CSF)
   - Granulocyte macrophase colony-stimulating factor (GM-CSF)
   - Thrombpoietin

   There are enough data for EPO, G-CSF and GM-CSF for their clinical use.
2. **Reduce blood loss** :
   - **DDAVP**
   - Topical agents - Fibrin glue, fibrin gel
3. **Agents that preserve platelet function**
   - Dipyridamole
   - Prostacylin
   - Heparin
4. **Antifibrinolytic agents**
   - Epsilon-Aminocorproic acid
   - Tranexamic acid
   - Aptotinin
5. **Blood substitutes or oxygen carriers**
   - Perfluocarbons
   - Hemoglobin solution
   - Liposome encapsulated hemoglobin

Recombinant Human Erythropoietin (EPO)

EPO is produced by kidney. Under normal conditions, EPO assures the survival of erythroid progenitor cells to replace red cells. When EPO level decreases, some of EPO-dependent progenitor cells die, and RBCs production is decreased. Half-life of EPO is 4-13 hours. Reombinant erythropoietin (EPO) is prepared by recombinant DNA technology.

Indications of EPO Therapy

- Anemia in renal failure (when creatinine > 1.8 mgm%)
- Patient receiving dialysis

- Anemia with HIV infection undergoing treatment with Zidovudine (AZT) (effective when erythropoietin level < 500 u/L)
- Anemia in cancer patient undergoing chemotherapy (erythropoeitin level < 200 u/L)
- Anemia in chronic diseases (e.g. rhumatoid arthirits)
- Pre-deposit autologous blood donation
- Pre-operative anemia

Indications under investigation

- Surgical anemia
- Bone marrow transplantation
- Anemia of pre-maturity
- Myelodysplastic syndrome
- Sickle cell anemia

Doses :

In renal failure - 50-100 U / Kg body weight, intravenously, or subcutaneously, three times weekly with dose reduction once the patient's Hct. reaches 0.30 - 0.34.

Patient undergoing dialysis - 34 U / Kg, three times weekly

Granulocytes and Granulocytes Macrophase Colony- Stimulating Factors

G-CSF and GM-CSF stimulate the proliferation of neutrophil progenitor cells and mobilize granulocytes from the marrow. GM-CSF if given repeatedly, will also increase eosinophils and monocytes. They are clinically used in:

- Chemotherapy induced neutropenia, it causes:
  - Decrease duration of neutropenia
  - Increase tolerance to cytotoxic drugs
  - Reduce need of granulocytes transfusions
- GM-CSF for the use in patients having autologous marrow transplant
- Both are used in patients undergoing allogeneic marrow transplant
- Patient having bone marrow suppression due to anti-viral agents
- Stimulate and increase the yield of peripheral blood stem cells (PBSCs)
- Both may be effective agents to increase granulocytes yield, G-CSF results collection upto 10 x 10^{10}rapheresis

Doses :

For increasing granulocytes:

G-CSF - < 5ug/Kg body weight per day

GM-CSF dose may be limited due to side effects.

G-CSF - 10 - 16 <s>g / Kg body weight per day to mobilize PBSCs

ADJUVENT THERAPY TO REDUCE BLOOD LOSS :

Desmopressin acetate (DDAVP) - [1-deamino, 8-D arginine, vasopressine] is a synthetic analog of antidiuretic harmone (vasopressin)

DDVAP increases the levels of Factor VIII and von Willibrand's factor (vWF)
Dose: 0.3 mgm / Kg body weight, intravenously.

Used in :

- Mild Hemophilia A
- von Willibrand's disease
- To improve or correct bleeding time in patients with acquired platelet defects as in uremia, cirrhosis, aspirin ingestion
- Topical agents

Fibrin glue and fibrin gel are blood derivatives rather pharmacological agents, and are used as intervention in surgical hemostasis. The agents are applied directly to wounds displaying bleeding or can be used to seal grafts.

Fibrin glue is derived from a source of fibrinogen and Factor XIII (Fibrin stabilizing factor), in which a solution of fibrinogen is mixed with a solution of bovine albumin and applied to surgical field. Alternatively autologous plasma can be used as a source of fibrinogen and factor XIII.

Fibrin gel consists of platelet-rich-plasma, which is mixed with a solution of bovine albumin and applied to surgical field. Platelet-rich plasma can be autologous.

Anti-coagulation of Extracorporeal Circulation
Coagulation activation in extracorporeal circulation in coronary-bypass (CBP) can be reduced using thrombin inhibition (e.g. hirulog and hirudin), thrombin inhibitor peptide and factor Xa inhibition with low molecular weight heparin.

Agents that preserve platelet functions
Infusion of the antiplatelet agent dipridamole has been reported to reduce activation and depletion of platelets during CPB causing significant reduction in postoperative blood loss and transfusion requirement.

Prostacylin has a platelet sparing effect during CPB, presumably because of inhibition of platelet aggregation.

There are no satisfactory studies that support their regular use.

ANTIFIBRINOLYTIC THERAPY
Three antifibrinolytic agents commercially available are:

- Epsilon - aminocarporic acid (EACA)
- Tranexamic acid
- Aprotinin

They are particularly useful in the control of bleeding in oral cavity e.g. extraction of permanent teeth, tongue and mouth laceration and oral surgery.
Doses: E-aminocarporic acid - 75 mgm / Kg every 4-6 hours orally
Tranexaminc acid - 25 mgm / Kg every 6-8 hours

There are no satisfactory studies that support the regular use of pharmacological agent for anti-coagulation of extacoporeal circulation, preservation of platelets and anti-fibrinolytic therapy.

Blood Substitutes or Oxygen carriers

Many blood substitute solutions for carrying oxygen like perfluocarbons emulsions, cell-free hemoglobin solutions and liposome-encapsulted hemoglobin, have been tried but none is available for satisfactory clinical use. Research in the blood substitutes has a long way to go.

Perfluoro Compounds

Perfluorocarbons (PFCs) are relatively simple, inert material (e.g. Teflon) that have the ability to dissolve large amount of oxygen. Most of them cause unpleasant side effects. However, one emulsion (Flusol-DA) has been licensed for limited use in precutaneous transluminal coronary angioplasty (PTCA). Newer emulsions that contain more PFC (and therefore more oxygen) are now in human clinical testing. A significant limitation to PFC emulsion is that in order to carry amount of oxygen similar to hemoglobin, a patient has to breath high concentration of oxygen.

Recently, a number of much improved stable emulsion of a variety of perfluoro compound have been developed with higher oxygen carrying capacity. However, none of these are presently available for clinical use.

Hemoglobin Solutions

In the past several efforts have been made to develop cell-free hemoglobin solutions. Such Hb solutions will eliminate the dependence on oxygen breathing as in PFC emulsions because Hb binds oxygen chemically. However, Hb solution itself is not suitable for direct infusion as it is rapidly cleared from the circulation, and its oxygen affinity is too high when stripped of its protective cell membrane. Efforts have been made by modifying Hb by pyridoxylation which lowers its oxygen affinity and by subsequent conjunction of the Hb with various biocompatible polymers or by polymerization of Hb itself which increase the size of Hb molecule complex to a point that will not filter out in renal glomerules.

Alternatively efforts have been made to detach heme moiety from hemoglobin and attach to polymer of appropriate size. This permit the use of heme of animal origin and prepare synthetic hemes. However, as yet there is no report of the use of modified Hb in human.

Encapsulated Hemoglobin

Hemoglobin can be enclosed in artificial liposomes. Such encapsulated Hb has been shown in rats as effective as red cells in transporting oxygen. However the half-life of liposomes in the circulation is short.

Encapsulated hemoglobin has never been administered in humans and its future as a blood substitute depends on the development of a membrane of superior composition.

Thus, at present, the concept that one would simply choose “artificial” over real blood for transfusions in human being is naive. It is likely that the blood transfusion services of future may provide an array of products - some natural, some synthetic - to be used for oxygen transport.

INTRODUCTION

Apheresis (or hemapheresis) is a Greek word that means to separate or remove. In apheresis blood is withdrawn from a donor or patient in anticoagulant solution and separated into components. One (or more) component is retained and the remaining constituents are returned to the individual.

Any one of the components of blood can be removed and the procedures are specified for the component selected. The process of removing the plasma from red cells is termed plasmapheresis. Similarly terms are given to the removal of other components, including platelets (plateletpheresis), red cells (erythrocytapheresis) or leukocytes (leukapheresis). Apheresis can be non-therapeutic or therapeutic. It can be done manually or using automated equipment.

Automated cell-separator machines are used for both component preparation and therapeutic applications. In manual apheresis whole blood is collected in multiple bags system and centrifuged off-line. It involves great care that the bags are labeled correctly and its contents are returned to ihe correct donor. With the current available automatic technology now the manual process is seldom used.

The issues of safety and quality, which are the basic requirement in blood banking, are met by the use of single-donor apheresis components. The advantages of single-donor components are:

Reduced multiple donors exposure	- Reduced risk of alloimmunization - Reduced incidence of transfusion-transmitted diseases

2.	Full and effective transfusion dose	
3.	Higher quality product	
4.	Purer product	leukocytes-reduced products
5.	Ability to match donor to patient	Platelet matched or HLA matched
6.	Fewer donor reaction due to return of fluid	- Crystalloids and anticoagulants - Longer period in apheresis help refilling of intravascular compartment, with fluid from interstitial spaces
7.	Smaller needle technology	Venous access injuries are less

Automated Separation Techniques By Centrifugation

In most apheresis instruments, centrifugal force separates blood into its components based on differences in specific gravity. Blood is drawn from individual with the assistance of a pump. Anticoagulant (ACD) is added to the tubing to prevent clotting. Anticoagulated blood from a donor or patient is pumped into a rotating bowl, chamber, or tubular rotor in which layering of components occurs based on density. The desired component is retained and the remaining elements are returned to the donor or patient by intermittent or continuous flow.

All systems have prepackaged disposable sets of sterile bags, tubing, and centrifugal devices unique to the instrument. Each has a mechanism to rotate and separation device without twisting the attached tubing. In the intermittent flow method, the centrifuge container is alternately filled and emptied. Most equipment in use now has a continuous flow of blood through the separation chamber. With both methods, single- or dual-vein access techniques are possible. Depending on the procedure and device used process time varies.

Each manufacturer supplies detailed information and operational protocols. It should be available to nursing and technical personnel.

Separation By Membrane Filtration

Filtration of plasma through a membrane allows collection of plasma from healthy donors or therapeutic removal of abnormal plasma constituents. Most of the instruments have the membrane arranged as hollow fibers, but some have flat plates. Inner membrane surface repel cellular elements in the flow of blood so that platelets are not activated and red cells survival is not shortened.

The Most Commonly Available Apheresis Devices And Their Functions:

Manufacturer	**Name of Equ.**	**Method**	**Products**
Haemonetic	- Haemonetic MCS	IFC	PI, P, L, PBSC
Corporation	- Haemonetic MCS Plus	IFC	PI,P, L, PBSC, RC.
	- PCS/PCS 2	IFC,MF	Plasma
Baxter (Fenwal)	- Autopheresis C	IFC,SM	Plasma
“	- CS-3000PIus	CFC	P1,P,L,PBSC
“	- CS-3000 + AMS	CFC	P1,P,L,PBSC,
“	- Amicus	CFC	P1,P,PBSC,RC
Gambro (Cobe)	- Spectra	CFC	PI,P,L,PBSC,RC
“	- Type	CF,MF	Plasma
Fresenius	AS-104	CFC	PI, P. L, PBSC

CFC-continous flow centrifugation; IFC-intermittent-flow centrifugation; MF-membrane filtration; SM-spinning membrane; Pl-platelet; P-plasma; PBSC-peripheral blood stem cells; RC-red cells; L-leucocytes.

Haemonetic Technology

Intermittent-flow-centrifugation cell separators, mainly produced by Haemonetic Corporation, use a fixed speed, separating the components according to their specific gravity (Fig. 15.1). The anticoagulated blood is pumped into a rotating bowl, separating the incoming blood in such a way that red cells move to periphery and plasma to the inside of rotating bowl, and white cells and platelets layers between the red cells and plasma (Fig, 15.2). Using optical detectors and a fluid surge elutriation process, desired blood component is retained and the remaining constituents of blood are returned to the donor or patient. A plateletpheresis procedure usually takes 6-8 cycles to collect a therapeutic dose.

Haemonetic Cell Separator (MCS)

Intermittent-flow-centrifugation (I.F.C.) cell separator (MCS), manufactured by Haemonetic Corp. has a rotary seal and it is a closed system. I. F. C. procedure is done as one -arm procedure and it is convenient to the donor. In I. F. C. process, extracorporeal blood volume is more and it takes longer time in comparison to continuous-flow centrifugation process because of single-arm venous access for both blood draw and return.

MCS Plus of Haemonetic is new cell separator of intermittent-flow technology (Fig. 15.1).

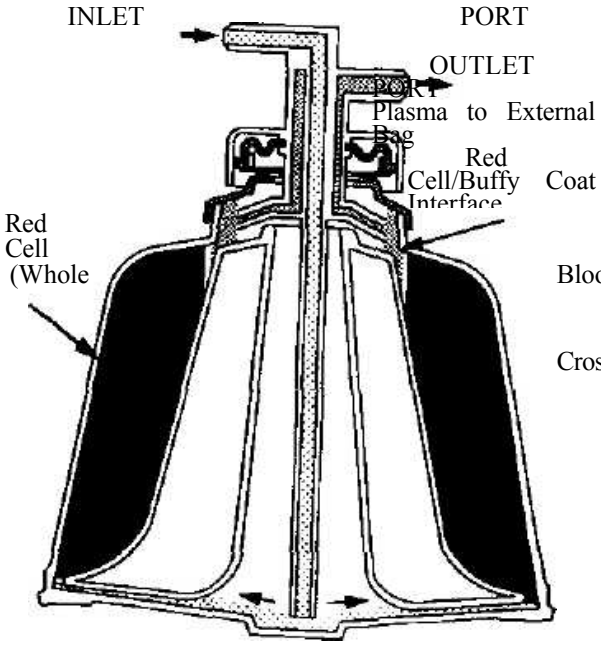


Fig.15.2

Cross-section of Haemonetics centrifuge bowl (IFC procedure)

It is very light. MCS Plus has been developed for automated red cells collection also and has the capability of using smaller drawing needle and a metered anticoagulant for the collection of red cells.

Both machines MCS and MCS Plus are capable of collecting single-donor platelets with or without extra plasma that can be used as fresh- or fresh-frozen plasma for transfusion.

The Plasma Collection System (PCS/PCS2) of Haemonetic is designed on centrifugation and membrane filtration technology to collect plasma for transfusion or source plasma. It has specially designed disposable bowl to collect plasma and 500-600 ml plasma can be collected. It can be used for therapeutic plasma exchanges also.

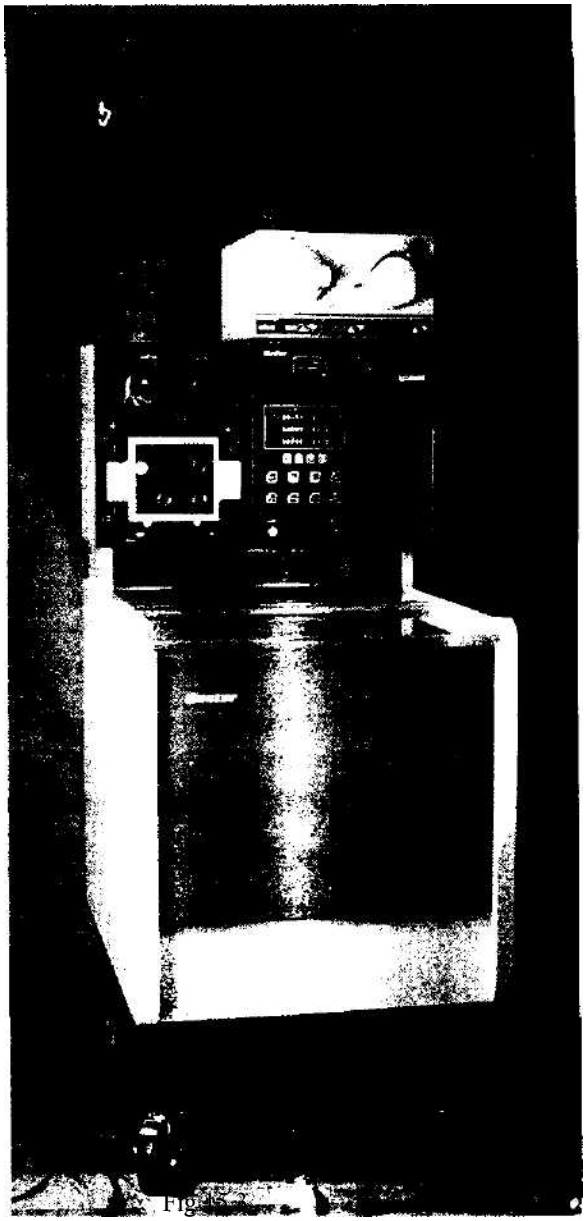


Fig. CS-3000 Plus Separator

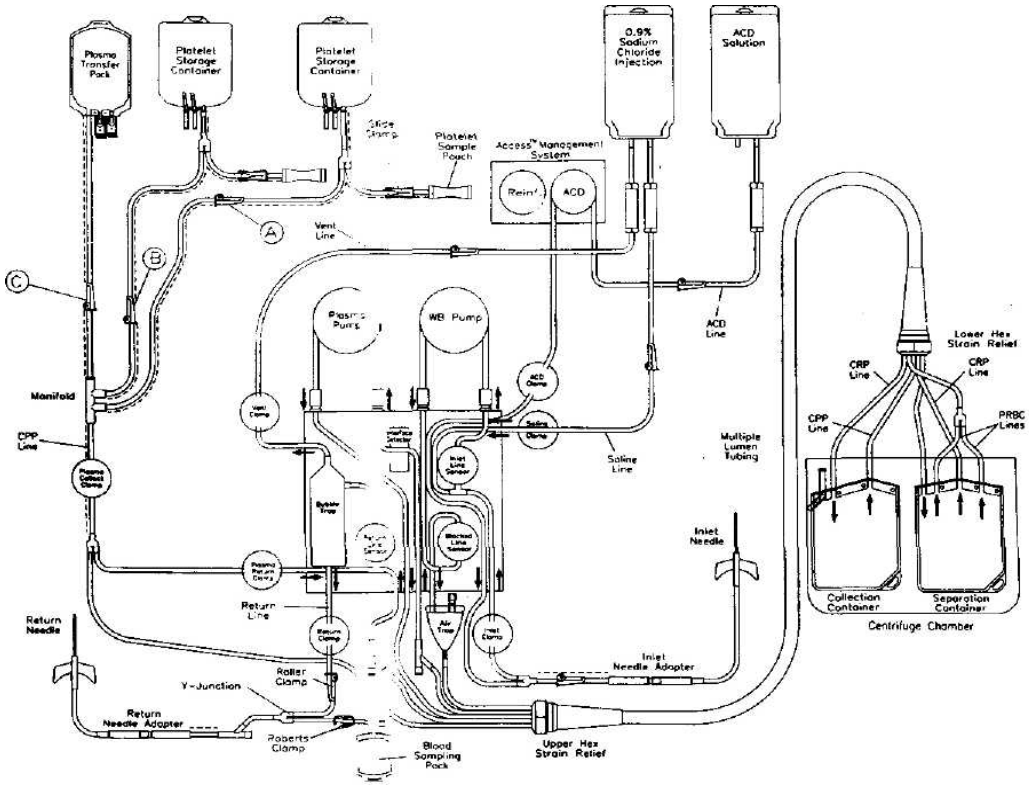
Gambro (Cobe) Technology

Cobe technology uses continuous-flow centrifugation technology and two arm venous access for blood draw and return. Originally technology was developed by IBM (IBM 2997 cell separator) and used a rotary seal and a rotating channel resembling a belt as the initial separation chamber. An improved dual channel system allowed a lower WBCs and RBCs contamination in the platelets. The Cobe Spectra has succeeded the IBM- 2997 and incorporated a similar channel designed as Cobe-2997. It is seal less system with the separation interfaces controlled automatically. It allows its use as two-arms procedure.

Cobe has also developed membrane cell separator, called Cobe TPE. This is continuous-flow device using a flat membrane to separate the cells of blood from the plasma. It is used to do the therapeutic plasma exchange (TPE) or to collect plasma from the donor.

Baxter (Fenwal) Technology

Fenwal uses continous-flow technology and a seal less system. The device is fully automated and computerized as in MCS or Cobe. An enhancement of the CS 3000 has two separation container holders (1) TNX - 6™ separation container holder has allowed collection of leukocytes reduced platelets and (2) another separation container holder labeled GRANULO used for white cells collection. This is called CS 3000 plus.



Note : Tubing segments identified with dashes (……)

Clamp

Fig.15.4

Flow Chart of CS-3000 Plus Blood Cell Separator

CS-3000 plus has micro-processed controlled and continuous-flow system (Fig. 15.3). It uses interface detector, which senses the optical density to limit cross contamination. There is always low extracoporeal volume and the process take less time in comparison to single arm procedure. It is fully closed system.

During operation anticoagulated blood is pumped into spinning separation container. Red cells are packed by centrifugal force towards the outer edges of the container. Then the red cells exit the separation container. The lower density component such as plasma, platelets or white cells is removed by plasma pump and enters the spinning collection container where platelets or white cells are packed by centrifugal force towards the outer edges of the container. The separated component remains packed in the container while other constituents of blood are returned to the donor/patient (Fig. 15.4).

It is capable of collection of single donor platelets without or with extra plasma for transfusion as fresh plasma or fresh-frozen plasma.

CS 3000 Plus has additional facility of Access Management System (AMS) Control Panel which has a control and indicator display for monitoring the auto-cuff, ACD pump and re-infusion pump. It can be used for therapeutic apheresis also.

Recently Baxter has introduced new automated cell separator called Amicus (Fig. 15.5). In Amicus separator short procedures times, single/double needle procedure, individual anti-coagulant control and various safety features contribute to an overall donor comfort and safety. It is both single or two arm procedure. Amicus cell separator has a rotating channel resembling a belt as the initial separation chamber It has a quiet, high efficiency centrifuge which provide consistent product volumes with in shorter collection time. Fig. 15.6.

Baxter has developed a machine for collection of plasma called Autopheresis-C (Fig. 15.8). It uses spinning membrane, thus incorporating both membrane and centrifugation technology. It can be used for the collection of 500-990 ml plasma. This system is intermittent-flow system and usually one-arm procedure. This collects the blood in a small rotating cylinder, forcing plasma through a polycorbonate membrane.

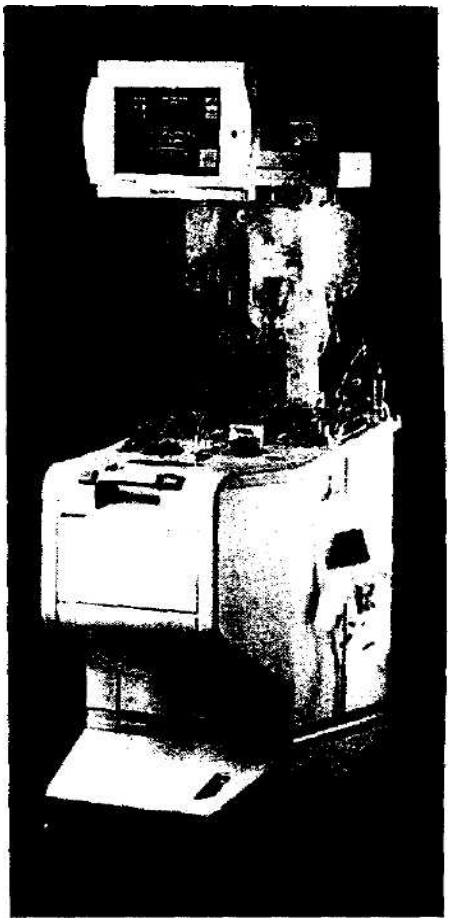


Fig.15.5
The Amicus
Separator
BAXTER

Apheresis-Drived Blood Components :

Products	**Quantities Collected**
Platelets	3.0-8.0 x 10^{11} in 180-200 ml plasma
Plasma	400 - 600 ml
RBCs	180 - 200 ml absolute RBCs
Granulocytes	1.0 - 3.0 x 10^{10} in 200-300 plasma
PBSCs	variable

Donors Reactions

Reaction	**W.B.**	**Plasma**
Vasovagal	Occasional	Rare
Hypvolemia	Occasional	Rare
Citrate effect*		Rare
Citrate toxicity	-	Very rare

* Citrate effect - numbness and tingling sensation around the mouth.

** Citrate toxicity - muscle cramps, shivering, nausea, vomiting and tetany

WB - whole blood

Amicus Single Needle Closed System Apheresis Kit

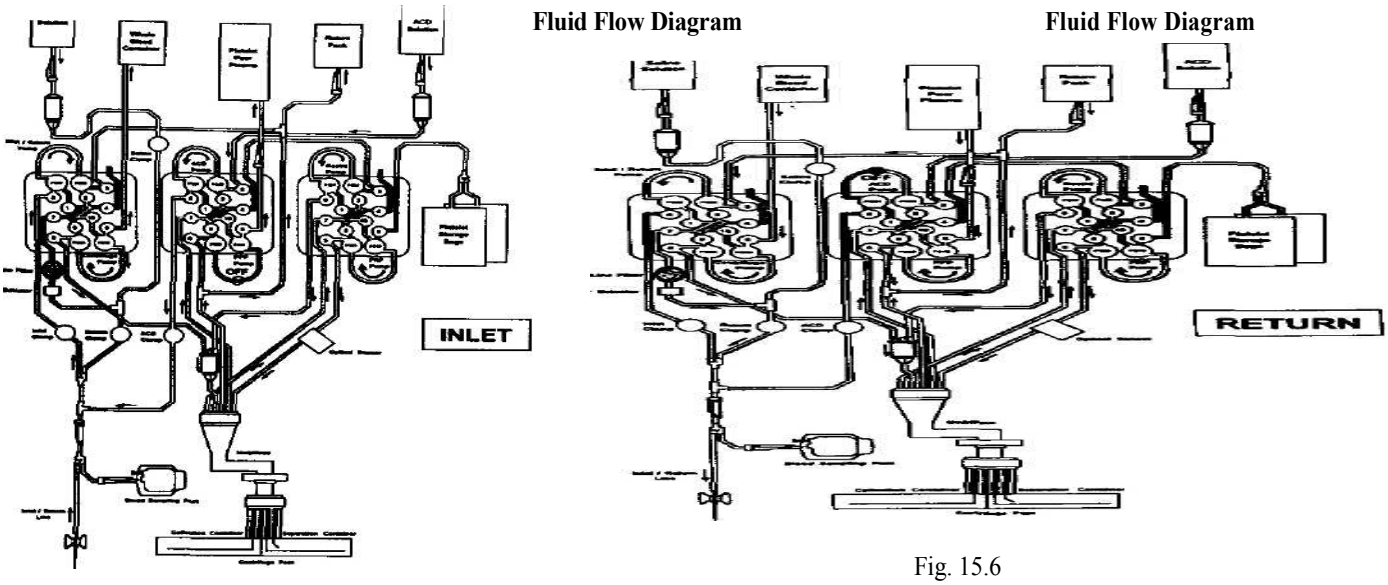


Fig. 15.6
Flow chart of AMICUS Separator Single Needle

General Requirements For Apheresis

1. A qualified, licensed physician is responsible for all aspects of the apheresis program.
2. Equipment should be good, reliable and in proper working condition.
3. Well-trained and motivated staff is essential to an effective apheresis program.
4. Operator (nursing or technical personnel) of apheresis machine must know all aspects of its operation and trouble shootings.
5. An apheresis operator must be friendly and must be able to relieve the anxiety of the donor/patient.
6. There must be a manual readily available to nursing and technical personnel, giving detail description of each type of procedure, and trouble shootings specific for the machine.
7. Records for laboratory findings and data for each apheresis procedure should be kept.

General Criteria For Selecting Apheresis-donor

1. Donor undergoing an occasional apheresis procedure (performed not frequently than once every 4 week) must meet the same criteria as a whole blood donation.

2. Donor should be preferably repeat donor - might have given blood 1-2 times earlier.
3. Written consent of the donor is taken after explaining the procedure in detail, time taken, and about possible hazards and benefits.
4. Venous access is an important consideration in apheresis-donor and veins should be examined at the time of the selection of a donor as:
   i). long needle-in and needle-out times
   ii). prolonged flow rate
   iii). frequent need for two venipunctures with continuous-flow equipment
5. Donor should be screened prior to apheresis for markers of infectious diseases transmitted by the transfusion of blood and its components in the same manner as for the whole blood. Each donor must be tested prior to each apheresis unless the donor in undergoing repeated procedures, in such cases testing for the markers of diseases need be repeated at 30 days interval.
6. Tests for hemoglobin, ABO group, Rh type, and screening for unexpected antibody are done.
7. More stringent regulations govern the donor who participate in serial apheresis program (procedure performed more frequently than every 4 weeks).
   i) Interval between two procedures should be at least 48 hours and the loss of red cells should not exceed 25 ml per week,
   ii) If donor's red cells could not be reinfused during a procedure, or if the participant donates a unit of whole blood, 12 weeks should elapse before subsequent apheresis procedure,
   iii) Careful monitoring of weight, blood cells count, serum protein levels and quantitation of immunoglobulins is required.
   - Age should be between 18-50 years.
   - Weight be 60 Kg or more.
   - Hemoglobin - 12.5 g/dl or more

PLATELETPHERESIS

In a plateletpheresis procedure, a portion of the donor's platelet and some plasma is removed with the return of the donor's RBCs, WBCs, and remaining plasma. A routine pletletpheresis procedure usually takes 1 to 1.5 hours. The product is prepared in closed system and can be stored for 5 days. Platelets can be prepared without or with extra plasma in a separate bag which can be transfused or stored as fresh-frozen plasma (FFP). If extra plasma is collected during plateletpheresis, all precautions are taken as in the plasmapheresis. Routinely, the number of platelets in an apheresis product is equivalent to 6 to 8 random platelet concentrates.

Specific Criteria for the selection of donor for plateletpharesis:

1. Donors who have taken aspirin containing medication within 36 hours are usually deferred.
2. Platelet may be collected from donors who do not meet the requirement if the component is of particular value to the patient - HLA matched donors.
3. The interval between procedures should be at least 48 hours. A donor shall not undergo the procedure more than 2 times in a week or 24 times in a year.

4. A platelet count is not required prior the first procedure or if the interval between plateletpheresis procedures is at least 4 weeks.
5. If plateletpheresis is performed more frequently than every 4 weeks, a platelet count should be done and must be more than 150,000/µl prior to performing subsequent plateletpheresis.
6. Some advocate the platelet count may be done before all plateletapheresis so that donors' health is not compromised.
7. If extra plasma is collected and if the procedure is performed more than once every 4 week, the procedure should not be done if the total serum protein is less than 6.0 g/dl or if there has been an unexplained weight loss.

LEUKAPHERESIS

Occasionally granulocytes are needed in neonates and adult with neutropenia and sepsis (gram-negative infection), not responding to antibiotics. To collect adequate number of granulocytes - usually 1.0 - 3.0 x 10^{10}, apheresis technique is applied.

A daily dose of at least I x 10^{10} granulocytes and HLA matching is necessary to achieve therapeutic effect. Granulocytes concentrates can induce HLA immunization, may transmit cytomegalovirus infection, and if not irradiated may cause graft-vs-host disease in susceptible recipients. HLA-incompatible granulocytes may cause respiratory distress.

There is renewed interest in granulocyte transfusion therapy in neonates and adult with sepsis and neutropenia not responding to the antibiotics because much larger cell doses can be obtained when cells are collected from donors stimulated with granulocyte colony-stimulating factors or Corticosteroids.

Collection of adequate dose of granulocytes needs administration of drugs or infusion of certain drugs during the col lection process. The donor is stimulated pre-donation with steroids to increase the circulating pool of granulocytes. A protocol using 20 mg of oral prednisone at 17, 12, and 2hours before donation gives better granulocytes yield with minimum systemic steroid activity. Before administration of eorticosteroids, donors should be asked about the history of hypertension, diabetes, and peptic ulcer.

Hydroxlethyl starch (HES), in low- or high- molecular weight form, is infused during the collection procedure to increase RBC sedimentation and to facilitate the separation and collection of granulocytes.

Recombinant hematopoietic growth factors, specially granulocyte colony-stimulating factor (G-CSF) effectively increase granulocyte yield. Hematopoietic growth factors can result in collection of up to 10 x10^{10} granulocytes per apheresis procedure.

All laboratory tests, ABO and Rh, antibody screening and infections disease markers are tested before phlebotomy. Red cells in granulocyte concentrates are always present, red cells should be compatible with the recipient's plasma, and if more than 2 ml red cells are present in granulocyte concentration it should be cross matched. Ideally D-negative recipient should receive granulocyte concentration from D-negative donor.

ERYTHROCYTAPHERESIS

Recent advancement in apheresis involves the collection of RBCs by automated apheresis. Many the automated apheresis machines have been improved and allow the collection of either two

may occur. In such cases the serial plasmapheresis is deferred for 12 weeks. Red cells loss must not be more than 25 ml per week.

4. It takes more time than automated plasmapheresis.

Plasmapheresis Using Automated Equipment

Plasmapheresis with automated equipment is based on the principles of separation of plasma from other blood components by centrifugation and membrane filtration techniques.

There are several automated machines designed for plasmapheresis on the principles of centrifugation and filtration. These machines require special sterile disposable tubing and containers. The anticoagulant is added in a controlled way. They work on intermittent-or continuous flow system.

The machines for plasmapheresis working on the principle of centrifugation technique are Haemonetics MCS Plus and Plasma collection system (PCS/PCS 2) both manufactured by M/S Haemonetics Corporation. Autopheresis C (APC), manufactured by Fenwal (Baxter), works on the principle of both membrane filtration and centrifugation. All these machines have intermittent-flow system. Another machine TPE manufactured by Cobe works on membrane filtration and continuous-flow system.

Autopheresis C is intermittent flow machine in which blood is collected in a small cylinder that is rotating and forcing plasma through a polycarbon membrane (Fig. 15- 8). There are both membrane filtration and centrifugation technology in Autopheresis C. Filtration has the advantage over the centrifugation that it collects cell-free plasma.

Advantages of automated plasmapheresis are:

1. Donors are never disconnected from their own red cells, thus eliminates the risk of transfusion of the red cells of another donor.
2. The speed of collection is considerably faster than in manual plasmapheresis 500-600 ml plasma can be collected within 30 minutes.
3. Donors prefer automated procedure to manual plasmapheresis
4. Total extracorporeal volume at any time, particularly with filtration plasapheresis, is less than that in double manual plasmpheresis
5. Single venous access

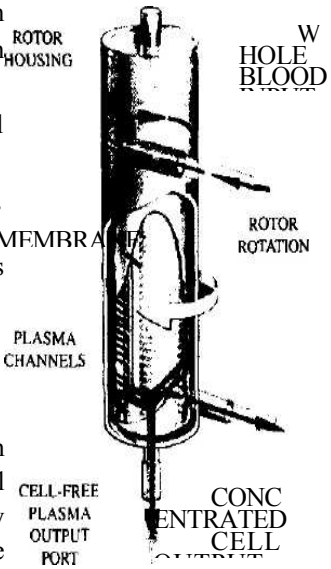


Fig- 15.8 The Autopheresis-C

Disadvantages of automated plasmapheresis

1. It is more expensive than the manual procedure.
2. Plasma separated from cell separators may contain platelets which are smaller than the average normal size and which if not removed, detract the quality of plasma intended for fractionation. At the same time these small platelets give a poor response to aggregation and arc not suitable for preparing platelets concentrates.

3. Membrane filters have potential problems such as leakage or damage to cells. The complement may activate.

Care of Plasmapheresis donors

1. The criteria for the acceptability of plasmapheresis donors are slightly sticker than that of routine blood donors.
2. Donors are informed about the procedure in details, about possible hazards, and written consent is taken.
3. Age should be between 18- 50 years
4. Weight should be 60 Kg or more.
5. Preferably donor should have given whole blood 1 -2 times earlier.
6. Total blood count and serum proteins should be with in normal limited, and tested periodically.

If the plasmapheresis is performed no more than once every 4 weeks, the criteria of selection that apply to whole blood donations are followed. However if donor is participating in a serial plasmapheresis program (e.g. plasma is donated more frequently than once every 4 weeks), procedure should not be performed if total protein is less than 6.0 g / dl or there has been unexplained blood loss. Every 4 months, all records and laboratory tests must be reviewed, and serum protein electrophoresis or immunoglobulin (IgM and IgG) levels must be determined.

It is safe to accept 600 ml plasma per session with an interval of two weeks between donations or 24 times in a year. It is better to have greater number of donors than to subject a small number of donors to more frequent donations.

Non-Therapeutic Plasmapheresis

1. To increase plasma inventory of FFP for transfusion
2. To collect plasma from IgA negative donor for transfusion.
3. To obtain plasma to prepare immunoglobulins to Rh, tetanus or HBsAg etc.
4. To collect plasma for preparing albumin, plasma protein factor (PPF) and other plasma components.
5. To prepare coagulation factors like Factor VIII, Factor IX complex etc.

Apheresis FFP (AFFP) differs significantly from whole-blood derived FFP (WBD-FFP).

	AFFP	**WBD-FFP**
Total volume	540 ml	200 ml
Absolute plasma*	486 ml	160 ml
Anticoagulant CPD/CPDA1	4% Sodium citrate	3% sodium citrate
Anticoagulant/	1:10	1:5
Anticoagulant plasma	0.4 g	0.6 g
Citrate/100 ml plasma		
Glucose	l00mg/dl	400-715 mg/dl
Cryoprecipitate	more	less
Residual platelets & WBCs**	less than WBD-FFP	

* Adequate dose affecting clotting in adult.

**Reactions caused by the release of cytokines by leukocytes are less in recipients by the transfusion of AFFP in comparison to WBD-FFP.

THERAPEUTIC PROCEDURES

Therapeutic apheresis does not cure a disease but can be very effective in alleviating the symptoms of the underlying disease. The efficacy of the procedure is enhanced by concomitant drug therapy, particularly immunosoppresive therapy in immune-mediated problems. The apheresis are classified by the component removed - cytapheresis if the component iscellular and plasma exchange if the offending substance in the plasma is removed.

THERAPEUTIC CYTAPHERESIS

Therapeutic Plateletpheresis

It can be used to treat patients who have abnormally elevated platelet count with related symptoms. This condition has been reported in patients with myeloproliferative disorders like polcythemia vera. Patients having count more than 1 ,000,000/μL may develop thrombotic or hemorrhagic complications. During apheresis procedure the platelet count can be decreased as much as one-third to one-half the initial value. The procedure can be repeated as frequently as necessary until drug therapy becomes effective and symptoms disappear.

Therapeutic Leukapheresis

It has been used to treat patients of leukemia, particularly with impending leukostasis, in which leukocytes aggregate and thrombi may interfere with pulmonary and cerebral blood flow. Leukapheresis is indicated if the leukocyte count is more than 100,000/ul. However the efficacy of such leukoreduction is unproved. The white cell count rises over weeks, and leukoreduction can be effected with the chemotherapy.

Therapeutic Eythrocytapheresis

Therapeutic platelet- and leuka-pheresis involve depletion of cellular constituents without replacement. Erythrocytapheresis is considered an exchange procedure. A predetermined quantity of blood is removed from the patient and replaced by homologous blood. It is useful to treat complications in sickle cell disease. It is also useful in patients with sever parasitic infection from malaria.

Therapeutic Plasmapheresis (Plasma Exchange)

Therapeutic plasmapheresis is not a cure for the underlying disease but rather a way to provide short term relief. The process of therapeutic plasmapheresis is actually a plasma exchange rather than apheresis. During therapeutic plasma exchange, the pathological substances in plasma are removed and replaced with a fluid. The replacement fluid may be plasma, albumin, saline or combination of albumin and saline. The plasma is constantly replaced with the fluid so patient's blood volume does not change.

The efficiency of a plasma exchange is related to the amount of plasma is removed. A procedure that removes the plasma equal to the patient's plasma volume is called one-volume plasma exchange. A one-volume plasma exchange reduces 30% of unwanted constituents of plasma. The second plasma exchange, as a part of the same procedure, reduce only 10% of unwanted constituents of plasma. It is recommended that approximately 1 to 1.5 L plasma may be exchanged per procedure. Pathological constituents present in intravascular and extravasclar spaces, effect the out come of plasma exchange. If the antibody, that is cause of the disease is IgM, apheresis is effective as IgM is primarily in intravascular. Where as IgG is equally present in intravascular and extravascular spaces

and apheresis is less effective and IgG synthesize and reappear quickly. Removal of IgG antibodies is more effective when combined with immunosuppresive drugs.

Plasmapheresis is indicated in the conditions mediated by plasma factors such as autoantibodies, immune complex, drugs or toxins bound to proteins, high cholesterol or triglycerides.

Plasma exchange is done on the request of the patient's physician.

Common Conditions for Therapeutic Plasmapheresis and Pathological Substances Removed

Conditions	Substances Removed
Waldenstrom's macroglobinaemia	Ig causing hyperviscocity
Myastheniagravis	Autoantibodies
Goodposture's syndrome	Autoantibodies
Hypercholesterolemia	Lipoproteins
Post transfusion purpura due to anti-PI^{A1}	Platelet antibodies
Acute Guillain-Barre syndrome	Uncertain
Lupus erythematosus	Immune complex
Factor VIII inhibitor	Auto-or allo-antibodies
TIP	Platelet-aggregating factors
Barbiturate poisoning	Protein-bound toxins

FLUIDS USED IN APHERESIS

All apheresis procedures use anticoagulant to prevent blood from clotting as it enters the separation machine. Most commonly used anticoagulant is ACD. Normal saline is used to prime the system to keep the line open and to help in maintaining fluid volume.

In therapeutic plasmapheresis (plasma exchange) procedure large volume of patient's plasma is retained and it has to be replaced with fluids to maintain adequate intravascular volume and oncotic pressure .

Comparison of the most commonly used Replacement Fluids:

Replacement Fluid	Advantages	Disadvantages
Crystalloid - Normal Saline	Least expensive Hypo-allergenic No risk of hepatitis & HIV	2-3 volumes required Hypo-oncotic No coagulation factors
Albumin in 5 % solution (NSA)	Mild hyper-oncotic Used in 1:1 ratio of the plasma removed. No risk of hepatitis & HIV	High cost No coagulation factors No immunoglobulins

5% Albumin with normal saline is generally preferred as replacement fluid. 5 % albumin is slightly hyper-oncotic and can be diluted with saline to 4.0-4.5 % for plasma exchange.

Fresh Frozen Plasma (FFP)

FFP contains all constituents of the removed plasma and this is optimal replacement fluid. FFP has the disadvantage that it may transmit transfusion-transmitted diseases, can cause allergic reactions, ABO incompatibility or sensitization to plasma proteins. It is the fluid of choice only when it is desired to replace clotting factors or to give some protein. FFP is recommended in plasma exchange in patients with thrombotic thrombo- cytopenia (TTP) or hemolytic urinary syndrome (HUS). FFP may provide some factors missing in patient with TTP or HUS such as precursor of prostacyclin or other anti-thrombolic factors.

Transfusion

Practice in

Clinical Medicine

Blood components transfusion practice requires the constant use of critical clinical judgement. The medical indications for every transfusion should be carefully evaluated, and each transufion should be monitered for therapeutic effectiveness. Adverse outcomes may follow hemotherapy, even when that therapy is indicated, transfusion should be undertaken only if the anticipated benefit outweighs the potential risks.

Principles of the clinical use of blood/blood products

1. The patient with acute blood loss should receive effective resuscitation (intravenous replacement fluids, oxygen, etc..) while the need for transfusion is assessed.
2. The specific clinical or laboratory indications for transfusion should be considered.
3. The patient's haemoglobin vlaue, although important, should not be the sole deciding factor in starting transfusion. The decision to transfuse should be supported by the need to relieve clinical signs and symptoms and prevent significant morbidity and mortality.
4. Transfusion should be prescribed only when the benefits to the patients are likely to outweigh the risks of transmitting HIV, hepatitis B & C, or other infectious agents through blood products.
5. Informed consent for transfusion of blood and its products should be taken. The physician should explain the risks and alternatives of transfuion to the recipient or responsible family

member and document in the medical record that it has been done. One time consent for repeated transfusion will suffice.

MULTIPLE TRANSFUSION

Multiple transfusion is the repeated transfusion of whole blood or red blood cells over a long period of time (months or year).

Indications:

- Hypoproliferative anemia:
  - Hypoplastic or aplastic anemias
  - Drugs or chemicals induced hypo or aplastic anemias
  - Radiation-induced marrow depression
- Hemoiytic anemias
  - Thalassemia major
  - Sickle cell anemia
  - Autoimmune hemolytic anemia (AIHA)
  - Paroxysmal nocturnal anemia (PNH)
- Anemia associated with chronic diseases:
  - Renal insufficiency
  - Chronic inflamation
  - Hepatic failure
  - Malingnancy

Guidelines for multiple transfusion:

- Packed red blood cells transfusion is preferred to whole blood transfusion.
- Leukocytes-poor red cells should be used in patients having febrile non-hemolytic transfusion reactions.
- Red cells should be of the same ABO and Rh (D) group as that of the patient and should be cross-matched by anti-human globulin test.
- Patients receiving regular multiple transfusions should be given iron-chelating agents to prevent hemosiderosis.

CONGENITIAL HEMOLYTIC ANEMIAS

The congenital hemoiytic anemias are due to hereditary abnormalities involving one of the three main components of erythrocytes - the membrane, hemoglobin and intracellular enzymes . Most common congenital hemolytic anemias relevant to the clinical practice of transfusion medicine are due to defects of hemogloublin

Defects of hemoglobin are :

- Structural anomalies - HbSS , HbS (Sickle Cells)
- Synthetic anomalies -failure to synthesize Hb adequately

β - Thalassemia

α-Thalassemia

other thalassemie syndrone

SICKLE CELL ANEMIA

Oxygen affinity of RBCs of sickle disease is decreased and 2, 3 - DPG levels are elevated. Symptoms of anemia are usually less.

Indications for transfusion therapy in sickle disease are:

- Severe Vaso-occlussive or painful crises refractory to medical management.
- Severe infection
- Acute splenic sequestration
- Stroke
- Liver infarction
- Chronic leg ulcer
- Priapism

Severe vaso-occlussive crises (painful crises)

Transfusion is given for painful crises unresponsive or refractory to conservative medical treatment. It is not given to treat the anemia but to reduce the proportion and number of sickle cells in circulation which ameliorate pain; the flow resistance of blood with sickle cells is reduced when a cells mixture of HbA, and HbS contains less than 40% sickle cells. Clinically when vaso-occulusion occurs the level for HbS usually exceeds 50%.

When atleast 60% of RBCs are replaced by normal HbA containing cells , the progress of symptoms usually ceases. Two therapeutic methods are available for achieving this objective:

Transfusion of fresher RBCs

- Fresher RBCs from non-sickle cells donors, 10-15 ml/kg body weight is given every 12 hours until the hemoglobin levels increase to 12-13 g/dl.
- Sickle cells, because of shorten survival, will disappear rapidly from circulation. Thereafter, small RBCs transfusion given every 2-3weeks will maintain the level of HbS below 40%, suppress HbS synthesis and ensure that the majority of circulating RBCs will be normal

Exchange transfusion:

- Simple transfusion is time consuming and increases the risk of fluid overload and congestive heart failure.
- So partial - exchange transfusion is advised as an alternative which is more rapid, and efficient method to decrease the level of HbS
- Partial-exchange transfusion in sickle cells anemia is indicated in
  - Painful crises
  - Heart disease
  - Cerebrovascular accidents (stroke)

Procedures

- Partial-exchange transfusion is done by transfusing RBCs, 15 ml/kg body weight, through one anticubital vein and simultaneously withdrawing. 20 ml blood /kg body weight, by gravity from the opposite anticubital vein.

- Exchange transfusion can be repeated after 24 hours.

Indications

- Transfusion is indicated in Sickle cell anemia with fatal pneumococol infections, such as sepsis, pneumonia and meningitis in addition to antibodies.
- Exchange transfusion may reduce immoglobulins, bacteria and endotoxins.

Splenic Sequestration Crises

- In children with homozygous sickle cell anemia and with sickle cell - B thalassemia associated with splenomegaly are at risk of sudden acute splenic sequestration crises.
- During the crises the spleen enlarges massively, Hb drops from a stable level of 7-8 g/dl to 2 g/dl or less and hypovolemic shock and death may occur.

Treatment

- Prompt whole blood transfusion will correct the hypovolemia and reverse the shock. It regresses the enlarged spleen.
- Splenectomy should be consider to prevent further crises.

Stroke

- Transfusion is given to decrease the level of HbS to 20% or less by simple RBC or exchange transfusions.
- Clinical improvement occurs in cases whose transfusions program maintains the Hb above 10g/dl and HbS level below 20-30% for 2 years.

Pregnancy

- Pregnancy with Sickle cell anemia is also life threatening
- Chronic transfusion program during the second or third trimister may reduce placental insufficiency, lower rate of still birth or prematurity
- Results of manual partial exchange transfusion or using continuous- flow automated erythrocytapheresis are also encouraging. Level of HbA is maintained at or greater than 40%. The procedure is repeated if the level of HbA is below 20% and the hematorcrit below 25 %.

Leg Ulcers and Priapism

The painful symptoms of leg ulcers and devasting sequely of priapism may be lessened by transfusion.

THALASSEMIA

Considerable progress has been made in transfusion therapy for patients with homozygous β- thalassemia (major thalassemia)

- Treatment has been mainly supportive and consisted of intermittent RBC transfusion to control severe anemia. Three programs of transfusion have been advocated.

  1. Transfusion program correcting severe anemia to a safe Hb level of 7 to 9 g/dl to avoid symptoms of anemia.

2. Hyper transfusion program to maintain Hb level between 10-12 g/dl is probably adequate. It decreases the effect of chronic anemia and prevents abnormal growth and development.
3. Super-transfusion program wherein Hb level is maintained at 12 g/dl is designed to completely suppress hematopoiesis. If this program is started from infancy, its advantages are:
    - Normal growth and development
    - Normal daily function and psychological well being
    - Transfusion requirement is less
    - Iron over load is also less
    - Pathological bone changes are less
    - Less cardiomegaly and hypersplenism

Blood Products used in thalassemics

- RBC should be 6 days old.
- Leukocyte -reduced red cells are preferred.
    - Washed red cells-Washing of red cells is a cumbersome process, and has risk of contamination.
    - Frozen - thawed and washed red cells - is costly process.
    - Transfusion with leukocytes reduction filters - cost effective but very efficient

Dose

- 10-20 ml /kg body weight every 3-4 weeks
- As body size increase, 1 -2 units every 3 weeks
- Pre-transfusion Hb level should be maintained 10g/dl

Iron over load

As a result of multiple transfusions , transfusion hemosiderosis develops. About 200 mgm of iron accumulates with each unit of transfused blood.

Continuous iron accumulation leads to:

- Hyperpigmentation
- Deposition of iron in heart, liver, pancreas and other endocrine glands, results in fibrosis and organ disfunction
- Serum ferintin is high.

Iron chelation in Thalassemia

- Desferrioxamine is currently the most effective iron chelating agent
- It is started as early as possible
- Daily 8 hours subcutaneous infusion of desferrioxamine 20-40 mg/kg body weight during sleep with portable battery operated infusion pump, 5 days in a week is sufficient therapy to achieve a negative iron balance.

Vitamin C

It converts ferric form of iron to the ferrous form and also facilitates mobilization of iron stores to free iron. Ferrous form of iron and the free iron get bound with desferrioxamine easily. Vitamin C upto 200 mgm/day is given along with desferrioxamine.

Splenectomy

Splenectomy should be performed when yearly transfusion requirement exceeds 200-250 ml/kg/ year. Following splencetomy all patients should be given a proplylactic antibiotic regime of penicillin (250 mgm twice daily) as well vaccinate aganist penumococcus 2-4 weeks prior splenectomy.

Neocytes

Young patients with certain hematologic disorders especially the thalassemia, often require continuous red cells therapy. Each millilitre of RBCs contains approximately 1 mg of iron, which accumulates in the tissues and causes hemosidrosis, its traditional therapy is an iron-chelating agent.

Another approach is to transfuse younger red cells (neocytes). The preparation of neocytes involves the selective removal of the donor's neocytes or younger red cells found in the upper portion the layer of red cells after centrifugation or neocytapheresis.

Half-life of young red cells i.e. neocytes is 90 - 100 days while that red cells is 60 days. Therefore some workers have attempted to use neocytes to (1) reduce blood requirement (2) to increase transfusion intervals and (3) to reduce iron overload. But neocytes therapy did not get wider acceptance because the preparation of neocytes by removing the upper layer of cells after centrifugation or neocytapheresis is time consuming and expensive.

NEONATAL AND PEDIATRIC TRANSFUSION PRACTICE

Transfusion practice in neonates and infants need special understanding due to their unique physiology. Pediatric anemia is due to the reduction of hemoglobin concentration or red cell mass below the normal values for normal infants /children.

Hemoglobin:

- Average Hb concentration of full term infants at birth is about 18.0 g/dl. All infants have a normal physiological decrease in Hb during the first 3 months.
- Average Hb concentration in normal child from 3 months to 6 years is 11.0 to 12.0 g/dl. From 7 to 13 years of age Hb is 13.0 g/dl.
- Most of the Hb in neo-born is fetal hemoglobin (Hb F) which delivers less oxygen than HbA.
- Hb level of healthy children above 14 years is the same as those of an adult.

Blood Volume :

- Blood volume of full term neonates is 85 ml/Kg/body weight, while that of premature neonates is 100 - 105 ml/kg/body weight.

Transfusion

Transfusions are given in small volumes (10-20 ml RBCs/kg body weight), increasing the risk of multiple donors exposure. Therefore, a single donor unit assigned to an individual new born and the use of sterile tube sealing device for acquisition of small volumes for transfusions over the shelf-life of the donor unit or the single unit multipack division technique can decrease the number of multiple donors exposure.

Blood should be as fresh as possible and not more than 6 days old to lesson the risk of hyperkalemia and to maximize the 2,3-diphophoglcerate (2,3-DPG) levels. Transfusions of red cells are preferred. However for limited donor exposure RBCs stored up to 42 days is acceptable unless hyperkalemia is a known problem.

Guidelines for Neonatal Red Blood Cell Transfusion

Transfuse red cells ≤ 20ml/kg body weight, not to exceed Hct. of 0.45 or Hb of 15 g/1.

1. Hct. ≤ 0.20 or Hb ≤ 7 g/dl and reticulocyte count < 4%
2. Hct. ≤ 0.25 or Hb ≤ 8 g/dl with any of the following conditions:
   i) Episodes of apnea/bradycardia ≥ 10 episodes/24 hours or ≥ 2 episodes requiring bag-mask ventilation,
   ii) Sustained tachycardia > 180 heart beats/min.
   sustained tachpynea > 80 breaths per min.
   iii) Cessation of adequate weight gain x 4 days (≤ 10 g/day)
   iv) Mild respiratory distress syndrome
3. Hct. ≤ 3.0 (Hb ≤ 10 g/dl with moderate respiratory distress syndrome.
4. Hct. ≤ 0.35 (Hb ≤ 12 g/dl) with severe respiratory distress syndrome or congenital heart disease associated with cyanosis or heart failure.
5. Acute blood loss with shock: blood replacement to establish adequate blood volume and Hct. 0.40.

Modified Blood/red cells is prefered

- Cytomegalovirus seronegative or leukocytes-reduced blood should be given to decrease the risk of cytomegalovirus transmission in preterm and immunodeficient infants.
- Units of blood can be irradiated to decrease the risk of graft-versus-host disease, particularly in premature neonates with suspected immunodeficiency or for neonates who are potential transplant candidates. Transfusions in a full term new born infants do not require irradiation.
- Irradiation is recommended for RBCs used in intrauterine transfusion.

RED BLOOD CELLS TRANSFUSION IN ANEMIA

Red Blood Cells Transfusion in Acute Anemia/Blood Loss.

The Hb concentration prior to the hemorrhage, the extent of hemorrhage, and the existence of other conditions which alter the physiologic response to acute blood loss may affect the decision to transfuse RBCs.

Patients almost always require perioperative RBCs transfusion when their Hb is less than 6 g/dl. and rarely when their Hb is greater than 10 g/dl. Between 6 and 10 g/dl Hb, transfusion

requirements depend on the extent of blood loss, underlying cardiac disease, and overall clinical condition.

In general, loss of less than 15% blood voloume results in minimal symptoms; 15% to 30% tachycardia; 30% to 40% increased signs of shock, and greater than 40% in severe shock.

Previously healthy patients can be treated with crystalloid alone for less than 30% blood volume loss. Patients with underlying disease may need transfusion at 30% blood loss, depending on the degree of anemia and the nature of disease.

Red Cell Transfusion Guidelines (Excluding Neonates) - Summary

Acute blood loss:

1. Evaluate the effects of blood loss and underlying diseases.
2. Estimate and/or anticipate degree of blood loss:

Loss of blood	Replacement Fluid
< 20% of blood volume	None
20 - 30% of blood volume	Crysralloids / Colloids
30 - 40% of blood volume	Red cells & crystalloids
> 40% of blood volume	Whole blood or Red cells & crystalloids

Hb Level

. >10g/dl,RBCs rarely needed

. < 6 g/dl, RBCs usually needed

. 6-10 g/dl, RBCs need depends on other factors

Measure vital signs and tissue oxygenation (useful when Hb is in the range of 6-10 g/dl and extent of blood loss is unknown)

Tachycardia, hypotension can not be corrected by volume replacement alone, RBCs needed when

Pvo_2 < torr, extraction ratio > 50%

Vo_2 < 50% of base line

Mote - Pvo_2 - oxygen tension of pulmunary arterial blood at the completion of oxygen unloading.

Vo_2 - oxygen consumption

Chronic Anemia

1. Treat with specific pharmacologic agents like vitmain B 12, folic acid, iron, recombinant human erythropoietin. if diagnosis indicates
2. Use specific strategies for sickle cell disease and thalassemia
3. Transfuse to minimize symptoms and risk of anemia at the Hb level of 5.8 g/dl

MASSIVE BLOOD TRANSFUSION

Several definitions of massive transfusion have been proposed:

- Massive blood transfusion is usually defined as the replacement of one or more blood volume(s) within 24 hours.
- Transfusion of about ten units of whole blood each of 450 ml of blood or 20units of red cells, within 24 hours.

- Replacement of more than 50% of the blood volume in 3 hours in an adult..

Normal blood volume usually being approximately :

- In adult about 70 ml per kg body weight or 7% of the body weight
- In children about 80 ml per kg. body weight or 8% of body weight
- In neonates 85-90 ml per kg body weight or 8.5 - 9.0% of body weight.

Massive transfusion may be required due to acute hemorrhage in :

- Surgical or medical emergencies (e.g. gastro-intestinal bleeding especially from varices.)
- Cardiac and vascular surgery
- Exchange transfusion in infants
- Obstetric cases.
- In multiple trauma
- Liver transplant

The blood transfusion strategy should be to maintain blood volume and its composition with in limits that are safe with regard to haemostasis, blood oxygen-capacity, and oncotic pressure and plasma biochemistry (Table 16.1). Blood sample should be sent to the laboratory at the earliest possible opportunity for blood grouping, antibody screening, compatibility testing, as well as baseline hematology, coagulation screening, including fibrinogen estimation and biochemistry investigation.

Table 16.1 Safe limits of Hb and Coagulation Factors

Investigation	**Target value**
Hemoglobin; (Hematocrit)	l0g/dl (0.33)
Platelet count	> 50 x 10^9 /L
Prothrombin time	< 1.5 x control
Partial thromboplastine time	< 1.5 x control
Fibrinogen	> 0.8 g/1

Management strategy for massive transfusion

1. Restore blood volume to maintain tissue perfusion and blood pressure
2. Maintain Oxygen carrying capacity
3. Treat any surgical source of bleeding
4. Correct coagulopathy by the judicious use of
   - Fresh frozen plasma
   - Platelet transfusion
   - Cryoprecipitate (if fibrinogen is low)
5. Prevent hypothermia

Restoration of blood volume to maintain tissue perfusion and blood pressure

Restoration of blood volume is utmost important to maintain tissue perfusion, blood pressure and hypovolemic shock, preventing tissue damage, and worsening of thrombocytopenia and coagulopathy.

Restoration of circulating volume is initially achieved by rapid infusion of crystalloid like Ringer's lactate solution and normal saline or colloids through large-bore (14 gauge or larger) peripheral cannulae. The use of albumin and non-albumin colloids versus crystalloids for volume replacement have recently been the subject of debate. The use of colloids is not recommended in the American college of surgeons Advanced Trauma life support guidelines.

Ringer's Lactate solution is rcommended as initial therapy and normal saline is an acceptable alternative to Ringer's lactate solution. Ringer's Lactate solution contain calcium, and if it is inadvertently mixed with a unit of blood, the blood may clot in the line or the bag.

Crystalloid is infused at 3:1 ratio for every unit of blood lost. Therapy is monitored by hemodynamic response and return of tissue perfusion (measured by mental status, urine output, capillary refill and absence of acidosis).

Resucitation then proceeds by the use of blood components, depending upon the patient's response. Patients who respond to about 2000 ml of crystalloid with return of stable vital signs and tissue perfusion do not generally require blood transfusion. Patients who do not respond to this, generally have major blood loss and require emergent red cells or whole blood transfusion and further fluid replacement.

Red cell transfusion is likely to be required along with fluid replacement when 30-40% of blood volume is lost. The loss of over 40% of blood volume is life threatening and whole blood may also be required.

Blood loss is usually underestimated and it must be remembered that hemoglobin and haemocrit values do not fall for several hours after acute hemorrhage. Determination of whether intermediate hemoglobin concentrations justify red blood cell transfusion should be based on patient's risk factors for complications of inadequate oxygenation such as:

- Rate of blood loss
- Cardiorespiratory reserve
- Oxygen consumption
- Measured cardiological variables, such as
  heart rate
  arterial pressure
  pulse rate
  cardiac output

These signs may assist in the decision making process, but it should be emphasized that silent ischaemia may occur even in the presence of stable vital signs.

Prevention of hypothermia

Hypothermia increases the risk of disseminated intravascular coagulation and other complications and may be prevented by prewarming the resuscitation fluids, patient-warming devices such as warm air blankets and use of temperature controlled blood warmers.

Massive transfusion of 100 ml/min or 1 unit of blood every 3 minutes requires blood warming otherwise recipient may develop hypothermia and arrhythmias.

Blood bank arrangements:

The degree of urgency for transfusion should be accurately conveyed to the blood bank.

1. Routine ABO and Rh(D) grouping, antibody screening and cross-matching by saline and Coombs' technique take about 3 hours.

2. If the blood is required urgently, the time of requirement should be mentioned on the requisition form. ABO and Rh typing is done by spin tube technique and cross-matching is done by saline spin technique or LISS method.
3. In very urgent cases uncorss-matched red cells of the same group as that of the patient, after doing ABO and Rh(D) typing of the patient are supplied with the concurrence of clinicians. Subsequently the cross-matching is done by saline spin tube method or by LISS technique.
4. In extreme urgent situation it may be necessary to supply uncross -matched group O red cells if the group of the patient is not known or there is no time to do ABO and Rh(D) grouping of the recipient.

   In an emergency, pre-menopausal females, whose ABO and Rh(D) blood group is not known, O Rh(D) engative red cells should be given to avoid Rh(D) sensitization and the risk of hemolytic diseases of new born in subsequent pregnancy.

   O Rh(D) positive red cells can be given to Rh(D) negative males and post-menopausal females patients if they do not have Rh antibodies in their blood and Rh (D) negative blood is not available.

   In case of (3) or (4) blood is supplied with the consent of the clinicians, and "Uncross-matched Blood" is written on the compatible report and on the label on the blood bag.

Hematological Monitoring:

The investigation shown in table 16.1 should be performed when the blood loss is substantially reduced to the order of (<0.5 L/h) and after the bleeding is under control. On the basis of laboratory findings correct haemostatic abnormalities.

1. Platelet Concentrates:

   Platelet concentrates are given to maintain platelet counts 50 x 10^9 /L or above, the minimum level required to achieve effective haemostasis when platelet function is normal. Expert consensus argues that platelets should not be allowed to fall below the critical level of 50x 10^9 / litre in acutely bleeding patients. A higher target level of l00x 10^9/litre has been recommended for those with multiple high-energy trauma or central nervous system injury.

   During cardiac bypass surgery bleeding may occur at counts of platelet higher than this, probably because of an acquired functional defect in platelets. Empirical platelet transfuion may be required when platelet function is abnormal, as is found during or after cardiopulmonary bypass.
2. Coagulation factor deficiency is the primary cause of coagulopathy.
   - Massive or large volume transfusion can result in disorders of co-agulation due to dilution of clotting factors and platelets.
   - Prolongation of activated partial thromboplastin time (aPTT) and prothrombin time (PT) to 1.5 to 1.8 times of the control values is correlated with an increased risk of clinical coagulopathy and requires correction.

     Fresh frozen plasma (15 ml/kg body weight) is given to correct coagulation abnormalities.

- Continued bleeding together with severely disturbed coagulation, needs more energetic therapy. Platelet concentrates, FFP or cryoprecipitate are given particularly when there is evidence of disseminated intravascular coagulopathy (DIC). Cyoprecipitate will replace fibrinogen and factor VIII.
  Infusion of FFP should be considered after one blood volume has been lost. FFP alone, if given in sufficient quality will correct fibrinogen and most coagulation factor deficiencies, but large volume may be required if fibrinogen volume remains critically low (<10 g/litre), and cryoprecipitate therapy should be considered.

Problems of Massive or large volume transfusion:

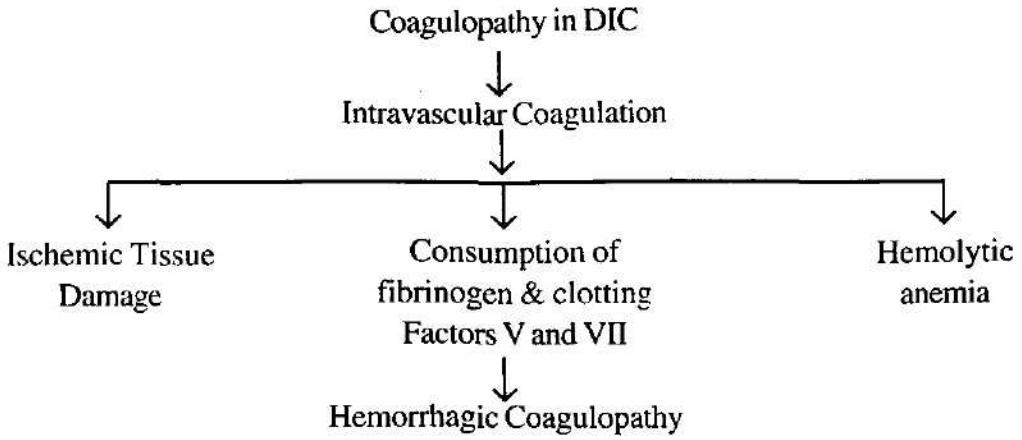
- ***Acidosis***
  During blood storage, red cells metabolism generates acids which result in reduction of its pH. Acidosis in a patient is more likely due to inadequate treatment of hypovolemia than due to the effects of transfusion.
  Usually body can neutralize this acid load from transfusion and the use of bicarbonate or other alkalizing agent is not required.
- ***Hyperkalemia***
  In stored blood there is small increase in extra cellular potassium. This rise is rarely clinical significant. As preventive measure use fresher blood preferably which is less than 7 days old.
- ***Citrate toxicity and hypocalcemia***
  Citrate toxicity is a rare problem in transfusion of large volume of whole blood. Citrate in anticoagulants binds serum calcium which reduces ionized calcium level in body. Hypocalcemia with acidosis and hypothermia can cause reduction in cardiac output, bradycardia and arrythymias.
  Most patients who undergo massive transfusion do not require calcium supplementation. In some cases, if calcium levels become critically low, calcium gluconate or calcium chloride can be given.
- ***Depletion of coagulation factors and platelets*** : Their management has already been explained earlier.

DISSEMINATED INTRAVASCULAR COAGUALTION

Disseminated intravascular coagulation (DIC) is an acquired coagulopathy. DIC is caused by the abnormal and uncontrolled activation and consumption of coagulation proteins, fibrinogen and platelets, causing small thrombi with in the vascular systems through out the body. Free thrombi in the vascular systems are the cause of DIC . There is over production of fibrinolytic enzymes which break down the clots formed, leading to an increase in fibrin degradation products. All the coagulation factors platelets, and fibrinogen are consumed faster than they are replaced, resulting in widespread bleeding.

Causes of DIC:

- Septicemia
- Disseminated malignancy
- Obstetric complications
  - Premature separation of placenta



Causes of **DIC** *(Contd...)*

- Retained products of conception
- Retained dead fetus
- Amniotic fluid embolism

- Trauma
- Hemolytic transfusion reaction
- Neonatal DIC secondary to sepsis and necrotizing entrocollitis
- Prolonged hypoxia or hypovolemia including shock.

Symptoms:

- Severe bleeding from many sites in the body.
- Sudden onset of blood from bruising
- Oozing from venipunture site.
- Microvascular thrombi may cause organs dysfunction:
  - Respiratory distress.
  - Renal failure
  - Jaundice
  - Coma

Laboratory investigation:

DIC is characterized by;

Reduced platelet count (thromboycytopenia)
Prolonged propthrombin time (PT) - 1.5 to 1.8 times of control (normal 10-14 sec.)
Prolonged activated partial thromboplastin time aPTT -1.5 to 1.8 times of control (normal 25-35 sec.)
Prolonged thrombin time - 1.5 times of control (normal 10±lsec)
Decreased fibrinogen concentration - < 1.0 g/L suggestive of DIC (normal 2-4 g/L)
Elevated fibrinogen degradation products (normal < l0mg/L)

Management:

Immediate and appropriate treatment or removal of the underlying condition is essential to prevent tissue ischemia and shock.

- Identify and treat or remove the cause of DIC
- Maintain blood volume

- If the patient is anemic give fresher whole blood available as it contains fibrinogen and other coagulation factors.
- Maintain hemostatic functions
  - If PT and aPTT are prolonged and the patient is bleeding, give FFP (15 to 20ml /kg body wt.)
  - If fibrinogen is low (< 80mg/dl), cryoprecipitate (6 to 12 units /kg body wt.) can be given.
  - If the platelet is count is less than 50 x 10^9 /L, and the patient is bleeding, give platelet concentrates 4-6 units or one unit of platelet apheresis.
- Exchange transfusion may be effective therapy for infants who suffer severe symptomatic DIC following birth asphyxia.
- Intravenous Heparin: Heparin inhibits fibrin formation and may result in increase plasma fibrinogen concentration but it does not inhibit the effect of fibrin split products. Heparin is not effective in gram - negative septicemia and in some other cases, Heparin is indicated in the cases of persistent thrombosis. The role of heparin is doubtful.
  In some cases heparin may be useful preceding the transfusion of FFP. The recommended does of heparin for an adult is a loading dose of 5000 units I. V. followed by 1500 units / per hour for 6-12 hours. If bleeding occurs due to heparin give protamine sulphate (heparin reversal).
  Heparin is contraindicated in surgery or already actively bleeding cases.

Monitoring of the case is done by estimating:

- Prothrombin time (PT)
- Activated partial thromboplastin time (aPTT)
- Fibrinogen level
- Hb and Hct.

IRRADIATION

Since past several years the use of irradiated blood products (primalary red blood cells and platelets) has increased dramatically. It has long been known that gamma irradiation inactivates lymphocytes in blood.

Viable lymphocytes in blood can be responsible for graft-versus-host disease (GVHD) in the recipient-host. The resulting disease has serious consequences like fever, skin rashes, hepatitis, diarrhea, bone marrow suppression and infection, all of them can progress to mortality.

Recipients at high-risk of GVHD are those who are severely immunsuppressed or immunocompromised, such as bone marrow transplant patients, neonates who have received intrauterine transfusion and exchange transfusion, and persons who are recipient of blood from first-degree relatives.

Indications for Transfusion of Irradiated Blood

Generally accepted indications :

- Bone marrow transplant or Peripheral blood stem cells recipients
- Neonates intrauterine transfusion recipients
- Neonatal exchange transfusion recipients
- Premature new born s (less than 1200g)
- Immunocompromised or immunosuppressed recipients

- Recipients of first-degree relative donors blood
- Recipients of HLA-selected platelets or platelets known to be HLA homozygous
- Patients with Hodgkin's disease or non-Hodgkins's lymphoma
- Granulocytes transfusion recipients

Indications under review

- Patients with hematological malignancies such as acute leukemia
- Term newborns on extracorporeal membrane oxy-generators
- Organ transplant recipients
- Patients receiving crossmatched compatible platelets

No established indications in:

- Patients with AIDS or HIV infection
- Most patients receiving chemotherapy
- Patients with aplastic anemia not receiving immunosuppressive therapy
- Full term neonates without other risks

Mostly either cesium-137 (^{137} Cs) or Cobalt-60 (^{60} Co) is used as the source of gamma rays. The usual dose is 25 Gray (Gy) to 35 Gy (1 Gray=100 rads), This dosage inactivates 85 to 95% of lymphocytes in the blood components without any adverse effect on other cellular components (red cells, platelets and granulocytes) of the blood,

The shelf-life of irradiated blood (RBCs) is 28 days from the date of irradiation or original expiration date of unit, whichever comes first. Studies have shown that irradiation causes a modest leakage of potassium, reducing their survival after transfusion. Therefore irradiated red blood cells are given in a reduced storage time. Some physicians believe that irradiated red blood cells for exchange transfusion should be washed to remove potassium, this step is not regarded as routinely necessary and should be done only in selected problem patients - for example, neonates receiving exchange transfusion with pre-existing hyperkalemia or renal failure. Other concerns about the effect of irradiation remain theoretical.

Products such as FFP and cryoprecipitate do not require irradiation because they do not carry viable lymphocytes.

Irradiation should not be performed on bone marrow or peripheral blood progenitor cells prior to their infusion.

TRANSFUSION IN OPEN HEART SURGERY

Transfusion of blood and its components is an important supportive therapy for patients undergoing open heart surgery requiring cardio-pulmonary bypass. The demand of blood and its components varies with each patient and depends upon:

- The patients peri-operative hematological status, such as anemia, thrombocytopenia, or coagulation disorder.
- Priming of pump-oxygenators with electrolytes or blood depends upon the condition of the patient and personal preference of surgeons and anesthetist. Generally priming of pump-oxygenators is now done with electrolyte solutions (e.g. Ringer's lactate or Hartman's solution). However, it may be necessary to prime pump-oxygenator with blood in exceptional circumctances e. g. in patients with sever renal failure or in neonates and small children.

Maximum Surgical Blood Order Schedule [MSBOS] (Continued...)

Cardiac- vascular	
Aortic aneurism resection	6
Aortic bypass with graft	4
Coronary artery bypass, adult	4(2)
Coronary artery bypass, children	2
Carotid endarterectomy	2
Femoral-popliteal bypass with graft	4
Urology	
Bladder, transurethral resection	T&S
Nephrectomy radical	3
Prostectomy, perineal	2
Prostectomy, transurethral	T&S
Renal transplant	2(2)
Obstretic & Gynecology	
Abortion, therapeutic	T&S
Cesarean section	T&S
D&C	T&S
Hystrectomy, abdominal/vaginal	T&S
Hystrectomy, radical	3
Labour/delivery, uncomplicated	T&S
Laproscopy	T&S
Tubal ligation	T&S
Tuboplasty	T&S
Vaginal repair	1
Vesico-vaginal or recto-vaginal-fistula repair	T&S

Note: Numbers may vary with institutional practice
T&S =Type and antibody screening

GENERAL TRANSFUSION PRACTICES

Filters for blood components

Generally two types of filters are used for blood transfusion: a 170-mm filter or a leukocyte-deletion filter. The 170-mm filter removes clots or cellular debris develop during storage from any blood products and is used in routine administration sets. A standard blood administration filter must be used for transfusion of all blood components.

Leukocyte-depletion filters are designed to remove white blood cells (WBCs). This filter is designed to prevent febrile nonhemolytic transfusion reactions, to prevent or delay the development of HLA antibodies, and to reduce the risk of CMV. Filtration in the blood bank seperatory. rather than at the bedside, is more reliable and better for reduction of leukocytes.

Administration of blood Products

A physician or a qualified nurse should administer blood and blood products.

2. Before starting transfusion, patient identity check should be done at the patient's bed side:
   - From the records of the patients
   - Ask the patient himself/herself the name, if he patient is unconscious identify the patient with wristband.
3. Check the following details on the compatibility report, and the compatibility label attached to the blood product:
   - Patient's name
   - Admission no.
   - Blood group
   - Donation no.
   - Collection and expiry dates

There should be no discrepancy

4. Check the blood pack:
   - Any sign of damage or leakage of the blood/its product.
   - Collection and expiry date on blood pack
   - Hemolysis in the plasma
   - Sign of contamination, such as change of colour in the red cells, which often look purple/black when contaminated.
   - Any clot

If the pack appear abnormal in any way, the unit must not be transfused and the blood bank must be informed immediately.

Time limits for infusion

Whole blood or red cells

1. The administration of whole blood or red cells should be started with in 30 minutes of issuing from the blood bank. If it is not required for transfusion it should be returned immediately to the blood bank with reasons.
   After 30 minutes of issuing it from the blood bank, it is not taken back in the blood bank.
2. Transfusion should be completed within 4 hours of starting the transfusion. These time limits have been determined for temperate climates where temperature in hospital building are generally between 22°Cand 25°C. If the ambient (room) temperature is very high, shorter 'out-of-refrigerator times' should be used.
3. Change the blood administration set after 12 hours, if the patient requires ongoing transfusion support.

Platelet Concentrates

1. Platelet concentrate should be administered as soon as they have been received.
2. Infusion should be compelted with in about 15-20 minutes.
3. Should be kept at room temperature 22°C to 24°C. Do not put in refrigerator.
4. Should be administered with transfusion set with filter
5. Platelets once issued are not taken back in the blood bank.

Fresh Frozen Plasma

1. FFP should be infused as soon as possible after thawing to avoid loss of labile clotting factors or thawed plasma stored at 2-4^{o}C should be used within 12 hours.

2. In adult, I unit of plasma should generally be infused with in about 15-20 minutes.
3. Thawed or partially thawed plasma is not taken back in the blood bank.

Disposable equipment for transfusion

1. Must be sterile and must never be reused.
2. Whole blood, red cells, platelet concentrate, plasma and cryoprecipitate are infused though sterile blood administration set containing 170-200 micron filter.
3. Use flexible plastic cannulae, if possible, as they are safer and preserve veins.
4. Leukocyte-depleting filters are expensive but they are effective in reducing the fibrile non-hemolytic transfuion reactions and the development of anti-leukocyte antibodies in multiple-iransfused patients.

Monitoring the transfused patients

A physician or a qualified and trained nurse should administer blood and blood products

1. **For each unit of blood transfused, monitor the patient at the following stages:**
   - Before starting the transfusion
   - As soon as the transfusion is started
   - For 15 minutes after starting transfusion
   - At least every hour during transfusion
   - On completion of the transfusion
   - 4 hours after completing the transfusion
2. **At each of these stages, record the following information** :
   - Patient's general appearance
   - Temperature
   - Blood pressure
   - Respiratory rate
   - Sign of any adverse reaction - these signs are fever with back pain (acute hemolytic transfusion reaction), anaphylaxis, hives or pruritis (urticaria! reaction). Congestive heart failure (volume over load) and fever alone (febrile non-hemolytic transfusion reaction).
3. Monitor the patient carefully during the first 15 minutes of the transfusion to detect any early signs and symptoms of adverse effects. The adverse effects of transfusion are usually dose-realted. therefore, every slow transfuion rates are used at the start, 15 to 50 ml are given during the first 15 minutes. Once the transfusion is progressing satisfactorily, the infusion rate can be increased so that the product is transfused within a reasonable time depending on the clinical condition of the patient.
4. Only isotonic (0.9 percent) saline or 5% albumin should be used to dilute blood components or can be infused with the transfusion set used for blood products transfusion, because other I.V. solutions like dextrose solutions such as 5% destrose in distilled water may damage the red cells and cause hemolysis or calcium containing solutions such as lactated Ringer's solution initiate coagulation in the infusion set. In addition, many drugs will cause hemolysis if injected through the blood infusion set.

Blood Warming

Routine warming of blood is not needed; infusing 2-4 units of refrigrated blood over several hours causes no harm. Patients who may need benefit from warmed blood include:

Adults receiving multiple transfuin at rates greater than 50 ml/kg/hr

2. Children receiving transfusion at rates greater than 15 ml/kg/hr.
3. Infants receiving exchange transfusion
4. Patients receiving rapid transfusion through central venous catheter
5. Patients with cold agglutinins

The rapid and massive transfusion of cold blood (2-6^{o}C) is associated with an increased risk of ventricular fibrillation and cardiac arrest.

Blood warming procedure

- It is carried our using approved blood warmer devices, such as the Level 1 Hot line.
- Blood is not warmed above 37°C. Excessive warming can cuase hemolysis and endanger the patient. If blood warmers are being used they should be tested before use to ensure that the temperature regulators are operating properly. The temperature of the blood should also be monitored.
- Do not immerse whole unit of blood or red cells in a water bath, or run blood through an extended tubing or coil in water bath.

Pressure Devices for rapid infusion

Pressure devices or pumps are some times used to achieve very fast flow rates in rapid transfusion:

1. Transfusion with 18 gauge needle or cannula is recommended
2. A device of a pressure bag with a sphygmomanometer is required in emergency situation when blood has to be transfused rapidly (about 5 minutes per unit). The bag should be inflated to about 200 mm Hg till blood flow through the drip chamber is continuous. Pressure of 300 mm Hg may cause the red cells to hemolyse and the blood bag seams to split.

Transfusion Reactions

Any adverse effect caused by transfusion may be considered a transfusion reaction. Some are mild, others are life-threatening. All reactions should be documented and reported.

Guidelines for the recognition of transfusion reactions are :

Category 1 - Mild reaction

Sign	Symptoms	Possible cause
▪ Localized - Urticaria - Rashes	▪ Itching	▪ Hypersensitivity (mild)

Category 2 - Moderately Severe

Sign	Symptoms	Possible cause
▪ Rushing ▪ Urticaria ▪ Rigor ▪ Fever ▪ Restlessness ▪ Tachycardia	▪ Anxiety ▪ Pruritus (itching) ▪ Palpitations ▪ Mild dyspnoea ▪ Headache	▪ Hypersensitivity (Moderately severe) ▪ Febrile non-haemolytic transfusion reations: - Antibodies to while blood cells, platelets. - Antibodies to proteins, including IgA ▪ Possible contamination with pyrogens and/or bacteria.

Category 3 - Life-Threatening

Sign	Symptoms	Possible Cause
▪ Rigors ▪ Fever ▪ Restlessness ▪ - Hypotension (fall of > 20% in systolic BP) ▪ Tachycardia (rise of >,*20%* in heart rate) ▪ Haemoglobinuria (red urine) > Unexplained bleeding ▪ (DIC)	▪ Anxiety ▪ Chest pain ▪ Pain near infusion site ▪ Respiratory distress/shortness of breath. ▪ Loin/back pain ▪ Headache ▪ Dyspnoea	▪ Acute intravascular haemolysis ▪ Bacterial contamination and septic shock. ▪ Fluid overload ▪ Anaphylaxis. ▪ Transfusion - associated lung injury.

Note**:** If an acute transfusion reaction occurs, first check the blood pack labels and the patient's identity. If there is any discrepancy, stop the transfusion immediately and consult the blood bank.

In an unconscious or anaesthetized patient, hypotension and uncontrolled bleeding may be the only signs of an incompatible transfusion.

In a conscious patient undergoing a severe haemolytic transfusion reaction, signs and symptoms may appear quickly - within minutes of infusing only 5-10 ml of blood. Close observation at the start of the infusion of each unit is essential.

Management in Adverse Transfusion Reactions

Category I: Mild

1. Slow the transfusion.
2. Administer antihistamine IM (e.g. Chlorpheniramine 0.01 mg/kg or equivalent)
3. If no clinical improvement within 30 minutes or if signs and symptoms worsen, treat as Category 2.

Category 2: Moderately Severe.

1. Stop the transfusion, replace the giving set and keep I.V. line open with normal saline.
2. Notify the doctor responsible for the patient and blood bank immediately.
3. Send blood unit with giving set, fresh blood samples (1 clotted and 1 in EDTA) from vein opposite infusion site with appropriate reaction form to blood bank for investigations.
4. Administer antihistamine IM (e.g. chlorpheniramine 0.01 mg/kg or equivalent) and oral or rectal antipyretic (eg. paracetamole 10 gm/kg; 500 mg - lg in adults). Avoid aspirin in thrombocytopenic patients.
5. Give I.V. corticosteroids and bronchodilators if there are anaphylactoid features (eg. broneospasm. stridor).
6. Collect urine for next 24 hrs for evidence of haemolysis and sent to laboratory.
7. In clinical imporvement, restart transfusion slowly with new blood unit if required and observe carefully.

8. If no clinical improvement with in 15 mintes or if signs and symptoms worsen, treat as Category 3.

Category : 3 Life-threatening

1. Stop the transfusion, replace the giving -set and keep I.V. line open with normal saline.
2. Infuse normal saline (initially 20-30 ml/kg) to maintain systolic BP.
3. Maintain airway and give high flow oxygen by mask.
4. Give adrenaline (as 1:1000 solution) 0.01 mg/kg body weight by intramuscular injection in severe allergic reaction.
5. Give I.V. corticosteroids and bronchodilators if there are anaphylactoid features (e.g. broncospasm, stridor)
6. Give diuretic: e.g. frusemide 1 mg/kg I.V. or equivalent (initially 40 mgm i.v., upto 250 mgm over 4 hours)
7. Notify the doctor responsible for the patient and the blood bank immediately.
8. Send blood unit with giving-set, fresh blood samples (1 clotted and 1 in EDTA) form vein opposite to the infusion site, with appropriate reaction form to blood bank for investigation.
9. Check a fresh urine specimen visually for signs of hemoglobinuria (red ro pink urine).
10. Start at 24 hour urine collection and fluid balance chart and record all intake and output. Maintain fluid balance.
11. Assess for bleeding form puncture sites or wounds. If there is clinical or laboratory evidence of DIC, give platelets (adult 5-6 units) and either cryoprecipitate (adult 12 units) or fresh frozen plasma (adult: 3 units)
12. Reassess. If hypotension persists:
    - Give further saline 20-30 ml/kg.
    - Give Inotrope (Dopamine, IV infusion, 1 gm/kg/min.)
13. If urine output falling or laboratory evidence of acute renal fialure (rising K+, urea, creatinine):
    - Maintain fluid balance accurately
    - Give further frusemide.
    - Consider dopamine infusion.
    - Seek expert help: the patient may need renal dialysis.
13. If bacteraemia is suspected (rigors, fever, collapse, no evidence of a haemolytic reaction), start broad spectrum antibiotics I.V. to cover pseudomonas and gram positives organisms.

17

Autoimmune Hemolytic Anemia

Immune hemolytic anemia is a condition in which the life-span of red cells is shortened due to presence of a humoral antibody in the circulation that is reactive with an antigen on the red cell. Broadly the immune hemolytic anemia can be classified into:

1) alloimmune
2) autoimmune

This chapter focuses on the autoimmune hemolytic anemia.

Autoimmune hemolytic anemia (AIHA)

Normally an individual does not form antibodies against 'self i.e. against one's own cells. In autoimmune hemolytic anemia due to some failure of mechanism regulating the immune system the individual produces humoral antibodies reactive with antigens on one's own red cells. These are referred to as autoantibodies and can be elicited by the positive direct antiglobulin test (DAT). In AIHA the red blood cells survival is shortened. Depending upon the balance between the severity of red cell destruction and the individual's capacity to increase red cell production, the patient may have mild to severe anemia or may even be non-anemic. Red cells are coated with IgG antibody with or without anti-complement antibodies. Splenic macrophages and to a lesser extent the liver Kupfer cells have receptors for Fc fragment of IgG and for C3b/C3bi and C4b. The IgG-coated cells along with C3b/C3bi act as opsonins and increase phagocytosis, which may be complete or partial. When partial it causes loss of cell membrane and hence spherocytosis. Spherocytes are rigid cells and lack deformibility. They get trapped and phagocytosed in the splenic sinusoids.

In warm-antibody type of hemolytic anemia direct complement-mediated hemolysis is rare. In cold-reactive AIHA, however, direct complement-mediated hemolysis may occur leading to intravascular hemolysis - hemoglobinemia and hemoglobinuria.

Classification: (Table: 17-1)

The AIHAs are classified in two main groups, depending on the optimal temperature of reactivity. About 48-70% of cases of AIHA are those that react at warm temperature (37°C) and are called warm AIHA (WAIHA); while about 16-32% of cases of AIHA react at cold temperature (4-24°C) and are termed as cold-agglutinin syndrome (CAS). Mixed-type autoimmune hemolytic anemia, which has the characteristics of both WAIHA and CAS is uncommon (7-8%). The frequency of paroxysmal cold hemoglobinuria is about 2% and that of drug-induced AIHA is about 12-18%. AIHA may be primary or idiopathic and secondary. Approximately half the cases are primary and others secondary.

Table: 17-1 Incidence of various kinds of AIHA

Type of AIHA	Incidence (%)
Warm autoimmune hemolytic anemia	48 to 70
Cold agglutinin syndrome	16 to 32
Mixed-type autoimmune hemolytic anemia	7 to 8
Paroxysmal cold hemoglobinuria	Rare in adults In children 2
Drug-induced	12 to 18

Laboratory findings:

In hemolytic anemia the hemolysis is accelerated (i.e. the life span of the red cells is shortened). Hemolysis may be intravascular or extravascular.

In AIHA hemolysis is predominantly extravascular. Accelerated hemolysis is characterised by the presence of reticulocytosis, spherocytosis, unconjugated hyperbilirubinemia, elevated serum lactate dehydrogenase (LDH), depletion of serum haptoglobin and erythroid hyperplasia.

In intravascular hemolysis, hemoglobinemia, hemoglobinuria and depletion of serum hemopexin may occur.

Direct antiglobulin test (DAT): (Table: 17-2)

DAT is positive in practically all patients with AIHA. Limitations of the test must, however, be understood:

1. The incidence of positive DAT has been reported to be 1 in 10,000 in healthy blood donors with no evidence of accelerated hemolysis; whereas in hospitalised patients the incidence of positive DAT is 0.3 to 1% with IgG antiglobulin reagent and 1.5% with polyspecific ('broad-spectrum') reagent. The higher frequency of reaction with the polyspecific reagent is probably due to binding of anti-complement antibodies to the red cells. Therefore, a positive DAT is diagnostic of AIHA only if there is evidence of accelerated hemolysis.

   When the DAT is positive with polyspecific reagent, monospecific reagents such as IgG. anti-complement and rarely IgA or IgM are used to determine nature of autoantibodies.

2. Rarely the DAT may be negative in AIHA. More sensitive techniques have shown that the red cells are coated with fewer antibodies, which are below the detectable limit of the test but are capable of causing accelerated hemolysis.
3. When red cells are heavily coated with autoantibodies the same may elute into plasma. Most of these autoantibodies are against common red cell antigens and give a positive indirect antiglobulin test (indirect Coombs' test) with donor cells during compatibility testing.
4. Autoantibody is adsorbed on the red cells and may be in small quantity in the serum, while alloantibody, may or may not be adsorbed on the red cells but always present in serum, therefore:
   - DAT is more strongly positive than I AT if autoantibody is present.
   - IAT is more strongly positive than DAT if alloantibody is present

Table 17- 2 Characteristics of DAT in AIHA & Drug-induced IHA

	IgG	C3	Serum	Elute	Specificity
WAIHA					
24 to 76%	+	+	IgG warm autoantibodies	IgG, & reactive with normal cells	Mostly anti-Rh, others include anti - LW, U, Wr^b, En^a, Kidd, Kell,Ge.
20 to 66%	+	0			
7 to 13%	0	+			
Cold agglutinin	0	+	IgM autoantibody	Non-reactive	Mostly anti-I Can be an ti-i Rarely anti-Pr
Mixed-type AIHA	+	+	IgG&IgM autoantibodies	IgG & reactive	Unclear, can be anti-I, -i or an other cold agglutinin
Paroxysmal cold hemoglobinuria	0	+	IgGbiphasic hemolysin (Donath-Landsteiner antibody), Antibody reacts in cold and hemolysis occurs, at 37°C in presence of complement	Non-reactive	Anti-P
Drug-induced AIHA	+	+	IgG autoantibody	Non-reactive	Often Rh related

Nature of autoantibodies:

Mostly the autoantibodies formed are against the high-incidence antigens and therefore, react with red cells of most random donors blood causing problems in selection of a suitable blood for transfusion. The autoantibodies coat the patient's red cells and are also present in the serum. Consequently, the interpretation of cell typing, compatibility testing, antibody detection and "antibody identification may be difficult by routine procedures.

2. Rarely the DAT may be negative in AIHA. More sensitive techniques have shown that the red cells are coated with fewer antibodies, which are below the detectable limit of the test but are capable of causing accelerated hemolysis.
3. When red cells are heavily coated with autoantibodies the same may elute into plasma. Most of these autoantibodies are against common red cell antigens and give a positive indirect antiglobulin test (indirect Coombs' test) with donor cells during compatibility testing.
4. Autoantibody is adsorbed on the red cells and may be in small quantity in the serum, while alloantibody, may or may not be adsorbed on the red cells but always present in serum, therefore:
   - DAT is more strongly positive than IAT if autoantibody is present.
   - IAT is more strongly positive than DAT if alloantibody is present

Table 17- 2 Characteristics of **DAT in AIHA & Drug rug-induced IHA**

	IgG	C3	Serum	Elute	Specificity
WAIHA					
24 to 76%	+	+	IgG warm	IgG, & reactive	Mostly anti-Rh, others
20 to 66%	+	0	autoantibodies	with normal cells	include anti - LW, U, Wr^b,En^a, Kidd,
7 to 13%	0	+			Kell,Ge.
Cold agglutinin	0	+	IgMautoantibodies	Non-reactive	Mostly anti-I Can be anti-i Rarely anti-Pr
Mixed-type AIHA	+	+	IgG& IgM autoantibodies	IgG & reactive	Unclear, can be anti-I, -i or an other cold agglutinin
Paroxysmal cold hemoglobinuria	0	+	IgGbiphasic hemolysin (Donath-Landsteiner antibody), Antibody reacts in cold and hemolysis occurs, at 37°C in presence of complement	Non-reactive	Anti-P
Drug-induced AIHA	+	+	IgG autoantibody	Non-reactive	Often Rh related

Nature of autoantibodies:

Mostly rhe autoantibodies formed are against the high-incidence antigens and therefore, react with red cells of most random donors blood causing problems in selection of a suitable blood for transfusion. The autoantibodies coat the patient's red cells and are also present in the serum. Consequently, the interpretation of cell typing, compatibility testing, antibody detection and antibody identification may be difficult by routine procedures.

2. If result is D-negative, testing for partial or weak D is not necessary as the patient can safely receive ABO compatible D-negative blood.

Typing for other red cell antigens:

This is beyond the scope of this book. Transfusion centres interested in developing these techniques should refer to AABB Technical Manuals and Clinical Practice of Transfusion Medicine, Third Edition, by Lawrence D. Petz, Scott N. Swisher, Steven Kleinman. Richard K. Spence and Ronauld G. Strauss. Churchill Livinstone, New York, **1**996.

Serum Studies:

Detection and identification of autoantibody and/or alloantibody in WAIHA:

In the event that transfusion therapy is-needed or autoantibodies and/or alloantibodies identification is needed in a patient with WAIHA, adequate assessment of the patient's serum for the presence and type of antibody is essential. Difficulties may arise due to the following possibilities:

- In addition to autoantibody the patient may have alloantibody formed from previous transfusion or pregnancy. A positive DAT may result from coating of red cells by an autoantibody or an alloantibody.
- Serum may contain autoantibody below the detectable limit. The amount formed may be small and absorbed on to the red cells in vitro.
- Clinically significant alloantibodies may be masked by the autoantibody.

An approach to the assessment of the patient's serum for the presence of type of antibody is given in Figure 17.1A/B.

1. If the antibody screening is negative, additional studies with the patient's serum are not indicated.
2. If the antibody screening is positive, antibody identification studies to determine the presence of autoantibody and/or alloantibody is performed. The most appropriate course of action is to perform adsorption studies.
3. If the patient has not been transfused with in 2-3 months and if the patient's red cells volume is sufficient, antibody screening after autoadsorption is the method of choice Fig. 17-1/A.

(a) Initially autoantibodies bound to red cells are removed from the patients red cells in order to optimize the autoadsorption procedure.

(b) Different methods for removing the aoutoantibodies are (i) elution at 56°C with enzyme pretreated cells (ii) ZZAP (dithiothreitol and cysteine-activated papain) (iii) chloroquine diphosphate. For details see chapter on Preparation of Solutions and Special Methods. It is efficacious to pretreat two aliquots of the patient's cells and autoadsorb the serum twice.

(c) Following autoadsorption an antibody screening should be performed with the adsorbed serum, if no reactivity is noted, units of blood should be selected and pretransfusion testing is done. If reactivity in the antibody screening is noted, antibody identification is done. If alloantibody is detected , antigen negative units are selected and pretransfusion testing is done for transfusion.

4. If the patient has been transfused with in 2-3 months or the sufficient volume of the

patient's cells is not available, allogeneic adsorption is done to determine if significant alloantibodies are present with warm autoantibodies Fig. 17.1/B.

Fig. 17.1/A Antibody Screening after Autoadsorption

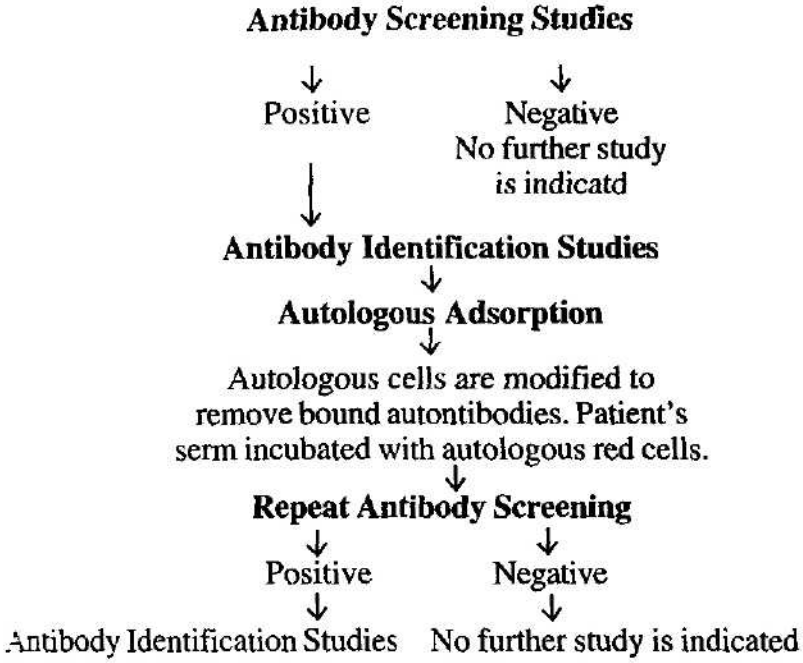
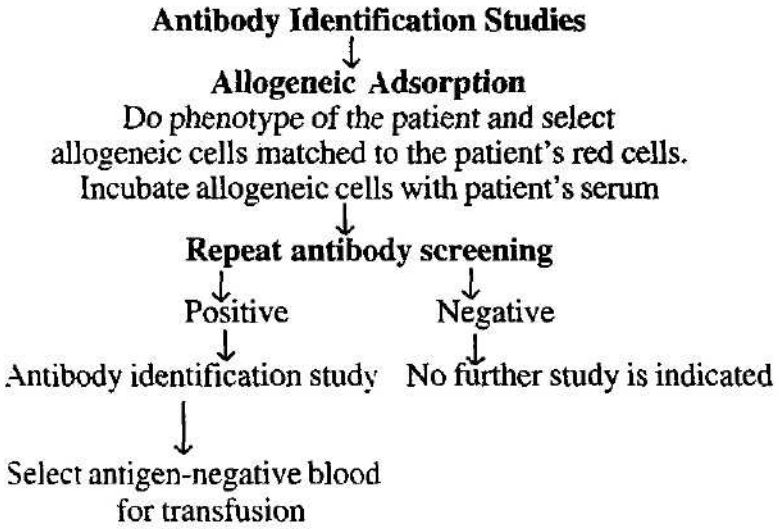


Fig. 17.1/B Antibody Screening after Allogeneic Adsorption



Antibody Screening after Allogeneic Adsorption (figure 17.1/B)

13 Determine patient's phenotype for common anigens (Rh, Kell, Duffy. Kidd, MNSs). Absorb tion antibody with allogeneic cells matched with patient's cell.

ft Repeat antibody screening studies, if negative no further studies are needed. Select units of blood and do pretransfusion testing. If reactivity in the antibody screening is noted, antibody identification is done. If antibody is detected, antigen negative units are selected and pretransfusion testing is done.

Note: For antibody screening and identification see Chapter 9.

Treatment:

- Transfusion in patients with WAIHA is avoided whenever possible because the majority of patients can be managed successfully without red cells transfusion, with prednisone, 60 mg per day given in three doses for 10-14 days. The majority of the patients will have marked improvement, with decrease in hemolysis and stabilization followed by rise in Hct. Then prednisone is gradually reduced.
- Splenectomy is considered when patient does not respond to prednisone treatment and subsequently prednisone doses more than 15-20 mg/day are also used used in maintain remission.
- Blood transfusion- RBCs transfusion is given to increase oxygen-carrying capacity of blood. Transfusion is reserved for the patients with underlying heart disease, cerebro-vascular ischemia or life-threatening anemia.
  The most compatible red cells should be given and the patient is closely monitored for any sign of adverse transfusion reaction. The transfused red cells are usually destroyed as rapidly as patient's own red cells, unless the patient has responded to prednisone.

AIRA due to Cold-reactive autoantibodies:

AIHA due to cold-reactive autoantibodies are of two types:

1. Mediated by cold agglutinins:
   a. Idiopathic or primary cold agglutinin disease (chronic disorder)
   b. Secondary cold-agglutinin hemolytic anaemia
      i. Post-infectious e.g. *Mycoplasma pneumoniae* and *infectious mononucleosis* (transient disorder)
      ii. Malignant lymphoproliferative disorder
2. Mediated by cold hemolysins of Donath-Landsteiner type
   a. Idiopathic or primary cold paroxysmal haemoglobinuria (very rare)
   b. Secondary
      i. Hemolytic anemia commonly seen in children due to viral infections
      ii. Congenital or tertiary syphilis

Pathogenesis:

Mos sold antibodies do not agglutinate RBC above 30°C and are harmless; higher the thermal ampitude of their action greater is the degree of pathogenecity (Table 17.3).

Table 17.3 Characteristics of benign and pathologic cold-reactive autoantibodies:

Parameter	**Benign autoantibodies**	**Pathologic autoantibodies**
Thermal amplitude	4°C (rarely weak reaction up to 24°C)	Broad amplitude 30 - 37°C
Titer	<16 (sometimes up to 32)	>1000at4°CinCAS. In Mixed-type AIHA, titer at 4°C may be <64
Reaction enhanced by	None	Albumin
Direct antiglobulin test	Neg. or weak +ve with polyspecific AHG	Positive
Clonality	Polyclonal	Monoclonal when idopathic, Polyclonal when secondary to infection
Immunoglobulin class	IgM	IgM & rarely IgA or lgG
Common antibody specificity	Anti-I	Anti-I, rarely anti-i, anti-IH or anti-Pr

The immunoglobulin class of cold autoantibody is mostly IgM, however IgA and IgG cold autoanibodies have been reported. The causative autoantibody of PCH is always IgG and is characterized by biphasic or bithermic hemolysis. Autoantibody binds to red cells at low temperature in the extremities and then causes hemolysis via the activation of complement as the blood warms to 37°C, and causes hemolysis and hemogloninuria.
Benign autoantibodies are present mostly in healthy individuals and are clinically silent. Pathologic autoantibodies are associated autoimmune hemolytic anemia.

Problems encountered in blood banking procedures due to cold-reactive autoantibodies:

ABO typing:

The autoagglutination is visible on naked-eye examination of the blood specimen collected in EDTA tube at RT. It is more evident on cooling to 4°C. Autoagglutination disappears when red cells are warmed to 37°C. Autoagglutination may be seen in peripheral blood films as well. Red cells that are heavily coated with cold autoantibodies agglutinate spontaneously giving false-positive results with anti-A and anti-B and anti-D sera. These discrepancies can be removed using the patient's red cells washed twice with normal saline warmed to 37°C or by collecting and keeping patient's blood sample at 37°C

Example: Patient blood % group O

Forward Grouping:

	Agglutination with	
	Anti-A	Anti-B
Patient's cells in own serum	+	+
Cells washed with saline warmed to 37°C	0	0

Reverse grouping:

	A,Cells	B Cells	O Cells	Autologous cells
Patient's serum	+	+	+	+
Prewarmed serum at 37°C	+	+	0	0

If the elution of antibodies does not occur by washing cells with saline warmed to 37°C, washing with saline warmed to 45°C may be successful. Sometimes elution with thiol reagent may be necessary. Warming of the serum or auto absorption resolves discrepancies of serum grouping.

Rh (D) typing:

Rh (D) typing generally gave false positive reactions when enhanced anti-D (high protein) reagents were in use. The Rh control may be positive, rendering the test invalid. Most reagents these days are monoclonal anti-D (low-protein), which normally give valid results; a negative result with any of the ABO reagents serves as a control for D typing. Red cells washed with warm saline are used if a discrepancy in ABO typing is observed. If washing of cells give unsatisfactory results, thiol reagents are used to remove the autoantibodies.

Cold agglutinins activate complement in vitro that attaches to the red cells. When test for weak D is performed using a polyspecific antiglobulin reagent a positive reaction is obtained. The Lest is negative with mono-specific anti-IgG or when specimen is collected in EDTA, which interferes with in vitro binding of the complement.

Similar problems occur with tests for other phenotypes (e.g. K, Fy^a) where antiglobulin reagent is used. This is also resolved by use of anti-IgG antiglobulin reagent or EDTA collected sample.

Direct antiglobulin test:

It is positive when cells are obtained from clotted blood or a polyspecific or anti-C3 reagent is used.

This problem can be resolved by:

- Washing the cells with normal saline warmed to 37°C.
- By collecting sample in EDTA, which interferes binding of complement in vitro.
- Using mono-specific IgG AHGS
- If the cells washed with saline warmed to 37°C gives unsatisfactory result, thiol reagents are used to remove autoantobodies.

Serum Studies:

- Unlike warm autoantibodies, cold autoantibodies nearly always are demonstrable in the serum.
- Autoantibodies in CAS commonly include anti-I, followed by anti-i and anti-IH.
- In PCH, autoantibodies to P, I, i and Pr have also been reported.

As with warm autoantibodies, cold autoantibodies may present serological problems if transfusion therapy is indicated: The reasons are:

- Cold autoantibodies may mask the presence of clinically significant alloantibodies.
- Autoantibody is capable of activating complement in vitro.
- Antiglobulin serum containing both anti-IgG and anti-C3 may give false result.

The problems can be resolved by:

- Prewarming of serum and red cells to 37°C, followed with an antiglobulin procedure that avoids temperature below 37°C, may alleviate such problems.
- In other patients adsorption procedures are needed before serum studies.
- Using IgG anti-human globulin serum.

Adsorption Studies :

Adsorption studies with autologous and allogeneic cells are done in the same way as in WIHA.

Autologous adsorption -

- If the patient has not been transfused within 2-3 months, autoadsorption is the method of choice.
- Adsorption at 4°C is better if patient's red cells pretreated with enzyme are used, but certain autoantibodies e.g. anti-P are denatured, and cannot be adsorbed. In such cases adsorption is repeated with untreated cells.
- Following adsorption. Antibody screening studies are done to evaluate the presence of any alloantibody.
- If antibody screening tests are positive, antibody identification studies should be performed.
- Tests with autoadsorbed serum may also resolve ABO reverse grouping.

Allogeneic Adsorption -

- If the patient has been given transfusion recently with in 2-3 months, allogeneic adsorption may be done.
- Allogeneic cells should be selected as described in the section of warm autoantibodies
- Enzyme treated cells facilitate the process.
- Following adsorption, antibody screening studies should be performed.
- Select antigen-negative blood for pre-transfusion testing and transfusion.

Treatment

- Generally anemia is mild and symptomatic treatment is given.
- Patients are advised to keep themselves warm, particularly the extremities.

- Folic acid 1 mg/day is given.
- In patients with severe anemia
  (a) Chlorambucil or cyclophosphamide is given. This result in decreased titer of cold agglutinins and less hemolysis.
  (b) Prednisone and splenectomy is generally not effective with some exceptions. Prednisone is beneficial in patients with low titer of IgM cold agglutinins having high thermal amplitudes and in patients with IgG cold agglutinin. In the later group splenectomy is also effective.
- Transfusion:
  (a) It is rarely required. Patients whose cardiovascular or cerebro-vascular systems are significantly compromised by anemia may require blood transfusion.
  (b) Washed red ells are given to avoid transfusion of complement component.

Paroxysmal Cold Hemoglobinuria(PCH) :

- **PCH** was associated with syphilis but due to effective treatment of syphilis there has been marked fall in incidence of PCH.
- It is now a rare disorder and generally occurs in children with viral infections.
- Acute intravascular hemolysis occurs on exposure to cold and is characterized by onset of fever, chills, malaise, abdominal cramps and back pain. All signs of intravascular hemolysis are evident, along with hemoglobinuria, hemoglobinemia, bilirubinemia depending on the severity and frequency of the attacks and depletion of serum haptoglobin levels.
- Diagnosis is confirmed by positive Donath-Landsteiner (D-L) test. The D-L antibody is an IgG antibody. Collect two blood sepcimens from the patient, one specimen, the control, is maintained at 37°C for 60 minutes after collection. The second sample is colled at 4°C for 30 minutes and then incubated at 37°C for an additional 30 mintes. Both samples are then centrifuged and obseved for hemolysis. In a positive Donath-Landsteiner test, hemolysis is seen in the sample placed at 4°C and then at 37°C, whereas no hemolysis is observed in the control sample.
- DAT is positive for C3d.

Treatment

- PCH is usually a self-limiting disease, with recovery in a few days or a week.
- Patients are given symptomatic treatment.
- Patients are kept warm.
- Prednisone and splenectomy are not effective.
- Syphilis should be excluded.

Drug-Induced Immune Hemolytic Anemias

Drug-induced immune autoantibodies may result in decreased red cells survival and may complicate pre-transfusion testing.

Three mechanisms have been described, which cause drugs induced-IHA : (Table 17-4)

- Drug or hapten adsorption
- Immune complex between drug, red cell membrane and antibody
- Induction of autoantibody

A fourth mechanism of non-immunological adsorption of proteins to the red cells (membrane modification) may produce positive DAT but does not cause hemolytic anemia e.g. with cephalothin (Keflin).

Table: 17.4 Drug Induced Hemolytic Anemia

Mechanism	**Protein defected by DAT**	**Detection of Antibody**	**Clinical Features**
Hapten or drug adsorption (e.g. penicillin)	IgG C3d*	Ab reacts with drug - coated red cells	Moderate degree of hemolysis usually extravascular
Immune complex type (Drug-Antibody-Target cells complex) e.g. quinine, quinidine phenacetin	C3d	Ab + drug+red cell sensitization, agglutination or hemolysis of red cells. Ab is IgG or IgM: elute is negative	Abrupt onset of severe intravascular hemolysis, renal failure
Autoimmune-induction (e.g. methyldopa)	IgG rarely complement	Autoantibodies against red cells; elute reacts with red cells	Mild to moderate degree of extravascular hemolysis

* present in about 40% of penicillin - induced IHA.

Treatment:

- The degree of hemolysis is usually mild to moderate, and anemia develops gradually. Hemolysis usually regresses within a few days after the drug is stopped but may continue for longer periods.
- In most patients no treatment is required
- Prednisone may shorten the period of recovery but usually is not required.

18

Autologous Blood Transfusion

Blood collected from a patient for re-transfusion at a later time into the same individual is called "autologous blood". The patient who receives his or her own blood gets the safest possible blood because no foreign antigens infused, no infectious diseases other than the patient may already have are transmitted. Its use has increased with the awareness of infections particularly human immuno-defficiency virus (HIV) transmitted through allogenic (homologous) tranfusion.

The general categories of autologous transfusions are:

(1) Preoperative donation of blood - 2 or more units blood are drawn and stored prior to anticipated need.

(2) Intra-operative blood collection - blood is collected in operation theatre prior to surgery or during surgery and include:

a) Perioperative hemodilution - (acute normo volemic hemodilution) 1 or 2 units of blood is drawn before surgery and concomitantly replaced with crystalloid or colloid solution.

b) Intraoperative blood - blood is collected (salvage) from the surgical field, then processed and returned.

(3) Post operative blood collection

General advantages and disadvantages of autologous transfusions are summarized below:

Advantages

- Prevents the possibility of transfusion-transmitted infections like HIV, HBsAg, HCV and Treponema Pallidum (syphilis).
- Prevents alloiminimization to red cells, leukocytes, platelets , and plasma proteins.
- Supplements blood supply - adds to blood inventory.
- Prevents adverse transfusion reactions especially allergic and febrile reactions.
- Provide blood to patients having antibodies against common antigens.
- Provides blood to patients who refuse homologous blood transfusion because of religious belief.
- Preoperative autologous donation stimulates the bone marrow to increase cells production.

Disadvantages

- Preoperative autologous blood donation subjects to patient to anemia and hypovolemia.
- Consequences of transfusing incorrect unit due to the clerical error.
- Careful management of labeling, storing, and reinfusion of blood/its products is necessary.
- Increase complexity of providing transfusion.
- Preoperative autologous blood donation is inconvenient to patient-donor.
- There may be unnecessary loss of blood if operation is postponed or transfusion is not needed.
- More costly than allogenic blood.
- Risk of adverse reactions during donation.

PREOPERATIVE AUTOLOGOUS DONATIONS

Preoperative autologous blood donations is most feasible for patients likely to require transfusion during elective surgery scheduled to take place with in 35 - 42 days (the shelf life of blood stored in liquid state). Long time storage in frozen state is expensive and ineffective. Each patient for preoperative autologous donation must be carefully evaluated by his or her physician and the blood bank consultant. Autologous donation requires the written advice of patient's physician. Patients are required to sign a consent acknowledging that they have been informed and understand the risks and advantages of autologous donation. See Annexure in the end of the chapter.

Each unit drawn from the patient is assigned a number that is placed on the bag and donors-patients' records in the blood bank. This allows the unit to be tracked to its final disposition. A label stating "For Autologous Use Only" must be placed on the bag. Autologous blood units should be stored in a separate shelf of the blood bank refrigerator.

Indications for Predeposit Autologous Donations :

Predeposit autologous donation is indicated in elective surgical procedures with reasonable probability for transfusion and for which there is sufficient time to obtain one or more units of blood with minimum risk and without creating significant hemoglobin deficit in patient-donor. Examples are orthopedic surgery (joint replacement), plastic and reconstructive surgery, cardio-vascular surgery, major abdominal surgery (splenectomy), and in obstetrics & gynaecological conditions - particularly women having multiple antibodies or antibodies to high frequency antigens.

Contraindications for Predeposit Autologous Donations:

1. Bacteremia and acute localized infection
2. Myocardial infarction in the past 6 months
3. Unstable angina
4. Aortic stenosis
5. Congestive heart failure
6. Significant ventricular arrythmias
7. Marked uncontrolled hypertension
8. Cerebrovascular accident with in 6 months

Eligibility of predeposit autologous donation

Patient-donor, for autologous donation need not meet all the criteria of homologous blood donation. Whenever requirements for donor selection or blood collection of homologous blood donations can not be applied, suitable guidelines for the individual patient-donor should be established in consultation with patient-donor's physician/surgeon and must be recorded. Major guidelines are:

Hemoglobin: Acceptable at 11 gm/dl or 33 per cent (0.33) hematocrit or higher. Below this level phlebotomy should not be done, except in special circumstances with the approval of patient's physician but it should not be done if hemoglobin is less than 10 g/dl.

Age: There is no upper or lower limit of age. Pediatric patients undergoing elective surgery can benefit from autlogous blood.

Weight and Volume of blood withdrawn: Donors weighing 60 kg or more can donate 450 ml of blood and donors weighing less than 60 kg may donate proportionately smaller volume of blood but no more than 8-9 ml/kg body weight. In pediatric patient of 8 years of age the weight should be 27 kg and no more than 10% of the patients blood volume should be drawn at each phlebotomy.

Frequency of donation: Donations are often scheduled weekly or even at 4 days intervals with the last phlebotomy performed 72 hours or more before the operation. This allows patient-donor plasma to return to normal before surgery. Oral iron (325 mgm of ferrous sulfate three times a day) is given to accelerate the restoration of hemoglobin to predonation levels. Use of erythropoietins along with iron is the most effective way to enhance the chances of successful predonation program, but it is expensive.

Use of Autologous Donation for Homologous use

A policy must be made for the final disposition of predeposit autologous donations and allowing it to crossover for allogenic (homologous) use. The unused unit of pre-operative autologous blood donation may be transferred (cross over) for allogeneic (homologous) use only if it meets all standard criteria of blood donors like Hb 12.5 g/dl and negative for various transmissible - infections like HIV 1 &2, Hepatitis B & C and treponema pallidum. Hower, the policy of allowing autologous blood to cross over for allogeneic use is controlversial and now it is not permissible becuase of doner - Patient's associated medical illness, medication, and intermittent bacteremia and other conditions.

Laboratory Testing

Minimum laboratory testing requirements, for the autologous unit are ABO group and Rh typing. Testing of autologous blood donations for infectious diseases markers is controversial. The rational for testing markers of diseases is to protect the hospital staff rather than the intended recipient. Some advocate that the first unit of autologous blood should be tested for the markers of the transfusion-transmitted diseases. If any infection disease test is positive, a biohazard label must be applied to the unit(s) and the patient's physician must be informed.

PERIOPERATIVE AUTOLOGOUS BLOOD DONATION

PERIOPRATIVE HEMODILUTION

Acute normovolemic or isovolemic hemodilution entails the removal of predetermined volume of blood from the patient, either immediately before or shortly after the induction of anesthesia in operation theatre, and its simultaneous replacement with blood volume expanders (colloid 1 ml or crystalloid 3 ml for every 1 ml of blood collected). The patient's hematocrit is lowered to about 20% (1-3 g/dl).

The volume of blood to be collected for a given hematocrit can be determined by the following formula:

$$\text{Vol. of blood may be remved} = \frac{\text{Estimated blood vol.} \times (\text{initial Hct - desired Hct})}{\text{Average of initial \& desired Hcts}}$$

Estimated blood volume = Body weight(kgs) × 70 in adults
= Body weight (kgs) × 80 in children

Example:
Weight of patient - 70kg
Estimated blood vol. - 5000 ml approx.
Initial Hct-45%; desired Hct-30%
Vol. of blood may be removed = 5000x([0.45-0.30]) / 0.0375
= 2000 ml

The total volume of blood collected should not exceed 40% of the patient estimated blood volume.

The advantages of the procedure include:

1. Surgical bleeding occurs at lower Hct., and therefore the loss of RBCs is less.
2. The blood flow through microcirculation is improved because of the reduced Hct.
3. The donated blood that can be used during or immediately after surgery is very fresh and contains viable platelets, adequate protein levels and good levels of all plasma clotting factors.

The collected blood usually does not leave the operation theatre. It can be stored at conditioned temperature in operation theatre or in the refrigerator at 2-6°C for upto 24 hours. All procedures and policies must ensure proper collection, handling, storage, identification, and transfusion or disposition.

Indications for Peri-operative Hemodilution

Surgical procedures where expected loss of blood is more than 1 L.

1. Cardiovascular surgery
2. Vascular surgery
3. Spinal surgery for scoliosis
4. Total hip or knee joint replacement

Contraindications for Peri-operative Hemodilution

1. It is usually inappropriate to do hemodilution when the hemoglobin is less than 11 g/dl as it decrease immediately 1 g/dl for each unit of blood removed.
2. Patients with impaired renal function who can not excrete the large volume of infused fluid.
3. Patients who have preoperative deficiencies of coagulation factors as hemodilution further reduces them.
4. Sever obstructive and/or restrictive pulmonary disease is a contraindication as near-normal oxygen transport is essential. Pulmonary function should be carefully evaluated.
5. Impaired myocardial infarction as a result of previous infarction or therapy with calcium channel or B-adrenergic blocking agents may limit the ability of the heart to respond to hemodilution with usual increase in out put. The same is applicable in severe aortic stenosis.
6. Bacteremia
7. Pregnancy with anemia

INTRAOPERATIVE BLOOD COLLECTION (Salvage)

Intraoperative blood salvage is the collection of sheded blood from a closed wound or body cavity during surgery and its subsequent transfusion into the same patient. Surgical indications for intraoperative blood salvage include any surgical procedure in which blood loss more than IL is anticipated and if there is no contamination (sepsis or penetrating wound) of surgical site or contamination with malignant tumor cells. Intaoperative autotransfusion can decrease the need for homologous transfusions.

The procedure can be used in many surgical procedures:

- Cardiovascular
- Vascular
- Orthopedic procedures (especially total hip replacement and spinal surgery)
- Liver transplant
- Ruptured ectopic pregnancy
- Trauma

Contraindications of Intraoperative Blood Salvage

- Infection - reinfusion of contaminated, even washed, blood may lead to bacteremia.
- Malignancy (malignant cells) - reinfusion of malignant cells may lead to metastatic spread.
- Fecal contamination.

Procedure

Many intraoperative autotransfusion devices are available. The basic principles involved are suction driven aspirator, which collects sheded blood from wound or body cavity during surgery, anticoagulation; filtration to remove debris, and fibrin; centrifugation and washing the red cells with normal saline and transfer into a separate pack for reinfusion.

Newer devices (e.g. Sorenson Autotransfusion Systems, Haemonetic's Cell Saver) that transfuse salvaged whole blood or washed red cells have proved to be safer and no major complications develop. Air embolism has never been reported with a newer autotransfusion device. The devices are costly and the process is cost effective.

All intaoperative salvage techniques must confirm to the safety requirements. Salvaged blood must be clearly labeled having name of the patient, identifying number, date and time of collection. Blood should be reinfused within six hours from the start of collection and should remain with the patient until reinfused**.**

Anticoagulants

Blood salvaged from a serosal cavity is frequently deficient in fibrinogen and platelets and will not clot. It is feasible to transfuse such blood with minimal or no added anticoagulant. For most surgical situations, however the patient is either systemically anticoagulated (for example, in cardiac surgery), or anticoagulant is added to the salvaged blood. In the later case, citrate anticoagulants are preferable to heparin, which at low doses, can cause paradoxical platelet activation and clotting.

Complications from the use of intraoperative salvaged blood are:

- Hemolysis
- Disseminated intravascular coagulation (DIC)
- Sepsis
- Air embolism

POSTOPERATIVE BLOOD SALVAGE

Techniques available for collecting the postoperative drainage are usually of value within 24 to 48 hours after surgery in patients actively bleeding into a closed site (e.g. after cardiopulmonary bypass, blood from the chest following traumatic hemothorax, joint cavity drainage). This procedure is also contraindicated where there is evidence of infection or malignant tumor cells in the site from which blood is being salvaged or when the rate of blood loss is less than 50 ml per hour. The blood collected from postoperative drainage is sterile and defibrinogenated. It will not clot.

It utilizes the **same** devices for collecting and processing blood that are used in intraoperative autotransfusion. It must be filtered (washing is optional) before it is returned to the patient. The blood must be reinfused with in six hours from the start of collection in order to minimize the proliferation **of** bacteria. The blood bag should be labeled having patient's name and an identifying number.

Conclusions

Autologous blood is generally accepted to be the safest type of blood for transfusion. It decreases the demand of banked blood. Logistical problems can be minimized by protocols developed in hospitals for successful autologous transfusion programs.

ANNEXURE

CONSENT FOR PREDEPOSIT AUTOLOGOUS BLOOD DONATION

1. I, Mr./Mrs./Miss……..…son/daughter/wife of…….have been explained fully the purpose and the procedure of the autologous transfusion, and the possibility and the nature of its complications.
2. I consent for withdrawl of my blood by an authorized member of the staff of the blood bank for autologous transfusion. If I do not require transfusion of the blood withdrawn for autologous transfusion, it may disposed off as per the hospital policy.

Date: Patient- Donor signature

Witness signature

Parent/Guardian's signature
(if patient is minor)

19

Hematopoietic Stem Cells and Progenitor Cells

There are certain cells in the bone marrow and peripheral blood whose only function is to produce different types of cells in blood. These cells are called hematopoietic stem cells and when they divide, they produce daughter cells called progenitor cells. Both the stem cells and progenitor cells are equally important in the patient's recovery process following a stem cell transplantation.

The hematopoietic stem cells transplantation regenerates patient's bone marrow and **immune** systems that have been destroyed by chemo-and/or radiation therapy to kill cancer cells. The stem cells also have the ability to regenerate themselves and develop into functional **blood** cells population made of eight distinct cell types - erythrocytes, granulocytes (neutrophils, eosinophils, basophils), macrophages, megakarocyte (platelets), and T and B lymphocytes. See figure **19-1.**

Sources of Hematopoietic Stem Cells.

There are three main sources of stem cells.

- Bone Marrow
- Peripheral blood
- Blood in umbilical cord and placenta

From these sites stem cells can be collected for hematopoietic transplantation.

Indications of hematopoietic stem cells :

Malignant hematological diseases

- Leukemia

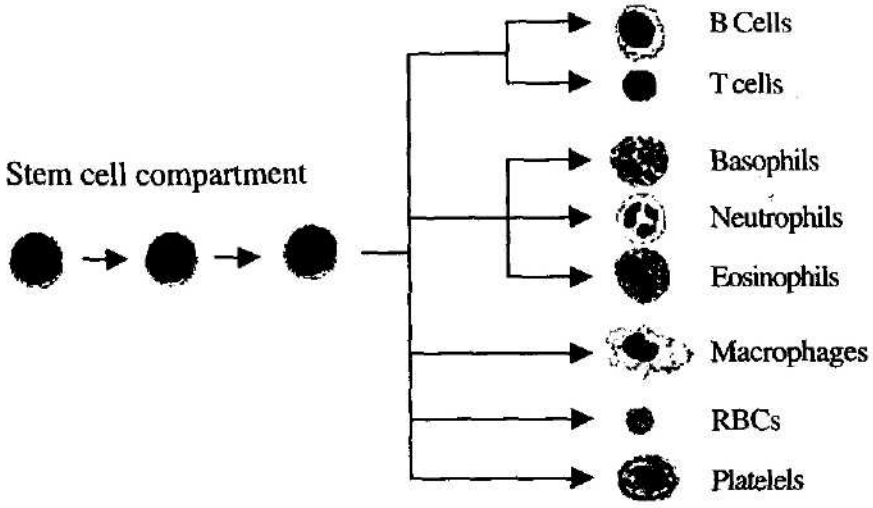


Fig. 19-1

HEMATOPOIESIS (The development of blood)

- Hodgkin's disease
- Non-Hodgkin's lymphomas
- Multiple myeloma

Hemoglobinopathies

- Thalassemia
- Sickle cell disease

Selected solid tumors

- Breast cancer
- Neuroblastoma

Non-malignant hematological diseases

- Congenital immunodeficiency
- Aplastic anemia

Types of Stem Cells Transplantation

There are three types of hematopoietic progenitor cells transplantations;

- Autologous
- Allogeneic
- Syngeneic

Autologous Transplantation

In autologous transplantation, patients own bone marrow or peripheral blood stem cells (PBSCs) are collected and later transfused into their own circulation to restore the hematopoietic function after chemo- and/or radiation-therapy to cure malignant disorders.

Allogeneic Transplantation

In allogeneic transplantation the hematopoietic stem cells from individuals (usually siblings), who are human leukocyte antigens (HLA) identical at the A, B, and DR loci, are used for transplantation. When such match could not be found, related donors mismatched at I or 2 HLA loci have been used.

Syngeneic Transplantation

In this the hematopoietic stem cells from identical twin are used for transplantation.

BONE MARROW COLLECTION

Bone marrow is usually collected, during the period of recovery from chemo- and/or radiation therapy, under spinal or general anesthesia. The iliac crests (pelvic bone) are punctured and aspirated multiple times in order to collect between 0.4 and 1.0 liter of fluid containing bone marrow and blood cells. The aliquots of marrow are immediately mixed with anticoagulant usually heparin and tissue culture medium. The aliquots of the bone marrow are pooled in a sterile beaker or flask and then filtered through series of filters or stainless sieves of 300 and 200-<W>m mesh size, to filter out most of the bone chips, clots, and fibrin. The collection is then transferred to one or more sterile transfer bags. The collected marrow may be infused directly into the patient or further processed for frozen storage.

In the bone marrow the cells that lead to hematopoietic reconstitution are found among the mononuclear cells (MNCs). Other cell population in the graft is superfluous.

The processing can require cell separation, RBC removal, buffy coat concentration, and mononuclear cell purification. In addition, malignant cells in the marrow may be purged by monoclonal antibodies. The bone marrow is processed by using cell separator to remove 95% of red cells while recovering more than 75% of colony forming unit granulocytes-macrophage (CFU-GM) or CD34 + cells in the final product containing more than 80% of mononuclear cells (MNCs). The concentration of nucleated cell is usually adjusted before cryopreservation to 3-4 x 10^7 cells/ ml.

The usual target of nucleated cells is 1-2 x 10^8 nucleated cells/kg body weight of recipient or 1000 colony-forming unit-granulocyte-macrophage (CFU-GM). These levels are usually achieved by collecting 10-20 ml of marrow per kg body weight of the donor. However more quantity may be required if purging of tumor cells is required from the marrow.

Bone marrow is generally cryopreserved in 20% dimethyl sulfoxide (DMSO) [Final concentration 10%] and stored in liquid nitrogen until used. Freezing and storage of the bone marrow is given in detail later.

Following marrow ablation with chemo- and/or radiation therapy, the bone marrow is rapidly thawed and infused into the blood stream of the patient to repopulate the marrow.

Bone marrow can be obtained either from an HLA-matched or partially matched donor (allogeneic) or from the patient himself (autologous).

Procedure for Processing Bone Marrow

- Harvest 2 x 10^8 nucleated cells per kg body weight
- Filter to remove bone chips and clots
- Reduction in red cell contamination and concentration of the nucleated cells by preparation of buffy coat or Ficoll-Hypaque density centrifugation
- Addition of autologous plasma and cryoprotectant (DMSO) to the marrow suspension

- Controlled-rate freezing
- Storage in liquid nitrogen

PE.RAL BLOOD STEM CELLS

Stem cell and progenitor cells have the capacity to migrate from the peripheral circulation to the bone marrow and vice versa. The primitive cells that could be used for transplantation circulate in the peripheral blood at concentration approximately 2% of those in the bone marrow. Peripheral blood stem cells transplants were initially used as an alternative to bone marrow in patients who have had irradiation in the pelvic area where bone marrow is normally harvested or have extensive cancer-cell contamination of the bone marrow. PBSCs are collected using a blood processing machine (cell separator) by a process known as apheresis. As large volume of blood is processed per collection, long term venous access through a catheter is required.

It was later discovered that, by unknown mechanism, the stem cells and progenitor cell contents of the peripheral blood are markedly increased during recovery from myelosuppressive chemotherapy (particularly cyclophosphamide), and/or following administration of recombinant hematopoietic growth factors particularly G-CSF and GM-CSF). Peripheral blood stem cells are now routinely mobilized by hematopoietic growth factors (with or without chemotherapy), which include G-CSF and GM-CSF or a combination of the two. G-CSF is given in the doses of 10-22 ug/kg/day for 5-7 days. This practice requires fewer apheresis sessions (1-3 vs 7-10) to collect enough PBSCs for transplantation.

Most transplant centers collect minimum of 1-2 x 10^6 CD34+ cells/kg or 10 x 10^4 CFU-GM/kg. or mononuclear cells 2xl0^8/kg body weight. Immediately after collection, the mononuclear cells, with or without Percoll or Ficoll-hypaque sedimentation, are suspended in autologous plasma, mixed with dimethyle sulphoxide (DMSO) and stored frozen in liquid nitrogen.

CD34+ cell selection is accomplished by immunomagnetic bead separation or avidin-biotin immunoaffinity column or using a machine called flow - cytometer.

The use of mobilized PBSCs led to significant recovery of neutrophil and platelet levels after transplantation. With PBSCs transplantation, neutrophil recovery occurs within 8-12 days, while platelet recover averages 8-15 days.

Mobilized PBSCs transplants are predominantly performed in the autologous setting due to initial concerns that the increased numbers of T-lymphocytes might increase the frequency and severity of GVHD. However, numerous recent reports have demonstrated successful allogeneic mobilized PBSCs transplants without increase rates of GVHD.

Abbreviation:

G-CSF - Granulocyte - colony stimulating factor
GM-CSF - Granulocyte macrophase - colony simulating factor
CFU-GM - Colony forming unit - granulocyte macrophase

Procedure for processing Peripheral blood progenitor cells

- Collection of 10 x 10^4 CFU-GM/kg or 2 x 10^6CD34+ cells/kg or mononuclear cells 2x 10^8/kg by apheresis
- Addition of autologous plasma and cryprotactant (DMSO)
- Controlled-rate freezing
- Storage in liquid nitrogen

Advantages of PBSC transplantation compared with bone marrow transplantation are:

Comfort and convenience to the donor/patient

- Procedure is less complex, has lower risks, is less painful and can be done on an out-door basis
- Donor does not undergo anesthesia or hospitalization
- Free from the effects of prior chemotherapy/radiotherapy
- Procedure take as little as 3 hours
- Harvest and transplant can be performed as out-patient procedure.

Peripheral blood stem cells are cleaner

- A graft is free from cancer cells
- Bone and tissue debris from bone marrow harvesting is eliminated

Patients rebound more quickly

- Rate of post-therapy recovery of bone marrow is accelerated
- Fewer number of red cell and platelet transfusions may be required
- Patient susceptibility to infection is reduced
- Incidence of GVHD with HLA matched PBSC transplantation is almost the same as with autologous bone marrow transfusion.

STEM CELL ASSAY

There are two methods that can be used to determine the adequacy of the stem cells collection for transplantation. These assays are the colony-forming unit (CFU) and CD34+ analysis.

Colony Forming Unit (CFU) Assay

The CFU assay involves growing a small portion of stem cell collection in a culture. A single stem cell or progenitor cell will form a group of mature blood cells and these groups may be counted using microscope. The CFU assay is the best indicator of the ability of stem cells to grow in the patient after transplantation, but major drawback of this assay is that the results are not available for 2 weeks. The CFU test cannot be used to determine on day to day basis.

CD34+Cell Assay

The CD34+ test is more prominent test for measuring the stem and progenitor cell contents of the collection. The stem cells are distinguished from the majority of the cells in the bone marrow or blood because the stem cells display a protein on their surface designated as CD34+. This exclusivity allows to count CD34+ cells using a technically complex machine called a flow-cytometer.

- The major advantage of CD34+ testing over the CFU test is that CD34+ results can be available within 24 hours.
- This rapid CD34+ testing can help to prevent the patient/donor from undergoing a needless second stem cell collection in cases where the first collection contained enough stem cells needed for transplantation. A number of studies have reported an adequate transplantation dose consists of between 2-5 million CD34+ cells/kg of patient's weight.

- The CD34+ analysis can also be used to check the patient's or donor's readiness to begin the stem cell apheresis collection.

STEM CELL SELECTION

Advance Cells Selection Technology for Isolating CD34 + Cells

Cells Selection is a specialized laboratory process used to reduce non-stem cell contaminants, including tumor cells, from transplant products to yeild CD34 + stem cells in a purified and concentrated form.

During the transplantation therapy, stem cell transplant product (called graft), derived from either bone marrow or peripheral blood, is harvested and processed in the laboratory and later re-infused into the patient intravenously following chemotherapy and/or radiation therapy.

This transplant is essential to regenerate the destroyed bone marrow from the effects of toxic drugs and radiation. The cells which are required to regenerate the marrow are CD34+stem cells (cells which express the CD34 marker Ag on the surface). Hence it is necessary to collect their optimum and pure dose for a successful transplant.

However, the harvested stem cell product contains a significant number of tumor cells, T cells and other contaminants along with the CD34+cells. The tumor cells present in the transplant may increase the tumor burden in the patient reducing the efficacy of the transplant with increased risk of relapse and the T cells present in the product may increase the incidence of GVHD. Hence it is extremely vital to purge or remove the tumor cells, and non-stem cells from the transplant.

Cells Selection performs this function of tumor cells purging as well as removing the T cells and other contaminants.

Cells Selection Applications:

There are various processes of cell selection such as Centrifugation, Ficoll Density Separation and Antibody mediated or immuno selection. Among these, Antibody mediated (Immuno) selection is commonly used owing to its high specificity and efficacy. It involves the actual physical selection of cells.

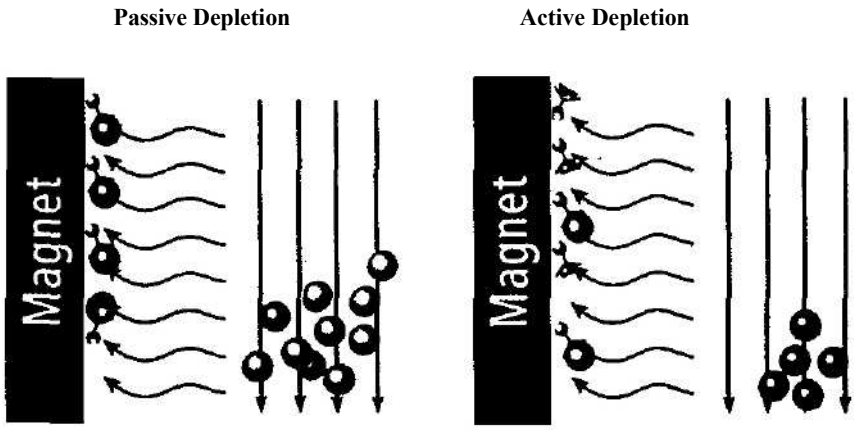
The applications of Antibody mediated Cell Selection are:

1. Cell Selection with the help of Columns.
2. Immunomagnetic Cell Selection (Cell selection with Magnets).
3. Cell Selection with Density Gradient Centrifugation (Cells are isolated directly from Whole Blood).
4. Antibody Based Enrichment System with Buoyant Density Centrifugation. (Density of unwanted cells is increased and they are removed, thereby enriching the stem cell product).

In the immunomagnetic stem cell selection there is simultaneous Positive and Negative selection in a single automated step.

1. **Positive Selection**: (Passive Depletion) Here the CD34+cells are magnetically retained and unwanted cells are removed.
2. **Negative Selection**: (Active Depletion) Tumor cells are magnetically retained and the desired cells are released and collected.

Some of the advanced machines that provide Immunomagnetic Cell Selection available are Isolex, Clinimacs, Seprate.



Isolex Magnetic Cell Selection System from Baxter:

An advanced, fully automated, closed system cell selection device used to specifically isolate or select stem cells from the transplant obtained from the patient. Through an immunomagnetic process, unwanted contaminants, including tumor cells, are removed leaving enriched stem cells.

Reagent Kit for Isolex 300 i contains:

- One vail of Anti-CD34 Monoclonal Antibody
- One vail of Dynabeads M-450 Sheep anti-Mouse IgG
- One vail of PR 34+Stem Cell Releasing Agent

Mechanism: The antibody mediated immunomagnetic technology of Isolex 300i Magnetic Cell Selector specifically recognizes and capture stem cells. In positive selection (passive depletion), the selection is accomplished using stem cell antibodies (protein molecules that bind only to the stem cells) while most other cells (T cells and tumor cells) are removed. Passive Depletion is simultaneously combined with Negative Selection (active depletion) wherein remaining tumor cells are magnetically retained while purified CD34 cells are released and collected.

The Key steps in the selection process are :

1. **Sensitization**: Anti-CD34 monoclonal antibody is mixed with cells to bind to CD34+ cells.
2. **Rosette / Capture** : Following washing, Magnetic beads (Dynabeads) coated with sheep anti-mouse IgG are added to remove unbound antibody. They recognize the murine-derived anti-CD34 antibody, leading to the formation of bead-target cell rosette complexes.
3. **Separation** : A magnetic field is applied enabling the CD34+ cell bead complexes to be separated magnetically from the rest of the cell suspension.
4. **Release**: After removing the non-target cells by washing, stem cell releasing agent is added to separate the bead/antibody from the CD34+cells.

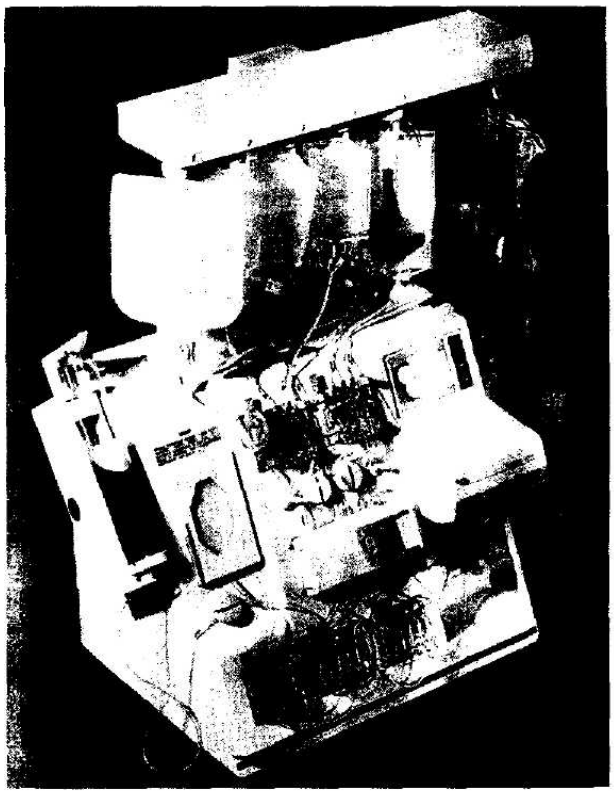
The Separated CD34+ cells are then washed to remove residual reagents and collected.

Salient Features of Isolex 300i Magnetic Cell Selector:

1. Baxter's Isolex is the only CE (Europe) marked and FDA (USA) approved CD34 selection system.
2. The only system to provide simultaneous Positive and Negative selection of cells in a single step.
3. Provides more than 90% CD34 + cell purity and more than 60% CD34 + cell yield.
4. High levels of tumor cell and T cell depletion (4.2 log).
5. It is costly procedure.

Indications and Usage

1. Indicated for processing autologous peripheral blood progenitor cell (PBPC) products to obtain a CD34+ cell enriched population intended for hematopoietic reconstitution after myeloablative therapy.
2. Isolex processing reduces the number of non-CD34+ (non-target) cells, including tumor cells, in the autologous graft as compared with an unselected PBSC.
3. Consistently reduces tumor cells in PBSC collection harvested from patients with leukemia, multiple myeloma, breast cancer etc.
4. Non CD34+ selected products are contaminated with tumor cells and associated with poor clinical outcome
5. Use of cells selected with Isolex would serve to reduce the incidence of GVHD
6. Patients show rapid engraftment with selected cells



ISOLEX 300 i System

Fig. 19.3

7. Role of CD34 + selected cells in Gene Therapy:
   - Replace missing or defective genes in stem cells to express normal protein (Hb)
   - Introduce therapeutic genes into stem cells

CORD BLOOD STEM CELLS

Cord blood is defined as blood contained within umbilical cord and in the placental circulation. Like embryos, cord blood cells contains primitive stem cells that give rise to red blood cells, white blood cells, platelets and immune system. Collection of cord blood for harvesting stem cells should be performed in a manner which would not alter the delivery of the infant and would not be harmful to the infant and mother.

Donor evaluation

Informed consent

Informed consent for collection of cord blood prior to delivery must be taken preferably from both parents or at least one parent or a legal guardian after explaining the purpose and process of cord blood collection.

Donor suitability

- A personal and family medical history of the mother; and father also if available, should be obtained and documented before or within 48 hours of the collection of blood. Such medical history should include an assessment of genetic disorder affecting mother, father and siblings of the new bom.
- A sample of blood from the mother should be collected 48 to 72 hours prior to delivery for testing for anti-HIV 1 &2, HBsAg, anti-HCV, syphilis; and anti-HBc.
- If any of the test is positive for any of these markers, cord blood should be prepared after consent from the parents and the transplant physician that if it could be used for autologous transplant in the infant if required. Otherwise, cord blood should not be collected for allogeneic transplantion.

Testing of the cord blood

A sample of cord blood should be kept separate at the time of collection and tested for:

- Cord blood cells should be tested for ABO and Rh group.
- For community cord blood collections (allogeneic transplant), Class 1 HLA typing and preferably Class 11 HLA typing also should be performed shortly after collection. It will be necessary to determine HLA compatibility with allogeneic related recipient to reduce the chances of GVH disease. It is not required for autologous transplant.
- An analysis of CD 34 + cells or CFU-MG or mononuclear cells is done to evaluate the progenitor cells contents. Mononuclear cells can be counted in peripheral blood smear.

Facility of notification and authorization

An agreement/acknowledgement between the administration of the hospital and collecting faculty should be established. The blood bank should have license from the licensing authority for processing the cord blood.

Cord blood Collection; Processing Method and Procedures

Cord blood collection personnel:

- Cord blood collection should be performed by the trained gynecologist/obstetrician or trained nurse, conducting the delivery, in collecting the blood from the umbilical cord.
- They should have experience in venipuncture. infection control, handling of bio-hazardous material.

Collection method

- Procedure used should be documented to result in retention of adequate sterility and stem cells viability.
- The collection of cord blood should not result in any deviation from normal obstetric procedures (e.g. time of clamping the cord).
- Both in utero (prior to placental delivery) and ex-utero (following placental delivery) collection methods are acceptable and have comparable efficacy. Use of a semi-closed or closed system (heparinized syringe or bag with ACD/CPD anticoagulant) by venipuncture of the umbilical vein under aseptic conditions is recommended.
- Use of open vessels for collection is unacceptable.
- The collection procedure should not be harmful either to mother or infant or compromise the cord blood collection.
- About 80-120 ml of cord blood can be collected.

Procedure:

- Umbilical cord is clamped and cut as in routine manner.
- Insertion site on the umbilical vein is prepared by cleaning with betadine and alcohol.
- Sterile needle of collection bag is inserted into the umbilical vein pointing towards placenta allowing the cord blood to flow into the collection bag by gravity. Once the vein collapses or blood ceases to flow, the collection procedure is complete.
- On average 60-120 ml of anti-coagulated cord blood can be collected.
- Alternatively heparinized syringe can be used for the collection of cord blood. About 85 ml of blood can be collected with syringe method and contamination rate is less than 1%.
- The procedure can be performed in vaginal or caesarian deliveries including multiple births.

Processing and storage method

Collection and storage *vessels:* Cord blood should be collected and stored in vials, bags, or other containers approved for cryopreservation of hematopoietic progenitor cells or validated by the cord blood bank.

Sterility testing: Sterility testing for bacterial and fungal contamination should be performed on a sample collected after addition of the cryoprotectant mixture and the results evaluated as a quality control procedure.

Cryopreservation: Cells should be cryopreserved in 20% dimethyl sulfoxide (DMSO) [10% final concentration].

Labeling: The final product should be labeled and/or tagged and should contain donor's identification number and date of collection. The label should be legible and indelible.

Uses of cord blood:

- Stems cells are primarily used in transplant medicine to regenerate a patient's bone marrow and immune system after they have been treated with chemotherapy and/or radiation to destroy the cancer cells. These stem cells also migrate to the patient's bone marrow and have the ability to regenerate themselves where they reproduce, creating a new blood and immune system for the patient.

- In the treatment of a variety of cancers and blood diseases.
- Stem cells are used for own use (antologous), in case of future need.
- Lower yield of $4xl0^6$ hematopoietic progenitor cells, restricts currently use of cord blood stem cells in children.
- Recently successful engraftment of cord blood stem cells from unrelated donors in recipients weighing 45 kg have been reported.

Advantages of cord blood cells over bone marrow

- Bone marrow is difficult to match between donor and recipient. Chances of bone marrow match within family are 25% whereas in cord blood cells chances are up to 50%.
- Collection of bone marrow is invasive and can be painful, as it requires many insertions to extract the marrow from the donor's bone with a needle and syringe. This is a lengthy process that requires general anesthesia. It is also an expensive procedure.
- Cord blood transplant may have higher survival rate, a higher quality of life after transplantation and less frequent hospitalization due to fewer complications such as Graft vs Host Disease (GVHD) due to the inability of juvenile cord blood cells to mount an attack against the recipient.
- Cord blood has been found to contain fewer viruses than in old donors' bone marrow.
- This make overall cost of cord blood transplantation less than traditional bone marrow transplants.

FREEZING OF HEMATOPOIETIC PROGENITOR (STEM) CELLS

Freezing of hematopoietic progenitor (stem) cells collected from bone marrow, circulating peripheral blood and umbilical cord differs from freezing of red cells with glycerol. Dimethyl sulfoxide (DMSO) has been found to be valuable cryoprotectant for many tissues and stem cells. DMSO rapidly traverses the cell membrane with minimum osmotic stress and does not need its removal before transfusion. The concentration of 1.5 M (DMSO) or less appears to be relatively non-toxic. The temperature of storage must be enough low to prevent the formation of extracellular ice, which may lead to dehydration injury.

The standard mixture contains 20% DMSO [final concentration 10%], an isotonic saline or electrolyte solution, and a source of protein, usually autologous plasma or human serum albumin. Stiff et al modified the standard freezing technique to incorporate a low-molecular weight fraction of hydroxyethyl starch (Pentastarch, McGaw chemical co., Irvine CA) as an additional cryoprotectant. This modification makes it possible to use half the amount of DMSO (final concentration 5%) with comparable recovery and viability to standard method. A lower concentration of DMSO reduces the unpleasant side effects of DMSO which are occasionally experienced, when large volume of thawed cells are infused.

At room temperature 10% DMSO may be toxic to the progenitor cells, so the freezing process must not be delayed. Each bag is placed in a metal container or between metal plates and is put in programmable freezer to cool at the control rate of -1 to-5 °C per minute to-80 to-100 °C or in vapour of nitrogen. Then transferred to liquid nitrogen freezer for storage at-196°C.

FREEZING OF BONE MARROW

Controlled-Rate Freezing of Bone Marrow

After harvesting, bone marrow is extensively processed before freezing. The bone marrow is put in stainless steel beakers with tissue culture medium and heparin for dilution and anticoagulation. The various culture media used for cryopreservation are MEM, RPMI, HBBS and TC199. Then the marrow is pressed through stainless steel mesh screens, of 300 and 200 μm mesh size, to filter out most of the bone chips, clots, fat and fibrin. The collection is then transferred to sterile blood transfer pack and transferred to laboratory. In the laboratory the bone marrow is processed by using cell separator to remove 95% of red cells while recovering more than 75% of colony forming unit-granulocytes macrophase (CFU-Gm) or CD34 + cells in the final product containing more than 80% of mononuclear cells (MNCs). The concentration of nucleated cells is usually adjusted before cryopreservation to 3-4 x 10^7 cells/ml.

Equal volume of 20% DMSO and cells suspension are mixed vigorously at 0-4 °C and transferred in 50-100 ml aliquots to polyolefin bags and immediately frozen at controlled rate of-1 to -5 °C per minute to -80-100 °C in mechanical freezer or in the vapour of nitrogen and stored at -196 °C in liquid nitrogen.

FREEZING OF PERIPHERAL BLOOD STEM CELLS (PBSCS)

Peripheral blood stem cells are harvested with automatic cell separator. The concentrations of PBSCs having 3 x 10^8 cells/ml in autologous plasma can be frozen and stored as bone marrow stem cells.

FREEZING OF CORD BLOOD

50 ml cord blood is taken in freezing bag and chilled to 4 °C. A 20% solution of DMSO in saline is brought to 4 °C in a separate container and kept in ice bath. The cord blood is vigorously mixed with an equal volume of the 20% DMSO solution and the bags are frozen as described for bone marrow and PBSCs.

Uncontrolled-Rate Freezing of Bone Marrow Stem Cells

A mixture of 10% DMSO and 12% HES is added to an equal volume of the cells suspension in human serum albumin. Stem cells preparation that has DMSO at 5% concentration, hydroxyethyl starch (HES) at 6% and human serum albumin at 4% in glucose saline can be frozen directly at -70 to -80°C without controlled temperature and does not cause damage during short period. However for longer storage it has been advised to store at -135°C.

Thawing and Transfusion

Cryopreserved progenitor cells can be thawed by immersion of the bag into well-stirred water bath at 30-37 °C. This is done at or close to the patient's bed side, and the thawed cells should be immediately transfused to avoid clumping in the material.

20

Transfusion in Transplantation

The importance of organ transplantation as a clinical activity has increased in recent years. Kidney transplantation still accounts for the vast majority of transplant operations, besides autologous and allogeneic bone marrow and peripheral blood stem cells transplantation are now well established procedures in the treatment of certain hematologic malignancies. In addition, transplantation of liver is now done with growing success and that of other solid organs including, heart, lung and pancreas is carried out, at least on experimental basis.

The blood bank plays a vital role in transplantation programs in transfusion of blood and blood products in transplantation.

BONE MARROW TRANSPLANTATION

Bone marrow transplantation is used to treat patients with various hematologic disorders like :

- Various leukemias
- Aplastic anemia
- Hemoglobulinopathies like thalassemia major
- Immunodeficiency syndrome
- Malignant diseases

Bone marrow transplant recipients present different type of problems than solid organs transplant recipients :

- The bone marrow transplant recipient is totally ablated before receiving an allogeneic marrow, due to it the recipient is at risk of opportunistic infections.

- Allogeneic bone marrow graft has immunologically potent lymphocytes, and the donor lymphocytes can recognize and respond to the HLA antigens in the recipient, resulting in graft-versus-host disease (GVHD). Graft rejection and GVHD are more severe with mismatched HLA antigens. HLA-A, HLA-B, HLA-C and HLA-DR matching of donor and recipient is important.
- Another difference is that the ABO antigens do not serve as a transplant barrier for marrow recipients. The donor-recipient ABO incompatibility does not increase the incidence of graft rejection or GVHD. The data shows that ABO antigens are not present on stem cells

Red Blood Cells Transfusion in BMT

- Major ABO-compatible transplant recipients require few RBCs transfusion than in ABO-incompatible transplant. ABO antibodies can cause delay in donors RBC production, necessitating more RBC transfusion. The median time for incompatible antibody disappearance in ABO - incompatible transplant is about 38.5 days (range 0 -116 days).
- During the transplant, the recipient's bone marrow hematopoietic stem cells and immune system are ablated and reconstituted with the donor's cells. This causes problems in blood bank related to transfusion support and the selection of blood during engraftment. It becomes difficult to decide whether to use blood components of donor's or recipient's ABO blood group.
- For major ABO-incompatible marrow recipients, recipient type RBCs are provided until there are no detectable incompatible plasma antibodies and the DCT test is negative. Any plasma containing products should be donor type, to prevent passive transfer of incompatible A and B antibodies.

  For example, in group O patient who received group A bone marrow transplant, the patient continues to produce anti-A and anti-B. In this situation group O red cells transfusion is given until anti-A is no longer detected. Components containing significant amount of plasma, such as platelets and FFP must be compatible with the donors blood groups. In this case group A platelets or FFP should be used.
- For minor ABO-incompatible marrow transplants, (recipient A/B blood group and donor O group marrow) RBCs should be donors type, and plasma products should be recipient type until recipient RBCs are no longer detected.
- For transplant that are both major and minor ABO-incompatible, RBCs should be group O and plasma products should be of AB group.

  For example, in case B group marrow is transplanted in A group recipient or reverse, then group O red cells and AB group plasma or platelets are used.

Granulocytes Transfusions in BMT

Granulocytes transfusions are beneficial in granulocytopenic patients (< 0.2×10^9 granulocytes/ L) who have infection unresponsive to antibiotics. However, certain problems are associated with granulocytes transfusions like:

- Insufficient granulocytes collection for effective transfusion dose.
- Short intravascular survival time of granulocytes (about 6 hours), suggesting daily administration of granulocytes is too frequent.
- Antibodies from prior RBCs transfusions may limit effective granulocytes transfusions of even HLA matched or partial HLA-matched donors.

- Increase incidence of CMV infection after granulocytes transfusions.

Granulocytes transfusions should be continued till the count of granulocytes is more than 0.2 x 10^9 /L. However more rapid WBC engraftment rates with the use of GM-CSF and G-CSF reduce the period of granulocytopenia; granulocytes transfusions have limited use.

Platelets Transfusions in BMT

Indications of platelets transfusions are:

- When platelets count falls to < 15 to 20 x $l0^9$/litre (spontaneous bleeding usually does not occur until platelet counts are < 5 to 10 x 10^9/litre).
- When platelets count > 20 x $l0^9$/litre with bleeding or associated with GVHD or viral infection.

The adult dose of platelets is 2.4-3x10^{11} platelets. This can be provided by infusing platelets separated from 5 to 6 units of whole blood (450 ml) or obtained from one donor by plateletpheresis. Alloimmunization is delayed and/or decreased by the use of single-dolor platelets. Leukocyte - poor blood products can decrease the incidence of alloimmunization and refractoriness to platelet transfusion.

Complications and their management:

- BMT patients are often immnuosuppressed and may be at the risk of graft-versus host disease (GVHD), which is a potentially fatal complication by transfused lymphocytes. Treatment of red cells and platelets by gamma irradiation under controlled conditions inactivates the lymphocytes and reduces the risk of GVHD.
- Some immunosuppressed patients are at the risk of cytomegalous virus (CMV) infection transmitted by blood transfusion. This can be avoided or reduced by transfusing blood that is tested and contains no CMV antibodies or by the use of leukocytes-depleted blood components.

Conclusions

Transfusions Prior to BMT

- Transfusion of blood or components before BMT diminishes the success of long term engraftment.
- Transfusion from blood relative, specially relative who serve as marrow donor, is likely to immunize the recipient.
- Lower risk of immunization with random donor blood.

To eliminate immunization give:

- Leukocytes-depleted cellular components (red cells / platelets)
- Single donor component (e.g. platelets prepared from apheresis)
- Gamma irradiated blood cellular components
- HLA-matched blood cellular components

Autologous Blood for Marrow Recipient

- Autologous pre-donation for blood replacement after marrow harvest is very useful and safe procedure and significantly reduces blood transmitted diseases and immunization.
- Bone marrow aspirate and blood loss is 300-1,800 ml (average 1,000 ml). Bone marrow donor suffers a loss of 20 - 30% blood volume.
- Hemoglobin after donation should be 12.5 g/dl. Iron is given if needed.

Scheme of Autologous Pre-deposit for Morrow Donor

Donation	Pre-dohation Hb.g/dl	Days prior to Surgery	Vol. of Donation
First	>13	14 (9-27)	300 (200-350)
Second	>12	8 (4-20)	340 (200-450)

Transfusions after BMT

- Red cells transfusion support is required until
  - Engraftment occurs
  - Blood cells production is adequate
  - To maintain Hb 9-10 g/dl
- To prevent GVHD:

  Blood cellular components (except autologous blood) must be irradiated.
- To prevent CMV:

  If the recipient is negative for CMV
  - Use components from CMV sero-negative donor
  - Or use highly efficient leukocyte reduction filters

Platelets Transfusion :

- Platelets are given every 2-3 days to maintain platelet count of >10,000/ul
- If patient becomes refractory to random donors platelets, HLA matched platelets, prepared from apheresis, are given.

Granulocytes Transfusion:

- Prophylactic granulocytes transfusion is not given.
- Therapeutic granulocytes are rarely needed.

TRANSFUSION IN SOLID ORGANS TRANPLANTATION

Solid organ transplantation is the ideal therapy for organ system failure in certain diseases. Allogeneic transplantation of foreign tissue can induce a cellular and humoral response that often leads to graft rejection. The severity of rejection often can be reduced by selecting HLA-matched organ for the recipient

The ABO system antigens are also a barrier to organ selection for transplantation. All normal individuals possess isoagglutinins to those antigens that they lack. Therefore, the same rules that govern the selection of blood for transfusion apply to the selection of most renal and heart transplant.

In addition to laboratory testing, graft rejection can be minimized with immunosuppressive therapy. Steroids decrease the humoral response and the cellular response can be inhibited with cyclosporine and azathioprine.

The third mechanism that influence is based upon the induction of tolerance to donor-specific antigens. The transfusion of blood from a potential donor promote graft acceptance through tolerance induction if the patient is not sensitized after donor-specific transfusions.

BLOOD TRANSFUSION IN LIVER TRANSPLANTATION

Liver transplantation is a lengthy, complex operation. It is done in patients who are generally in the end-stage of liver failure and frequently have coagulation deficiencies and thrombocytopenia.

The transfusion needs of these patients are more than any other transplantation due to:

- Risk of excessive bleeding due to the presence of pre-existing hemorrhagic disorders such as esophageal varices.
- Technical factors associated with the lengthy and complicated surgery.
- Disease associated hemostatic dysfunctions (multiple coagulation defficiency and thrombocytopenia).
- Removal of liver during surgery leave the patient ahepatic for some time, which further complicates the coagulation. This phase is associated with:

  Decrease in Factors VIII and V
  Decrease in fibrinogen
  Increase in fibrinolysis

  This period continues until approximately 30-60 minutes after donor liver revascularization and during this period it is very difficult to maintain hemostasis.
- The post operative period is often associated with hypercoagulable state, perhaps due to slow recovery of normal levels of anticoagulant proteins such as antithrombin III and protein C and S after surgery.

Requirement of blood and blood products:

- On average liver transplant patients require 15 to 30 units of whole blood or red cells, 20 to 30 units of fresh frozen plasma (FFP), 10 to 20 units of random-donor platelets (or equivalent by plateletpheresis) and 8 to 10 units of cryoprecipitate during the operation and in the immediate post operative period.
- Intraoperative salvage may reduce the need of units of blood during operation.
- In early phase of operation the use of whole blood may be optimum strategy because it provide necessary volume support, oxygen-carrying capacity and stable coagulation factors.
- During ahepatic phase as there is cessation of the production of coagulation factors and enhanced fibrinolysis. FFP and cryoprecipitate(factor VIII) along with red cells are required.
- Pediatric liver transplant may require on average 4 units of red cells (0.6 units of RBCs /Kg body weight), 7 to 8 units of FFP, 3 to 4 units of platelets, and 1 to 2 units of cryoprecipitate.

Monitoring of coagulation :

- Coagulation is monitored hourly with rapidly assayed prothrombin time, partial thromboplastin time, fibrinogen level, platelet count and other key coagulation factors (e.g. V and VII) if indicated.
- Aprotinin is bovine serine protease inhibitor, which reduces fibrinolysis and protects platelet function during surgery. However, its dosage regime has not been standardized, anaphylactic reactions and thrombosis have been reported during liver transplant.

Considerations of ABO and Rh:

Except in emergencies, donors livers should be ABO-compatible with the recipient and ABO-idcntical red blood cells and fresh forzen plasma is used. In case of massive transfusion group A/ B recipients can be sitched to group O RBCs and group AB recipients can be switched to group A

RBCs. If the supply of AB FFP is insufficient, early use of A group RBCs followed by a switch to group A FFP is appropriate. A gneral rule for massive transfusion is to switch red cells first, then switch plasma, and reverse the order when returning to the patient's original blood type.

Transfusion support of RH(D)-negative patients not immunizd to the D antigen is not standardized. Mostly it is preferred to provide D-negative blood to D-negative premenopausal females, if needs are excepted to be moderate. If massive blood loss occurs, the patient could then be switched intra-operatively to D-positive blood, if necessary. Production of anti-D occurs less frequently in D-negative liver transplant patients. D-negative males and post-menopausal D-negative females without anti-D in their blood can be transfused with D-positive blood.

Coagulation Considerations:

During surgery, hemodilution, platelet consumption, disordered thrombin regulation, and fibrinolysis derange the hemostatic process. The coagulopathy is specially severe during the anhepatic and early reperfusion stage. Hematocrit guides the use of RBCs, colloid and crystalloids; the platelet count guides transfusion of platelets; the prothrombin time and activated partial thromboplastin time guide use of FFP; fibrinogen determinations guide the use of cryoprecipitated AHF and atifibrinolytic agents.

Serologic problems in liver transplantation:

- Positive DAT and alloantibodies prior to surgery, as many patients for liver transplant may have already received many blood transfusions, may cause problem in pretransfusion serological evaluation.
- Donor lympocytes transplanted with the organ may continue to survive and function in the recipients. Transplanted B lymphocytes may continue to produce antibody(ies) that they may produce in the donor.

The production of donor-derived red cells antibody(ies) occur more frequently in the recipient who receive ABO incompatible liver. In such cases donor-derived antibody(ies) develop in 1 to 2 weeks and can persists for 6 months.

Donor-derived antibody(ies) can result in DAT positive. During this period blood selected for transfusion should be compatible with both the recipient and donor-derived antibody(ies).

- Large quantity of blood transfusion may cause irnmunosuppression in the patient.
- Patients who receive large volume of blood may have higher rate of bacterial infection and transmission of CMV.

Blood Transfusion in Kidney Transplantation

The renal transplantation is simpler and faster surgical procedure than other solid-organ transplants. The best graft survival rates are obtained when kidneys are obtained from HLA-identical, ABO-compatible siblings but such donors are available for few cases only. Three general strategies are considered by transplantation surgeons and immunologists before transplant to minimize graft rejection ;

(1) The use of immunosuppressive agents like azathioprine, prednisone and cyclosporine.
(2) The matching of donor and recipient antigens to minimize graft 'foreignness'. Antigen disparities that effect graft rejection include the ABO blood group antigens and HLA antigens. It is not clear what combination of HLA gene products matching promotes

optimal graft survival. HLA-DR is the most critical for good graft survival. In highly sensitized recipients, it is necessary to match for HLA-A and HLA-B because of the presence of class 1 antibodies. In highly sensitized recipients, identification of HLA serum antibodies is also important.

(3) The induction of tolerance to donor-specific antigens : Several studies have shown that donor-specific blood transfusion and transplants from living related individuals have potential benefits.

The effects of blood transfusion on the success of renal transplantation are complex and paradoxic. The transfusion of blood may lead to HLA antibody immunization. Yet graft survival rates are improved with pre-transplant blood which does not result in forming HLA antibodies. It has been observed that the transfusion effect is lost with the newer immunosuppressive agents. Preferably transfusion of leukocyte-reduced red cells with leukocyte-deplated filters should be given to reduce the chances of immunization.

The data for re-combinant Epo, G-CSF, and GM-CSF led its routine use in patients with renal failure. Therapeutically effective levels are maintained after single intravenous or subcutaneous injection for 24 hours. An increase in reticulocytes counts occur with in 10 days if EPO is given daily. Epo avoids viral exposure and reduces HLA sensitization associated with blood transfusion.

Perioperative Transfusion

With overall change in intraoperative surgical practice, lower hematocrit levels (30%) are accepted without transfusion. Some workers have found a higher incidence of delayed graft function when patient's Hct level is more than 30%. This has been attributed to sludging of blood in microcirculation. Minimum amounts of red cells are used during kidney transplant, and other components are seldom required. Mostly two cross-matched units are kept for the kidney transplant. Many institution simply use type-and-screen procedure.

TRANSFUSION IN HEART TRANSPLANTATION:

Heart transplantation is done to treat cardiomyopathies and end stage of ischemic heart disease. As heart has extremely short total ischemic time (3 hours for heart, compared with 72 hours for kidneys) HLA matching is not feasible. Total ischemic time is the duration during which there is no blood flow through organ. The single most important HLA test performed pretransplant is the HLA antibodies screening. Recipients with no HLA antibodies receive transplants without HLA - matching. Those with HLA antibodies require pretransplant cross-matches for class 1 and 11 HLA match to determine recipient-donor compatibility.

Heart transplants need on average 5 to 6 units of RBCs, 4 units of FFP and Ito 2 units of platelets.

TRANSFUSION IN LUNG **TRANSPLANTATION:**

Majority of cases with emphysema and cystic fibrosis need double-lung transplants. The indications for single-lung transplant are pulmonary fibrosis and emphysema. In addition, more patients now undergoing single-lung transplant for primary hypertension, previously treated by heart-lung transplantation.

Short ischemic time like heart, HLA matching is not possible. However, the HLA matching between recipient and donor play important role in live-donor transplant in graft survival rate.

The requirement of blood and its components is similar to that of heart transplant.

TRANSFUSION IN PANCREAS TRANSPLANTATION:

The primary indication of pancreas transplant is diabetes. The majority of pancreas transplants are simultaneous pancreas/kidney transplants, equal numbers of pancreas transplants following kidney transplant and few pancreas transplants alone. Increased long survival rates of transplant have been reported with HLA-DR matching.

Pancreas transplants alone need about 2 units of RBCs and pancreas-kidney transplants recipients need on average 3-4 units of RBCs.

Because of risks of cardiac complications with pancreas transplantation, islet cell transplantation has been actively pursued.

21
Preparation of Solution & Methods

The definitions, calculations given below will serve as simple principles necessary for preparing
solutions.

Definitions :

Mole, gram-molecular weight

Weight, in grams, of a substance is equal to the molecular weight of the substance.

Molar Solution

A one molar (IM) solution contains one mole of solute in a liter of solution. The solvent is generally distilled water unless otherwise indicated.

Gram-equivalent weight:

Weight in grams of a substance, which will produce or react with one mole of hydrogen ion.

Normal Solution :

A one normal (IN) solution contains one-gram equivalent weight of solute in a liter of solution.

Percentage solution :

The percent of solution gives the weight or volume of solute present in 100 units of total solution. Percentage can be experessed as :

- Weight/weight(w/w) - grams of solute in 100g of solution

- Volume/volume(v/v) - milliliters of solute in 100 ml of solution
- Weight/volume(w/v) - grams of solute in 100 ml of solution

Atomic weights (rounded to whole numbers) :

- H=l; 0=16; Na=23; Cl=35; K=39; P=31; S=32

Molecular weights:

- NaH_2PO_4 = 23+2+31+64 = 120g
- KH_2PO_4 = 39+2+31+64 = 136 g

Molar solutions:

- 1MKH_2PO_4
  - Molecular weight of KH_2PO_4 = 39+2+31 +64 = 136g
  - 1M KH_2PO_4 requires 1 x 136= 136 g of solute KH_2PO_4 made upto 1000ml of distilled water.
- 0.15M$NaH_2PO_4.2H_2O$
  - Molecular weight of $NaH_2PO_4.2H_2O$=23+2+31+64+36=156 g
  - 0.15M $NaH_2PO_4.2H_2O$ requires 0.15 x 156 = 23.4 g of solute
  - $NaH_2PO_4.2H_2O$ made upto 1000ml of distilled water

Normal solutions:

- **IN NaOH**
  - Molecular weight of NaOH = 23+16+1= 40g
  - IN NaOH requires 1x40 =40 g of solute (NaOH) made upto 1000 ml of distilled water.
    One mole of NaOH dissociates into one mole of H+,so gram-equivalentweight and gram-molecular weight are same.
- **1NHCL**

  Molecular weight of HCL = 36g

  IN HCL requires 36 g solute made upto 1000 ml of distilled water.
  One mole HCL dissociates into one mole H+,so gram-equivalent weight and gram-molecular weight are same.
- **1NH_2SO_4**

  Molecular weight of H_2SO_4=98g

  1 N H_2SO_4= (98 ±*2)* = 49g salute made up to 1 L distilled water. One mole H_2SO_4 dissociates to give 2 moles of H^+, so the gram- equivalent weight is half of the gram- molecular weight i.e. 49g.

Percent Solution:

0.9% NaCL is Normal Saline

Normal saline requires 0.9 g solute (NaCL) made upto 100 ml of distilled water, pH 7.2 - 7.4

Phosphate buffer, Iso-osmotic

Prepare two stock solutions

(A) 0.15M$NaH_2PO_4.2H_2O$ 23.4 g/L

(B) 0.I5MNa_2HPo_4 21.3 g/L

Prepare working buffer solutions of the desired pH by mixing appropriate volumes of the two solutions. A few examples are:

pH	Solution A	Solution B
7.0	32.0 ml	68.0 ml
7.2	24.0 ml	76.0 ml
7.4	18.0 ml	82.00 ml
7.6	13.0 ml	87.0 ml
7.7	9.5 ml	90.5 ml

Normal human serum has an osmolarity of 289 ± 4 mM. Hendry (1961) recommended slightly different concentrations of the stock solution, namely 25.05 g/L NaH_2PO_4.2H2O and 17.92g/L Na2HPO4, for an iso-osmotic buffer.

Phosphate-buffered saline

- Take equal volume of iso-osmotic buffer, pH 7.4 and 9g/L normal saline solution.

  Buffered saline is certainly better than normal saline for suspension of red cells for use over 12-24 hours period and storage at 4°C.

HEMOGLOBIN ESTIMATION OF BLOOD DONORS

COPPER SULPHATE SOLUTION SPECIFIC GRAVITY METHOD

The method is based on specific gravity and is reasonably reliable method for determining the hemoglobin of blood donors. It is indirect measure of the Hb value.

Preparation of Copper Sulphate Solution

$CuSO_4$ solution of sp. gr. 1053 is used for Hb estimation.

Method -1 Preparation of $CuSO_4$ Solution

Prepare stock solution of copper sulphate (sp.gravity 1.100)

- Dissolve 159.63 of pure air dried crystals of copper sulphate($CuSO_4$ $5H_2O$) in distilled water and make upto exactly 1000ml at 25°C. The specific gravity of the solution must be 1.100.

Preparation of $CuSO_4$ solution of specific gravity 1.052-1.055

Sp.gr	Stock solution	Distilled water	Hb equivalent
1.052	51ml	100 ml	12.0
1.053	52ml	100 ml	12.5 g
1.054	53ml	100 ml	13.0 g
1.055	54ml	100 ml	13.4 g

The solution should be stored at room temperature in tightly capped containers to prevent evaporation.

Method-2

Dissolve 8.33 gm of pure air dried crystals of copper sulphate ($CuSO_45H_2O$) in 100 ml of distilled water. The Specific gravity of the solution must be 1.053.

Quality Control

Functional validation of copper sulphate solution

Procedure:

1. Every batch of $CuSO_4$ should be checked with blood samples of known hemoglobin in a range around 12.5 g/dl, (e.g. 12 g/dl; 12.5 g/dl; 13.0 g/dl and 13.5 g/dl)
2. Gently place a drop of each blood sample into $CuSO_4$ solution of sp. gr. 1.053.
3. Drops of all blood samples with hemoglobin of 12.5g/dl or above will sink and those with Hb level below 12.5g/dl will float.

Measurement of Specific gravity of $CuSO_4$ solution

The specific gravity of the CuSO can be measured directly with a calibrated hydrometer, a specific gravity 1.053 + 0.0003 g/ml renders the CuSO4 solution acceptable for use in donor screening.

Procedure

A $CuSO_4$ solution of specific gravity 1.053 is used for determining haemoglobin level of 12.5g/dl.

1. Dispense 30 ml of copper sulphate solution (sp.gr 1.053) into appropriately labeled clean, dry, tube or bottle. Change the solution daily or after 25 tests and be sure that the solution is properly mixed before use.
2. The site of skin puncture i.e. finger tip is cleaned with antiseptic solution and allowed to dry.
3. Sterile disposable lancet is used for prick and there should be free flow of blood.
4. The drop of blood is collected either in capillary tube or pipette and allowed to fall gently from a height of lcm above the surface of the copper sulphate solution.
5. The drop gets enclosed in a sac of copper proteinate, which prevents any change in specific gravity for about 15 seconds. If the drop of blood remains at the surface, or rises from the bottom of the solution, the drop is lighter than the CuSO4 solution and the hemoglobin content of the blood is less than 12.5g/dl. However if the drop sinks within 15 seconds, Hb of blood is 12.5 g/dl + 0.19 or more.

Note: If plasma protein level of donors is on lower limit of normal, it is possible a donor may be rejected though he/she may be having required Hb.

Remedial source of errors in Hb estimation by Copper Sulphate method;

- Taking first drop of blood from finger prick.
- Squeezing the finger because the blood not flowing freely.
- Dirty pipette (pipette not flushed out properly every time).
- Chip of delivering end of pipette.

CYANMETHEMOGLOBIN (HICN) METHOD

Principle

The basis of the method is dilution of blood in a solution containing potassium cyanide and potassium ferricyanide. Hb, Hi and HbCO (but not SHb) are all converted to HiCN.

The absorbance of the solution is then measured in photoelectric colorimeter at a wave length of 540 nm or with a green filter.

Reagents Required

- Modified Drabkin's reagent (diluent)
- Cyanmethemoglobin (HiCN) standard solution.

Modified Drabkin's solution for estimating Hb by (HiCN) method :

Potassium Ferricyanide	200 mg
Potassium Cyanide	50 mg
Potassium Dihydrogen Phosphate	140 mg
Nomidet P40 (Shell Chemical Co.)	1 ml
Distilled water	1L

Other non-ionic detergents which can be used in place of Nomidet include Sterox SE (Harleco) 0.5 ml, Triton x - 100 (Rohm and Hass) 1 ml or Saponic 218 (Alcoac Inc.) 1 ml. The reagent should be clear and pale yellow in color and pH should be 7.0 - 7.4.

When measured against water as blank in a photoelectric colorimeter at a wave length of 540 nm. The absorbance must read zero. Drabkin's solution is commercially available.

Cyanmethemoglobin (HiCN) standard solution

- **Standard** solution is available commercially.
- **The** unused solution should be discarded at the end of the day on which the ampule is opened, to avoid contamination.
- The HiCN standard solution is used for direct comparison with blood which is also converted to HiCN in Drabkin's solution.

Blood Sample

- Measurement can be carried out on blood which has been stored in EDTA (1.5 mgm EDTA/ml)at4°C.
- Fresh capillary blood from finger prick can also be used if added immediately to reagent solution.

Method

1. Switch on the photoelectric colorimeter and wait for 15-20 min to warm up before use.
2. Add 20 |il of blood to 4ml of diluent. Stopper the tube containing the solution and invert it several times. Allow to stand at room temperature for 5-10 min to ensure the completion of the reaction. This solution of HiCN is ready to be compared with the standard.
3. Select filter of wave length 540 nm.
4. Set the colorimeter at zero against blank (Drabkin's Sol.).

5. Measure the absorbance of the HiCN standard solution (brought to room temperature if previously stored in refrigerator) in the colorimeter against the blank.
6. Observe the absorbance value of the test solution prepared as in Step 2.

Interpretation

- Record the absorbance (A) value directly in the colorimeter calibrated for direct reading of Hb in g/dl.
- If the colorimeter is not meant for taking direct reading of haemoglobin g/dl record the observance readings and haemoglobin can be calculated from the following formula:-

$$\text{Hb g/ dl} = \frac{\text{A}^{540}\text{of the test}}{\text{A}^{540}\text{of the std}} \times \frac{\text{Conc.of Std.} \times \text{Dilution factor}(\text{e.g. 201})}{1000}$$

Note: Absorbance, formerly called optical density

Precautions

- Blood should not be clotted.
- The reagent should be discarded, if it becomes turbid.
- The mixture of blood and reagent should be clear. Turbidity is due to contamination and give false result.
- Pipette should be accurate to take 20 ul blood.
- Standard solution should be discarded at the end of day on which the ampoule is opened.

HEMOCUE HEMOGLOBIN SYSTEM

Hemocue hemoglobin system is precise, accurate method for measuring hemoglobin.

It consist of:

1. Self -filling disposable microcuvette with reagent in dry form. It serve as a pipette, test tube measuring cuvette all in one.
2. Control cuvette is supplied with each photometer for verifying the calibration of the photometer.
3. Photometer: Calibrated at factory against the cyanmethemoglobin (HiCN) method

- Measurement at 570 nm and 880 nm
- Automatically zeroes itself after measurements
- Automatically checks the intensity of light and operation of photocells
- Chemical reaction takes place in the cuvette and the photometer automatically displays the result in less than 60 seconds.
- Hemoglobin measuring range 0-25.6 g/dl

Advantages:

- Quick safe and hygienic handling
- Accuracy is ± 1.5 %
- Microcuvette automatically draws precise volume of blood
- No blood dispensing, pipetting, or mixing of blood with reagent

LECTINS

They are saline extracts of seeds having agglutinating property with specific antigens and are useful as group typing reagents.

Extract of Dolichos biflorus → Lectin Anti-A_1 agglutinates A_1, cells but not A_2 cells

Extract of Ulex europaeus → Anti-H. It reacts with cells having H antigen activity

Vicia graminia →Anti- N. It reacts with cells having N antigen

Anti-A_1, and anti- H are commercially available.

POLYAGGLUTIEVATION

Mostly polyagglutination is associated with bacterial or virus infections and disappears when the infection is eliminated. Microbial enzymes alters the normal structure of the red cell membrane and exposes hidden receptors such as T, Tk, Th and VA. All normal adult human sera contain anti-T and react with the exposed T cryptantigen. Most cord sera lack anti-T. Therefore, ABO-compatible cord sera may be used as typing reagent. Monoclonal anti-A and anti-B can also be used to obtain valid results with polyagglutinable cells. Lectins (seeds extracts) can also be used to detect polyagglutination. (see table 19.2)

A somatic mutation (Tn) is persistent and the inherited forms (e.g. Cad) are permanent.

Table 19.2 : Reaction (polyagglutination) of red cells with lectins

Lectins	T	Tk	Th	Tn	Cad
Archis hypogaea (pea nut)	+	+	+	-	-
Dolicus	-	-	-	+	+
Glycine Soja (soyabean)	+	-	-	-	+
Salviasclarea	-	-	-	+	-

SALINE METHOD FOR IgM ANTIBODIES TESTING

Saline method is the simplest serological technique for determining the reaction of IgM antibodies against ABO, H, Ii, MN, Lewis (Le^a, Le^b), Lutheran (Lu), and P blood groups antigens. The IgM antibodies are approximately 750 times more efficient than IgG in agglutination reaction. The IgM pentamer, with diameter of 1000 A, has the ability to easily bridge the distance between red cells and cause agglutination. The IgM antibodies react at lower temperature (4-27°C).

IgM antibodies are capable of agglutination red cells suspended in 0.85% saline solution.

Method

1. Put 2 drops of test serum or known serum in properly labeled tube.
2. Add 1 drop of 2-4% suspension of appropriate red cells in saline and mix.
3. Incubate at R.T. for 30-45 min. In urgent cases, centrifuge the tubes at 1000 rpm for 1 min. after 5-10 min. incubation at room temperature (Spin method)
4. Observe the supernatant fluid for the presence of hemolysis against a well lighted background.
5. Gently disperse the cells button and check for agglutination against a well lighted background.

6. Where no agglutination is seen visually, examine the contents under microscope.
7. Record the result immediately.

Interpretation

Agglutination or haemolysis is a positive result.

METHODS FOR IgG ANTIBODY TESTING

IgG antibodies are acquired (immune) antibodies and are clinically significant. They are responsible for hemolytic transfusion reactions and hemolytic diseases of the new born. To detect the presence of IgG antibodies several media are used to enhance and potentiate the antibody-antigen reaction. Several enhancement media/ reagents are aimed at reducing the net negative charge (zeta potential) on the surface of red cells, for more details see the chapter on Basic Principles of Immunology. The enhancement media/ reagent generally used for detecting IgG antibodies are 22% albumin, enzymes, low ionic strength solution (LISS), and antihuman globulin sera.

ALBUMIN TESTS FOR IgG

Albumin is used in blood group serology as

(i) 22% concentration for enhancing the agglutination of red cells coated with IgG antibodies
(ii) concentration of 1-7% as a stabilizer in other reagents, specially those to be stored at 4°C.

Mostly 22% bovine albumin is used to enhance the agglutination of red cells coated with IgG antibodies for detection and identification of IgG antibodies, cross- matching and cell typing.

Method

A One Stage Method (Albumin used as additive)

1. Add two drops test serum or known serum to a labeled tube
2. Add one drop of 2-4% suspension of appropriate RBCs in saline
3. Add two drops of bovine albumin 22%
4. Mix and incubate at 37°C for 30-60 min
5. Centrifuge at 1000 rpm for lmin
6. Gently resuspend the cell button and observe for agglutination/ haemolysis
7. Confirm all negative results under microscope
8. Agglutination or hemolysis is a positive result

Two Stage Albumin Method (Albumin layering)

1. Add 2-3 drops of serum to a labeled tube
2. Add 1 drop of 2-4% red cell suspension in saline in the tube
3. Mix and incubate at 37°C for 30 minutes
4. Centrifuge at 1000 rpm for 1-2 minutes
5. Without disturbing the cell button, gently add two drops of 22% albumin to run down the inside of the tube. Albumin will form a layer on the top of the cells. Do hot mix
6. Incubate at 37oC for 10-20 minutes
7. Gently resuspend the cells and observe for agglutination/ hemolysis
8. Confirm all negative results under microscope

One-stage technique (additive method) takes less time and is more frequently used, but the two-stage technique (layering method) is more sensitive.

Note: Manufacturer's instructions should be followed:

Agglutination or hemolysis is a positive result.

ENZYMES

The reaction of certain blood group antigen and antibodies are selectively enhanced by the use of enzymes. The enzymes enhance antibody reactivity to Rh. Kidd, P, Lewis and I antigens and destroy or depress reactivity to red cells antigens Fy^a, Fy^b M, N, S. Some of the enzymes used in detection of antibodies are:

- Papain (from papaya),
- Ficin (from figs),
- Bromeline (from pineapple)
- Trypsin (from pig stomach).

Enzymes reduce red cell surface negative charge and zeta potential. For more detail see 'Factors effecting antigen-antibody reactions' in the chapter on Basic Principles or Immunology.

Papain, Ficin or bromelin are usually used as 0.5% solution in phosphate-buffered saline.

There are two enzymes techniques:

- One- stage enzyme technique: enzyme is added directly to serum and red cells mixture.
- Two- stage enzyme technique: red cells are pretreated with enzymes before addition of serum.

Preparation of Papain Cysteine solution for 1-Stage **tecnique**

The concentration of papain in Low's papain solution is 1 % in phosphate buffer pH 6.2, accompanied by L-cysteine hydrochloride as an activator.

A. Preparation of phosphate buffer (pH 6.2)

1. Phosphate buffer stock solution
   (a) M/15 $Na_2HP0_4.\ 2H_20$ 11.866 gm + 1000 ml D.W.
   (b) M/15 KH_2P0_4 9.066 gm + 1000 ml D.W.

   Filter and keep them separately in sterile condition in refrigerator at 4-6°C.
2. Preparation of working solution of phosphate buffer at the time of preparing papain cystein solution having pH 6.2

M/15 $Na_2HP0_4.\ 2H_20$	M/15 KH_2P0_4
1 Vol	4Vol for pH 6.2
4Vol	1 Vol for pH 7.4
0.4 Vol	9.6 Vol pH 5.4

B. Preparation of papain cysteine reagent

1. 10 gm of papain (BDH/Merck 1: 350) is dissolved in 250 ml of phosphate buffer (pH 6.2)
2. Mix. grind if necessary.
3. Centrifuge the solution after 15 min., separate the supernatant and discard deposit, filter through Whatman's filter No. 1.

4. 4.85 gm of L-cystein Hcl (German) is added in 25 ml of phosphate buffer (pH 6.2).
5. Mix papain solution and cysteine solution and add phosphate buffer (pH 6.2) to make the volume 1000 ml.
6. Incubate at 37°C for 1 hour.
7. Adjust pH to 6.2, if less it can be increased by adding 5N - NaOH (10 gm of NaOH in 50 ml H_20). If pH is more it is discarded.
8. Store in small aliquot at -20°C.

Preparation of Papain solution for 2-Stage technique

A. Stock solution of papain solution

(1) Suspend I g of papain (BDH/Merck 1: 350) in 100 ml of phosphate-buffered saline (This solution can be kept for several months at 4°C, but it can be kept better at -20°C).

(2) If desired, this suspension can be centrifuged after storage for 24 hours at 4°C with occasional agitation. The clear supernatant is slightly less active than the original suspension.

B. Working solution of Papain solution

For use, 1 volume of the stock solution is added to 9 volumes of phosphate-buffer saline, pH 7.3, prepared by adding 3 volumes of M/15 Na2HPO4 (9.46 g/L) to 1 volume ofM/15 $KH_2 P0_4$ (9.07g/L)

Enzyme Tests for IgG

Methods

1. One stage method
2. Two stage method

The two-stage enzyme technique is more sensitive than the one-stage enzyme technique. Two stage method is used for detection and identification of IgG antibodies. One-stage method is generally used for cross matching and cell typing as it is very convenient. It can also be used for detection and identification of IgG antibodies in serum.

One Stage Enzyme Method

1. Take 1 vol serum in a test tube.
2. Add 1 vol of papain cysteine reagent.
3. Add 1 vol of *2%* cell suspension in saline.
4. Incubate at 37°C for 30-45 min.
5. See for agglutination. Confirm negative result under microscope.

NOTE: Autocontrol should be put.

Two Stage Enzyme Method

First stage • pretreatment of RBCs

1. Take 0.1 ml of 3 times washed cells in a test tube.
2. Add 0.2 ml of papain working solution.
3. Incubate at 37°C for 15-30 min.

4. Wash the cells 3 times with normal saline.
5. Make 2-4% suspension of sensitized cells in normal saline.

Second stage-antigen-antibody reaction

1. Take 2 volumes of serum in a test tube.
2. Add 1 volume of papainised red cells.
3. Incubate at 37°C for 30 min.
4. See for agglutination. Confirm negative result under microscope.

NOTE: Autocontrol should be put.

LOW IONIC STRENGTH SALT SOLUTION (LISS)

Low ionic strength solution media generally contain 0.2% sodium chloride. It result in an increased rate and degree of antibody uptake two to four times as compared to normal saline during sensitization, with an incubation time of 5-15 minutes. However, all antibodies are not equally responsive to LISS. Anti- A and anti-B remain uneffected.

Uses of LISS

- Screening, identification and quantitation of antibodies
- It is useful in emergency cross-matching due to short incubation time
- Useful in elective surgery, patient serum is kept after doing blood group, antibody screen and cross-match is done on request at the time of operation

Preparation of Low Ionic Strenght Salt Solution (LISS)

LISS described by low and Messeter consists of:

0. 17 M Saline	180 ml
0.15 M Phosphate buffer, pH 6.7	20 ml
0.3 M Sodium glycinate, pH 6.7	800 ml

Procedure for preparation of one litre of LISS

1. 18 g of glycine is dissolved in about 500 ml of distilled water (sodium glycinate is not available commercially).
2. The pH is adjusted to 6.7 by drop wise addition of 1 N NaOH.
3. 20 ml of phosphate buffer (0.15 M) pH 6.7 is added to the glycine solution. Phosphate buffer (0.15 M) pH 6.7 is prepared by adding 0.15 M $NaH_2PO_4.2H_2O$ (23.4 g/L) to 25 ml of 0.15 M Na_2HP0_4 (21.3 g/L) until the pH is 6.7. Approximately equal volumes of the both solutions are needed.
4. 1.79 g of NaCl dissolved in 100 ml of distilled water is added to the solution.
5. The solution is made up to one litre with distilled water, mix thoroughly.
6. Adjust pH to 6.7 with 1 N NaOH.
7. It is sterilized by seitz filteration or autoclaving to avoid bacterial growth. Alternatively low concentration of sodium azide 0.1 g/L may be added.
8. Dispense 100 ml in small sterilized vials under laminar flow. Store at 4°C or at -20°C to avoid bacterial growth.

Quality Control

Non- Serological

- pH should be within the range of 6.65-6.85,
- Conductivity should be 3.6-3.7 mmho/cm at 23°C.
- Osmolarity 270-285 mmol.

Serological

A weak IgG anti-D (0.25 iu/ml) should give a +/++ reaction with R_1r red cells by the routine LISS-AHG test. This examination should be carried out in parallel tests using the previous batch
of USS.

Alternative method for preparation of LISS

1. Add 1.75 g NaCl and 18 g glycine to a 1 litre volumetric flask.
2. Add 20 ml of phosphate buffer prepared by combining 11.3 ml of 0.15M KH_2P0_4 and 8.7 ml of 0.15 M Na_2HP0_4.
3. Add distilled water to the 1000 ml mark.
4. Adjust pH to 6.7 with IN NaOH.
5. Add sodium azide 0.1 g/L as a preservative.
6. Dispense 100 ml in sterile vials under laminar flow. Store at 4°C or -20°C.

NOTE: Adding sodium azide in this quantity raises the ionic strength from 0.0355 to 0.043. This does not affect the serologic behaviour of the solution.

Method (LISS -IAT technique)

1. Wash the red cells twice in normal saline.
2. Wash these cells once in LISS.
3. Make 2-3% cell suspension in LISS.
4. Take equal volume (2 drops) of serum and (2 drops) LISS- suspended cells in tube.
5. Incubate at 37°C for 15 min in routine and 5 min in emergency.
6. Centrifuge at 1000 rpm for one minute
7. Examine the supernatant for hemolysis, resuspend the cells and observe for agglutination, and record results. A positive result (agglutination and/or hemolysis) indicates incompatibility.
8. If there is no haemolysis or agglutination seen in the above test, wash the cells three times in large amount of normal saline. Decant supernatant in each wash as completely as possible.
9. Add 1-2 drops of AHG serum.
10. Centrifuge at 1000 rpm for 1 min (500 g for 15 second.).
11. Gently shake the tube to dislodge the button and examine for agglutination, using optical aid and record the result.
12. Add 1 drop of IgG coated red cells to any test that is non -reactive. Mix, centrifuge at 1000 rpm for I min. Look for agglutination. If a negative result (no agglutination) is obtained, the test result is invalid and the whole test should be repeated

Interpretation

- It is same as in DAT or IAT.
- Hemolysis or agglutination is a positive result.

Precautions

For routine work LISS should be at ambient temperature as cold LISS straight from 4°C storage increases unwanted cold antibody reaction.

- Red cells should be washed twice in normal saline to free them from serum before adding LISS to them. Traces of residual serum will result in non-specific uptake of autologous serum complement on the red cells in LISS.
- Equal volume of serum and LISS suspended 2-3% cell should be used.
- The tube should be shaken gently to see agglutination.
- Bottle of LISS in use should be discarded after 48 hours.

AM1HUMANGL0BULIN TEST FOR IgG ANTIBODIES

See chapter on 'Antihuman Globulin Test'

ANTIBODYTITRATION

Titration is semi-quantitative technique of measuring the concentration of an antibody in a serum. The titre of an antibody is usually determined by testing the serial two-fold dilution of the serum against appropriate red cells.

- Meticulous pipetting technique is necessary for meaningful titration results. Mouth pipetting should not be done and the tip of the pipette should not be broken. Semi-automatic pipette with disposable tips are recommended. New disposable tip should be used for each serum.
- Optimum incubation time, temperature, and appropriate method depending on the type of the antibody should be used. IgM antibodies are tested by saline method and IgG antibodies are tested by albumin or enzyme or AHG method.
- The age and concentration of red cells may effect the results. Freshly drawn and prepared cells suspension should be used. When the titers of an antibody in two or more sera are compared, all sera should be tested concurrently against the red cells from the same donor.
- When sequential prenatal serum samples are tested for changing antibody titer, samples should be stored frozen for comparison with subsequent specimens. Each specimen should be tested along with immediate preceding sample.
- Only a titer change of two tubes or more is significant.
- Results should be read macroscopically. The **prozone phenomenon** may produce weaker reactions in the first one or two tubes than in higher dilutions, so the entire series of tubes should be evaluated.

Titration Technique (Double Dilution)

1. Label a row of test tubes, according to the serum dilution, usually 1:1 through 1:512.
2. Deliver 0.1 ml or 1 volume of saline into all tubes except the first tube.
3. Add 0.1 ml or 1 volume of serum to tubes 1 and 2 (dilution 1:1 and 1:2).

4. Mix the contents of tube 2 with a clean pipette and then transfer 0.1 ml or 1 volume of the mixture of tube 3(1:4 dilution).
5. Continue the same technique, through all dilutions and remove 0.1 ml or 1 volume from the dilution tube with dilution of 1:512 and discard or save for further dilution if required.
6. Add 0.1 ml or 1 volume of 2-5% saline suspension of appropriate red cells to each tube.
7. Incubate at the appropriate temperature according to the antibody being tested. In case of anti- A and anti-B, incubate at room temperature for 30-45 minutes.
8. Gently dislodge the red cells and observe macroscopically for agglutination. The agglutination titer is recorded. Results are expressed as the reciprocal of the highest serum dilution that causes macroscopic agglutination. Thus a serum which gives visible agglutination at 1:256 is said to have a titer of 256. In describing the titer of the serum, it is usual to ignore the diluting effect of the cell suspension.

NOTE

- For IgG antibody titer add 0.1 ml or 1 volume of bovine albumin 22% or papain cystein after step 5.
- Incubate at 37°C for 30-40 min.
- Observe for the agglutination.
- IgG antibody titer can also be done by I AT.

AVIDITY

Speed and strength of agglutination is termed as avidity. The test is done by mixing two drop of anti-serum with one drop of 40-50% cell suspension on a slide or tile and rocking gently at room temperature (RT). The time for a clearly visible reaction (+1) and then for strong (+4) reaction to occur are recorded with the help of stop watch.

GRADING OF AGGLUTINATION REACTION

+4 Single clump of agglutination with no free cells
+3 Three or four individual clumps with few free cells
+2 Many fairly large clumps with many free cells
+1 Fine granular appearance visually, but definite small clumps (10-15 cells) per low power field
+W 2 to 3 cells sticking together per low power field, uneven distribution
Visually no agglutination
- All cells are free
H Hemolysis (partial or total) must be interpreted as positive

SALIVA TEST FOR A,B.H SUBSTANCES

(Inhibition Technique)

Approximately 75% of individuals possess the Se gene that governs the secretion of water soluble ABH antigens into all bods fluids with the exception of cerebrospinal fluid. These secreted antigens can be demonstrated in saliva by inhibition test with ABH antisera.

Collection and Preparation of Saliva

- After rinsing the mouth collect 3-5 ml of saliva in a clean wide-mouthed container. To increase salivation ask the person to chew a rubber band or keep few grains of salt in the mouth.
- Keep the container in a boiling water-bath for 10 minutes to inactivate enzymes.
- Collect clean supernatant after centrifugation at high speed for 10 minutes.
- Discard the opaque or semi-solid material.
- Keep supernatant in the refrigerator at 4-6°C if the test is to be done within few hours; freeze it if test is not done the same day.

Saliva Dilution

Saliva from adults should be diluted 1:2, undiluted saliva contains non-specific glycoproteins which can cause erroneous results.

Dilution of Anti-Serum

Titer anti-A, anti-B and anti-H: depending on the final titer, dilute serum in saline to a titer of 1:8 to 1:16 (example: if the final titer of anti-Ais 1:256 and the desired titer is 1:8, dilute anti-A in 32 (256÷8 = 32).

Method

Set up the tubes and control as follows:

TestA	**TestB**	**TestH**
2 drops saliva	2 drops saliva	2 drops saliva
+	+	+
2 drops of diluted anti-A	2 drops of diluted anti-B	2 drops of diluted anti-H
Control A	**Control B**	**Control H**
2 drops of saline	2 drops of saline	2 drops of saline
+	+	+
2 drops of diluted anti-A	2 drops of diluted anti-B	2 drops of diluted anti- H

Additional saliva controls from known secretors and non-secretors are desirable.

- Mix and allow the tubes to stand at room temperature for 20 minutes.
- Add 1 drop of 5% saline suspension of A cells to tube A, B cells suspension to tube B, and O cells suspension to tube H.
- Mix and incubate all tubes at room temperature for 30-60 minutes.
- Centrifuge at 1000 rpm for 1 minute.
- Read results with naked eyes.

Interpretation

- No agglutination in the test sample and agglutination in the corresponding control tube indicates that the antiserum has been neurteralized by the blood group specific substance A, B, or H and the individual is secretor.
- Agglutination in all the test samples and control tubes indicates the absence of blood group substances and the person is non-secretor or Bombay phenotype (oh).

Example

	Test A	**Test B**	**Test H**
Agglutination	-	+	-
	Control A	**Control B**	**Control H**
Agglutination	+	+	+

This example indicates the presence of A and H substances in the saliva and the individual is blood group A. Similarly B group secretor will secrete B and H substances and O group secretor will only have H substance in the saliva.

TEST FOR HEMOLYSINS ANTI- A **AND ANTI-B**

The hemolysin test detects immune anti-A or anti-B which hemolyses appropriate red cells in the presence of complement. Hemolysin test is used to determine the ability of anti-A and anti- B in group O subjects to cause the hemolysis of cells when O group is given to an individual of another blood group.

Method

1. Place 2 drops of fresh test serum, not more than 24 hours old, in each of the two tubes labeled A and B. If serum is unavoidably more than 24 hours old, add an equal volume of fresh human AB serum free from lysins, as a source of complement, which is important to reaction.
2. Add 1 drop of fresh 2-5% saline suspension of washed A_1 cells to tube A and 1 drop of B cell suspension to tube B.
3. Mix gently and incubate at 37°C for 2 hours.
4. Centrifuge and examine the supernatant against a well lighted white-back ground to detect hemolysis.
5. Score the degree of lysis according to the intensity of the supernatant colour (pink to red) and cell button size.
6. Any shade of pink or red indicates hemolysis.
7. Hemolysis in the tube containg A_1 red cells indicate the presence of anti-A hemolysins and hemolysis in the tube containg B red cells indicates the presence of anti-B hemolysin.

N.B.: Use of weaker cell suspension or larger amount of serum will increase hemolytic activity.

Alternate Method

One volume of each 50% suspension of washed A, and B cells is put in two tubes labeled A and B having 9 volumes of fresh test serum of group O blood. The mixture is incubated at 37°C for 2 hours, then centrifuged and the supernatant is inspected for hemolysis. The result is interpreted as above.

ELUTION

A positive DAT result indicates that RBCs are sensitized with IgG antibody and/or complement in vivo, and there may be no or little antibody in the serum. Positive DAT may be caused by autoantibodies, alloantibodies (in HTR or HDN) or antibodies to certain medicines. Tests with monospecific AHG reagents can determine if IgG or complement component or both are coating the red cell, but the antibody specifity can not be determined which is bound to the red cells membrane by DAT.

Elution is a technique used to dissociate antibody from sensitized red cells and to recover antibody in an inert diluent such as normal saline or 6% albumin, and is called elute. This elute can be tested, like serum, to determine the antibody specificity.

The objective of such procedures is to recover antibody bounded to red cells in elute for detection and identification and some elution procedures recover antibody free red cells in useable form for phenotyping and or absorption of auto-antibody.

Elution can be performed by a number of following physical and chemical procedures

CHANGE IN THERMODYNAMICS (temperature)

Changes in thermodynamic reverse attractive forces between antigen and antibody, or disturb the structural complementarity of the antigen and antibody.

Heat Elution

(Landsteiner and Miller 1925)

Application

This is useful in eluting anti-A and/ or anti-B from red cells in the diagnosis of ABO-HDN or for eluting other antibodies which bound more strongly at low temperature. It is not much efficient in recovering IgG antibodies. This method has the advantage of speed and simplicity.

Materials

- 6% Bovine albumin, prepared by diluting 22% bovine albumin with saline (1.4ml 22% bovine albumin + 3.6 ml normal saline) or AB serum.
- Antibody coated red cells, DAT positive (2ml)
- Supernatant saline from last wash from antibody coated cells.

Methods

1. **Preparation of supernatant from last wash**
   - Wash the antibody coated red cells (from which the elute is to be made) six times in large volumes of normal saline.
   - The final wash should be performed by adding saline equal to the volume of washed packed red cells. Centrifuge and recover the supernatant of the last wash and test it in parallel with the elute.
   - This last wash is an important negative control that demonstrates that the residual serum antibody has been removed before the elute is prepared from the washed red cells.

2. **Preparation of Elute**
   - Add saline (or AB serum or 6% albumin) equal to the volume of washed packed cells.
   - Place the tube in a 56°C water bath for 5-10 minutes. Agitate frequently during incubation.

- Immediately centrifuge at 1000 rpm for 2 minutes. A heated centrifuge maintained at 56°C is ideal, but if this is not possible then warm the cups of the centrifuge in water at 56°C and centrifuge quickly.
- Immediately transfer the supernatant elute into another test tube and test against a panel of ;cells for the presence of antibody in parallel with the last wash supernatant.
- If elute used same day, it can be prepared in saline, if storage is anticipated elute is prepared in AB serum or in 6% albumin.

N.B.: Last wash supernatant is tested for antibody in parallel with elute to ensure that any eluted antibody is not contiminants from residual serum.

Freeze-thaw (Lui)

It is a method of eluting ABO antibodies by freezing washed antibody coated red cells in 5% albumin and then thawing them. After removal of stroma by centrifugation the elute is ready for testing antibodies.

ELUTION BY THE USE OF ORGANIC SOLVENT (ETHER, XYLENE)

Organic solvent disruct antibody- antigen bonds by lowering the surface tension of the liquid media, thereby reversing forces needed to hold antigen and antibody together. Organic solvent are more sensitive because they remove more antibodies from RBCs in elute for testing antibodies.

ELUTION BY ETHER

It is useful in separatice of warm-reactive auto- and alloantibodies (IgG) from red cells which are positive to direct antiglobulin test (DAT) viz. in Rh- HDN, immune hemolytic anemias and suspected transfusion reactions etc.

Materials

- Diethyl ether reagent grade or anesthesiologic grade).
- Packed DAT positive red cells (2 ml), washed 6 times in saline.
- Supernatant m last wash from antibody coated red cells.

Method

1, Preparation of supernatant from last wash

- Wash the antebody red cells from which the elute is to made, six times in a large volume of saline.
- Recover the lest wash as describe above in step 1 in Heat Elution method.

2. Preparation of elure

1. Add an equal volume of saline to the washed packed red cells and mix.
2. Add ether in an amount equal to the total volume of red cells plus saline. The ether must be fresh; the appearance of brown colour of the ether indicates that the ether is oxidized and the elute will be useless.
3. Stopper and mix the tube by repeated inversion for 1 min.
4. Carefully remove the stopper to release the volatile ether.
5. Incubate at C for 30 min. This step is optional, but it will result in a more potent elute if included.

6. Centrifuge the tube at 1000x g (3400 rpm) for 10 min. The tube will then contain 3 layers- clear ether on the top, red cell stroma in the middle and haemoglobin strained elute at the bottom.
7. Aspirate and discard the top layer.
8. Carefully pierce the middle stromalayer with a pipette and aspirate the bottom hemoglobin stained layer below the stromal layer into a clean tube.
9. Incubate the elute in the unstoppered tube at 37°C for 15 min and periodically bubble air through elute by pipette to drive off residual ether.
10. Test the elute for the presence of antibody in parallel with the last wash supernatant.

Last wash supernatant is tested in parallel with elute to ensure that any eluted antibody is not contaminants from residual serum.

ELUTION BY XYLENE

Reagents

- Reagent grade xylene.
- Packed DAT positive red cells, washed 6 times in saline.
- Supernatant saline from last wash from antibody coated cells.

Method

1. Mix equal volume of red cells, normal saline and xylene in a test tube.
2. Stopper the tube and mix the contents for 2 minutes.
3. Remove the stopper. Place the tube at 56°C for 10-15 minutes. Stir the contents of the tube.
4. Centrifuge the tube at 1000 rpm for 10 minutes.
5. Carefully remove and discard the upper layer of xylene and the stoma.
6. Transfer the elute in a clean tube, and test in parallel with the last supernatant wash.

ACIDELUTES

In acid elutes techniques the use of acid solution reduces the pH and disrupts the complementarity of antigen- antibody bonds. The optimal pH for antigen-antibody binding is 6.8-7.2. Acid elutes reduce the pH to 3 or less by mixing the sensitized cells with an acid solution. The antibody is released from the RBCs membrane. The acid solution becomes elute. Citric acid, glycine, and digiton acid are used in acid elution techniques.

These techniques are fast and sensitive and do not need hazardous chemicals.

CITRIC ACID ELUTION

Reagents

- Eluting solution: Citric acid (monohydrate), 1.3 g; KH_2P0_4, 0.65 g; Saline to 100 ml,: Store at 4°C.
- Neutralizing solution : $Na,P0_4$, 13.0 g: distilled water to 100 ml.: store at 4°C.
- Packed DAT positive cells, washed 6 times in saline.
- Supernatant saline from last wash.

Method

1. Chill all reagents at 4oC before use.
2. Take 1 ml of packed red cells in a tube and add 1 ml of eluting solution. Note the time.
3. Stopper the tube and mix by inversion for 90 seconds.
4. Remove stopper and immediately centrifuge the tube at 1000 g for 45 seconds.
5. Transfer the supernatant to a clean tube and add 5-6 drops of neutralizing solution: save red cells for use in adsorption if needed.
6. Check pH; adjust, if necessary, to pH 7.0 by adding more neutralizing solution.
7. Centrifuge at 1000xg for 2-3 minutes to remove precipitate that forms after neutralization. Remove the supernatant elute and test in parallel with the supernatant saline wash from the final wash.

NOTE

- Once the red cells have been rendered DAT- negative, they may be used for phenotyping, except the Kell system. Antigens of the Kell systems are weakened after citric acid treatment.
- Citric-acid modified red cells may be treated with enzymes and used in autologous adsorption.

COLD-ACID ELUTION

Reagents

- Glycin-HCL (0.1 M, pH 3.0) is prepared by dissolving 3.75 g of glycin and 2.992 g of sodium chloride in 500 ml of distilled water. Adjust pH to 3.0 with 12 N HCL. Store at4°C.
- Phosphate Buffer (0.8 M, pH 8.2) is prepared by dissolving 109.6 g of $Na,HP0_4$ and 3.8 g of KH_2P0_4 in about 600 ml of distilled water. Adjust pH with IN NaOH or IN HCl if required. Add distilled water to make final volume of 1L. Store at 4°C.
- Normal saline, at4°C.
- Packed DAT positive red cells, washed 6 times in saline.
- Supernatant saline from final wash.

Method

1. Place the red cells in a test tube and chill in an ice bath for 5 minutes.
2. Add 1 ml of chilled saline and 2 ml of chilled glycin-HCl to 1 ml of washed red cells.
3. Mix and incubate the tube in ice bath for 1 minute.
4. Quickly centrifuge the tube at 1000xg for 2-3 minutes.
5. Transfer the supernatant elute to a clean test tube and add 0.1 ml of Phosphate bufferPH 8.2 for each 1 ml of elute.
6. Mix and centrifuge at 1000 rpm for 2-3 minutes.
7. Transfer the supernatant elute in a clean test tube and test the elute in parallel with the supernatant saline from the final wash.

NOTE

- Keep glycin in ice bath during use, to maintain correct pH.
- Phosphate buffer will crystallize during storage at 4°C.Redissolve at 37°C before use.

- Addition of phosphate buffer restores neutrality to the acid elute. Unneutralized acidity may cause hemolysis of the reagent red cells used in test in the elute.

The above techniques, sometimes appropriately referred to as 'total elute' for use in antibody --identification studies; RBCs from which elute is prepared are usually rendered useless for any purpose. "Partial elutes' to remove antibody from sensitized RBCs may be performed using ZZAP or chloroquine disulphate solutions so that RBCs may be used for autoabsorption or phenotyping respectively.

ELUTION OF IgG ANTIBODY BY USING CHEMICAL TREATMENT

Antibody dissociation from antibody coated red cells can be accomplished by the use of chemical reagents capable of splitting immune complex without denaturing the red cells membrane. The aim of these elution techniques is to elute warm antibody from red cells so that cells can be used for typing antigens like Rh, Fy Kell, Kidd etc. by IAT and for adsorption of warm auto-and-alloantibodies. Two commonly used chemical reagents for obtaining free intact red cells are:

- Chloroquine diphosphate
- ZZAP reagent, (a mixture of proteolytic enzyme and reducing agent dithiothreitol).

Removal of red cells bound IgG with Chloroquine diphosphate

Chloroquine diphosphate dissociates IgG from antibody coated red cells with minimal damage to the integrity of red cells membrane. Prolonged treatment may result in haemolysis and loss of red cells antigen. Some denaturation of Rh- antigen may occur. Treated cells may give weaker reaction than expected or false negative reaction, specially when saline-reactive or chemically modified anti-Rh typing reagents are used. Therefore, Rh phenotyping should be done with high protein slide/ rapid tube reagents and appropriate manufacturer's diulent control. Chloroquine diphosphate does not dissociate complement component from red cells, therefore, monospecific anti- human globulin serum (IgG) should be used and not polyspecific antiglobulin reagent.

Materials

1. Chloroquine Diphosphate Solution
   - 20 g chloroquine diphosphate (Sigma chemical) is dissolved in 100 ml of 0.01 M phophate buffered saline, pH 7.2 (200 mg chloroquine disphosphate per ml. of phosphate buffered saline).
   - The pH is adjusted to 5.0-5.1 using 5 N NaOH. The reagent is stored at 4°C.
2. Test red cells (antibody coated cells).
3. Antiglobulin reagent (anti- IgG).

Procedure

1. To one volume of washed packed red cells added 4 volumes of chloroquine diphosphate solution.
2. Mix by inversion. Then leave the sample at room temperature for 2 hours with periodic mixing
3. After incubation, the chloroquine treated red cells are washed 4 times with large volumes of isotonic saline and then make 2-5% cell suspension of washed cells in saline for testing.
4. Take one volume of *2-5%* treated cell suspension and 1 volume of mono-specific anti-IgG reagent (AHGS) and perform DAT

5. If reactive, repeat steps 1-3, till cells are non- reactive to DAT.
6. Make the cell suspension as in step 3. The cells can be used for antigen typing or auto-absorption procedure.

N.B.: All IgG sensitised cell cannot be rendered non- reactive, even after prolonged treatment and occasionally red cells get hemolysed.

Removal of Red Cells Bound IgG with ZZAP Reagent

(ZZAP is a mixture of Cysteine-activated papain and dithiothreitol)

This results in complete dissociation of antibody and complement component from red cells, but MNSS, Duffy and Kell antigens are destroyed. It uncovers antigenic sites on red cells which are capable of binding autoantibody in the serum. ZAAP treated red cells are specially suited for autoabsorption procedures because treatment results in intact RBC that have reduced IgG. ZAAP treated red cells are usually not used for typing (except Rh typing).

Reagents Required

- 1 % cysteine-activated papain; store at-20°C (ficin, bromelin, or trypsin may be substituted for cysteine- activated papain).
- 0.2 M dithiothreitol (DTT); store at -20°C, (0.2 M 2- mercaptoethanol may be substituted for 0.2 MDTT).
- PBS, 0.01 M, pH 7.3; store at 4°C.
- Test cells coated with antibody.

Preparation of ZZAP Reagent

(0.2 M DTT + 0.**1** % Cysteine- Activated Papain)

Method

Preparation of ZZAP reagent

1. Mix 2.5 ml of 0.2 M DDT, 0.5 ml of **1** % cysteine- activated papain and 2ml PBS buffer, pH 7.3
2 Invert several times to mix; pH should be 6.0-6.5. The reagent is stable for at least 5 days at4°C.

ZZAP Treatment of Red Cells

1. Take 1 ml packed red cells in a tube to be treated.
2. Add 2 ml of ZZAP reagent.
3. Mix, and incubate at 37°C for 30 minutes with periodic mixing.
4. Wash red cells 3-4 times with isotonic saline, and remove supernatant as much as possible.
5. Resuspend ZZAP treated cells to 2 - *5%* in saline for use. The cells can be used for auto-absorption.

ABSORPTION

Absorption: This technique is used for the removal of an antibody from the serum by the attachment of the specific antibody to the corresponding antigen on the red cell surface under optimal conditions (opposite of elution), often used interchangeably with *adsorption.*

Application

- Separation of antibody in sera containing multiple antibodies.
- Removal of unwanted antibody, specially anti-A or anti-B from a sera that contains an antibody suitable for reagent.
- Removal of auto-antibody to permit the detection of coexisting allo-antibody(ies).
- Confirmation of the presence of antigens on red cells through their ability to remove specific serum antibody.

ALLO-ANITBODY ABSORPTION TECHNIQUE

Application

To remove an unwanted antibody from serum for the purpose of antibody identification or to prepare reagent antisera.

Method

1. Wash a large volume of red cells to be used in the absorption, three times in normal saline. These cells must possess the antigen corresponding to the antibody that is to be absorbed and shall lack the antigen corresponding to another antibody, if any, that is not to be absorbed (i.e. to remain in the serum).
2. After the final wash .Centrifuge the cells so that they are tightly packed and remove as much of saline as possible.
3. Divide the cells into two aliquots.
4. Add serum equal to the volume of packed cells in one aliquot.
5. Incubate at optimal temperature, at which antibody to be absorbed will react, for 30-60 min, mixing occasionally.
6. Centrifuge and recover the serum.
7. Test the serum to see that absorption is complete., if not, repeat the procedure using another aliquot of packed red cells. Always test to ensure that the antibody that is to remain in the serum is sufficiently reactive before continuing with repeat absorption.

COLD AUTO-ABSORPTION TECHNIQUE

Application

This technique is indicated when antibody reactivity is seen at room temperature with screening cells and with the patient's autocontrol. If the patient has been transfused recently, the procedure should be used with caution, since alloantibodies may be removed in addition to autoantibodies.

Blood Samples

- For better absorption results, fresh blood samples should be obtained in anti-coagulant solution and plain vial separate..

  Serum for absoiption: Allow sample to clot in the ice bath or the refrigerator, remove serum and maintain at cold temperature.

- Cells for absorption : Anti-coagulated sample is placed at 37oC. Allow cells to settle by gravity. Remove plasma, wash remaining cells three times with warm (37°C) saline.

Method 1 (Using untreated cells)

1. Wash the red cells three times in saline (this can be omitted if specimen has been collected as described above).
2. Remove the supernatant saline after last wash
3. Divide the red cells into two equal aliquots.
4. Add patient's serum equal to the volume of packed cells in one aliquot.
5. Mix and incubate in refrigerator for 30-60 min. Mix frequently during incubation for maximum absorption.
6. Centrifuge and separate the serum.
7. Test the absorbed serum against autologus cells to ensure that absorption is complete .If absorbed serum is still reactive with the autologous cells, absorption is not complete and should be repeated with the second aliquot of washed packed red cells.

Untreated red cells may be used for homologous absorption, but they are less effective than enzyme treated red cells in removing autoantibody, as enzyme treated cells take up more antibodies.

Certain antigens, viz. M,N,S,s and Duffy are destroyed or modified by enzyme.

Method II (Using enzyme treated red cells)

1. Add **I** volume of 1 % papain solution to 1 volume of washed, packed autologous red cells.
2. Mix and incubate at 37°C for 15 min.
3. Wash red cells 3 times with normal saline. Remove the supernatant saline from the last wash.
4. Divide red cells into two aliquots.
5. Add patient's serum equal to the volume of packed cells in one aliquot.
6. Mix and incubate in refrigerator for 30-60 min. Mix frequently during incubation for maximum absorption.
7. Centrifuge and separate the serum.
8. Test the absorbed serum against autologous cells to ensure that absorption is complete If absorbed serum is still reactive with the autologous cells, absorption is not complete and should be repeated with the second aliquot of washed packed enzyme treated red cells.

WARMAUTO-ABSORPTION TECHNIQUE

Application

This technique is indicated when antibody reactivity is seen at 37°C with screening cells and with patient's autocontrol. The procedure should be used with caution if the patient has been transfused in the last 2-3 months.

Method -1

Heat and Enzyme Method

1. Collect the patient's sample in the anticoagulant and another sample in a plain vial at 37°C and separate serum at 37°C.

2. Wash the patient's red cells with warm saline (37°C). Remove the supernatant saline of the last wash.
3. Suspend the washed patient's red cells in saline at 1:1 ratio.
4. Elute the warm autoantibodies from the cells into equal volume of saline at 56°C for 3 to 4 minutes (or at 44°C for 60 min if red cells are fragile) mixing constantly, centrifuge and remove the supernatant.
5. Wash the cells again 3 to 4 times in warm saline.
6. Spin and remove the excess saline.
7. Add one volume of 1% papain solution to one volume of washed packed autologous red cells.
8. Mix and incubate at 37°C for 15min.
9. Wash the cells three times with normal saline. Remove the supernatant saline.
10. Divide red cells into 2 aliquots.
11. Add patient's serum equal to the volume of packed cells in one aliquot.
12. Mix and incubate at 37°C for 30-60 min. mixing occasionally.
13. Centrifuge and recover serum
14. Test the absorbed serum against the autologous cells.

If absorbed serum is still reactive, absorption is not complete and repeat steps 11-13 with the second aliquot of washed packed enzyme treated cells.

DONATH -LANDSTEINER TECHNIQUE (QUALITATIVE).

It is a diagnostic test for Paraoxysmal cold haemoglobinuria (PCH)

Method: Direct Test

Collect two blood specimens from the patient, one specimen, the control, is maintained at 37°C for 60 minutes after collection. The second sample is cooled at 4°C for 30 minutes and then incubated at 37°C for an additional 30 minutes. Both samples are the centrifuged and observed for hemolysis.

Interpretation

In a positive Donath-Landsteiner test, hemolysis is seen in the sample placed at 4°C and then at 37°C, whereas no hemolysis is observed in the control sample.

Method 2: Indirect test

1. Separate the serum from the clotted blood at 37°C, with precaution that the sample of blood should not be allowed to cool.
2. Add nine drops of serum from the individual under test to one drop of washed packed red cells (group O) in tube 1.
3. In the second tube take equal parts of serum from the individual undertest, fresh normal serum to act as a source of complement and washed packed red cells (group O)
4. Place the first tube and the second tube at 0°C in crushed ice for 30 minutes. Negative controls are provided by keeping a duplicate of tubes 1 and 2 at 37°C throughout the test. Transfer the tubes at 0°C to the water bath (37°C) and keep for one hour.
5. Examine for hemolysis.

Interpretation

A positive test shows hemolysis in tubes 1 and 2 (or in 2 only, if the patient is deficient in complement). No hemolysis should be observed in the negative controls.

Donath -Landsteiner Technique (Quantitative)

Method

1. Make serial doubling dilutions of the patient's serum in fresh normal AB serum.
2. Add an equal volume of a washed 2 percent suspension of group O red cells to each tube.
3. Immerse the tubes in crushed ice at 0°C for 30 minutes, then transfer theme to a 37°C water bath for one hour.
4. Centrifuge and examine each tube for the degree of hemolysis .

   Read according to the following scale:

+4	=	complete hemolysis
+3	=	deep red supernatant
+2	=	red supernatant
+1	=	pale pink supernatant
±	=	weak hemolysis

Interpretation

The hemolysis titer is the reciprocal of the highest dilution giving a ± reaction.

ABSORPTION AND ELUTION

(for weak subgroups of A or B)

1. Wash 1 ml. cells to be tested at least three times with saline. Discard the supernatant after last wash.
2. Add 1 ml. of anti -A to red cells if weak variant of A is suspected, or 1 ml of anti -B if weak variant of B is suspected.
3. Mix the cells with anti-sera and incubate at room temperature for one hour.
4. Centrifuge the mixture and discard the supernatant anti-sera
5. Wash the remaining red cells for minimum five times with a large volume of saline(10 ml or more). Save the supernatant of the fifth wash to test for free antibody.
6. Add an equal volume of saline to the washed and packed cells and mix.
7. Elute the absorbed antibody by placing the tube 56°C water bath for ten minutes and mix the red cells saline mixture at least once during this period.
8. Centrifuge and remove the cherry colored elute and discard the cells.

Testing of Elute

1. If anti-A was used, test the elute against three different samples of A, cells and three group O cells at room temperature, at 37°C and with antiglobulin serum.

2. It anti -B was used, test the elute against three samples of B cells and three group of O cells at room temperature, at 37°C and with antiglobulin serum.
3. Test the fifth saline wash in the same manner to show that washing has removed all antibody, not bound to the cells.

Interpretation

If the elute agglutinates or react with specific A or B cells and does not react with 0 cells, the cells tested have active A ro B antigen on their surface capable of binding with specific antibody. If the elute reacts also with O cells, it indicates non-specific reactivity in elute, and the results are not valid.

If the fifth saline wash material is reactive with A and B cells,the results of the test made on the elute are not valid, as it indicates that active antibody was present in the medium unattached to the cells being tested.

MICROPLATE TECHNOLOGY

The use of microplate has been successfully applied in blood bank laboratory. All routine tests of blood grouping, anti body screening and cross matching can be done in microplate without loss of sensitivity of anitbodies or any problem in blood grouping.

Advantages of Microplate Technology:

- **It** is cost effective for testing large number of blood samples.
- Enhanced sensitivity of reactions.
- Saving in reagents and equipment.
- Less laboratory space required.
- Less time for technologists.
- Results can be observed visually or can be evaluated with automatic photometeric readers which eliminates human error.

Microplate :

These are polystyene plates having 96 wells arranged in 8 rows (lettered A to H) each having 12 wells (numbered lto 12). Fig. 20-1

	1	2	3	4	5	6	7	8	9	10	11	12
A	O	O	O	O	O	O	O	O	O	O	O	O
B	O	O	O	O	O	O	O	O	O	O	O	O
C	O	O	O	O	O	O	O	O	O	O	O	O
D	O	O	O	O	O	O	O	O	O	O	O	O
E	O	O	O	O	O	O	O	O	O	O	O	O
F	O	O	O	O	O	O	O	O	O	O	O	O
G	O	O	O	O	O	O	O	O	O	O	O	O
H	O	O	O	O	O	O	O	O	O	O	O	O

Fig. 21-1 Microplate

These are polystryene plates, having 96 wells arranged in 8 rows (lettered A to H) each having 12 wells (numbered 1 to 12). Microplate with U shaped or V shaped wells, each having capicity of 25 microns can be used in blood group serology,. Plates have electrostatic properties . It is important to select the plates with low static properties. The addition of bovine albumin and for Tween 20 to diluents and wash solutions may help to eliminate static effect produced by polystyrene. Even then it is advisable to prepare plates before use. It can be done by rinsing the plates with Tween 20, after which they are shaken and dried over night at 37°C. Microplates are disposable.

Principle

Microplate is a matrix of 96 "short" test tubes. Principle of hemagglutination by test tube method is also applied here. Microplate may be rigid or flexible, with either U shaped or V shaped wells. U-bottom plates are more widely used because result can be-read by observing the characteristics of resuspended RBC's or the streaming pattern of RBC's when the plate is placed at an angle.

Agglutination strength can be estimated by automatic photometric devices also, that read results by the light observance in U-bottom wells to differentiate between positive and negative results.

U wells and V wells

In blood group serology the plates with U wells or V wells can be used equally well. In V shaped wells when low concentration (0.1 -0.2 %) of red cells are used, the sensitivity is more, while in U shaped well red cells suspensions of 2-3 % are used.

Equipment

1. ***Dispensers-semi automated*** devices for dispensing equal volumes to a row of wells.
2. ***Washers semi-automated*** are normally used to wash specimens before adding antiglobulin serum. U bottom plates are preferred for washing RBC's because the samples can be re-suspended more easily.
3. ***Microplate readers*** - Automated devices are available that read microplate by photometer; differentiation between positive and negative test is accompanied by light observance in U -bottom wells. 96 observance value can be processed in less than 1 minute and the results interfaced with microprocessor. The automated reader passes light beams through the bottom of the wells. Negative results produce high optical density readings because the RBC's dispersed over the well bottom absorb more light than the concentrated button of cells obtained in a positive results. The microprocessor component of the reader interprets the reactions and the blood group results are printed for the laboratory record.
   RBC's control must be run with automated reader to enable the reader to differentiate false, positive reaction due to nonspecific aggregation. For accurate results, the RBC's concentration of routine test samples must be carefully controlled (0.5 to 2 % give the best sensitivity) and centrifugation and resupension must be performed in very uniform manner.
4. ***Centrifuges for microplate*** - Appropriate centrifugation conditions must be established for each centrifuge. Consult the manufactures for specific information.

Personnel

Before personnel have sufficient experience, the microplate procedure should be run in parallel with the standard procedure in tubes to confirm that if the results of microplate procedure are same as that of standard procedure in tubes. All discrepant results obtained in the parallel evaluation should be recorded with the summary of the investigations undertaken to resolve each discrepancy.

Diluent and wash solutions

In all techniques 0.02% albumin in saline is recommended as a red cells diluent and wash solutions. This is prepared by adding 6 ml of 30% albumin to one litre of normal saline , pH6-8. Alubumin limits the electrostatic properties of polystryene and prevents monolayering which is important when diluted red cells suspensions are used . Alternatively, red cells may be diluted in low ionic strength solutions.

For enzyme method cells are pretreated with 1% papain or 0.05% bromeline and cells suspensions is made in normal saline or in low ionic strength solution.

Standardization of anti-sera

1. Make two-fold master dilutions of the anti -A, anti -B, anti-AB and anti -Rh (D) monoclonal antibodies.
2. Dispense *25µL* volumes of each dilutions into U well plates and add 25µL of 2-3 % suspension of appropriate red cells in albumin saline.
3. Incubate the plate at R.T for 20-30 min.
4. Centrifuge the plate at 100 g (about 500 rpm) for 40 seconds.
5. For agglutination, there are various methods for reading :
   (i) Shake the plate on agitator for the minimum period necessary to disperse negative controls. After shaking, each plate is placed immediately on an angled plate stand at 60° -70° from the horizontal. The reaction can be read macroscopically; in a negative reactions red cells trail from the center of the well and in a positive reactions cells remain in the center or fall in a discrete button to the bottom of the well.

   Alternatively after placing the microplate at angled stand for atleast one minute (and not longer than 5 min) the result can be obtained by automatic reader at 570 nm wave length.

   (ii) After centrifuging the plate (step 4), place the plate at an angle plate stand at 60°-70° from the horizontal for 2-3 min and than reverse for reading. The reactions are read macroscopically from back side of plates. In a negative reactions red cells trail from the centre of well and in positive reaction cells remain in the centre or fall in a discrete button to the bottom of the well.
6. The greatest dilution giving macroscopic +++ agglutination, and a clear negative control reaction is selected for use:

Note: Test the anti - A against A_1 A_2, A_1B. A_2B, B and pooled O cells ;anti -B against B, A_1 B, A_2B and pooled O cells. Anit -Rh(D) against O R_1R_2 O R_2R_2 It,, and Orr cells.

Alternate method to make working dilution of anti-sera

Double dilution from 1 in 2 to 1 in 1024 are prepared of anti-A, anti-B, anti-Dl and anti-D2 in normal saline in tubes. These are tested against reagents A cells, B cell and D positive cells.

Clear positive reaction with reagent cells and 2-3 dilutions short of the end point are selected as the working dilution.

Tests:

Saline Technique

1. 25µL Anti-sera (-A, -B, -AB, & -D) of the appropriate dilution are dispensed into wells
   of microplate. In ABO and Rh typing the plates can be stored in sealed plastic bag at 4°C.
2. Add 25µL of a 3% cell suspension of cells to anti-sera (-A, -B, -AB & -D) in wells.
3. Incubate the plate for 30 min to 1 hour depending on the anti-sera at the appropriate
   temperature.
4. Centrifuge the plate at 100 g for 40 seconds.
5. Examine for agglutination either after agitation or after tilting to about 60°-70°

from
horizontal until the negative controls begin to trail as described above.

MICROPLATE FORMAT
ABO and Rh(D)Taping

			1	2	3	4	5	6	7	8	9	10	11	12
ABO	Anti-A	A	O	O	O	O	O	O	O	O	O	O	O	O
Cell	Anti-B	B	O	O	O	O	O	O	O	O	O	O	O	O
Typing	Anti-AB	C	O	O	O	O	O	O	O	O	O	O	O	O
ABO	A Cells	D	O	O	O	O	O	O	O	O	O	O	O	O
Serum	B Cells	E	O	O	O	O	O	O	O	O	O	O	O	O
Typing	O Cells	F	O	O	O	O	O	O	O	O	O	O	O	O
Rh(D)	Anti-Dl	G	O	O	O	O	O	O	O	O	O	O	O	O
Typing	Anti-D2	H	O	O	O	O	O	O	O	O	O	O	O	O
Anti-body Screening														
OR_1R_2	O	O	O	O	O	O	O	O	O	O	O	O	O	O
OR_2R_2	O	O	O	O	O	O	O	O	O	O	O	O	O	O

(Fig-21.2 Microplate Formate)

Enzyme Techniques

One stage enzyme technique

1. Dispense 25pL serum,25pL Low's papain and 25uL 3% cells suspension in saline or LISS to the appropriate wells of microplate.
2. Incubate the plate at 37° C for 20-25 minutes.
3. Centrifuge the plate at 100 g for 40 seconds.
4. Examine for agglutination either after agitation or after tilting to about 60°-70^{p} until the negative control begin to trail as described before.

Two -stage enzyme technique

Pre -treatment of red cells

Reagent required : 1 % papain or 0.5 % bromelin

1. Incubate one volume of washed, packed cells and 2 volumes of papain solution (see pretreatment of red cells) at 37°C for 15-30 minutes.
2. Wash the cells three times in saline.
3. Make 2-4% red cells suspension in normal saline or LISS.

Method:

1. Dispense 25|iL of serum, 25uL of enzyme pre-treated cells in wells.
2. Incubate the plate at 37°C for 20-25 min (covered with lid).
3. Centrifuge the plate at 100 g for 40 seconds.
4. Examine for agglutination either after agitation or after tilting to about 60°-70° until the negative control begin to trail as described before.

Indirect antiglobulin test (IAT)

1. Dispense 25uL of serum and 25(iL of a 5 % suspension of washed red cells in saline or LISS in appropriate wells.
2. Incubate at 37°C for 30 min (covered with a lid)
3. Fill the well with saline.
4. Centrifuge the plate at 200 g (1000 rpm) for 40 seconds.
5. Decant the saline by flicking the plate.
6. Resuspend the cells and mix on the plate shaker for 40-60 seconds, repeat the process of washing four times and leave the packed cells after the last wash.
7. Add 50uL of undiluted polyspecific anti -human globulin serum (AHG serum) to the cells in each well.
8. The contents of the wells are mixed by shaking the plate on the plate shaker for one minute at low speed.
9. Centrifuge the plate at 60 g for 40 seconds.
10. Examine for agglutination either after agitation or after tilling to about 60°- 70° until the negative control begin to trail as described earlier.
11. Add ldrop of IgG sensitized cells to all negative reactions.
12. Re-centrifuge the plate and read results. All negative reactions should now be positive.

Direct antiglobulin test (DAT)

1. Dispense 25 uL of a 5% suspension of red cells in saline.
2. Fill the well with saline.
3. Wash the cells four times as described in indirect antigloubulin test (step .5,6).
4. Perform the comb's test as in indirect antiglobulin test (Step 7-12)

GELTECHNOLOGY

Gel Technology DiaMed ID Microtyping System developed by Dr. Yves Lapierre of France, gives more reproducible and standardized test results Antiglobulin test can be performed without multiple washing of red cells before adding into anti human globulin (AHG) serum and without the need to add sensitized control cells to all negative AHG tests. Gel technology can be used for any immunohematological test that has hemagglutination as its endpoint, such as:

- ABO &Rh typing
- Typing for other blood group systems
- Antibody screening & identification
- Compatibility testing including cross-matching

The DiaMed ID gel test is provided as gels held in microtubes contained in a plastic card. Each microtube contains about 35mL of sephadex (dextran acrylamide) gel prepared in a buffer solution such as LISS or saline. The gel may contain other elements : preservatives such as sodium azide, specific regents such as AHG or other RBC-specific antisera for grouping, sedimenting agents like bovine serum albumin etc. These reagents are added to the gel at the time of manufacture. Thus. the reagent is uniformly dispersed throughout the length of the gel column. Six of these microtubes are embedded in a plastic card to allow ease of handling, testing, reading and disposal. A wide range of test systems is available including red cell typing, DAT. antibody screening and identification and compatibility testing.

Principle :

The basic principle of the gel test is that, instead of a test tube, the serum and cell reaction takes place in a microtube consisting of a reaction chamber that narrows to become a column (fig-21.3). Red cells or mixture of cells & serum (as appropriate) are dispensed into the gel. The cells are *always* added prior to the serum - so that the serum does not come into contact with the gel, which is very important in IAT. This eliminates the need for the *'Wash phase'as* in conventional techniques. Incubation takes place, followed by centrifugation under strictly controlled conditions.

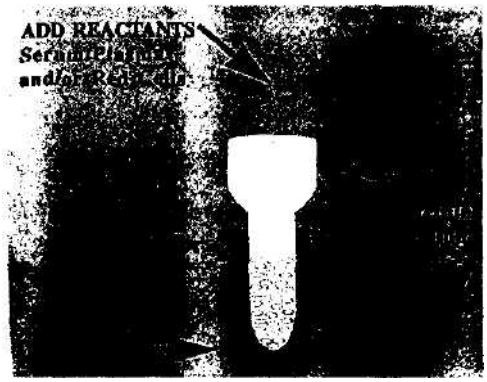


Fig. 21.3
Microtube with Gel

Methods

Forward Grouping

ABO and Rh (D) Determination

- A 5 % suspension of the test Red cells is prepared inLISS.
- 501 of this suspension is added to each of the microtubes (1- 4 having Anti-A, Anti-B, Anti-AB and Anti-D).
- After incubation at room temperature for 10 minutes, the card is centrifuged.
- Read results.

ABO Serum Grouping
(Reverse Grouping)

Reverse grouping is generally done along with forward grouping.

- For reverse grouping 50uL of known 5% cell suspension of A1 and B cells are added to the respective microtubes. (5 & 6 respectively)
- 25uL each patients serum is added to microtubes 5 & 6.
- Incubate at room temperature for 10 minutes.
- The card is then centrifuged and the results are read.

DAT

DAT is performed using the LISS/Coombs' cards. Each card contains six microtubes containing gel with polyspecific AHG serum incorporated into it. Each card can be used to perform 6 tests.

- A 0.8% suspension of the patient's red cells is prepared by adding 10UL of patient's red cells to 1 ml of LISS solution.
- 50^L of this suspension is added to one microtube.
- The card is then centrifuged and results are read.

IAT

The cards used for performing IAT are LISS/Coombs' cards containing six microtubes with polyspecific AHG incorporated into the gel. Each card can be used to perform 6 tests.

- Appropriate red cells are added to the gel first.
- This is followed by the addition of the patient's serum.
- Incubate at 37°C for 15 minutes.
- Centrifuge and read results.

Antibody Screening and Identification

Antibody screening and identification utilizes both AHG and neutral gels cards. Prepapainized
cells are used with the neutral gel cards and LISS suspended cells are introduced into the gels
containing AHG.

Test Reactions

Agglutination reactions are graded similar to that in test tube hemagglutination.

4+ reaction is represented by a solid band of agglutinated red cells at the top of the gel column. Usually no red cells are visible in the bottom of the microtube.

3+ reaction is represented by a predominant amount of agglutinated red cells toward the top of the gel column with a few agglutinates staggered below the thicker band. The majority of agglutinates are observed in the top half of the gel column.

2+ reactions is characterized by red cell agglutinates dispersed throughout the gel column with few agglutinates at the bottom of the micro tube. Agglutinates should be distributed through the upper and lower halves of the gel.

1+ reactions is characterized by red cells agglutinates predominantly observed in the lower half of the gel column with red cells also in the bottom. These reactions may be weak, with a few agglutinates remaining in the gel area just above the red cells pellet in the bottom of the microtube.

Negative reaction is represented by red cells forming a well- delineated pellet in the bottom

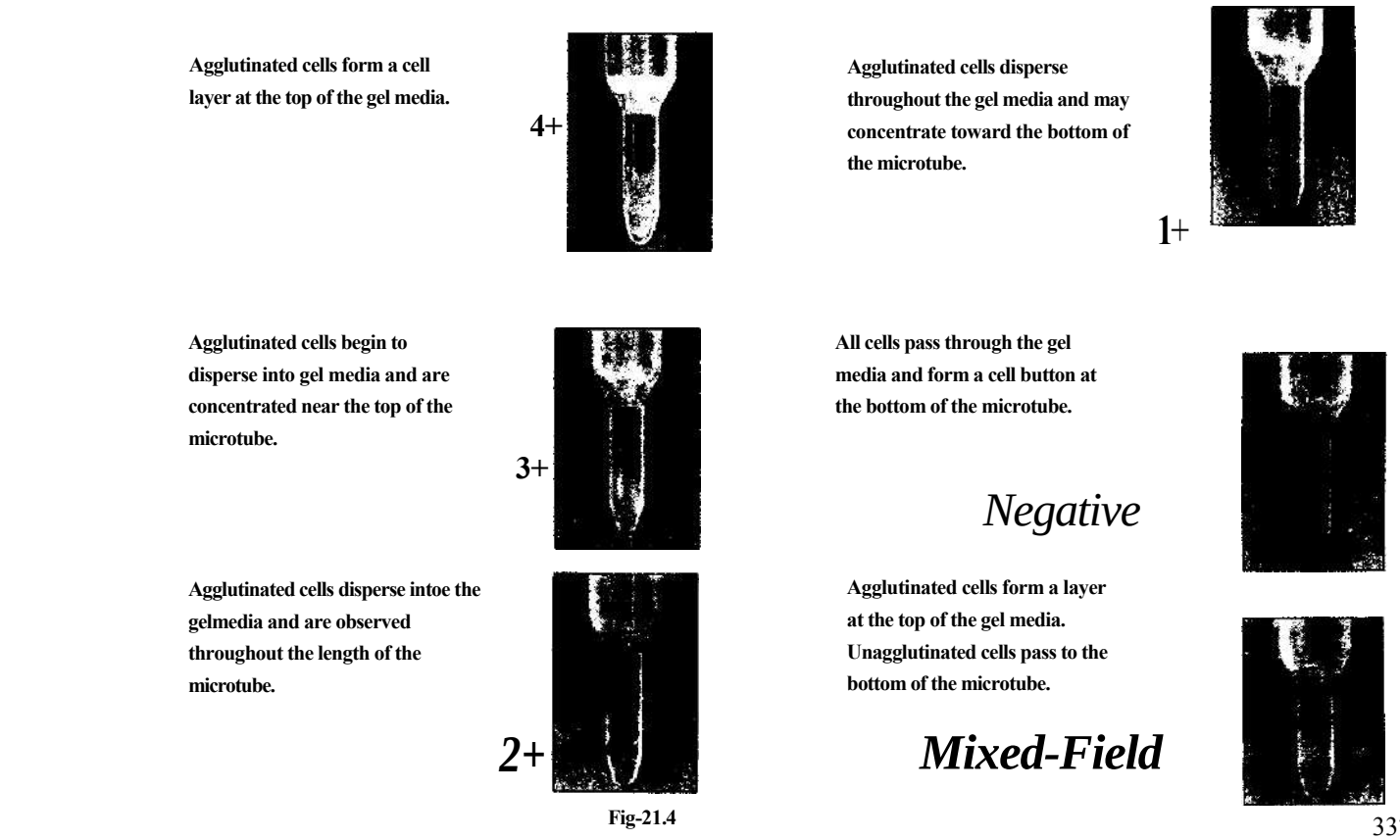


Fig-21.4

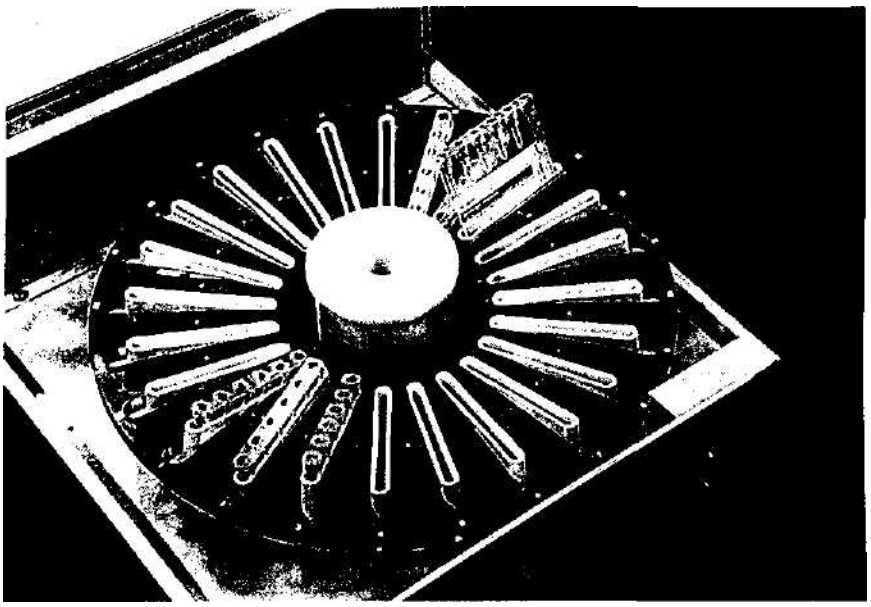


Fig-21.5
Centrifugation of microtubes

of the microtube . The gel above the red cell pellet is clear the free of agglutinates.

Mixed-field reactions may be recognized as a layer of red cell agglutinates at the top of the **gel** accompanied by pellet of unagglutinated cells in the bottom of the microtube.

Advantages of Gel Technology

- **The** gel technique is simple, reliable, rapid to use, reproducible and very sensitive.
- Greater uniformity between repeat tests.
- Decrease specimen volume are needed to perform a large number of tests.
- No tube shaking or resuspension of red cells button leading to variation among technologists in reading and grading the agglutination.
- In AHG test no wash steps, and no need to use sensitized red cells in negative AHG tests
- The provision of centrifuge (Fig. 21.5) calibrated to spin at optimal speed for fixed and correct length of time, reduce the potential of errors during this phase of the test.
- More objective, consistent and reproducible interpretation of results.
- The cards have a shelf life of 1 year and easy storage at room temperature (18 - 25 °C).

Disadvantages of Gel Technique

The following equipment are required:

- Special centrifuge to accommodate the microtube cards (Fig.21.5)
- Special incubators to incubate the microtube cards.
- Pipette to dispose 25uL of serum.
- Pipette to dispense 50uL red cells suspension.
- It is expensive.

GLASS MICROBEADS TECHNOLOGY

This system for irregular antibody screening was described in 1993 by Reis K. J. This test is performed in a microcolumn prefilled with glass microbeads in suspension of antihuman globulin serum, any diagnostic reagent or neutral isotonic solution. The sensitized cells are trapped by the microbead suspension during column centrifugation. See Figure 12.6.

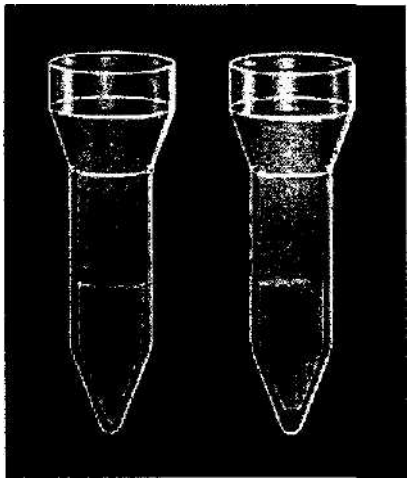


Fig-12.6

The detection of sensitized red cells is based on the sieving effect of glass microbeads. Red cells and serum are incubated at the upper part of a column over the glass microbeads suspension. These microbeads are calibrated and during the centrifugation step they retain the agglutinates and the unsensitized cells sediment at the bottom. Kits are manufactured by Ortho Diagnostic System and marketed by the name of Ortho Bio Vue system. See Figure 12.7.

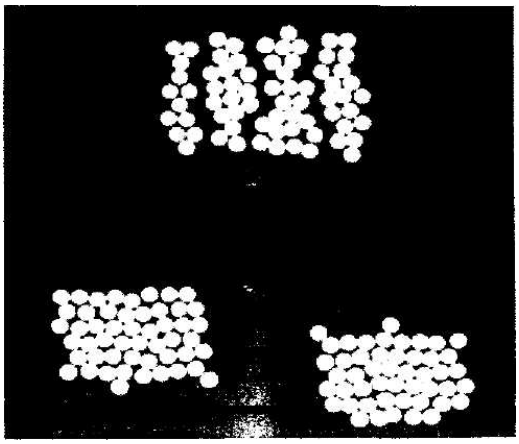


Fig-12.7

Cassettes of different program are available like:

1. ABO-Rh(D) typing with ABO reverse grouping
2. AHG polyspecific (anti-IgG + anti-C3d)
3. AHG - IgG monospecific
4. Crossmatching
5. ABO-Rh(D) typing in new born

Column Agglutinate Technology:

Test	Pipette	Incubation	Centrifug
ABO &Rh(D) typing	10µl 3-5% red cells suspension	-	5 min.
ABO reverse typing	A_1 and B cells + 40 µI patient/donor serum	-	5 min.
Direct AHG test (DAT)	10 µl 3-5% red cell	-	5 min.
Indirect AHG test (IAT)	50µl LISS + 10ul3-5% red cells suspension + 40 µl patient serum	10-30 min	5 min.

Advantages of Glass beads technology

1. Minimum incubation time of 10 minutes for antibody screening or cross matching
2. Biphasic centrifugation time is only 5 minutes
3. In AHG test there is no need to wash cells or to use sensitized cells for confirmation
4. No tube shaking or re-suspension of cells button leading to variation in reading and grading the agglutination
5. The provision of centrifuge calibrated to spin at optimal speed for fixed and correct length of time, reduce the error during this phase of the test
6. More objective, consistent and reproducible interpretation of results

Disadvantages of Glass Beads Technology

1. Special centrifuge to accommodate glass beads cassettes (Bio Vue TM Centrifuge)
2. Special incubators to incubate the glass breads cassettes (Bio Vue TM Incubator 32)
3. Pipettes to dispense 10 p.1, 40 µJ, 50µl
4. It is expensive

22
Quality Assurance In Blood Transfusion

The blood transfusion services should provide blood and blood products that are safe, pure, potent and effective. To provide a high level of assurance of safe blood and transfusion practices to blood donors, physicians, patients and patients' families, a quality philosophy must be evolved by blood transfusion services

The method of bringing this quality philosophy into operation includes, quality control, quality assurance and continuous quality improvement.

Assurance of a quality product includes issues of safety of patients and blood donors, reagent quality control, monitoring of equipment repair and maintenance, competence of personnel, and testing of a defined number of units of each product for the appropriate parameters and hazardous waste management. Inherent in this goal is the provision of a safe work environment.

Adequate record keeping is essential to the process of quality and safety assurance. Besides, appropriate data collection, retrieval and analysis are also essential to the process of monitoring quality.

Blood and blood components are biological products and considered as Drugs. Blood bank compliance with federal regulations and standards prescribed in Drugs and Cosmetic Acts is the statutory requirement of the Drugs Controller General of India.

Quality control, quality assurance and continuous quality improvement are complementary, yet distinctly different concepts

QUALITY CONTROL

Quality control is management of the testing process.

It includes :

- Assessment of the accuracy and reproducibility of a test.
- Equipment or instruments used to perform tests must Be monitored to determine if they are functioning properly.
- Reagents must be tested to determine if they have maintained their specificity and sensitivity.
- Quality control of the products

QUALITY ASSURANCE

- Quality assurance is the term used for all the measures from the recruitment of donors to transfusion of blood or blood products, to ensure that the products are of the quality required for their intended use, and that the laboratory results are reliable. This ensures that the patient receives a positive, defined advantage from a particular product, that donor, patient and staff are not harmed. Some activities are entirely within the control of blood transfusion services while others (e.g. collection blood sample and administration of blood) may be outside of its immediate control, although even these should meet the requirements set by the transfusion service.
- Quality assurance entails defining, controlling and documenting every aspect of a process or procedure in such a way that compliance with determined standards can be predicted. Thus quality control is the integral part of quality assurance.

CONTINUOUS QUALITY IMPROVEMENT

- Continuous quality improvement is a broad term that involves reviewing whether appropriate and efficient care was provided to the patient. It also involves the process of reducing rework, inappropriate patient care and waste.
- In all of these quality process, observations are made and conclusions are drawn. Corrective measures are taken if expectations are not met.

PRINCIPLES OF QUALITY ASSURANCE IN BLOOD TRANSFUSION

For safe transfusion practice, quality assurance requires an organized scheme of management, with clearly identified levels of individual responsibility, properly trained staff and adequately designed and equipped laboratories.

Depending on the level of operation, a section with specific responsibility for quality control under one senior trained technologist may be created.

The main areas requiring attention in quality assurance program include:

- Premises
- Personnel
- Specifications and quality control of
    - blood and blood products
    - reagents
    - equipment

- Manual of Standard Operating Procedures (SOP)
- Documentation
- Educational, research and development programmes.
- Introduction of automation and computing (where possible)

Premises

- The design and construction of blood transfusion premises are important aspects of good manufacturing practice.
- There must be adequate space for work and movement of the staff, overcrowding must be avoided.
- Furnishing, fittings, and floor material must be carefully selected so that it can be cleaned, and convenient for working.
- Lighting must be adequate throughout the premises, and proper lighting may be necessary in bleeding room and the areas where tests are carried out.
- Attention should be given for proper ventilation.
- Adequate power and water supplies, facilities for waste disposable and strict adherence to standards of sanitation are essential.
- Premises should be air-conditioned, if it is not possible, at least bleeding room, laboratories, and component preparation room should be air-conditioned.
- Provision of generator for continuous power supply is essential.

Personnel

There must be adequate personnel with appropriate educational qualification, training, and experience to ensure competent performance of assigned duties applicable to all categories of the staff working in the department of blood transfusion services including laboratory staff, social workers.

Job description should exist for all personnel involved in blood transfusion service including donor center.

The staff personnel should be courteous, interested, cheerful and friendly, as well as professional and efficient.

Technical persons

- The technical persons must have basic educational qualification in medical laboratory technology along with experience of working in blood transfusion service.
- They should be given in-house training for laboratory procedures once they are hired.
- The competence of personnel to continue to perform their assigned job functions must be periodically evaluated and recorded.
- There should be provision for continuous medical education (CME) program to upgrade the knowledge in recent techniques.
- Supervisory personnel must periodically review the results and evaluations, to ensure that all persons adhere to testing standards. Corrective actions should be taken, if needed.

Social workers and donor organizers:

The blood donor organizers, who may be social workers, should have all information about voluntary blood donation, need of blood and blood products, and scientific and technical advances in blood

banking. There should be meetings for the social workers and donors organizers at which they can express and exchange views about their work.

The donor organizers should be courteous, interested, cheerful and friendly, as well as professional and efficient,

QUALITY ASSESSMENT AND UTILIZATION REVIEW

Quality assessment and utilization is accredited by:

Director of Blood Transfusion Service

Ensures that the technicians do procedures according to the standard operative procedures and quality control is maintained.

Representatives of the State/ Central Drugs Controller

Ensures that the blood transfusion service follows the rule and regultions laid down in Part XII B of Drugs and Cosmetics Acts and Rules notified by the Drugs Controller General (India).

Hospital Transfusion Committee

Hospital transfusion committee assesses the adequacy of the blood transfusion service as well as the performance of the clinicians in using blood and its components appropriately. It has representatives from the disciplines who are maximum users of blood, medical superintendent as chairperson, in-charge of blood transfusion service as member - secretary, and nursing superintendent.

Role of transfusion committee

- Review the appropriateness of ordering policies of blood/ red cells/ components.
- Review handling and administration of blood and its components.
- Review medical record randomly selected from the list of transfused patients.
- Review summary report of usage statistics such as -
  - Total number of units of blood/ component ordered or cross-matched.
  - Total number of units of blood/ component tranfusion given.
  - Cross-match: Transfusion ratio
    It is calculated by dividing cross-matched units by the number of units transfused. If it is more it is an indicator that too much units are requested to 'on hold'.
- Number of waste and out dated units: Its high number indicates
  - Inadequate management of blood transfusion service
  - Over ordering of blood/components practice of clinicians
- Number of autologous transfusion given
- Review of transfusion reactions (where necessary, institute corrective measures).
- To promote continuous medical education in transfusion medicines for hospital staff.
- To prepare maximum surgical blood order schedule (MSBOS) for surgical procedures.

An efficient hospital transfusion committee will contribute to increase safety of blood and blood products and its proper utilization.

Standard Operating Procedures (SOPs), Including Documentation

Every blood transfusion service should have its own standard operating procedures (SOPs) manual. It should have instructions in detail about the techniques, procedures and other activities in the blood bank laboratory to maintain uniform standards of procedures, and quality control. Every activity should be carried out according to the clearly written instruction in SOPs. The standard operating procedures should be revised as often as necessary; and the date of revision should be clearly recorded. Obsolete operating procedures must be removed from circulation.

SOP should be available at each working areas.

Standard operating procedures manual should include:

- Donor selection by questionnaire
- Blood collection (Phlebotomy)
- Procedures of testing and processing the blood donations.
- The title, and the brief explanation of the purpose of the procedure.
- Details of the methods, and the example of work protocols.
- The reporting procedure for results, and the action to be taken if problem occur.
- Specifications of reagents and equipment.
- Procedures of preparation of blood components and their quality control.
- Specific quality control procedures.
- Training requirements for staff to perform the procedures.
- Biosafety in the transfusion laboratory

Advantages of SOP.

- **It** ensures that specified standards are met uniformly and at all times.
- It helps to standardize and monitor the performance of all the staff members, particularly technical personnel.
- It helps in training the staff particularly newly appointed ones.
- It helps the staff in performing assigned job.

SPECIFICATION AND QUALITY CONTROL OF REAGENTS

General Principles

- The reagents must be of high quality and have a shelf -life of at least one year for use.
- All anti-sera must comply with the standards laid down for potency (titre and avidity) and specificity.
- All blood grouping and anti-human globulin (AHG) reagents should contain a preservative to minimize bacterial and fungal growth.
- They should be kept in the refrigerators at 2°C-6°C, however, freezing is not recommended. Manufacturer's instructions should be followed for storage.
- All reagents should be clearly labeled with the batch number, expiry date and storage temperature; instructions for use are enclosed with each reagent packing.

- All reagents should be used according to the manufacturer's instructions. They must be re-standardized if they are to be used by alternative techniques, or in a diluted form (e.g. ABO and Rh typing in microplates or automatic system)
- New reagents should not be introduced into routine work until internal serological assessments have confirmed that the new reagents are satisfactory.
- Polyclonal (human serum) reagents should carry a statement saying that the individual donations used to prepare the product have been tested and found negative for **HIV** antibody, HBsAg and HCV antibody. However, since no test offers the complete assurance that products derived from human blood will not transmit infection, it should be handled with care.

REAGENTS

Standardization of blood group reagents is carried out by the manufacturers and they should meet established requirements. The quality control at the user laboratory should be done to check new batches to ensure that they comply with all biological standards.

Almost all laboratories use reagents red cells, ABO and Rh typing sera, anti-human globulin (AHG), one or more proteases, bovine serum albumin (BS A), isotonic saline and low ionic strength salt solution (LISS). Their quality control is very essential.

Table 22.1: Quality control of Reagent red blood cells

Parameters	Quality requirements	Frequency of controls
Appearance	No hemolysis or turbidity in supernatant by visual inspections	Each day
Reactivity and specificity	Clear cut reactions with known sera against red blood cells antigens	Each day

If reagent red cells are slightly hemolysed, the cells can be washed once with saline. If the supernatant becomes clear after one wash, and they are reactive, the cells are acceptable for use. Hemolysed and discoloured red cells are discarded.

QUALITY CONTROL OF ANTIBODY REAGENTS

Quality control of ABO reagents:

The main criteria for the quality of ABO antibody reagents are testing for specificity, and potency (avidity, and titer). There should be no reaction with negative control, no cross reactive and no rouleaux or prozone phenomena.

The reagent should be clear on visual inspection daily.

Table 22.2 Quality Control of ABO reagent (anti-A, anti-B, and anti-AB)

Parameter	Quality requirments	Frequency of control
Apperance	No turbidity, precipitate, particles or gel formation by visual insepction.	Each day
Specificity	Clear cut reaction with red cells having corresponding antigen(s); and no reaction with negative control.	Daily and of each new lot.
Avidity	Macroscopic agglutination with 50% red cells suspension in homologous serum/normal saline using the slide test; 10 seconds for anti-A, anti-B and anti-AB with A_1 and/or B cells at R.T; 20seconds with A_2 and A_2B cells.	Daily & each new lot
Reactivity	No immune hemolysis, rouleaux formation or Prozone.	Each new lot. •
Potency	Undiluted serum should give +++/C reactions in saline tube test using a 3% red cells suspensions at R.T., titer should be at least 128 for anti-A, anti-B, and anti-AB with A, and/or B cells, 64 with A_2 and A_2B cells.	Each new lot

Table 22.3 Acceptable Titer and Avidity of ABO reagents

Antisera	Type of the reagent	Type of red cells (2-3% cells suspension)	Titer	Avidity Time	Intensity
Anti-A	Polyclonal	A	1:256	10-12 sec	+++
		A_2	1:128	15-18 sec	++to+++
		A_2B	1:64	15-18 sec	++
		O			
		B			
	Monoclonal	A_1	1:256	3-4 sec	+++
		A_2	1:128	5-6 sec	++to+++
		A_2B	1:64	5-6 sec	++++
		O	-	-	-
		B	-	-	-
Anti-B	Polyclonal	B	1:256	10-12sec	+++
		A_1B	1:128	12-15sec	+

Acceptable Titer and Avidity of ABO reagents *(continued)*

		O	-	-	-
		A_1	-	-	-
	Monoclonal	B	1:256	3-4sec	++++
		A_1B	1:128	5-6sec	+++to+++
		O	-	-	-
		A_1	-	-	-
Anti-AB	Polyclonal	A	1:256	10-12sec	+++
		B	1:256	10-12sec	+++
		A_2 O	1:64	15-18sec	++to+++
	Monoclonal	A_1	1:256	3-4sec	++++
		B	1:256	3-4sec	
		A_1	1:128	5-6sec	+++
		O	-	-	-

Quality Control of Anti-D sera

Rh(D) is most immunogenic and has a higher frequency distribution among all the five varieties of Rh. Quality control of anti-Rh (D) regents are more complex than ABO reagents because of the great variety of reagents, methods of use and the necessary control procedure. The quality control of main types of anti -D reagents are given in table 22-4 and 5.

Table 22.4 Quality Acceptable of Rh anti sera **(Anti-D)**

Parameterl	Quality requirement	**Frequency of control**
Appearance	No turbidity, precipitiation, particles or gel formation by visual inspection	Each day
Specificity	Clear cut reaction with R1,r cells	Each day and each new lot. and no reaction with IT cells:
Avidity	Visible agglutination with 40% red cells suspension in homol-ogous serum using the slide test.	Each day and each new lot
Reactivity	No immune hemolysis, rouleaux formation or prozone phenomenon.	Each new lot
Potency	Undiluted serum give +++ reactions in designated test for each serum and a titer 32-64 for anti-D, anti-C anti -E, anti-CD anti-DE using R,r,R, r, red cells.	Each new lot

Table 22-5 Acceptable Titer & Avidity of Anti-D in Anti-Rh (D) Reagent

Type of reagent	Type of red cells	Titer +		Avidity	Intensity
		Immediate spin	After 30-45 min incubation		
IgM Monoclonal	OR, r or R_1R_2cells	1:64-1:128	1:128-1:256	5-10 Sec	+++
Blend of lgM +IgG monoclonal	OR,ror R_1R_2cells	1:32-1:64	1:128-1:256	10-20 Sec	+++
Blend of monoclonal IgM+ Polyclonal (human) IgG.	Same as above	Same as above	Same as above	Same asabove	+++
Polyclonal (Human) anti-Rh(D)	OR,ror R_1R_2cells	-	1:32-1:64 InAlb/Enz/ AHGtest	60 Sec	+++

For reliable Rh(D) typing the following requirements must be met:

1. Use two distinct anti-Rh(D) reagent of two different manufacturers or use two distinct anti-Rh (D) reagents of two different batches of the same manufacturer.
2. Incorporate Rh- positive (group OR_1r) and Rh- negative (IT) control cells with test.
3. Each test sample must give a negative 'auto' test (own cells and own serum). Auto controls are necessary to minimize false positive results due to auto-agglutination or the presence of sensitized cells in vivo..
4. Monoclonal Rh (D) reagents are unreliable for the detection of D^u.
5. Blend of IgM and IgG monoclonal or blend of monoclonal IgM and polyclonal (Human) IgG can be used for AHG test to identify weak D antign (D^u).

Polyspecific Anti -human Globulin (AHG) reagents.

Polyspecific anti-human globulin reagents have anti-complement C3b and C3d as well as anti-IgG acitvity.

For quality control testing:

- Each vial of a new batch is tested for its specificity and sensitivity with IgG (anti-D) coated OR_1r red cells as positive control and non sensitized OR_1r cells as negative control.
- For complement acitivity, check with red cells coated with C3b, C3d or red cells sensitizied with complement binding (anti -Le^a).
- The potency of anti-IgG of AHG reagents can be estimated by titration using IgG (anti-D) sensitized OR_1r cells.

Table 22.6 Acceptable quality of anti-globulin reagent:

Parameter	Quality requirement	Frequency of control
Appearance	No precipitate, particles or gel formation by visual inspection.	Each day
Reactivity and Specificity	• No prozone phenomenon	Each new lot.
	• No hemolysis or agglutination of unsensitized red cells	Each new lot.
	• Agglutination of red cells sensitizied with anti-D serum containing not more than 0.2 mg/ml antibody activity.	Each day and each new lot
	• Agglutination of red cells sensitized with a complement binding antibody (e.g.anti Le^a).	Each new lot.
	• Agglutination of red cells coated with C3b and C3d, and no/ weak agglutination with C4 coated red cells.	Each new lot.

Minimum requirements for Quality Product of AHG are :

Anti-IgG	**1:64**
Anti-C3/C4	**1:4**

For more details - see chapter on Antihuman globulin (Coombs' Serum).

ENZYME REAGENT

Protease enzymes (e.g. papain and bromelin) are widely used for antibody detection. Their suitability is generally based on the demonstration of satisfactory serological activity with weak IgG anti-Rh (D). For quality control of protease (Enzymes) see table 22.7

Table 22.7 : Quality Control of Proteases (Enzymes)

Parameter	Quality requirements	Frequency of control
Reactivity	• No agglutination or hemolysis using inert AB serum.	Each day
	• Agglutination (+++/C) of cells sensitizied with a weak IgG (Anti-D).	Each batch
Potency	• An IgG antibody, preferabely anti-D standardized to give a titer about 32-64 by the protease technique, should show the same titer on repeated testing with different batches.	Each batch
	• The 2-stage enzyme titer should atleast be equal to the titer obtained with IgG (anti-D) by AHG test.	Each batch

The one stage technique, in which the enzyme, antibody and red cells are all mixed is satisfactory but not as sensitive as 2-stage technique.

BOVINE SERUM ALBUMIN (BSA)

It is usually used as

1. 22% albumin as an enhancer of agglutination.
2. 1-7 % albumin as a stabilizer in other reagents specially those to be stored at 4° C.

Table 22.8 : Quality control of 22% bovine serum albumin (BSA)

Parameter	Quality requirement	Frequency of control
Appearance	No precipitate, particles or gel formation by visual inspection.	Each day
Purity	> 98% albumin, as determied by electrophoresis.	Each new lot
Reactivity	No agglutination of unsensitized red cells; no hemolytic activity; no prozone phenomenon.	Each new lot
Potency	IgG anti-D should give a titer of 32-64 with red cells R_1r	Each month

Quality control of LISS (Low Lonic Strength Saline)

For preparation and quality control of list see chapter on Special Methods.

Table 22.9 : Quality control of Normal Saline

Parameter	Quality requirement	Frequency of control
Appearance	No turbidity or particles by visual inspection	Each day
NaCl content	0-154 mol/l(=9g/I)	Each new batch
$_P$H	6.0-8.0	Each new batch
Hemolysis	Mixture of 0.1 ml saline and 0.1 ml of 5% red cells suspension centrifuged after 10 min, no hemolysis.	Each new batch

Table 22.10 : Quality control of Distilled water

Parameter	Quality requirement	Frequency of control
Appearance	Clear, no particles on visual inspections	Each day
PH	6.0-7.0	Each new batch

Quality control of Copper Sulphate ($CuSO_4$) specific gravity 1.053
See chapter on preparation fo Solution and Methods

QUALITY CONTROL OF VARIOUS BLOOD COMPONENTS

Quality control of blood and its products depends upon;

- Selection of donor
- Quality of the container and anticoagulant preservative solution
- Technique of phlebotomy
- Storage temperature

Quality control of Whole Blood

- Quality control of whole Blood volume: weigh atleast 1 % of donations and calculate the volume from the formula given below:

$$\text{Vol (ml)} = \frac{\text{Wt. of bag + blood (g) - wt of empty bag (g)}}{1.05}$$

- Presently varying amount of blood 350/ 450 ml is collected at various centers. Centers preparing blood components take 450 ml of blood.
- Most of the centers use equipment for automatic shaking and weighing the blood collected.

Table 22.11 The quality control of Whole Blood.

Parameter	Quality Requirement	Frequency of Control
Volume	350/450 ml ± 10 %	1 % of all units
Anticoagulants*	49/63 ml	All units
PCV(Hct)	30 to 40%	4 units per month
HBs Ag	Negative by ELISA	All units
Anti-HCV	Negative by ELISA	All units
Anti-HIVI&II	Negative by ELISA	All units
Syphilis	Negative by Screening test	All units
Sterility	By culture	Periodically (1% of all units)

* Volume of anticoagulant should be proportionate to the volume of blood.
14 ml of CPD/ CPDA -I anticogulant solution is required for 100 ml of blood.

Table 22-12: Quality control of red cells concentrate (Prepared from 450ml Blood)

Parameter	Quality requirement	Frequency of control
Volume	280 ± 40 ml	1 % of all units
PCV (Hct)	*109c ± 59c*	Periodically

Table 22-13: Quality control of red cells in preservative sol. (Adsol/SAGM)

Parameter	Quality requirement	Frequency of control
Volume	350 ± 20 ml	1% of all units
PCV(Hct)	55-65%	Periodically

Leucocytes -Poor Red cells

Leucocytes poor red cells have at least 70% of the white cells removed while retaining at least 70% red blood cells.

Table: 22-14 Quality control of Leucocytes- poor red cells

Method of Prepartion	Parameter	Quality requirement	Frequency of control
Leucocytes poor red cells modified by centrifugation	White cells removed Residual red cells Remaining	< 70% leucocytes of original quantity >70% of the original quantity	4 units a month
Washed red cells	Plasma removed	99%	
	RBCs loss	20%	
	Leucocytes removed	85%	
Leucocytes poor red cells modified	White cells removed Red cells remaining by leucocyte filter	99% removed 90-95%.	4 units a month

Table: 22-15 Quality control of platelet concentrate.

Quality control of platelet concentrate prepared from 450 ml of whole blood

Parameter Quality	Requirements	Frequency of control
Volume	50-70 ml	All units
Platelets count	$>5.5x10^{10}$	4 units per month
pH	>6.0	4 units per month
RBC contamination	0.5 ml	4 units per month
WBC contamination	$5.5x10^{7}$ - $5x10^{8}$	4 units per month

Quality control of platelet concentrate prepare from buffy coat

Parameter	Quality requirement	Frequency of control
Volume	70-90 ml	4 units per month
Platelet count	$6\text{-}9X10^{10}$	4 units per month
PH	>6.0	4 units per month
WBC contamination	$>5.5X10^{6}$	4 units per month
RBC contamination	Traces to 0.5 ml	4 units per month

On visual inspection unit which does not have a pink or red discoloration may be assumed to to contain insufficient red cells to cause immuniztion.

Table: 22-16 Quality of Platelet Concentrate by Apheresis

Parameter	Quality Requirement
Volume	200-300 ml
Platelets count	>3.0-7.0x10^{11}
pH	>6.0
Residual leucocytes	< 5.0 x 10^{6}
Red cells	traces to 0.5 ml

Table: 22-17 Quality control of Fresh Frozen Plasma (FFP)

Parameter	Quality control	Frequency of control
Volume	200-220 Plasma	4 units per month
Stable coagulation factors	200 units of each factor	4 units per month
Factor **VIII**	0.7 units/ml	4 units per month
Fibrinogen	200-400mg	4 units per month

Table : 22-18 Quality Control of Cryoprecipitate (Factor VIII)

Parameter	Quality control	Frequency of control
Volume	10-20 ml	Occasionally
Factor VIII	80-120 units	Occasionally
von-Willebrand factor	40-70% of the original	Occasionally present.
Factor XIII	20-30% of the original	Occasionally
Fibrinogen	150-250 mg	Occasionally
Fibronectin	55 mg	Occasionally

75% units sampled and tested should have the values indicated above.

Table: 22-19 Quality Control of Plasma. (Frozen)

Parameter	Quantity requirement
Volume	200-220 ml
Stable coagulation factors	200 units of each factor
Factor V, VII, fibrinogen	Reduced

Table :22-20 Quality control of Granulocytes

A: Granulocytes prepared from hemapheresis

Parameter	Quantity requirement
Granulocytes	1 x 10^{10}
Other leucocytes	0.1 x0.7xl0^{9}
Platelets	2-10 x 10^{11}
Red cells	5-50 ml
Plasma	200 - 400 ml.
HES if used	6-12 % of volume

B: Granulocytes prepared from single unit of blood

Parameter	Quantity requirement
Volume	200-250 ml
Granulocytes	0.5-1 x 10^{9}

Quality Control of Equipment.

Selection and Evaluation

1. All equipment used in blood bank should meet mandatory technical, electrical and safety standards.
2. Preferably equipment should be purchased for which expertise for maintenance and repair is available locally with the manufacturer/ supplier.
3. Installation should be formalized with the assistance of commercial installation staff in conjunction with hospital engineering departments, to ensure compliance with electrical safety standards.
4. Once the equipment has been installed and calibrated according to the supplier's specifications, it should be confirmed that its performance meets required standards. It may then be introduced into routine work.
5. Each equipment should be checked and assessed for its proper working after repair and its record should be maintained.

General Quality Control of Equipment

1. The performance of laboratory equipment must be monitored periodically, the result must be recorded and adjustment be made if necessary.
2. Program of preventive maintenance, including cleaning and recalibration is mandatory. This program should be planned, in conjunction with hospital maintenance engineer or annual maintenance contract specialists, in order to minimize disruption of services.
3. Laboratory staff should do cleaning of the equipment, and periodic verification of speed by tachometer and temperature by thermometer.

Quality control and maintenance of equipment:

Refrigerator for storage of blood :

- Read recording temperature chart and digital temperature frequently, atleast once a day, the proper temperature range is between 2-6°C.

- The clockwise mechanism driving the temperature recorder should wound regularly and the chart is changed weekly.
- Periodically, temperature inside the cabinet should be counter checked with the help of precision thermometer.
- Alarm system should be battery operated and independent of the main electric supply. The alarm system should be set in such a manner as to make sound when the temperature is out side the required range of 2^0- 6^0C. The system should be checked once a week by immersing the sensor in ice water (for low temperature) and in water at 15°-20°C (for higher temperature). If alarm system is not working properly corrective measures should be taken.
- Blood refrigerator must be clean and well lit.

Freezers:

- Quality control of freezers is similar to blood bank refrigerator. Check the temperature-chart and digital temperature frequently atleast once a day.
- Periodically check the temperature of digital system by precision thermometer kept inside the cabinet periodically.
- If there is no automatic defrosting system it should be defrosted whenever needed.

Refrigerated Centrifuge:

- This should be checked by the service engineer every 3-4 months.
- Accuracy of speed and time should be checked with the precision rpm meter (tachometer) and stopwatch.
- Temperature inside the centrifuge bowl should be recorded by a temperature tester with the lid closed and the rotor stationery.

Laboratory Centrifuge (Bench Top):

- This should be checked every 3-4 months for accuracy of the speed and time with the precision rpm meter (tachometer) and stopwatch.
- It should be cleaned regularly.

Water bath and Incubator:

- They should be kept clean.
- The temperature should be checked daily.
- Accuracy of the thermometer should also be checked periodically
- Water should be changed frequently.

Microscope:

- Keep the microscope covered when not in use
- Lubricate coarse adjustment of rack and condenser every six months.
- Keep stage clean, clean condenser, lenses, with moistened lens paper frequently and clean lenses immediately if accidentally become dirty.

Automatic cell washer:

- Automatic cell washer is centrifuge programmed to add saline automatically to test tubes, then centrifuge them and decant the saline. They can be set to perform this function 1-4 times.

- The cell washer should be checked periodically to determine whether the tubes get correctly filled with saline, emptied and proper cell button is performed.
- A kink in the saline entry tubing, blockage of the saline port due to salt crystals or bent or misaligned saline port may give uneven fills and inadequate washes.
- Plastic tubing should be checked monthly for any deterioration.
- Perform duplicate titration of standards antisera, e.g Anti-D (IgM+IgG)
- against D positive cells in saline. Wash one set of titer with cell washer and another set manually, test both set by antiglobulin serum. Score should not differ significantly.

Automated equipment for shaking and weighing blood bag:

- It should be checked daily for its performance.
- Check its weighing system with a known weight in grams.

QUALITY CONTROL OF TECHNIQUES

- The objective of quality control of techniques is to ensure a consistently high standards of performance of the most commonly used techniques such as ABO and Rh typing, anti-human globulin test, detection and identification of irregular antibodies of clinical significance and compatibility test.
- Techniques which have been validated for accuracy, reliability and sensitivity are subject to quality monitoring by the use of positive, negative and auto- controls.
- Technique errors may be due to:

    - Lack of proper reagents
    - Lack of attention to incubation time or temperature, or to centrifugation speed and time,
    - Insufficient washing of cells.
    - Too strong or too weak cells suspension, haemolysis read as a negative result
    - Failure to confirm negative results under microscope.

BIO SAFETY IN THE TRANSFUSION LABORATORY

- It is essential that safe working practice are instituted and maintained as the staff in blood transfusion laboratories are constantly at risk of infection from the blood that they handle every day.
- Microbiological test HBsAg, antibodies to HIV and HCV must be performed in a separate room.
- For disinfection of viral and bacterial contamination 1% hypochlorite solution is the disinfectant of choice for use in blood bank. All used glass tube and slides must be disinfected in hyprochlorite solution before washing, and plastic tubes should be soaked in hyprochlorite before disposal, holding time of the infected material in hyprochlorite solution should be atleast 30 minutes.
- For disinfecting blood spillage and heavily soiled equipment, hyprochlorite solution of 10 times of the strength (1%) should be used.
- Infected material should be decontaminated by autoclaving, before disposal or incinerated.

General Safety Precautions are:

- No eating, drinking , smoking or application of cosmetics in the laboratory.
- Mouth pipetting is forbidden.
- Any break in the skin tike cut, puncture wound or skin eruption must be kept covered with water proof dressing.

- Protective coats must be worn in the laboratory and must be removed before leaving the laboratory
- Laboratory staff should wear disposable gloves.
- Hands must be washed properly before leaving the laboratory.

Record Keeping System

Blood bank and blood transfusion service must have a record keeping system manual, computerized or a combination of both. Records must be indelible, legible handwritten or typed or they may be retained electronically, provide they can be easily retrieved for reference of review and adequate backup exists in case of system failure. Records must be stored in a manner that protects them from damage and from accidental or un-authorized destruction or modification. Records should be maintained for 5 years as required under Drugs and Cosmetics Acts and rules.

A blood bank must be able to trace a unit of blood/component from collection/preparation to final disposable in a timely manner.

Efficient documentation requires appropriate design of forms, labels and registers for each activity of blood bank. Record should be legible and correction should be initialed.

Minimum Record keeping requirements are:

Record of Blood Donors

Donor registration form have:

- Blood donor consent for donation
- Donor's name and father/husband name
- Donation - voluntary or replacement
- Date of birth (age), Sex and weight
- Address (office & residence) and telephone number
- History of illness

Record of blood donation

- Date of blood donation
- Donation number (Identification number)
- Physical examination record - pulse, temperature, and blood pressure
- Hemoglobin
- ABO and Rh(D) group
- Results of HBsAg, anti-HCV, anti-HIV 1&2. VDRL/RPR and malaria tests
- Disposal: issued for transfusion or discarded

Record of Blood component Preparation

- Name of component
- Donation number
- ABO and Rh(D) group
- Date of preparation
- Result of tests of markers of transmissible infections
- Disposal: issued for transfusion or discarded

Recipient's requisition form

- Patient's name with father/husband's name
- Admission/Registration number
- Age and Sex
- Hospital name - room/bed number
- Name of clinician attending the patient
- Diagnosis and reasons of transfusion
- Number of units of blood/component required
- Date and time of requirement
- Requirement is routine or emergency

Compatibility Test Record on the Requisition Form

- Recipient's ABO and Rh(D) group
- Antibody screening in patient's blood
- Donor's unit donation number
- ABO and Rh(D) of donor' unit
- Cross-matching for IgM&IgG
- Result of compatibility : compatible or uncompatible
- Initial of technician performing the test

Blood /Blood Components Issue Record:

- Serial number & Date
- Patient's name, Age & Sex
- Admission number & room / ward number
- ABO&Rh(D)
- Number of donation, ABO & Rh (D)
- Date of Collection & Date of Expiry
- Name of Components
- Compatibility for IgM & IgG
- Cross-matched by
- Issued by & time of issue

Record of Blood Transfusion Reactions and their Investigations

- Reported transfusion reaction cases should be investigated
- Record should be kept

Record of Infections Markers Tests:

- Anti-HIV 1& 2Test
- HBsAgTest
- Anti-HCVtest
- VDRL/RPR
- Malaria

Other Documents Required in Blood Transfusion Services:

- Stock register of consumable articles.
- Register of non-consumable articles.
- Daily stock register of blood and components
- Quality control record

For more details for documentation and record keeping see chapter on Blood safety and Federal Regularity Requirements

HLA SYSTEM

Laboratory

Techniques

The Major Histocompatibility Complex (MHC) exists in almost all animal species and in man is represented by the HLA system. The letter H in HLA designates human and L designates leucocytes, as these were the first cells shown to carry the antigens of this complex. The letter A was originally a locus designation. However, with the discovery of several more HLA loci, it is now taken to represent an antigen. HLA system is the most important genetic determinant of graft acceptance or rejection between genetically distinct individuals and so its products are known as the major histocompatibility molecules in contrast to other genetic loci, which exert only relatively minor effects on histocompatibility. Thus individuals who express the same MHC molecules accept tissue grafts from one another, while those who differ at their MHC loci reject such grafts.

I. GENETICS OF HLA

The HLA molecules are encoded by a cluster of genes on a small region of approximately 2cM (centimorgan) in length, on the short arm (p21.3) of chromosome 6. The region spans approximately 4 megabases or $4x10^6$ nucleotides and contains numerous genes, only some of which are concerned with Histocompatibility. The major biological function of the HLA genes concerns the presentation of foreign (or self) antigens to the immune system. Thus HLA molecules act as 'receptors' that capture peptide fragments of antigen which are displayed (or presented) on the cell surface where they can be recognized by appropriate T cells. In presenting foreign antigens

to T cells, HLA molecules evoke CTL (cytotoxic T lymphocyte) and helper T cell responses, which then regulate specific immunity.

The HLA system contains nearly 240 potential genes arranged so closely to each other that they are generally inherited together. A set of antigens on the same chromosome inherited enbloc from each parent is called a haplotype. Almost 40% of the total HLA genes have immune related functions. There are 6 major HLA loci encoded within the human MHC (Fig. 23.1). These genes encode structurally homologous products, which are classified into HLA class I and class II molecules based on their structure, tissue distribution, source of peptide antigen and the function of responding T cells.

The class I HLA molecules are encoded by three distinct genetic loci, known as HLA-A, B and C, clustered in the class I region of the MHC and spanning approximately 2 megabases. The class II molecules include HLA-DR, DQ and DP, each of which is encoded by distinct genetic loci clustered in the class II region spanning approximately 1 megabase. Each of the 6 linked loci encodes a distinct HLA product expressed on the surface of antigen presenting cells. The part of the MHC between the class I and class II regions is referred to as the class III or central region.

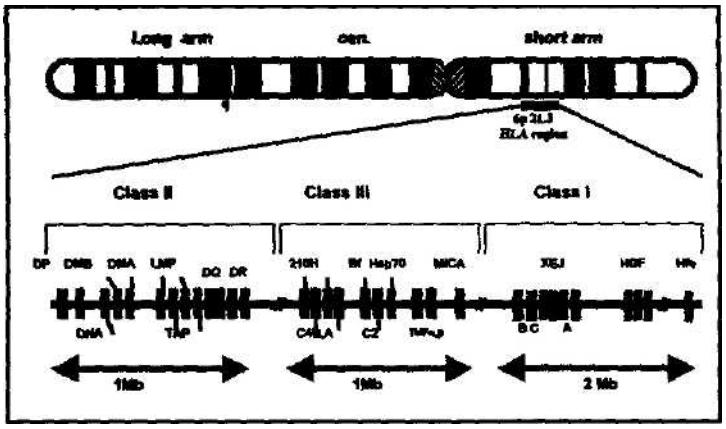


Fig 23.1 The human major histocompatibility complex: The human major histocompatibility complex (MHC) on the short arm of chromosome 6. The HLA class I, class II and Class III regions are shown spanning a 4000 Kb region. TAP- transporter of antigenec peptides; 21-OHOSteroid 21-hydroxylase enzyme; C4 A&B-complement C4 loci; Bf-properdin factor B the alternate complement patheway; C2- complement component C2; TNF- tumor necrosis factor, MICA-MHC class I chain related gene; Hfe- haemachromatosis gene. The classical HLA loci) A,B,C,DR,DQ,DP) are shown in solid bars

4.1.1 POLYMORPHISM IN THE MHC

A unique feature of the HLA system closely related to its biological significance is its , extraordinary polymorphism. Indeed, there are no other loci known to have similar degree of polymorphism, which means that exceptional inter- individual variability is found in DNA sequences of HLA molecules of a population.

All regions of the MHC are known to be highly polymorphic, constituting several closely linked loci each with a large number of genes that can be further split into many allelic types differing in their nucleotide sequences. Figure 23.2 shows the number of alleles over the years

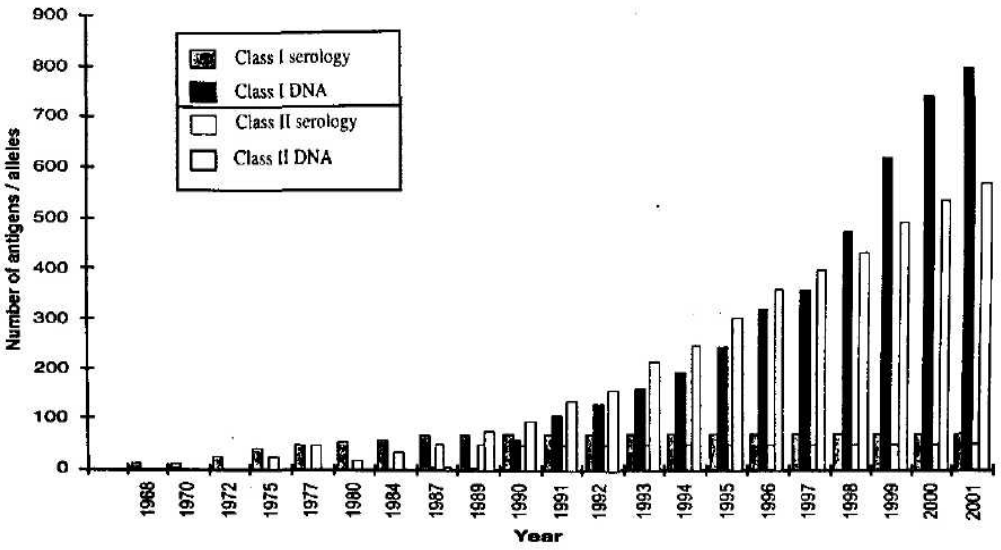


Fig. 23.2 : Ever increasing HLA diversity. The figure depacts the number of alleles in the HLA class I (A,B,C) and class II (DR,DQ,DP) loci identified by serology and by DNA based methods over the years.

defined separately by serological and DNA based techniques in the HLA class I (A, B, C loci) and class II (DR, DQ, DP loci) regions. By July 2001, more than a total of 1340 alleles have become known at the DNA level (IMGT/HLA database) (Robinson et al, 2000).

It is apparent that the determination of polymorphism depends strongly on the methodology employed for the study. Thus polymorphism detected by molecular technologies is much larger than that detected by serology (Table 23.1).

Table 23.1. Polymorphism in HLA. Number of alleles defined by serological and DNA based methods.

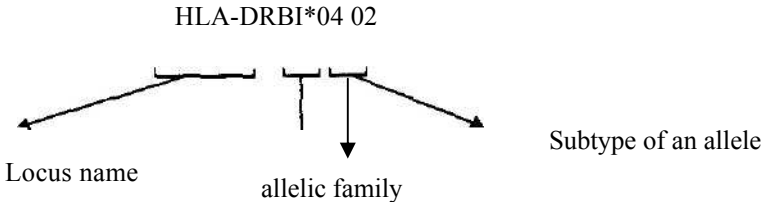
	A	B	C	DR	DQ	DP
No. of alleles						
Serology	23	47	8	14	9	6
Molecular	201	412	106	262	46	97

Theoretically several million phenotypic combinations (approximately 150 billion or even more) are possible in the HLA system. A close study of different population groups reveals that the degree of polymorphism in the HLA system may not be as extensive as indicated since certain alleles are associated with each other more frequently than would be expected on the basis of their individual gene frequencies. This non- random association of the alleles of two HLA loci found together on the same HLA haplotype is termed''Linkage disequilibrium' and is expressed in terms of delta. It is known to vary between different populations. Each individual inherits two antigens of each loci -A, -B, -C and -DR, one from either parent. A set of antigens on the same chromosome inherited enbloc from one parent is called a haplotype. The two HLA haplotypes in an individual form the genotype, whereas the total HLA antigen profile constitutes the phenotype.

According to the Mendelian pattern of random segregation, a child in the family has a 25% chance to be HLA- identical with one of his sibs, 50% chance to be HLA haplo-identical (sharing one parental haplotype only) and 25% chance to be HLA unidentical, i.e. a total mismatch. Since parents and offsprings share 50% of the genes, the HLA match in this category of transplants is a 'one-haplotype match', i.e. the donor and recipients are matched for one HLA chromosome.

3. NOMENCLATURE

The HLA antigens are designated numerically separately for each locus. Individual loci are given descriptive names in Roman letters, e.g. HLA-A26, HLA-B8, HLA-DR3, etc. A 'W prefix in the number indicates a workshop specificity, which, though recognized serologically, has not yet attained the optimal definition, e.g. HLA-BW22 or DRw6. With the introduction of molecular biological procedures, the nucleotide and amino acid sequences of almost all alleles in the HLA system have become known. Thus, a revised nomenclature has been introduced that takes into account alleles (and their splits) defined in each of the alpha or beta chains of individual loci. For example, the original DR4 has been split into at least 22 subtypes. These are designated as locus symbol (e.g. DRB1) followed by (*) and then the allele number. Accordingly, the new DR4 alleles and their subtypes are designed as DRB1, *040l. *0402, *0403 etc., indicating various molecular subtypes. Likewise, the 14 known subtypes of HLA-B27 are designated as B*2701, B*2702, B*2703, etc. An example of the new nomenclature of a DR4 allele is shown below.



4. LABORATORY METHODS OF HLA TESTING

HLA testing is carried out for patients requiring organ and bone marrow transplantation in order to find a suitable matched donor from among the family members or the unrelated donor pool. Donor matching is essential for transplantation to avoid the risk of graft versus host disease (GVHD) and graft rejection. A full house matched donor is essential for bone marrow transplantation. On the other hand, kidney transplants are found to function well even with a 50% mismatch with the recipient. Since HLA alleles determine disease susceptibility, their identification has been used for diagnostic and/or prognostic purpose. The most common example in this area is HLA-B27 testing of ankylosing spondylitis and related spondyloarthropathies. Accurate HLA testing is also essential for blood component therapy, anthropological studies involving different racial groups and for a variety of research applications including development of MHC based vaccines.

Materials required for HLA Testing

(h) Peripheral Blood : For serology testing on viable lymphocytes or for DNA extraction for PCR based tests.

b) Lymph nodes/ Spleen: For serology testing on extracted lymphocytes (required for cadaver donor typing) or for DNA extraction for PCR based tests.
c) Serum/ plasma: For detection of soluble HLA molecules and test for anti HLA antibodies
d) Tissue : Required for DNA extraction for PCR based tests.

Methods for HLA testing

Depending on the need and requirements, following methodologies can be followed for testing polymorphism of the HLA -A, -B, -C (class I), DR, -DQ and -DP (class II) loci.

(i) *Serological:* Microlymphocytotoxicity test (two stage)
ii) ***Cellular:*** Mixed lymphocyte culture (MLC), Primed lymphocyte testing (PLT).
iii) ***Biochemical:*** One-dimensional or two-dimensional gel electrophoresis.
iv) ***Molecular methods:*** Several techniques have become available, of which more than one technique is essential for optimal HLA testing. RFLP, PCR-SSP, PCR-SSOP, PCR-SSCP, ARMS PCR, RBH/RLS.
v) ***Sequence based typing*** (SBT)
vi) ***Microarray or*** *DNA chip technology.*

Serology relies on expression and identification of different motifs of the HLA molecule on the cell surface. On the other hand, the PCR based methodologies depend on the quality of DNA extracted and rely on identification of nucleotide differences between HLA alleles at various levels of resolution (low/ intermediate/ high) (Figure 23.3).

The usefulness of conventional serological assays for HLA typing has been limited by the non-availability of reliable allele specific sera. Indeed, it is often difficult to obtain sera that react solely with the product of one allele of one locus. Although hybridoma technology has helped in overcoming the problem to some extent, a complete set of monoclonal antibodies for detection of regular serotypes is still lacking. The main reason for this is the fact that most of these antibodies are produced in the mouse system and are therefore not identical to human alloantibodies. Also,

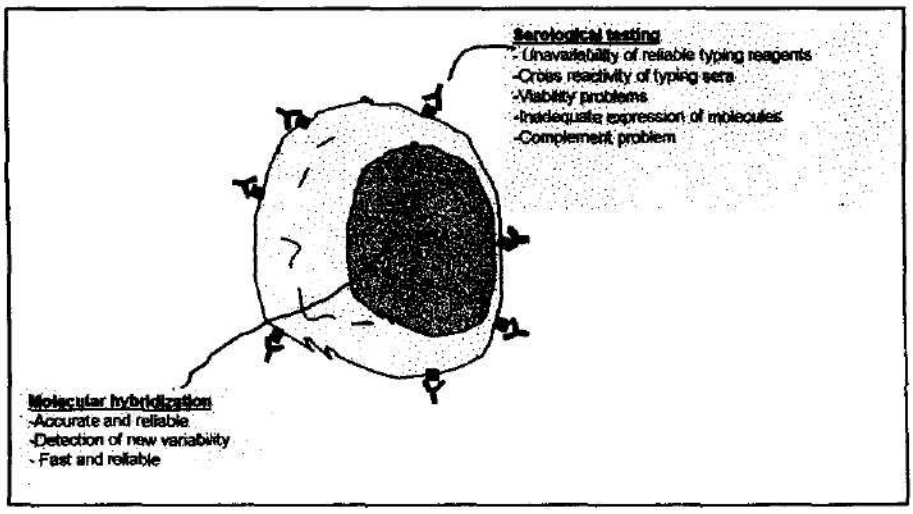


Fig. 233. Principle of serological and molecular HLA testing: Serology is based on the identification of expressed differences on cell surface while the DNA based methods identify differences at the nucleotide level, resulting in the depicted differences in

conventional serology is designed to identify broad allelic families (for example HLA-A2, B40). However monoclonal antibodies often recognize narrow determinants and therefore many of-them are not applicable for the serology test system. Figure 23.3 lists several other limitations of serology since the test is primarily dependent on expression of HLA molecules on the cell surface, requirements of viable cells and of complement. Molecular techniques on the other hand, are more accurate, reliable and detect differences even at a single nucleotide difference. These are therefore most suitable for defining several fold more number of alleles than serology

4.1.1 Serology

The conventional method for testing HLA class I and class II alleles is the complement dependent Microlymphocytotoxicity test (Terasaki and McClelland, 1964), involving mono or oligospecific sera. This is a two-stage test involving antigen-antibody reaction on the immune cell surface (1st stage), followed by complement-mediated lysis of the cells (2nd step). It is performed on a microtest tray with 60 or 72 wells that contains one ml of precoated allele specific antiserum defining a particular HLA specificity (Figure 23.4)**.** Generally, peripheral blood lymphocytes or purified T cell preparations are utilized for HLA-ABC typing, while for the class II testing of HLA-DR and DQ antigens, the nylon wool separated B lymphocyte preparations are employed. Approximately 2000 lymphocytes are added in each well and the tray incubated at room temperature for 1/2 hour. This is followed by a further incubation step of one hour using rabbit complement. The test is terminated by staining the dead cells with trypan blue or Eosin Y dye and fixing with formaldehyde. For class II typing, double incubation periods are used.

In this test, the discrimination of HLA antigens is based on recognition of different conformational structures of each antigen expressed on the cell surface. Several antisera are generally required to assign a specificity due to the complex patterns of serological cross reactivity between individual HLA determinants. Two antigens with the same amino acid sequences at positions critical for protein folding will have the same structural configuration and thus would

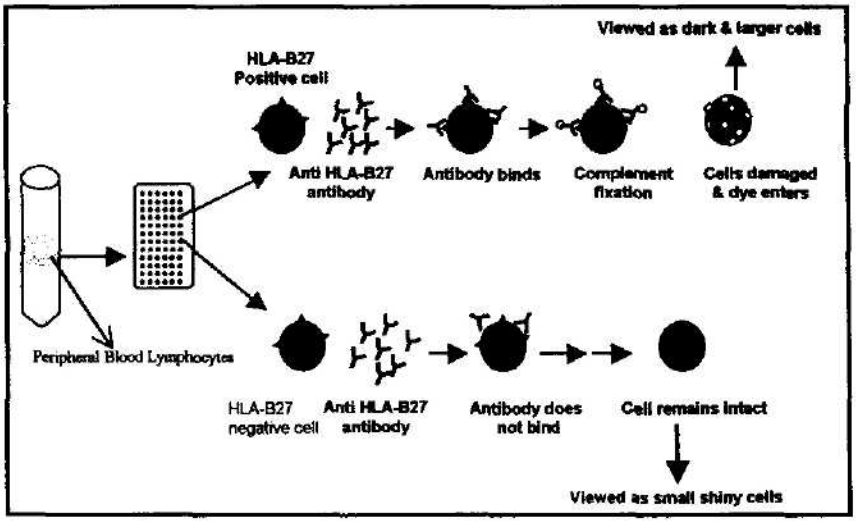


Fig 23.4. Principle of the microlymphocytotoxicity test. The dead cells are seen under the phase-contrast microscope, as larger and dark stained cells while the live cells appear as small, unstained shiny dots.

be identified by the same antibody, a phenomenon called *'crossreactivity'*. Alleles with different nucleotide sequences but with same conformational structure of the expressed protein cannot be distinguished by serology. Thus identification of new alleles is difficult by serological techniques.

HLA Class I results by serology are quite reliable for all broad specificities but it is often difficult to correctly assign class II specificities by serology. Indeed, only limited diversity in the HLA-DR, DQ and DP loci can be satisfactorily discriminated by this method. Since the technique is dependent on clear differentiation between live and dead cells, it has several limitations because of poor viability of B cells, processing defects, T cell background, variable cell surface expression of HLA molecules and failure of complement-mediated reactions. Though the technique is fast losing its reliability as a method to generate a fully characterized HLA typing result, it is still used for routine purposes owing to low cost of testing, nominal infrastructure and equipment requirements. It is also a useful tool for the identification of null or non-expressed alleles in conjunction with DNA based methods. The increasing discrepancy between results obtained by serological methods as compared to DNA based methods is slowly phasing out the former, particularly for class II typing. An analysis of transplant results has revealed upto 28% discrepancy between serology and DNA techniques (Opelz, 1990). Often information on linkage between broad serological alleles of DR and DQ loci is utilized to verify HLA-DR typing results. When haplotypes are encountered that do not harbor one of the known DR and DQ associations, a misinterpretation of typing results is imminent. This necessitates the use of alternative typing methods.

4.1.1 Serological Phenotyping Tests

i) **HLA-ABC phenotyping** : Since HLA-A, -B, -C alleles are expressed on all nucleated cells and quantitatively best expressed on lymphocytes, these cells are primarily employed for this test. Peripheral blood lymphocytes (PBLs) or purified T cell preparations are used in the complement dependent cytotoxicity (CDC) assay for the purpose of HLA matching in organ and bone marrow transplantation, disease association markers and for platelet transfusion purposes.

ii) **HLA-DR/ DQ phenotyping**: Serological typing of HLA Class II (DR & DQ) alleles is performed generally on nylon wool separated B lymphocytes by methods and purposes described above. Once the HLA class I and II antigens of all the family members have been defined by serology, the four parental HLA haplotypes can be deduced and analyzed for HLA genotype matching for organ and bone marrow transplantation.

4.1.2 Serological Crossmatch Tests

In addition to HLA matching, it is essential to test for preformed antidonor antibodies in the recipient's serum in situations involving organ transplantation. For this purpose, the microlymphocytotoxicity technique is applied to test the recipient (patient) serum with the donor cells. Any preformed antibodies in the serum would thus result in a cytotoxic reaction. Depending on the clinical requirements, the test is carried out using either peripheral blood lymphocytes (PBLs) or purified T or B cell preparations so as to identify antibodies to particular cell types. Often it is desirable to perform the test using recipient's own cells (autologous CXM) to determine the presence of autoantibodies. It is also necessary to use DTT (Dithiotheritol) absorbed serum to rule out the presence of IgM antibodes in the recipient serum. DTT binds to the pentameric structure of the IgM molecule and disrupts its structure leading to a negative cross match reaction a situations when only IgM type of antibodies are present in the serum.

4.1.3 Flow cytometry crossmatch (FCXM)

The flow cytometry crossmatch is often desirable in situations involving second grafts and in recipients with history of pre-sensitization due to other factors such as multiple blood transfusions and pregnancies. The test utilizes donor lymphocytes and recipient sera using a flurochrome labeled second antibody in order to detect IgG donor-specific antibodies against T and/or B cells in a flowcytometer. The FCXM is 10-100 times more sensitive than the conventional serological crossmatch test. Also it identifies both cell as well as type specific (IgM or IgG) antibodies of even low liters.

4.1.4 Anti-leucocyte antibody screening test

The serological technique is also used to detect antibodies directed to specific HLA type by testing the patient's (recipient's) serum with a panel of donor cells of known HLA types. This helps in selectively avoiding grafts positive for HLA alleles to which the recipient may have been previously sensitized. This test is called the '*Panel Reactive Antibody (PRA) screening'* for HLA Class I and/or Class II antibodies, using a randomly selected cell panel. It is essentially a complement dependent lymphocytotoxicity assay, although flow based PRA can also be employed for achieving greater sensitivity.

4.2 *Cellular Techniques :*

Antigens of the HLA-D locus and the DP antigens are defined by the cellular techniques of mixed lymphocyte culture (MLC) and primed lymphocyte typing respectively. This requires the use of homozygous typing cells (HTCs) having only one HLA-D specificity and/ or specific T cell clones. When lymphocytes from two individuals are cultured together, each cell population is able to recognize the nonself HLA class II antigens of the other. As a response to those differences, the lymphocytes transform into blasts with associated DNA synthesis that can be monitored by ^{3}H uptake. MLC serves as an equivalent of 'cellular crossmatch' prior to BMT and more interestingly serves as an in vitro transplant model for studying possible rejection or GvHD reaction. The cellular techniques have a number of intrinsic difficulties making them less applicable routinely. These are almost forgotten tests for most HLA laboratories.

4.3 Biochemical methods :

These methodologies are based on the principle that allelic variation in the HLA antigens are correlated with specific amino acid substitutions. The method of one dimensional gel electrophoresis (1D-IEF) is very efficient in detecting variants or subtypes of HLA-A and B antigens, but suffers from the drawback that the interpretation of IEF is dependent on the serological antigen definition. Serology and IEF together, on the other hand, provide a better definition of each allelic product and also can detect substantially larger number of class I alleles. The structural polymorphism of the class II antigens can be determined by two-dimensional gel electrophoresis. This technique however, is laborious, expensive and time consuming.

4.4 DNA based methods

As HLA alleles are defined by their nucleotide sequences, a typing methodology directly detecting variations in the sequence / polymorphic sequence motifs would define the encoded HLA molecules better than the serological techniques. The DNA based methodologies require amplification of DNA by the Polymerase Chain reaction (PCR) a process originally discovered

by Mullis and coworkers (1986) and subsequent differentiation using synthetic oligonucleotides to the nucleotide variations between different HLA molecules. The DNA typing methodology has superseded serology as a more efficient way for HLA typing in routine clinical testing laboratories and allows a better resolution of HLA phenotypes. These methods are based on discrimination of specific nucleotide sequence differences between alleles. In contrast to the rapid development of HLA class II typing strategies, DNA typing for HLA class I alleles has been slow due to the complexity of polymorphism in class I region, and high degree of homology and sharing of sequence motifs between loci. A variety of PCR based techniques are used depending on the resolution of testing desired and the number of samples to be tested. The techniques derive their names by the use of reagents employed, with PCR as the prefix.

HLA Class I genes

• Sequence specific primers	PCR-SSP
• Reverse Blot hybridization/ reverse line strips	RBH/RLS
• Single stand conformation polymorphism	PCR-SSCP

HLA Class II genes

• Restriction fragment length polymorphism	RFLP
• Sequence specific oligonucleotide probes	PCR-SSOP
• Sequence specific primers	PCR-SSP

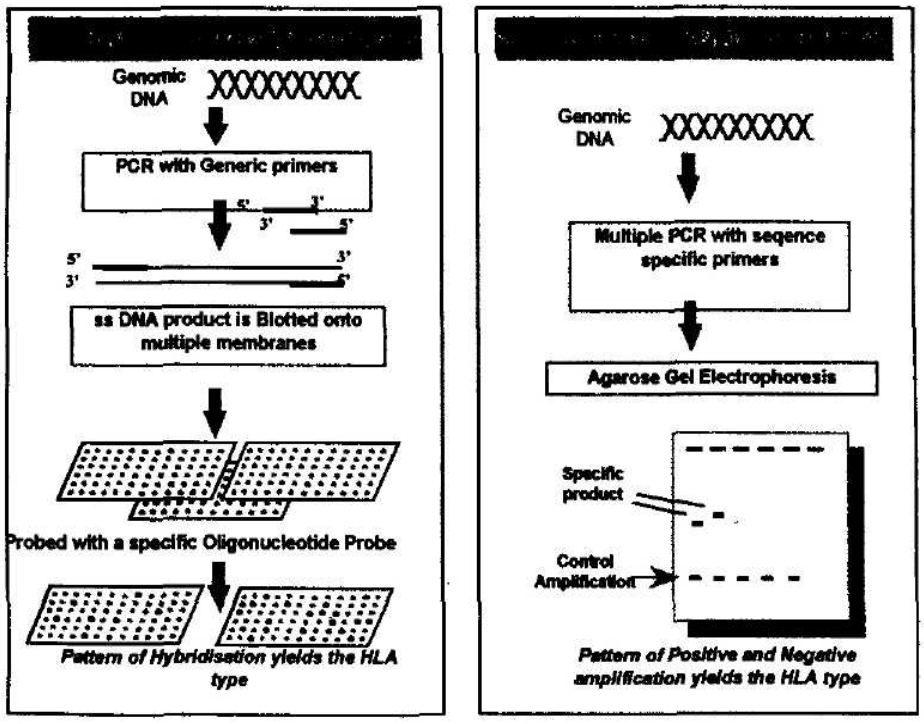
4.4.1 **RFLP**: Restriction fragment length polymorphism (RFLPs) or Southern blot hybridization was the first DNA based technology employed for HLA typing in the early 80's. It provided a new approach to studying HLA class II structural polymorphisms previously not recognized by serology. Briefly, DNA fragments of various lengths generated by digestion with specific restriction endonucleases are separated by gel electrophoresis and then hybridized using radio labelled DNA sequences as probes. Although RFLP provides a means of studying both the coding as well as non- coding regions of the HLA complex either in introns or in flanking regions, the complexities of banding patterns observed with this technology and inter locus cross hybridization of probes makes it less useful in routine practice. The polymorphism revealed by RFLP is limited and dependent upon the restriction enzymes and probes used. The technique is rarely used for routine HLA typing. Recently, an improvement on RFLP, combining it with PCR (PCR-RFLP) has been introduced.

4.4.2 **PCR-SSCP or RSCA** : The technique implies polymerase chain reaction based- single strand conformational polymorphism or reference strand mediated conformation analysis. The method is based on differences in the mobility of DNA molecules caused by non-complementary bases in two strands of the double helix. Thus when the PCR products of identical length from different alleles are annealed to a reference strand, they move different distances on the gel due to mismatches in the duplex formed with the reference strand and the test DNA.

4.4.3 **PCR-SSOP** : The PCR based sequence specific oligonucleotide probe technique is well established as a low to high resolution typing procedure for defining polymorphic HLA class II alleles. The resolution of the method depends on the type and number of probes used for testing. Essentially, it involves amplification of the locus to be tested using generic primers to all alleles constituting that locus and immobilization of the DNA covalently bound to nylon membranes by UV irradiation. The membrane is subsequently incubated

with an oligonulceotide probe to the polymorphic motif to be detected. A series of incubation and washing steps follow after which the bound oligonucleotide is detected by a detection system (chemiluminescent, colorimetric or radioactive). Only amplified DNA with complementary sequences bind to the probe and are thus detected as positive dot blots signals (Figure 23.5a).

The PCR-SSOP technique is specially suited to testing large numbers of samples at a time. A dot blot apparatus is used to immobilize upto 96 DNAs on a nylon membrane and hybridized with specific probes labeled with biotin or other detection systems. The efficiency of the technique depends on availability of ideal, monospecific probe sequences for identification of single alleles at high resolution, standardization of temperature of probe hybridization and critical washing steps to rule out false negative and positive reactions (Mehra et al, 1991,1994; Rajalingam et al, 1996). Although PCR-SSOP is a technique of very high sensitivity, it is not discriminatory in defining cis/ trans localizations of sequence motifs in heterozygous individuals. For example B*3501/ B*4901 cannot be distinguished from B*5001/ B*5301 heterozygotes since HLA-B sequences of both genotypes differ only with respect to the cis or trans positions of sequence motifs in the hypervariable region of exon 2 and exon 3. PCR-SSOP produces the same hybridization pattern for both the combinations. In such situations, PCR-SSP or nested PCR techniques are most helpful. PCR-SSOP needs further improvements to become a more reliable and accurate routine typing procedure. Recently, two other very similar techniques related to PCR-SSOP have been developed, both involving a reverse SSOP methodology using nylon strips with pre-immobilized probes (Reverse blot hybridization (RBH) and Reverse line strips (RLS)).



a b

Fig. 23.5: PCR-SSP AND SSOP methodologies: The figure depicts the principle of the two commonly employed *'PCR based techniques for HLA typing

4.4.4.1 PCR-SSP : The PCR based sequence specific primer technique is reliable for both class 1 and class II alleles. Here, oligonucleotides based on polymorphic sequences are used as set of primers pairs to amplify regions of differences between any two alleles. The number of different PCRs required to distinguish between a set of alleles being at least half the number of alleles to be typed. Only complementary oligonucleotides are able to prime a PCR reaction and can be visualized as an amplified band on an agarose gel (Figure 23.5b). Complete high resolution typing can be carried out using nested primers. The technique is more suitable for small number of samples and for quick results. Although cost is a limiting factor for PCR-SSP, it is the technique of choice for donor- recipient matching for bone marrow transplantation and for HLA-DR/DQ allele typing in organ transplant situations. A version of this technique called 'Phototyping' involves testing of alleles from the HLA class I as well as II region at high resolution using a large number of primers all standardized to work at similar PCR conditions (Bunce et al, 1995). Another variation of this technique using 'nested primers' helps to distinguish alleles at high resolution. It involves two **PCR** reactions on the same target DNA. In the first step, a variable sequence is amplified following which a set of primers internal to the ones used in the first step is used. This method help to resolve alleles that show extensive sequence homology to each other. In situations involving large sample numbers, as in population studies, it is advisable to use PCR-SSOP alongwith class I serology.

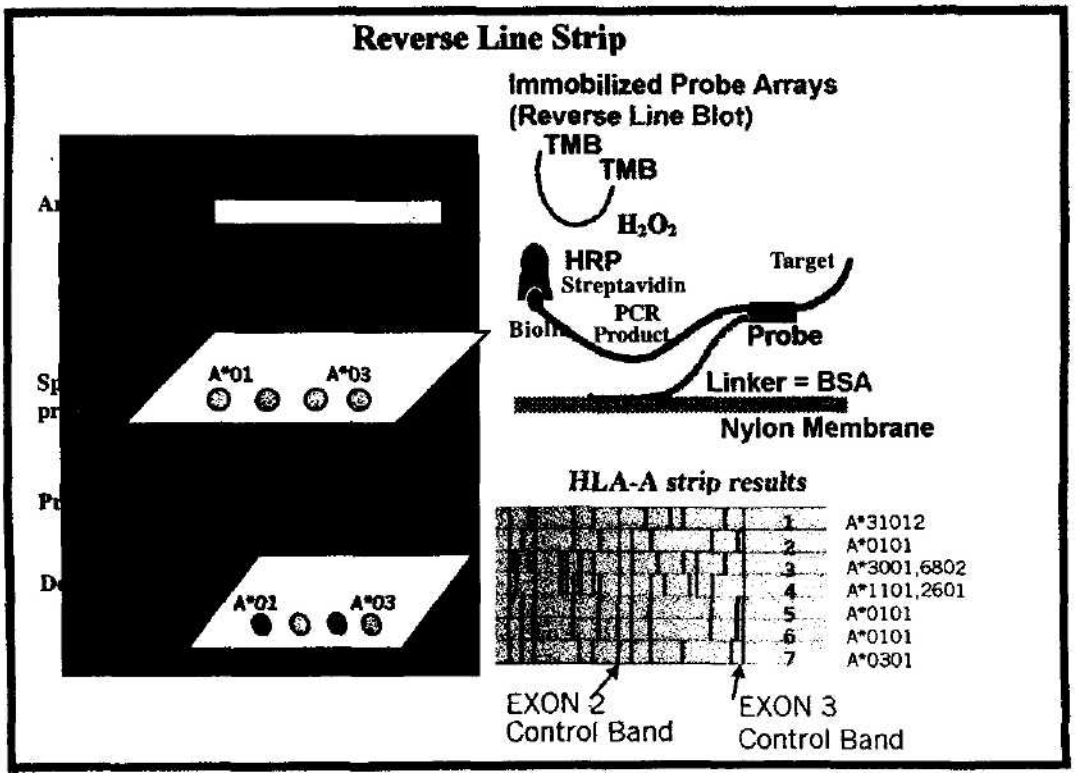


Fig. 23.6: Principle of the Reverse line strip methodology. DNA samples labeled by incorporation of biotinylated primers are hybridized to probes immobilized on nylon membranes with the help of chemical linkers. The hybridized DNA are then detected by addition of streptavidin conjugated with the Horse Radish peroxidase enzyme. Subsequent addition of substrate (3,3',5,5' Tetra Methyl Benzidine) leads to colour development on positive samples only.

4.4.5 RBH/RLS techniques: The reverse blot hybridization and reverse line strip techniques are fore runners to the DNA chip technology, where a set of oligonucleotide probes to defining specific polymorphic motifs are immobilized on nylon membranes and then hybridized with the amplified **DNA** to be tested. The technique is suitable for typing small number of samples as in forensic cases (Figure 23.6). The technique is an improvement over the PCR-SSP since it can define HLA-A,B,C at an intermediate to high resolution typing level; not available with any other similar technique for class IHLA typing.

4.4.6 ARMS-PCR technique : The technique is an amplification refractory mutation system defined originally by Newton and coworkers (1989). The method utilizes a combination of primers designed against group and allele specific sequence sites. The SSP design is based on the ARMS 1 system by which a mismatch at 3^1 residue of the primer inhibits non-specific amplification. The technique is particularly helpful for detection of HLA class I alleles at high resolution level, where the sequence variation is not restricted to specific regions of the genome being spread over the entire locus. HLA class I alleles show extensive homology between different alleles on the same locus as well as interlocus similarities. The special primer design in this technique enables correct allele assignment and inhibits false positive reactions (Figure 23.7).

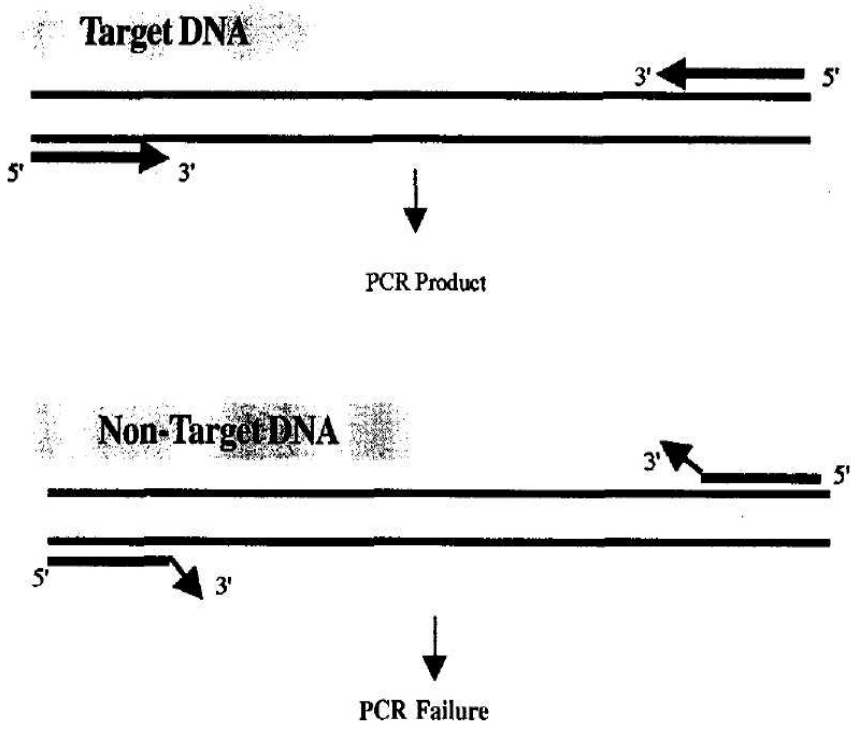


Fig. 23.7. Principle of the ARMS-PCR methodology: A positive amplification is achieved when the primer
is an exact complementary match to the target DNA sequence. On the other hand the primer does not bind to the non-target sequences resulting in failure of PCR amplification.

4.5 Sequence based typing (SBT): Sequence based typing has gained immense importance during the last few years as a fool proof technique to provide the ultimate high resolution typing

of HLA alleles. The technique is based on identification of the complete exonic sequence of the allele in question and thus allows no discrepancies. A PCR is performed that amplifies both the alleles in a heterozygous individual. The two alleles are then sequenced and positions of heterozygosity are analyzed using computerized software that aligns the sequences obtained with combination of all known alleles and thus assign possible HLA types. The technique is highly suitable for Class II typing, but class I alleles due to their extensive polymorphism place great demands on the quality of sequencing analysis. High degree of sequence homology among different HLA class I loci including the less polymorphic HLA-E, F, G, genes and several pseudogenes cause multiple problems if a particular locus has to be investigated. Considering the increasing number of newly detected alleles, sequencing is fast becoming the most reasonable and suitable typing approach for both HLA class I and II alleles.

As a result of application of DNA based techniques, a large number of new HLA alleles have been discovered and characterized. Several' novel alleles have been discovered in the Indian population and reported recently by our group (Mehra et al, 2001, Jainietal, 2001).

4.6 Limitations of PCR based techniques

i.) The cost of most PCR based technologies is prohibitive for use in routine service laboratories and hence these are used primarily in large referral centers, as secondary, confirmatory typing techniques, for transplant purposes and research applications.

ii.) Since the high-resolution methods are based on sequences available from studies carried out predominantly in Caucasoid and Oriental populations, identification of novel alleles found in other ethnic groups or of rare alleles could be missed unless more than one procedure is applied.

iii.) Some heterozygotes cannot be distinguished by the PCR based techniques depending on the sequences of the concerned alleles. Alternate methods then have to be utilized to resolve such discrepancies.

iv.) False positive and negative reactions due to failure of amplification and/or hybridization need laborious standardization of the techniques and careful analysis of results to avoid wrong assignment of alleles.

4.7 Future perspectives

Histocompatibility has changed a great deal over the years. From early days of comparatively simple serology, biochemical and cellular methodologies, HLA has come a long way to the present sophistication of molecular procedures including the recently defined sequence based typing (SBT). Due to the extraordinary important role that HLA molecules play in antigen presentation and stringency of the relationship between epitope and associated HLA alleles, high resolution typing is increasingly in demand in clinical and experimental settings. Functionally significant high resolution typing of HLA alleles is only achievable through molecular methods.

The completion of the human genome project offers the opportunity to identify genome variations in cells and tissues quantitatively, automatically and simultaneously using high throughput microarray analysis. DNA oligonucleotide based array technology (popularly called the DNA chip technology) is the technique of future for detecting single nucleotide polymorphisms (SNPs) relevant for HLA typing.

Plasma Derivatives &

Plasma Substitutes

PLASMA PROTEIN SOULTIONS (PSS)

Plasma protein solutions are prepared from pooled plasma after removal of factor VIII concentrate, fibrinogen and immunoglobulins either by Conn ethanol extraction method or by chromatographic method.

Albumin Preparations

Albumin is available for clinical use as;

1. Albumin 5% solution
2. Albumin 25% solution
   They contains 96% albumin and 4% globulin.
3. Plasma protein fraction (PPF) 5% solution
   It contains 83% albumin and 17% globulin.

Characteristics of Albumin Preparation

- The 5% solution are osmotically and oncotically equivalent to plasma, whereas the 25% solution is five times that of plasma
- Products are heated and/or chemically treated to reduce the risk of transmission of viral diseases.

- Shelf - life depends on the storage temperature:

Temperature	Shelf-life
Room Temperature (20-25°C)	3 Years
2°-8°C	5 Years

Indications for *5%* albumin and PPF:

- Used for blood volume expansion and colloid replacement
- Hypoproteinemia following burns and extensive surgery, albumin is administered to maintain the albumin level of 5.2 g/dl.
- The replacement fluid in therapeutic plasma exchange
- Hemorrhagic hypovolemic shock
- Burns
- Retroperitoneal surgery in which large volume of protein-rich fluid may pool in bowel

Indications for 25% albumin.

- Sever hypoproteinemia in acute nephortic syndrome and acute liver disease.
  - Adults should be given 100-400 ml of 25% albumin daily.
  - Children 1.5-6 ml /kg body weight in 24 hours.
  - Diuretics should also be given alongwith albumin if edema is present.

- Hyperbilirubinema in the new born: 5-10 ml of salt poor albumin is given along with blood for exchange transfusion. It binds excessive bilirubin and reduce incidence of kernicterus.
- Toxemia of pregnancy: 50 ml of salt poor albumin is given daily.

Contraindications:

- Hypoproteinemia in malnutrition.
- Chronic nephrotic syndrome.
- Cirrhosis of liver.

Adverse effect:

- Urticaria and anaphylactoid reactions
- Circulatory overload
- Febrile reactions
- Hypotension due to vasoactive substance in plasma, Sometimes it is seen with PPF when the rate of administration is more than 10 ml/min.

Note:

Process of heat treatment or chemical treatment of plasma derivatives to reduce the risk of transmitting viruses is very effective against viruses that have lipid envelops:

- HIV 1&2 viruses
- Hepatitis B virus
- Hepatitis C virus
- HTLV- 1 & 2 viruses

Inactivation of non-lipid enveloped viruses e.g. hepatitis A are less effective.

Factor VIII Concentrate:

Preparations available:

(1) Factor VIII prepared from large pools of plasma is sterile, lyophilized.

- Vials of freeze-dried protein labeled with content, usually 250 i.u. of factorVIII.
- It is reconstituted aseptically with the diluent provided with the vial.
- One i.u. is the factor activity present in 1 ml of normal pooled plasma less than 1 hour old.
- It is inactiveated for viruses by heat treatment, solvent/detergents or purified with monoclonal antibodies.

(2) Recombinant DNA technology is currently used to manufacture of factor VIII concentrate which is commercially available. It is clinically equivalent to factor VIII derived from plasma. It does not have the risk of transmitting viral infections.

Storage: Freeze-dried products are stored at 2-6°C up to stated expiry date on the label.

Doses: 10 - 20 i.u./kg body weight after every 12 hours. (see chapter 14 'cryoprecipitate Factor VIII).

aPTT can be used to monitor factor VIII levels.

Indications: • Hemophilia A, to prevent or control bleeding

- Hemophilia A with low levels of inhibitors to factor VIII
- von-Willibrand disease if the preparation contains von-Willibrand Factor (Factor VIII:vWF). Mostly factor VIII concentrates do not provide factor VIII:vWF levels needed for normal platelet function in von-Willibrand disease.

Inhibitors to Factor VIII

Approximately 10-15 per cent hemophiliacs develop inhibitor to factor VIII. In persons with hemophilia A, an inhibitor is suspected if administration of factor VIII concentrate no longer produces the expected therapeutic results. Persons who develop inhibitors can be divided into two groups: low and high responders. Low responders have a low concentration of antibody and can be treated with the standard or slightly higher than normal doses of factor VIII concentrate. High responders have high concentration of antibody to factor VII and are difficult to treat. Factor IX products have been found to be effective.

Plasma Derivatives Containing Factor IX

(1) Prothrombin Complex : (PCC)
Contains Factors II, IX and X and to varying degree of factor VII
[Anti-inhibitor coagulation complex (AICC) for the treatment of patients with high titers of antibodies to factor VIII]

(2) Factor IX Concentrate: contains Factor IX
Vials of both products are freeze-dried and virus-inactivated.

Indications:

- Treatment of hemophilia B (Christmas disease)
- PCC is also used to treat prolonged prothrombin time and patients with high titer of antibodies to factor VIII.

IMMUNOGLOBULINS PREPARATIONS

Immunoglobulin for intramuscular use:

A concentrated solution of the IgG antibody component of plasma, prepared from large pools of plasma of donors containing antibodies against infectious agents. They are inactivated for viruses and do not transmit viruses-transmitted infections.

Indications:

- Congenital hypogammaglobinemia: 0.7 ml/kg I.M.
- Persons exposed to diseases like: Prophylactic dose is
  Hepatitis A or Measles 0.02 to 0.04 ml/kg, I.M.

Immunoglobulin for Intavenous use

Prepared for safe I.V. administration.

Indications:

- Idiopathic autoimmune thrombocytopenic purpura and some other immune disorders.
- Treatment of immune deficiency state
- Hypogammaglobinemia
- Myasthenia gravis
- HIV-related diseases

Hyperimmune globulins

Used for prevention of diseases like: Hepatitis B
Varicella zoster
Rabies, mumps & others

Anti-Rh(D) Immunoglobulin (Anti-D RhIG)

Prepared from plasma containing high level of anti-Rh D antibody from previously immunized persons.

Indications: To prevent Rh (D) negative mother from Rh immunization who is pregnant with Rh(D) positive infant.

Table 24.1: Plasma derivatives and their uses: (Summary)

Product	Composition	Approximate Volume	Indication
Albumin	*5%* or 25%	varies	Volume expansion; fluid mobilization
Factor III Concentrates Recombinant humanF,VII)	Factor VIII some fibrinogen & von Willibrand factor		Hemophilia A; von-Willibrand's disease (selected products only)
Factor IX complex, X Factor IX conc.)	Facror II, VII, IX, minimal amounts of other proteins		Heredity factor II. IX, or X deficiency, factor VIII inhibitor

plasma derivatives and their uses *(Continued)*

lmmunoglobulin	IgG antibodies, for IV or IM use	varies	Treatment of hypoglobinemia or agammaglobinemia, Immune-thromboctopenia (IV Preparation only)
Rh immune globulin	IgGanti-D preparation	l ml	Prevention of HDN due to D antigen

PLASMA SUBSTITUTES

Those designed to provide colloid osmotic pressure or expand the plasma volume i.e. colloid and crystalloids.

Colloidal solutions are (Table 24.2)

1. Dextrans: They are mixtures of polysaccharide molecules of different molecular weight i.e. Dextran 40 and Dextran 70
2. Hydroxyethyl starch 450
3. Gelatine

Table : 24.2: Currently marketed colloidal solutions are:

Genetic name	**Contents**	Intrayescular half-life **Intrayescular ,half-life**
Dextron 40	10% polysaccharide (MW 40,000) with normal saline	4-6 hours
Dextran 70	6% polysaccharide (MW 70,000) with normal saline	6-8 hours
Hydroxyethyl starch 450	6 % solutions in 0.9% saline (MW 45,000)	24 hours
Gelatine (Haemacel)	3.5% gelatine polypeptide (MW 35,000) with Ringers solutions	3-5 hours

Dextran 40

It is 10 % polysaccharide (M.W.40,000) solution in normal saline. Dextran 40 increases the plasma volume comparatively rapidly due to its high concentration and low molecular weight. Its intravascular half-life is shorter (4-6 h) than that of hydroxyethyl starch or dextran 70 as it is rapidly excreted in urine. Infusion of dextran is associated with interstitial dehydration in patient if crystalloids are not given with it. It has also some role in the prevention of thrombosis. It is generally accepted that the dose of Dextran 40 should not exceed 500 ml in 24 h in adult.

Dextran 70

It is 6 % polysaccharide (M.W. 70,000) solution in normal saline. It has strong and comparatively long lasting effect (6-8 hours). Dextran 70 has significant antithrombotic action through interfering with the platelet- Factor VIII- endothelial interaction. The infusion of 500 ml Dextran 70 per 24 hours has been seen to offer significant protection against post-operative thromboembolic complication. Its use of more than 1000-1500 ml may cause abnormal bleeding in patients and due to this, use of Dextran 70 has reduced at some places.

Side effects of Dextrans1

- Circulatory over load, particularly in patients where anemia is more marked.
- Renal tubular obstruction has been associated with dehydrated patients. %
- Hemostatic defects as a results of inhibition of platelet function in higher doses.
- Anaphylactoid reactions.

Hydroxyethyl Starch 450 (HES)

HES is available as a 6% solution in 0.9% saline (M.W. 45000). Because of its more recent introduction, there have been few controlled clinical trials of this colloidal plasma substitute. Its expander effects are more modest than those of Dextran 70, but its intravascular half-life appears to be longer. Its initial half-life in the circulation is 24 h, but almost 20% of the infused HES remains in the body after one week after a single infusion of HES, and repeated administration has a cumulative effect.

Indication for use

- Expansion of blood volumes
- HES is used as an additive to increase granulocyte yiels in leucopheresis by cell separartor

Side effects:

- HES interferes to some extent with hemostatic mechanisms, through less than dextran.
- It has no antithrombotic property.
- Renal functions does not seem to be effected by HES.
- Severe anaplylactoid reactions have been observed.

Dose:

The tolerated does appears to be approximately 3000-4000 ml/24h

Gelatin (Haemacel)

Succinyl gelatin and partially degraded gelatin have molecular weight of 35,000. They are available as 3.5-4.0% solutions in 500ml bottles (Haemacel). Gelatin has a half-life in the circulation of three to five hours. Gelatin has no antigenic property and does not interfere with haemostasis.
The gelatins are the least potent of the colloidal plasma substitutes.
Blood volume expansion effects are satisfactory while awaiting blood. The dose is 500-1000 ml.

Side Effects:

- Acute circulatory overload.
- They have no adverse effect on renal functions.
- Show no interference with hemostasis in volumes upto 1000-1500 ml in 24 hours.
- They do not have anti-thrombotic effects.
- Anaphylactoid reactions have been observed.

Table- 24.3 : Advantages and disadvantages of various colloidal solutions

Name of Colloids	Intravascular Volume effects		Circulatory overload	Potential side effects		Anaphy-lactoid reactions	Maximum dose (ml). for a 70 kg"' adult
	Intia l	duration		renal damag	abnormal		
Dextarn 40	++++	++	++++	+	+	+	500
Dextarn70	+++	+++	+++	+	++		1000-1500
Hydroxyethyl Starch 450	++	++++	++	+	+	+	3000-4000*
Gelatin	+	+	+	+	+	+	1500*

* together with blood or red cell concentrates

Crystalloid Solutions

Replacement type crystalloid solutions are formulated to correct body fluid deficits. They have the capacity to expand the plasma volume temporarily since the intravascular aand extravascular concentration of electrolytes tend to equilibirate quickly.

Replacement crystalloid are isotonic with respect to sodium concentration and include Ringer's lactated and 0.9% sodium chloride solutions.

These fluids enter both the plasma and interstitial fluid compartment without shifting the osmotic balance. As a result large volume of these replacement fluids are required to expand the intravascular plasma volume. They should be judiciously administered to avoid flooding of the extravascular space, particularly in pulmonary edema. The advantages and disadvantages of colloids and crystalloid solutions in table 24.3

Table 24.4: Advantages and disadvantages of colloids and crystalloids

Solutions	Advantages	Disadvantages
Colloids	• Readily available • Storage & administration easy • Do not transmit diseases • Provide oncotic pressure	• Short half -life in circulation • Mid Immungenic • May interfere with hemostasis • May interfere with blood grouping and cross-matching • May delay replacement of albumin
Crystalloids	• Readily available • Storage & administration easy • Non-immunogenic • Do not transmit diseases • Do not inhibit synthesis of albumin • Cheap	Lack of oncotic pressure than plasma

25

Blood Transfusion Safety and Regulatory Requirements

Blood transfusion service is a vital part of the National Health Services and increasing advancement in the field of transfusion technology has necessitated to enforce regulations to ensure the safety and efficacy of biologies, which include blood and its components and diagnostic reagents and devices used by blood establishment. Drugs Controller General of India has formulated a comprehensive legislation to ensure better quality control system on collection, storage, testing and distribution of blood and its components.

Director General of Health Services carries out amendments from time to time relating to the requirements of blood transfusion services in the Drugs and Cosmetic Act, 1940 and Rules thereunder to meet the latest requirements for safe blood and its components.

The Drugs Controller General (India) issued a notification that the blood should not be collected from the paid professional blood donors w.e.f. January 1998, in order to supply more safe blood and its components to the patients, following an order from the Supreme court.

The problem of transfusion-associated acquired immuno-deficiency syndrome (AIDS) resulted in the introduction of several new policies and procedures related to blood safety. The Ministry of Health and Family Welfare (Government of India) issued a notification in the year 1989 under the Drugs and Cosmetics Acts and Rules and made the test for human immunodefiiency virus (HIV) mandatory.

For strict compliance, the Drugs and Cosmetics Rules were again amended (Rules 68A, Part XB and Part XIIB of Schedule F) in the year 1992-93 and the Drugs Controller General (India)

was vested with the power of Central Licence Approving Authority (CLAA) to approve the licence of notified drugs viz. blood and blood products, I. V. fluids, vaccines and sera.

Central Government further amended the Drugs and Cosmetics Rules, 1945 and notified **the** Drugs and Cosmetics (2nd Amendment) Rules, 1999 and came in force and have been highlighted in this chapter.

Concurrent new data and heightened concern for blood safety provoked the introduction of testing for marker of hepatitis C virus (HCV) and test of antibody to HCV has been made mandatory in 2001 under the Drugs and Cosmetics Rules.

Human blood is covered under the definition of "Drugs" under Section 2 (b) of Drugs and Cosmetics Act. Hence it is imperative that the blood banks need to be regulated under the Drugs and Cosmetics Act and Rules thereunder and the licence is granted for operating a blood bank by the State Licensing and Central Licence Approving Authorities after inspection.

PARTXB

REQUIREMENTS FOR THE COLLECTION, STORAGE, PROCESSING AND DISTRIBUTION OF WHOLE BLOOD, HUMAN BLOOD COMPONENTS BY BLOOD BANKS AND MANUFACTURER OF BLOOD PRODUCTS

122-EA. Definition - In this part and in the Forms contained in Schedule A and in Part XII B and Part XIIC of Schedule F, unless there is anything repugnant in the subject context:

(a) "blood bank" means a place or organization or unit or institution or other arrangements made by such organization, unit or institution for carrying out all or any of the operations, for collections, apheresis, storage, processing and distribution of blood drawn from donors and /or for preparation, storage and distribution of blood components.

(b) "donor" means a person who voluntarily donates blood after he has been declared fit after a medical examination, for donating blood, on fulfilling the criteria given hereinafter, without accepting in return any consideration in cash or kind from any source, but does not include a professional or a paid donor;

Explanation- For the purposes of this clause, benefits or incentives like pins, plaques, badges, medals, commendation certificates, time- off from work,membership of blood assurance program, gifts of little or intrinsic monetary value shall not be construed as consideration.

(c) "replacement donor" means a donor who is a family friend or a relative of the patient - recipient.

(d) "professional donor" means a person who donates blood for a valuable consideration, in cash or kind, from any source, on behalf of the recipient - patient and includes a paid donor or a commercial donor"

(e) "blood" means and includes whole human blood, drawn from a donor and mixed with an anti-coagulant.

(f) "autologous blood" means the blood drawn from the patient for re-transfusion into himself/ herself later on.

(g) "blood component" means a drug prepared, obtained, derived or separated from a unit of blood drawn from a donor.

(h) "aphersis" means the process by which blood drawn from a donor, after separating plasma or platelets or leukocytes, is retransfused simultaneously into the said donor.

(i) "plateletpheresis" means the process by which the blood drawn from a donor, after platelet concentrates have been separated, is retransfused simultaneously into the said donor.

(j) "plasmapheresis" means the process by which the blood drawn from a donor, after the plasma has been separated, is re-transfused during the same sitting in to the said donor.

(k) "leucapheresis" means the process by which the blood drawn from a donor, after leucocyte concentrates have been separated, is re-transfused simultaneously into the said donor

(l) "blood products" means a drug manufactured or obtained from pooled plasma of blood by fractionation, drawn from donors.

122-F. Form of application for licence for operation of Blood Bank/processing of whole human blood for components/manufacture or Blood Products for sale or distribution -

Application for the grant and/or renewal of licence for the operation of Blood Bank/processing of Human Blood for components/manufacture of Blood Products shall be made to the Licensing Authority appointed under Part VII in Form 27-C or Form 27-E (Appendices 1 & 2) as the case may be and shall be accompanied by licence fees of rupees six thousand and an inspection fees of rupees one thousand and five hundred for every inspection thereof or for the purpose of renewal of licence.

Provided that if the applicant applies for renewal of licence after the expiry but within six months of such expiry the fee payable for the renewal of the licnece shall be rupees six thousand and inspection fees of rupees one thousand and five hundred plus an additional fees at the rate of rupees one thousand per month or a part thereof in additional to the inspection fee.

Provided further that a licence holding a licence in Form 28 C or Form 28 E (Appendices 3 & 4) as the case may be for operation of blood bank/processing of whole human blood for components/manufacture of blood products shall apply for grant of licence for renewal under sub-rule (1) before the expiry of the said licenece on Form 27-C or Form 27-E as the case may be and he shall continue to operate the same till the orders on his application are communicated to him.

The licensing Authority shall inspect the establishment before renewing the licence and if satisfied, certificate of renewal of licence is issued on Form 26-G and Form 26-I (Appendices 5 & 6) as the case may be.

1. {EXPLANATION.- For the purpose of this rule, 'Blood Bank' means a place or organizational unit or an institution, or other arrangement made by such organizational unit or institution for carrying out all or any of the operations of manufacture of human blood components or blood products or whole human blood for its collection, storage, processing, distribution from selected human donors.}
2. A fee of rupees one thousand shall be paid for a duplicate copy of licence issued under this rule, if the original is defaced, damaged or lost.
3. Application by licensee to manufacture additional drugs listed in the application shall be accompanied by a fee of rupees three hundred for each drug listed in the application.
4. On receipt of the application for the grant or renewal of such licence, the Licensing Authority shall,-
   (i) Verify the statements made in the application form.
   (i) Inspect the manufacturing and testing establishment in accordance with the provisions of rules 122-I.

(i) In case the application is for renewal of licence, information of past performance of the licensee shall be verified.

5. If the Licensing Authority is satisfied that the applicant is in position to fulfill the requirements laid down in the rules, he shall prepare a report to that effect and forward it along with the application and the licence (in triplicate) to be granted or renewed, duly completed to the Central Licence Approving Authority.

Provided that if the Licensing Authority is of the opinion that the applicant is not in a position to fulfill the requirements laid down in these rules, he may, by order, for reason to be recorded in writing, refuse to grant or renew the licence, as the case may be.

6. If, on receipt of application and the report of the Licensing Authority referred to in Sub-rule 5 and after taking such measures including inspection of the premises, by the inspector, appointed by the Central Govt. under Section 21 of the Act, and/or along with expert in the field concerned if deemed necessary, the Central Licence Approving Authority, is satisfied that the applicant is in a position to fulfill the requirement laid down in this rule, licence is granted or renewed, as the case may be.

Provided that if the Central Licence Approving Authority is of the opinion that the applicant is not in a position to fulfill the requirements laid down in these rules he may, notwithstanding the report of the Licensing Authority, by order, for reason to be recorded in the writing, reject the application for grant or renewal of licence as the case may be and shall supply the applicant with a copy of the inspection report.

122-G. Form of licence for the operation of a Blood Bank/Processing of Whole Human Blood for components and manufacture of Blood products and the conditions for the grant or renewal of such licence

A licence for the operation of a Blood Bank or for processing Whole Human Blood for components and manufacture of blood products shall be issued in Form 28-C or Form 28 E or Form 26-G or Form 26-1. Before a licence in Form 28-C or Form 28 E or Form 26-G or Form 26-I is granted or renewed the following conditions shall be complied with by the applicant-

1. The operation of the Blood Bank and/or processing of whole human blood for components/ manufacture of blood product shall be carried out under the active direction and personal supervision of competent technical staff consisting of at least one person who is whole time employee and who is a Medical Officer and possessing -
   a) Post Graduate degree in Medicine-M.D. (Pathology/Transfusion Medicines); or.
   b) Degree in Medicine (M.B.B.S.) with Diploma in Pathology or Transfusion Medicine having adequate knowledge in blood group serology, blood group methodology and medical principles involved in the procurement of blood and/or preparation of its components; or
   c) Degree in Medicine (M.B.B.S.) having experience in Blood Bank for one year during regular service and also has adequate knowledge and experience in blood group serology, blood group methodology and medical principles involved in the procurement of blood and/or preparation of its components,

The degree or diploma being from a university recognized by the Central Government

Explanation- For the purpose of this condition, the experience in Blood Bank for one year shall not apply in the case of persons who are approved by licensing Authority and/or Central Licence

Approving Authority prior to the commencement of the Drugs & Cosmetics (Amendment) Rules,1999.

2. The applicant shall provide adequate space, plant and equipment for any or all the operations of blood collection or blood processing. The space, plant and equipment required for various operation is given in Schedule 'F', Part XII-B and / or XII-C.
3. The applicant shall provide and maintain adequate technical staff as specified in Schedule 'F', Part XII-B and/or XII-C.
4. The applicant shall provide adequate arrangements for storage of Whole Human Blood, Human Blood Components and blood products.
5. The applicant shall furnish to the Licensing Authority, if required to do so, data on the stability of Whole Human Blood, its components or blood products which are likely to deteriorate, for fixing the date of expiry which shall be printed on the labels of such products on the basis of the data so furnished.

122-H. Duration of Licence-

An original licence in Form 28-C or Form 28 -E or a renewed licence in Form 28-G or Form 28-I unless sooner suspended or cancelled shall be valid for a period of five years from the date on which the year in which it is granted or renewed.

122-I. Inspection before grant or renewal of licence for operation of Blood Bank, processing of Whole Human Blood for Components and Manufacture of Blood Products

Before a licence in Form 28-C or Form 28 -E is granted or a renewal of licence in Form 26-G or Form 26-I is made, as the case may be, the Licensing Authority and/or Central Licence Approving Authority, shall inspect the establishment in which Blood Bank is proposed to be operated/whole human blood for component is processed/blood products are manufactured shall be inspected by one or more inspectors, appointed under the Act and/or along with the Expert in the field concerned. The Inspector or Inspectors shall examine all portions of the premises and appliances/ equipments and inspect the process of manufacture intended to be employed or being employed for operation of blood bank/processing of whole human blood for components/ manufacture of blood products. The inspector (s) shall also inspect the testing facilities and also enquire into the professional qualification of the expert staff and other technical staff to be employed.

122-J. Report by Inspector

The Inspector or Inspectors shall forward a detailed descriptive report giving his finding on each aspect of inspection along with his recommendation in accordance with the provisions of Rule 122-I to the Licensing Authority or to the Central Licence Approving Authority.

122-K. Further application after rejection

If within a period of six months from the rejection of application for a licence the applicant informs the licensing Authority that the conditions laid down have been implemented and deposits an inspection fee of rupees two hundred and fifty, the Licensing Authority, may again inspect the establishment and if satisfied that the conditions for the grant or renewal of a licence have been complied with, shall grant or renew a licence in Form 28-C or Form 28 -E;

Provided that in case of drug notified by the Central Government under rule 68-A, the application together with the inspection report and the Form of licence (in triplicate to be granted

<w>

or renewed), duly completed shall be sent, to the Central Licence Approving Authority, who may approve the same and return it to the licensing Authority for issue of the licence."

122-L. Delegation of powers by the Central Licensing Approving Authority

The Central Licensing Approving Authority may, with the approval of the Central Government, by notification delegate his power of signing licences and any other power under rules to persons under his control having same qualifications as prescribed for Controlling Authority under Rule 50-A, for such areas and for such periods as may be specified.

122-M. Provision for appeal to the State Government by a Party whose licence has not been granted or renewed

Any person who is aggrieved by the order passed by the Licensing Authority or Central Licence Approving Authority, as the case may be, may within thirty days from the date of receipt of such order, appeal to the State Government or Central Government, as the case may be , after such enquiry, into the matter as it considers necessary and after giving the said person an opportunity for representing his view in the matter may pass such order in relation thereto as it thinks fit.

122-N. Additional information to be furnished by an [applicant] for licence or by a licensee to the Licensing Authority

The applicant for the grant of licence or any person granted a licence under the part shall, on demand furnish to the Licensing Authority, before the grant of the licence or during the period the licence is in force, as the case may be, documentary evidence in respect of the ownership or occupation, rental or other basis of the premises, specified in the application for licence or in the licence granted, constitution of the firm or any other relevant matter, which may be required for the purpose of verifying the correctness of the statement made by the applicant or the licensee, while applying for or after obtaining the licence, as the case may be.

122-O. Cancellation and suspension of licences

(1) The Licensing Authority or Central Licence Approving Authority may for such licences granted or renewed by him after giving the licensee an opportunity to show cause by such an order should not be passed by an order in writing stating the reason thereof, cancel a licence issued under this part or suspend it for such period as he thinks fit, either wholly or in respect of some of the substances to which it relates, [or direct the licensee to stop collection , storage, processing , manufacture and distribution of the said substances and thereupon order the destruction of substances and stocks thereof in the presence of an Inspector] if in his opinion, the licensee has failed to comply with any of the conditions of the licence or with any provision of the Act or Rules thereunder.

(2) A licensee whose licence has been suspended or cancelled may, within three months of the date of the order under sub-rules (1) prefer an appeal against that order to the State Government or Central Government, which shall decide the same.

122-P. Conditions of licence

"A licence in Form 28-C, Form 28-E, Form 28-G or Form 26-I shall be subject to the special conditions set out in Schedule F, Part XII-B and Part XII-C, as the case may be, which* relate to the substance in respect of which the licence is granted or renewed and to the following general conditions, namely:-"

(i)

(a) The licensee shall provide and maintain adequate staff, plant and premises for the proper operation of a Blood Bank for processing whole human blood, its components and/or manufacture of blood products.

(b) The licensee shall maintain staff, premises and equipments as specified in Rule 122-G. The licensee shall maintain necessary records and registers as specified in Schedule F, Part XII-B and XII-C.

(c) The licensee shall test in his own laboratory whole human blood, its components and blood products and maintain records and registers in respect of such tests as specified in Schedule F, Part XII-b and Part XII-C. The records and registers shall be maintained for a period of five years from the date of manufacture.

(d) The licensee shall maintain/preserve reference sample and supply to the Inspector the reference sample of the whole human blood collected by him in adequate quantity to conduct all the prescribed tests.

(ii) The licensee shall allow an inspector appointed under the Act to enter, with or [without] prior notice, any premises where the activities of the Blood Bank are being carried out, for the processing of Whole Human Blood and/or Blood Products, to inspect the premises and plant and the process of manufacture and the means employed for standardizing and testing the substance.

(iii) The licensee shall allow an Inspector appointed under the Act to inspect all registers and records maintained under these rules and to take samples of the manufactured product and shall supply to Inspector such information as he may require for the purpose of ascertaining whether the provisions of the Act and Rules thereunder have been observed.

(iv) The licensee shall from time to time report to the Licensing Authority any changes in the expert staff responsible for the operation of a blood bank/processing of whole human blood for components and/or manufacture of blood products and any material alterations in the premises or plant used for that purpose which have been made since the date of last inspection made on behalf of the Licensing Authority before the grant of the licence.

(v) The licensee shall on request furnish to the Licensing Authority, or Central Licence Approving Authority or to such Authority as the Licensing Authority, or the Central Licence Approving Authority may direct, from any batch unit of drugs as the Licensing Authority or the Central Licence Approving may from time to time specify, sample of such quantity as may be considered adequate by such Authority for any examination and, if so required, also furnish full protocols of the test which have been applied.

(vi) If the Licensing Authority or the Central Licence Approving Authority so directs, the licensee shall not sell or offer for sale any batch/unit in respect of which a sample is, or protocols are furnished under the last preceding sub-paragraph until a certificate authorizing the sales of batch/unit has been issued to him by or on behalf of the Licensing Authority or the Central Licence Approving Authority.

(vii) The licensee shall on being informed by the Licensing Authority or the Controlling" Authority that any part of any batch/unit of the substance has been found by the Licensing Authority or the Central Licence Approving Authority not to conform with the standards of strength, quality or purity specified in these Rules and on being directed to do so, withdraw, from sales and so far as may in the particular circumstances of the case be practicable recall all issues already made from that batch/unit.

(viii) No drug manufactured under the licence shall be sold unless the precautions necessary for preserving its properties have been observed throughout the period after manufacture. Further no batch/unit manufactured under this licence shall be supplied/distributed to any person without prescription of Registered Medical Practitioner.

(ix) The licensee shall comply with the provisions of the Act and of these Rules and with such further requirements, if any, as may be specified in any Rules subsequently made under Chapter IV of the Act, provided that where such further requirements are specified in the Rules, these would come in force four months after publication in the Official Gazette.

(x) The licensee shall maintain an Inspection Book in Form 35 to enable an Inspector to record his impressions and defects noticed.

(xi) The licensee shall destroy the stocks of batch/unit which does not comply with standard tests in such a way that it would not spread any disease/infection by way of proper disinfection method.

(xii) All bio-medical waste shall be treated, disposed off or destroyed as per the provisions of The Bio-Medical Wastes(Management and Handling) Rules 1996.

The licensee shall neither collect blood from any professional donor or paid donor nor shall he prepare blood components and/or manufacture blood products from the blood drawn from such a donor.

PART XII B OF SCHEDULE F
REQUIREMENTS FOR THE FUNCTIONING AND OPERATION OF A BLOOD BANK AND/OR FOR PREPARATION OF BLOOD COMPONENTS

BLOOD BANKS/BLOOD COMPONENTS

A. GENERAL

1. Location and Surroundings : The blood bank shall be located at a place which shall be away from open sewage, drain, public lavatory or similar unhygienic surroundings.
2. Building : The building (s), used for operation of a blood bank and/or preparation of blood components shall be constructed in such a manner so as to permit the operation of the blood bank and preparation of blood components under hygienic conditions and shall avoid the entry of insects, rodents and flies. It shall be well lighted, ventilated and screened (mesh), wherever necessary. The walls and floors of the rooms, where collection of blood or preparation of blood components or blood products is carried out shall be smooth, washable and capable of being kept clean. Drains shall be of adequate size and where connected directly to a sewer, shall be equipped with traps to prevent back siphonage.
3. Health, clothing and sanitation of staff: The employees shall be free from contagious or infectious diseases. They shall be provided with clean overalls, head-gears, foot-wears and gloves, wherever required. There shall be adequate, clean and convenient hand washing and toilet facilities.

A blood bank shall have an area of 100 square meters for its operations and an *additional* area of 50 square meters for preparation of blood components. *It shall be consisting of a room* each for -

(1) Registration and medical examination with adequate furniture and facilities for registration and selection of donors;
(2) Blood collection (air-conditioned);
(3) Blood component preparation. (This shall be air-conditioned to maintain temperature between 20°C - 25°C);
(4) Laboratory for blood group serology. (air-conditioned)
(5) Laboratory for blood transmissible diseases like Hepatitis, Syphilis, Malaria, HIV-antibodies (air-conditioned);
(6) Sterilization-cum-washing;
(7) Refreshment-cum-rest room (air-conditioned);
(8) Store-cum-records.

NOTES:

(1) The above requirements as to accommodation and area may be relaxed, in respect of testing laboratories and sterilization-cum-washing room, for reasons to be recorded in writing by the Licensing Authority and / or the Central Licence Approving Authority, in respect of blood banks operating in Hospitals, provided the hospital concerned has a pathological laboratory and a sterilization-cum-washing room common with other departments in the said hospital.
(2) Refreshments to the donor after phlebotomy shall be served so that he is kept under observation in the Blood Bank.

C. PERSONNEL

Every blood bank shall have following categories of whole time competent technical staff :-

(a) Medical Officer, possessing the qualifications specified in Condition (I) of rule 122-G.
(b) Blood Bank Technician(s), possessing -
  (i) Degree in Medical Laboratory Technology (M.L.T.) with six months' experience in the testing of blood and/or its components; or
  (ii) Diploma in Medical Laboratory Technology(ML T) with one year's experience in the testing of blood and/or its components, the degree or diploma being from a University/Institution recognised by the Central Government or State Government.
(c) Registered Nurse(s).
(d) Technical Supervisor(where blood components are manufactured), possessing -
  (i) Degree in Medical Laboratory Technology (M.L.T.) with six months' experience in the preparation of blood components; or
  (ii) Diploma in Medical Laboratory Technology (M.L.T) with one year's experience in the preparation of blood components, the degree or diploma being from a University/ Institution recognised by the Central Government or State Government.

NOTES:

(1) The requirements of qualification and experience in respect of Technical Supervisor and Blood Bank Technician shall apply in the cases of persons who are approved by the Licensing Authority and/or Central Licence Approving Authority after the commencement of the Drugs and Cosmetics (Amendment) Rules, 1999.

(2) As regards, the number of whole time competent technical personnel, the blood bank shall comply with the requirements laid down in the Directorate General of Health Services Manual.

(3) It shall be the responsibility of the licensee to ensure through maintenance of records and other latest techniques used in blood banking system that the personnel involved in blood banking activities for collection, storage, testing and distribution are adequately trained in the current Good Manufacturing Practices/Standard Operating Procedures for the tasks undertaken by each personnel. The personnel shall be made aware of the principles of Good Manufacturing Practices/Standard Operating Procedures that affect them and receive initial and continuing training relevant to their needs.

D. MAINTENANCE:

The premises shall be maintained in a clean and proper manner to ensure adequate cleaning and maintenance of proper operations. The facilities shall include-

(1) Privacy and thorough examination of individuals to determine their suitability as donors.

(2) Collection of blood from donors with minimal risk of contamination or exposure to activities and equipment unrelated to blood collection.

(3) Storage of blood or blood components pending completion of tests.

(4) Provision for quarantine, storage of blood and blood components in a designated location, pending repetition of those tests that initially give questionable serological results.

(5) Provision for quarantine, storage, handling and disposal of products and reagents not suitable for use.

(6) Storage of finished products prior to distribution or issue.

(7) Proper collection, processing, compatibility testing, storage and distribution of blood and blood components to prevent contamination.

(8) Adequate and proper performance of all procedures relating to plasmapheresis, plateletpheresis and leucapheresis.

(9) Proper conduct of all packaging, labeling and other finishing operations.

(10) Provision for safe and sanitary disposal of-

(i) Blood and/or blood components not suitable for use, distribution or sale.

(ii) Trash and items used during the collection, processing and compatibility testing of blood and/or: blood components.

E. EQUIPMENT:

Equipment used in the collection, processing, testing, storage and sale/distribution of blood and its components shall be maintained in a clean and proper manner and so placed as to facilitate cleaning and maintenance. The equipment shall be observed, standardized and calibrated on a regularly scheduled basis as described in the Standard Operating Procedures Manual and shall

operate in the manner for which it was designed so as to ensure compliance with the official requirements (the equipments) as stated below for blood and its components.

Equipment that shall be observed, standardized and calibrated with at least the following frequencies:-

EQUIPMENT	PERFORMANCE	FREQUENCY,	FREQUENCY OF CALIBRATION
1. Temperature recorder	Compare against thermometer	Daily	As often as necessary
2. Refrigerated centrifuge	Observe speed and temperature	Each day of use	As often as necessary
3. Hematocrit centrifuge	[1]—	—	Standardize before initial use, after repair or adjustments, and annually.
4. General lab. centrifuge	—	—	Check speed with tachometer every 6 months,
5. Automated Blood typing	Observe controls for correct results	Each day of use	—
6. Haemoglo-binomete	Standardize against cyanamethemoglo-bulin standard	Each day of use	—
7. Refractiometer or Urinometer	Standardize against distilled water.	—ditto —	—
8. Blood container weighing device	standardize against container of known weight	—ditto —	As often as necessary,
9. Water Bath	Observe Temperature	—ditto—	—ditto—
10. Rh view box (wherever necessary	—ditto —	—ditto-	—ditto—
11. .Autoclave	—ditto —	Each time of use	— ditto-
12. .Serologic rotators	Observe controls for correct results	Each day of use	Speed as often as necessary
13. .Laboratory thermometers	—	—	Before initial use
14. .Electronic thermometers	—	Monthly .	—

Standardization and caliberation of equipment *(continued)*

15. Blood agitator/ Weighing device	Observe weight of the first container of blood filled for correct results	Each day of use	standardize with container of known mass or volume before initial use, and after repairs or adustment

(F) **SUPPLIES AND** REAGENTS:

All supplies and reagents used in the collection, processing, compatibility, testing, storage and distribution of blood and blood components shall be stored at proper temperature in a safe and hygienic place, in a proper manner and in particular-

(a) All supplies coming in contact with blood and blood components intended for transfusion shall be sterile, pyrogen-free, and shall not interact with the product in such a manner as to have an adverse effect upon the safety, purity, potency or effectiveness of the product.

(b) Supplies and reagents that do not bear an expiry date shall be stored in a manner that the oldest is used first.

(c) Supplies and reagents shall be used in a manner consistent with instructions provided by the manufacturer.

(d) All final containers and closures for blood and blood components intended for transfusion shall be clean and free of surface solids and other contaminants.

(e) Each blood collecting container and its satellite container(s), if any, shall be examined visually for damage or evidence of contamination prior to its use and immediately after filling. Such examination shall include inspection for breakage of seals, when indicated, and abnormal discoloration. Where any defect is observed, the container shall not be used or, if detected after filling, shall be properly discarded.

(f) Representative samples of each lot of the following reagents and/or solution shall be tested regularly on a scheduled basis by methods described in the Standard Operating Procedures Manual to determine their capacity to perform as required:

Reagents and solutions	**Frequency of testing along with controls**
Anti-human serum	Each day of use
Blood grouping serums	Each day of use
Lectin	Each day of use
Antibody screening and reverse grouping cells	Each day of use
Hepatitis test reagents	Each run
Syphilis serology reagents	Each run
Enzymes	Each day of use
HIV I and II reagents	Each run
Normal saline (LISS and PBS)	Each day of use
Bovine Albumin	Each day of use

(G) GOOD MANUFACTURING PRACTICES (GMPs)/STANDARD OPERATING PROCEDURES (SOPs):

Written Standard Operating Procedures shall be maintained and shall include all steps to be followed in the collection, processing, compatibility testing, storage and sale or distribution of blood and/or preparation of blood components for homologous transfusion, autologous transfusion and further manufacturing purposes. Such procedures shall be available to the personnel for use in the concerned areas. The Standard Operating Procedures shall interalia include:

1.(a) criteria used to determine donor suitability.

(b) methods of performing donor qualifying tests and measurements including minimum and maximum values for a test or procedure, when a factor in determining acceptability;

(c) solutions and methods used to prepare the site of phlebotomy so as to give maximum assurance of a sterile container of blood;

(d) method of accurately relating the product(s) to the donor;

(e) blood collection procedure, including in-process precautions taken to measure accurately the quantity of blood drawn from the donor;

(f) methods of component preparation including, any time restrictions for specific steps in processing;

(g) all tests and repeat tests performed on blood and blood components during processing;

(h) pre-transfusion testing, wherever applicable, including precautions to be taken to identify accurately the recipient blood components during processing;

(i) procedures of managing adverse reactions in donor and recipient reactions

(j) storage temperatures and methods of controlling storage temperatures for blood and its components and reagents;

(k) length of expiry dates, if any, assigned for all final products;

(l) criteria for determining whether returned blood is suitable for reissue;

(m) procedures used for relating a unit of blood or blood component from the donor to its final disposal;

(n) quality control procedures for supplies and reagents employed in blood collection, processing and re-transfusion testing;

(o) schedules and procedures for equipment maintenance and calibration;

(p) labelling procedures to safe guard its mix-ups, receipt, issue, rejected and in-hand;

(q) procedures of plasmapheresis, plateletphersis and leucapheresis if performed, including precautions to be taken to ensure re-infusion of donor's own cells.

(r) procedures for preparing recovered (salvaged) plasma if performed, including details of separation, pooling, labeling, storage and distribution.

(s) all records pertinent to the lot or unit maintained pursuant to these regulations shall be reviewed before the release or distribution of a lot or unit of final product. The review or portions of the review may be performed at appropriate periods during or after blood collection, processing, testing and storage. A thorough investigation, including the conclusions and follow-up, of any unexplained discrepancy or the failure of a lot or unit to meet any of its specification shall be made and recorded.

2. A licensee may utilize current Standard Operating Procedures, such as the manuals of the following organizations, so long as such specific procedures are consistent with, and at least as stringent as, the requirements contained in this Part, namely :-
   (i) Directorate General of Health Services Manual.
   (ii) Other Organizations or individual blood bank's manuals, subject to the approval of State Licensing Authority and Central Licence Approving Authority.

H. CRITERIA FOR BLOOD DONATION:

Conditions for donation of blood :

(I) General -No person shall donate blood and no blood bank shall draw blood from a person, more than once in three months. The donor shall be in good health, mentally alert and physically fit and shall not be inmates of jail, persons having multiple sex partners and drug-addicts. The donors shall fulfill the following requirements, namely :-
   (a) the donor shall be in the age group of 18 to 60 years.
   (b) the donor shall not be less than 45 kilograms;
   (c) temperature and pulse of the donor shall be normal;
   (d) the systolic and diastolic blood pressures are within normal limits without medication;
   (e) hemoglobin which shall not be less than 12.5 grams;
   (f) the donor shall be free from acute respiratory diseases;
   (g) the donor shall be free from any skin diseases at the site of phlebotomy ;
   (h) the donor shall be free from any disease transmissible by blood transfusion, in so far as can be determined by history and examination indicated above;
   (i) the arms and forearms of the donor shall be free from skin punctures or scars indicative of professional blood donors or addiction of self injected narcotics

3. Additional qualifications of a donor. -

   No person shall donate blood, and no blood bank shall draw blood from a donor, in the conditions mentioned in column (1) of the Table given below before the expiry of the period of deferment mentioned in the column (2) of the said Table.

Table: Deferment of blood donation

CONDITIONS	PERIOD OF DEFERMENT
(l)	(2)
(a) Abortions	6 months
(b) History of blood transfusion	6 months
(c) Surgery	12 months
(d) Typhoid	12 months after recovery
(e) History of malaria and duly treated	3 months (endemic) 3 years (non endemic area)
(f) Tattoo	6 months
(g) Breast feeding	12 months after delivery

Deferment of blood donation *(continued)*

(i)	Immunization (Cholera, Typhoid, Diphtheria, Tetanus Plague, Gammaglobulin)	15 days
(j)	Rabies vaccination	1 year after vaccination
(k)	History of Hepatitis in family or close contact	12 months
(l)	Immunoglobulin	12 months

2. No person shall donate blood and no blood bank shall draw blood from a person, suffering from any of the diseases mentioned below, namely.-
   a Cancer
   b. Heart disease
   c. Abnormal bleeding tendencies
   d. Unexplained weight loss
   e. Diabetes-controlled on Insulin
   f. Hepatitis infection
   g. Chronic nephritis
   h. Signs and symptoms, suggestive of AIDS
   i. Liver disease
   j. Tuberculosis
   k, Polycythemia Vera
   I. Asthma
   m. Epilepsy
   n. Leprosy
   o. Schizophrenia
   p. Endocrine disorders

I. **GENERAL EQUIPMENT AND INSTRUMENTS**:

1. For blood collection room :
   (i) Donor beds, chairs and tables: These shall be suitably and comfortbly cushioned and shall be of appropriate size.
   (ii) Bedside table.
   (iii) Sphygmomanometer and Stethoscope.
   (iv) Recovery beds for donors.
   (v) Refrigerators, for storing separately tested and untested blood, maintaining temperature between 2 to 6 degree centigrade with digital dial thermometer, recording thermograph and alarm device, with provision for continuous power supply.
   (vi) Weighing devices for donor and blood containers.
2. For hemoglobin determination :
   (i) Copper sulphate solution (specific gravity 1.053)
   (ii) Sterile lancet and impregnated alcohol swabs.
   (iii) Capillary tube (1.3x1.4x96 mm or pasteur pipettes)
   (iv) Rubber bulbs for capillary tubings.

Sahli's hemoglobinometer/Colorimeteric method.

3. For temperature and pulse determination:

   (i) Clinical thermometers.

   (ii) Watch (fitted with a seconds-hand) and a stop-watch.

4. For blood containers :

   (a) Only disposable PVC blood bags shall be used (closed system) as per the specifications of IP/USP/BR

   (b) Anti-coagulants: The anti-coagulant solution shall be sterile, pyrogen-free and of the following composition that will ensure satisfactory safety and efficacy of the whole blood and/or for all the separated blood components.

      (i) Citrate Phosphate Dextrose solution (CPD) or Citrate Phosphate Dextrose Adenine- 1 (CPDA-1), 14 ml. solution shall be required for 100 ml of blood.

NOTE 1.

(i) In case of single/double/triple/quadruple blood collection bags used for blood component preparations, CPDA blood collection bags may be used.

(ii) Acid Citrate Dextrose solution (A.C.D with Formula-A). I.P., 15ml solution shall be required for 100 ml of blood.

(iii) Additive solutions such as SAGM, ADSOL, NUTRICEL may be used for storing, and retaining Red Blood Corpuscles upto 42 days.

NOTE 2.

The licensee shall ensure that the anti-coagulant solutions are of a licensed manufacturer and the blood bags in which the said solutions are contained have a certificate of analysis of the said manufacturer.

5. Emergency equipment/items.

   (i) Oxygen cylinder with mask, gauge and pressure regulator.

   (ii) 5 percent Glucose or Normal Saline,

   (iii) Disposable sterile syringes and needles of various sizes.

   (iv) Disposable sterile I.V. infusion sets.

   (v) Ampoules of Adrenaline, Noradrenaline, Mephentin, Betamethasone or Dexamethasone, Metoclorpropamide injections

   (vi) Aspirin.

6. Accessories :

   (i) Such as blankets, emesis basins, haemostats, set clamps, sponge forceps, gauze, dressing jars, solution jars, waste cans.

   (ii) Medium cotton balls, 1.25 cm. adhesive tapes.

   (iii) Denatured spirit, Tincture Iodine, green soap or liquid soap.

   (iv) Paper napkins or towels.

   (v) Autoclave with temperature and pressure indicator.

   (vi) Incinerator

   (vii) Stand-by generator.

Laboratory equipment:

(i) Refrigerators, for storing diagnostic kits and reagents, maintaining a temperature between 4-6^0 C + 2^0C with digital dial thermometer having provision for continuous power supply.

(ii) Compound Microscope with low and high power objectives.

(iii) Centrifuge (Table Model)

(iv) Water bath: having range between 37°-56°C

(v) Rh viewing box in case of slide technique.

(vi) Incubator with thermostatic control.

(vii) Mechanical shakers for serological tests for Syphilis.

(viii) Hand-lens for observing tests conducted in tubes.

(ix) Serological graduated pipettes of various sizes

(x) Pipettes (Pasteur)

(xi) Glass slides

(xii) Test tubes of various sizes/microwell plates (U or V type)

(xiii) Precipitating tubes 6mm x 50mm of different sizes and glass beakers of different sizes

(xiv) Test tube racks of different specifications,

(xv) Interval timer electric or spring wound,

(xvi) Equipment and materials for cleaning glass wares adequately,

(xvii) Insulated container for transporting blood, between 2 degree centigrade to 10 degree centigrade temperatures, to wards and hospitals,

(xviii) Wash bottle

(xix) Filter papers

(xx) Dielectric tube sealer.

(xxi) Plain and EDTA vials

(xxii) Chemical balance (wherever necessary)

(xxiii) ELISA reader with printer, washer and micropipettes.

J. SPECIAL REAGENTS:

(1) Standard blood grouping sera Anti-A, Anti-B and Anti-D with known controls. Rh typing sera shall be in double quantity and each of different brand or if from the same, supplier each supply shall be of different lot numbers.

(2) Reagents for serological tests for syphilis and positive sera for controls.

(3) Anti-Human Globulin Serum (Coombs' serum)

(4) Bovine Albumin 22 percent & Enzyme reagents for incomplete antibodies.

(5) ELISA or RPHA test kits for Hepatitis B & C and HIV I & II.

(6) Detergent and other agents for cleaning laboratory glass wares.

K. TESTING OF WHOLE BLOOD:

(1) It shall be the responsibility of the licensee to ensure that the whole blood collected processed and supplied conforms to the standards laid down in the Indian Pharmacopoeia and other tests published, if any, by the Government.

(2) Freedom from HIV antibodies (AIDS) Test -Every licensee shall get samples of every blood unit tested, before use, for freedom from HIV I and HIV II antibodies either from laboratories specified for the purpose by the Central Government or in his own laboratory. The results of such testing shall be recorded on the label of the container.

(3) Each blood unit shall also be tested for freedom from Hepatitis B surface antigen, and Hepatitis C Virus antibody, VDRL and malarial parasite and results of such testing shall be recorded on the label of the container.

NOTE:

(a) Blood samples of donors in pilot tube and the blood samples of the recipient shall be preserved for 7 days after issue.

(b) The blood intended for transfusion shall not be frozen at any stage.

(c) Blood containers shall not come directly in contact with ice at any stage.

L. RECORDS:

The records which the licensee is required to maintain shall include inter alia the following particulars, namely:-

(1) Blood donor record: It shall indicate serial number, date of bleeding, name, age, address and signature of donor with other particulars of age, weight, hemoglobin, blood grouping, blood pressure, medical examination, bag number and patient's detail for whom donated in case of replacement donation, category of donation (voluntary/replacement) and deferral records and signature of Medical Officer Incharge.

(2) Master records for blood and its components: It shall indicate bag serial number, date of collection, date of expiry, quantity in ml. ABO/Rh Group, results for testing of **HIV** I and HIV II antibodies, Malaria, V.D.R.L., Hepatitis B surface antigen and irregular antibodies (if any), name and address of the donor with particulars, utilisation issue number, components prepared or discarded and signature of the Medical Officer Incharge.

(3) Issue register: It shall indicate serial number, date and time of issue, bag serial number, ABO/Rh Group, total quantity in ml, name and address of the recipient, group of recipient, unit/institution, details of crossmatching report, indication for transfusion.

(4) Records of components supplied: quantity supplied; compatibility report, details of recipient and signature of issuing person.

(5) Records of A.C.D./C.P.D/CPD-A/SAGM bags giving details of manufacturer, batch number, date of supply, and results of testing.

(6) Register for diagnostic kits and reagents used: name of the kits/reagents, details of batch number, date of expiry and date of use.

(7) Blood bank must issue the cross matching report of the blood to the patient together with the blood,unit.

(8) Transfusion adverse reaction records.

(9) Records of purchase, use and stock in hand of disposable needles, syringes, blood bags, shall be maintained.

NOTE: The above said records shall be kept by the licensee for a period of five years.

M. LABELS:

The labels on every bag containing blood and/or component shall contain the following particulars, namely:

(1) The proper name of the product in a prominent place and in bold letters on the bag.
(2) Name and address of the blood bank
(3) Licence number
(4) Serial number
(5) The date on which the blood is drawn and the date of expiry as prescribed under Schedule P to these rules.
(6) A coloured label shall be put on every bag containing blood. The following colour scheme for the said labels shall be used for different groups of blood:

Blood Group	Colour of the label
O	Blue
A	Yellow
B	Pink
AB	White

(7) The results of the tests for Hepatitis B surface antigen, and Hepatitis C virus antibody, syphilis, freedom from HIV I and HIV II antibodies and malarial parasite.
(8) The Rh group.
(9) Total volume of blood, the preparation of blood, nature and percentage of anti-coagulant.
(10) Keep continuously temperature at 2°C-6° C for whole human blood and/or components as contained under III of Part XIIB.
(11) Disposable transfusion sets with filter shall be used in administration equipment.
(12) Appropriate compatible cross matched blood without a typical antibody in recipient shall be used.
(13) The contents of the bag shall not be used if there is any visible evidence of deterioration like haemolysis, clotting or discoloration.
(14) The label shall indicate the appropriate donor classification like "Voluntary Donor" or "Replacement Donor" in no less prominence than the proper name.

NOTES:

1. In the case of blood components, particulars of the blood from which such components have been prepared shall be given against item numbers (5), (7), (8), (9) and (14).
2. The blood and/or its components shall be distributed on the prescription of a Registered Medical Practitioner .

II. II. BLOOD DONATION CAMPS.

A blood donation camp may be organized by -

(a) a licensed designated Regional Blood Transfusion Centre; or
(b) a licensed Government blood bank; or

(c) the Indian Red Cross Society or
(d) a licenced blood bank run by registered voluntary or charitable organizations recognized by State or Union Territory Blood Transfusion Council.

NOTES:

(i) "Designated Regional Blood Transfusion Centre" shall be a centre approved and designated by a Blood Transfusion Council constituted by a State Government to collect, process and distribute blood and its components to cater to the needs of the region and that centre has also been licensed and approved by the Licensing Authority and Central Licence Approving Authority for the purpose.
(ii) The designated Regional Blood Transfusion Centre, Government blood bank and Indian Red Cross Society shall intimate within a period of seven days, the venue where blood camp was held and details of group wise blood units collected in the said camp to the licensing Authority and Central Licence Approving Authority.

For holding a blood donation camp, the following requirements shall be fulfilled/complied with, namely:-

(A) Premises, personnel etc

(a) Premises under the blood donation camp shall have sufficient area and the location shall be hygienic so as to allow proper operation,maintenance and cleaning.
(b) All information regarding the personnel working, equipment used and facilities available at such a Camp shall be well documented and made available for inspection, if required, and ensuring—
  (i) continuous and uninterrupted electrical supply for equipment used in the Camp;
  (ii) adequate lighting for all the required activities;
  (iii) hand-washing facilities for staff;
  (iv) reliable communication system to the central office of the Controller/Organiser of the camp;
  (v) furniture and equipment arranged within the available place;
  (vi) refreshment facilities for donors and staff;
  (vii) (vii)facilities for medical examination of the donors;
  (viii) proper disposal of waste.

(B) Personnel for Out-door Blood Donation Camp :

To collect blood from 50 to 70 donors in about 3 hours or from 100 to 120 donors in 5 hours, the following requirements shall be fulfilled/complied with :-

(i) One Medical Officer and two nurses or phlebotomists for managing 6-8 donor tables;
(ii) two medico social workers;
(iii) three blood bank technicians;
(iv) two attendants;
(v) vehicle having a capacity to seat 8-10 persons, with provision for carriage of donation goods including facilities to conduct a blood donation camp.

(C) Equipment:

1. BP apparatus.
2. Stethoscope.
3. Blood bags (single, double, triple, quadruple)
4. Donor questionnaire.
5. Weighing device for donors.
6. Weighing device for blood bags,
7. Artery forceps, scissors.
8. Stripper for blood tubing.
9. Bed sheets, blankets/matress.
10. Lancets, swab stick/tooth picks.
11. Glass slides.
12. Portable Hb meter/copper sulphate.
13. Test tube (big) and 12x100 mm (small)
14. Test tube stand.
15. Anti-A, Anti-B and Anti.AB, Antisera and Anti-D
16. Test tube sealer film.
17. Medicated adhesive tape.
18. Plastic waste basket
19. Donor cards and refreshment for donors.
20. Emergency medical kit
21. Insulated blood bag containers with provisions for storing between 2°-10° C
22. Dielectric sealer or portable tube sealer
23. Needle destroyer (wherever necessary)

III. PROCESSING OF BLOOD COMPONENTS FROM WHOLE BLOOD BY A BLOOD BANK

The Blood components shall be prepared by blood banks as a part of the Blood Bank services. The conditions for grant or renewal of licence to prepare blood components shall be as follows :-

(A) ACCOMMODATION:

(1) Rooms with adequate area and other specifications, for preparing blood components depending on quantum of work load shall be as specified in item B under the heading "I. BLOOD BANKS/BLOOD COMPONENTS" of this Part.

(2) Preparation of Blood components shall be carried out only under closed system using, double, triple or quadruple plastic bags except for preparation of Red Blood Cells Concentrates, where single bags may be used with transfer bags.

(B) EQUIPMENT:

(i) Air conditioner;
(ii) Laminar air flow bench;
(iii) Suitable refrigerated centrifuge;
(iv) Plasma expresser
(v) Clipper and clips and or dielectric sealer;

(vi) Weighing device; -
(vii) Dry rubber balancing material;
(viii) Artery forceps, scissors;
(ix) Refrigerator maintaining a temperature between 2°C - 6°C, a digital dial thermometer with recording thermograph and alarm device, with provision for continuous power supply;
(x) Platelet agitator with incubator (wherever necessary)
(xi) Deep freezers maintaining a temperature between -30°C to -40°C and -75°C to -80°C
(xii) Refrigerated Water bath for Plasma Thawing;
(xiii) Insulated blood bag containers with provisions for storing at appropriate temperature for transport purposes;.

(C) PERSONNEL:
The whole time competent technical staff meant for processing of Blood Components (that is Medical Officer, Technical Supervisor, Blood Bank Technician and Registered Nurse) shall be as specified in item C, under the heading "I. BLOOD BANKS/BLOOD COMPONENTS" of this Part.

(D) TESTING FACILITIES:
General: Facilities for A,B, AB and O groups and Rh (D) grouping. Hepatitis B Surface antigen and Hepatitis C virus antibody, VDRL, HIV I and HIV II antibodies and malarial parasites shall be mandatory for every blood unit before it is used for the preparation of blood components. The results of such testing shall be indicated on the label.

(E) CATEGORIES OF BLOOD COMPONENTS:
(I) CONCENTRATED HUMAN RED BLOOD CORPUSCLES:
The product shall be known as "Packed Red Blood Cells" that is Packed Red Blood Cells remaining after separating plasma from human blood.

General Requirements :
(a) Storage: Immediately after processing, the Packed Red Blood Cells shall be kept at a temperature maintained between 2 degree centigrade to 6 degree centigrade.
(b) Inspection: The component shall be inspected immediately after separation of the plasma, during storage and again at the time of issue. The product shall not be issued if there is any abnormality in color or physical appearance or any indication of microbial contamination.
(c) Suitability of Donor: The source blood for Packed Red Blood Cells shall be obtained from a donor who meets the criteria for Blood Donation as specified in item H under the heading "I. BLOOD BANKS/BLOOD COMPONENTS" of this Part.
(d) Testing of Whole Blood: Blood from which Packed Red Blood Cells are prepared shall be tested as specified in item K relating to Testing of Whole Blood under the heading "I.BLOOD BANKS/BLOOD COMPONENTS" of this Part.

(e) Pilot samples: Pilot samples collected in integral tubing or in separate pilot tubes shall meet the following specifications :

(i) One or more pilot samples of either the original blood or of the Packed Red Blood Cells being processed shall be preserved with each unit of Packed Red Blood Cells which is issued.

(ii) Before they are filled, all pilot sample tubes shall be marked or identified so as to relate them to the donor of that unit or Packed Red Blood Cells.

(iii) Before the final container is filled or at the time the final product is prepared, the pilot sample tubes accompanying a unit of Packed Red Blood Cells, shall be attached in a tamper-proof manner that shall conspicuously identify removal and re-attachment.

(iv) All pilot sample tubes, accompanying a unit of packed red blood cells, shall be filled immediately after the blood is collected or at the time the final product is prepared, in each case, by the person who performs the collection of preparation.

(f) Processing :

(i) Separation: Packed Red Blood Cells shall be separated from the whole blood,—

(a) if the whole blood is stored in ACD solution within 21days, and

(b) if the whole blood is stored in CPDA-1 solution, within 35 days, from the date of collection- Packed Red Blood Cells may be prepared either by centrifugation done in a manner that shall not tend to increase the temperature of the blood or by normal undisturbed sedimentation "method. A portion of the plasma, sufficient to ensure optimal cell preservation, shall be left with the Packed Red Blood Cells.

(ii) Packed Red Blood Cells Frozen : Cryophylactic substance may be added to the Packed Red Blood Cells for extended manufacturer's storage not wanner than minus 65°C provided the manufacturer submits data to the satisfaction of the Licensing Authority and Central Licence Approving Authority, as adequately demonstrating through in-vivo cells survival and other appropriate tests that the addition of the substance, the material used and the processing methods results in a final product meets the required standards of safety, purity and potency for Packed Red Blood Cells, and that the frozen product shall maintain those properties for the specified expiry period.

(xiv) Testing: Packed Red Blood Cells shall conform to the standards as laid down in the Indian Pharmacopoeia.

(2). PLATELETS CONCENTRATES:

The product shall be known as "Platelets Concentrates" that is platelets collected from one unit of blood and re-suspended in an appropriate volume of original plasma.

General Requirements :

(i) Source: The source material for platelets shall be platelet- rich plasma or buffy coat which may be obtained from the whole blood or by plateletpheresis.

(ii) Processing

(a) Separation of buffy-coat or platelet-rich plasma and platelets and re-suspension of the platelets shall be in a closed system by-centrifugal method with appropriate speed, force and time.

(b) Immediately after collection, the whole blood or plasma shall be stored at 20°-24°C. When it is to be transported from the venue of blood collection to the processing laboratory, during such transport action, the temperature as close as possible 20°-24°C shall be ensured. The platelet concentrates shall be separated within 6 hours after the time of collection of the unit of whole blood or plasma.

(c) The time and speed of centrifugation shall be demonstrated to produce an undamped product, without visible hemolysis, that yields a count of not less than $3.5x\ 10^{10}$ and $4.5x\ 10^{10}$ i.e. platelets per unit from a unit of 350 ml. and 450ml. blood respectively. One percent of total platelets prepared shall be tested of which 75 percent of the units shall conform to the above said platelet count.

(d) The volume of original plasma used for re-suspension of the platelets shall be determined by the maintenance of the pH of not less than 6 during the storage period. The pH shall be measured on a sample of platelets which has been stored for the permissible maximum expiry period at 20°C-24°C.

(e) Final containers used for platelets shall be colorless and transparent to permit visual inspection of the contents. The caps selected shall maintain a hermetic seal to prevent contamination of the contents. The container material shall not interact with the contents, under the normal conditions of the storage and use, in such a manner as to have an adverse effect upon the safety, purity, potency, or efficacy of the product. At the time of filling, the final container shall be marked or identified by number so as to relate it to the donor.

(iii) Storage

Immediately after re-suspension, platelets shall be placed in storage not exceeding for a period of 5days from the date of collection at 20°C-24°C. with continuous gentle agitation of the platelet concentrates.

(iv) Testing:

The units prepared from different donors shall be tested at the end of the storage period for -

(a) Platelet count:
(b) pH of not less than 6 measured at the storage temperature of the unit;
(c) measurement of actual plasma volume;
(d) one percent of the total platelets prepared shall be tested for sterility;
(e) the tests for functional viability of the platelets shall be done by swirling movement before issue;
(f) if the results of the testing indicate that the product does not meet the specified requirements, immediate corrective action shall be taken and records maintained.

(v) Compatibility Test:

Compatible transfusion for the purpose of variable number of Red Blood Cells, A,B, AB and O grouping shall be done if the platelets concentrate is contaminated with red blood cells.

(3) GRANULOCYTE CONCERNTRATES:

(i) Storage: It shall be kept at 20°C-24°C for a maximum period of 24 hours.
(ii) Unit of granulocytes shall not be less than 1x10'° when prepared on cell separator.
(iii) Group specific tests/HLA test wherever required shall be carried out.

(4) FRESH FROZEN PLASMA :

Plasma frozen within 6 hours after blood collection and stored at a temperature not warmer than 30°C, shall be preserved for a period of not more than one year.

(5) CRYOPRECIPITATE:

Concentrate of anti-hemophilic factor shall be prepared by thawing of the fresh plasma frozen stored at -30°C.

(a) Storage :

Cryoprecipitate shall be preserved at a temperature not higher than -30°C and may be preserved for a period of not more than one year from the date of collection.

(b) Activity :

Anti-hemophilic factor activity in the final product shall be not less than 80 units per bag. One percent of the total cryoprecipitate prepared shall be tested of which seventy five percent of the unit shall conform to the said specification.

F. PLASMAPHERESIS, PLATELETPHERESIS, LEUCAPHERESIS USING A CELL SEPARATOR.

An area of 10 square meters shall be provided for apheresis in the blood bank. The blood banks specifically permitted to undertake the said apheresis on the donor shall observe the criteria as specified in item H relating to criteria for blood donation under the heading "BLOOD BANK/ BLOOD COMPONENTS" of this part. The written consent of the donor shall be taken and the donor must be explained, the hazards of apheresis. The Medical Officer shall certify that donor is fit for apheresis and it shall be carried out by a trained person under supervision of the Medical Officer.

(A) PLASMAPHERESIS, PLATELET PHERESIS AND LEUCAPHERESIS:

The donors subjected to plasmapheresis, plateletpheresis and leucopheresis shall, in addition to the criteria specified in item H relating to the CRITERIA FOR BLOOD DONATION, under the heading "I BLOOD BANKS/BLOOD COMPONENTS" of this Part being observed, be also subjected to protein estimation on post-pheresis/ first sitting whose results shall be taken as a reference for subsequent pheresis/sitting. It shall also be necessary that the total plasma obtained from such donor and periodicity of Plasmapheresis shall be according to the standards described under validated Standard Operating Procedures.

NOTE:

(i) At least 48 hours must elapse between successive apheresis and not more than twice in a week.
(ii) Extracoporeal blood volume shall not exceed 15% of donor's estimated blood volume,
(iii) Platelet pheresis shall not be carried out on donors who have taken medication containing asprin within 3 days prior to donation,
(iv) If during plateletpheresis or leucapheresis, RBCs cannot be re-transfused then at least 12 weeks shall elapse before a second cytapheresis procedure is conducted.

(C) MONITORING FOR APHERESIS:
Before starting apheresis procedure, hemoglobin or hematocrit shall be done. Platelet count. WBC counts, differential count may be carried out. In repeated plasmapheresis, the serum protein shall be 6 gm./100ml.

(D) COLLECTION OF PLASMA:
The quantity of plasma separated from the blood of a donor shall not exceed 500 ml. per sitting and once in a fortnight or shall not exceed 1000 ml. per month,

PART XIIC

I. REQUIREMENTS FOR MANUFACTURE OF BLOOD- PRODUCTS

The blood products shall be manufactured in a separate premises other than that meant for blood bank. The requirements that are essential for grant or renewal of licence to manufacture blood products such as Albumin, Plasma Protein Fraction, Immunoglobins and Coagulation Factor Concentrates, shall be as follows namely:-

A GENERAL REQUIREMENTS :

1. Location and surroundings, buildings and water supply:
   The requirements as regards location and surrounding, buildings and water supply as contained in paragraphs 1.1.1,1.1.2,1.1.3 of Part I of Schedule M shall apply mutatis mutandis to the manufacture of blood products.
2. Disposal of waste and infectious materials :
   (i) The requirement as regards disposal of waste and infectious materials as contained in paragraph 1.1.4 of Part I of Schedule M shall apply mutatis mutandis to the manufacture of blood products.
   (ii) Proper facility shall also be provided for potentially infectious materials, particularly HIV I & HIV II, Hepatitis B surface antigen and Hepatitis C virus antibody through autoclaving, incineration or any other suitable validated methods.
3. Health, clothing and sanitation of personnel:
   (i) The requirement as contained in paragraph 3 of Part I of Schedule M shall be complied with.
   (ii) The personnel working in the manufacturing areas shall be vaccinated against Hepatitis B virus and other infectious transmitting diseases.
4. Requirements for manufacturing area for Blood Products.
   (i) For the manufacture of blood products; separate enclosed areas specifically designed for the purpose shall be provided. These areas be provided with air locks for entry and shall be essentially dust free and ventilated with an air supply. Air supply for manufacturing area shall be filtered through bacteria retaining filters (HEPA Filters) and shall be at a pressure higher than in the adjacent areas. The filters shall be checked for performance on installation and periodically thereafter, and records thereof shall be maintained.
   (ii) Interior surfaces (walls, floors and ceilings) shall be smooth and free from cracks, they shall not shed matter and shall permit easy cleaning and disinfection. Drains shall be excluded from aseptic areas. Routine microbial counts of the manufacturing

area shall be carried out during manufacturing operations. The results of such counts shall be checked against well documented in-house standards and records maintained. Access to the manufacturing areas shall be restricted to a minimum number of authorised personnel. Special procedures for entering and leaving of the manufacturing areas shall be prominently displayed.

(iii) Sinks shall be excluded from aseptic areas. Any sink installed in other clean areas shall be of suitable material such as stainless steel, without an overflow, and be supplied with water of potable quality. Adequate precautions shall be taken to avoid contamination of the drainage system with dangerous effluents and airborne dissemination of pathogenic micro-organisms.

(iv) Lighting, air-conditioning, ventilation shall be designed to maintain a satisfactory temperature and relative humidity to minimise contamination and to take account of the comfort of personnels working with protective clothing,

(v) Premises used for the manufacture of blood products shall be suitably designed and constructed to facilitate good sanitation,

(vi) Premises shall be carefully maintained and it shall be ensured that repair and maintenance operations do not present any hazard to the quality of products. Premises shall be cleaned and, where applicable, disinfected according to detailed written validated procedures,

(vii) Adequate facilities and equipments shall be used for the manufacture of blood products derived from blood plasma,

(viii) All containers of blood products, regardless of the stage of manufacture, shall be identified by securely attached labels. Cross contamination shall be prevented by adoption of the following measures, namely :-

(a) processing and filling shall be in segregated areas;

(b) manufacture of different products at the same time shall be avoided;

(c) simultaneous filling of the different products shall be avoided;

(d) ensure transfer, containers/materials by means of airlocks, air extraction, clothing change and careful washing and decontamination of equipment;

(e) protecting containers/materials against the risk of contamination caused by re-circulation of untreated air or by accidental re-entry of extracted air;

(f) using containers that are sterilised or are of documented low “bioburden”;

(ix) Positive pressure area shall be dedicated to the processing area concerned;

(x) Air-handling units shall be dedicated to the processing area concerned;

(xi) Pipe work, valves and vent filters shall be properly designed to facilitate cleaning and sterlisation. Valves on fractionation/reacting vessels shall be completely steam-sterlisable. Air vent filters shall be hydrophobic and shall be validated for their designated use;

5. Ancillary Areas:

(i) Rest and refreshment rooms shall be separated from other areas.

(ii) Facilities for changing and storing clothes and for washing and toilet purposes shall be easily accessible and appropriate for the number of users. Toilets shall not be connected directly with production or storage areas,

(iii) Maintenance workshops shall be separated from production areas. Wherever parts

and tools are stored in the production area, they shall be kept in rooms or lockers reserved for that use.

(iv) Animal houses shall be well isolated from other areas, with separate entrance.

B COLLECTION AND STORAGE OF PLASMA FOR FRACTIONATION:

A. Collection :

(1) Plasma shall be collected from the licensed Blood Banks through a cold chain process and stored in frozen condition not warmer than -20°C.

(2) Individual plasma shall remain in quarantine till it is tested for Hepatitis B surface antigen and Hepatitis C virus antibody HIV I and HIV It.

(3) A sample from pooled—lot plasma of about 10-12 units of different donors shall be tested for Hepatitis B surface antigen and Hepatitis C virus antibody, HIV I and HIV II and if the sample found negative, only then it shall be taken up for fractionation.

B. Storage Area:

(1) Storage areas shall be of sufficient space and capacity to allow orderly storage of the various categories of materials, intermediates, bulk and finished products, products in quarantine, released, rejected, returned, or recalled products.

(2) Storage areas shall be designed or adopted to ensure good storage conditions. In particular, they shall be clean, dry and maintained within temperature required for such storage and where special storage conditions are required (e.g. temperature, humidity), these shall be provided, checked and monitored.

(3) Receiving and dispatch bays shall protect materials and products from the weather and shall be designed and equipped to allow containers of incoming materials to be cleaned, if necessary, before storage.

(4) Where quarantine status is ensured by storage in separate areas, these areas shall be clearly marked and their access restricted only to.authorised personnel.

(5) There shall be separate sampling area for raw materials. If sampling is performed in the storage area, it shall be conducted in such a way so as to prevent contamination or cross-contamination.

(6) Segregation shall be provided for the storage of rejected, recalled, or returned materials or products.

(7) Adequate facility shall be provided for supply of ancillary material, such as ethanol, water, salts and polyethylene glycol. Separate facilities shall be provided for the recovery of organic solvents used in fractionation.

C. PERSONNEL;

1. Manufacture:

The manufacture of blood products shall be conducted under the active direction and personal supervision of competent technical staff, consisting of at least one person who shall be a whole time employee, with one year practical experience in the manufacture of blood products/plasma fractionation and possesses -

(a) Post-graduate degree in Medicine -M.D. (Microbiology/ Pathology/Bacteriology/ Immunology/Biochemistry); or

(b) Post-graduate degree in Science (Microbiology); or
(c) Post-graduate degree in Pharmacy (Microbiology), from a recognised University or Institution.

2. Testing:
The head of the testing unit shall be independent of the manufacturing unit and testing shall be conducted under the active direction and personal supervision of competent technical staff consisting of at least one person who shall be a whole time employee. The Head of the testing unit shall have eighteen months practical experience in the testing of drugs, especially the blood products and possesses -

(a) Post-graduate degree in Pharmacy or Science-(Chemistry/Microbiology/Bio-chemistry); or
(b) Post-graduate degree in Medicine -M.D. (Microbiology /Pathology/ Biochemistry), from a recognised University or Institution

D. PRODUCTION CONTROL :

(1) The production area and the viral inactivation room shall be centrally air-conditioned and fitted with HEPA Filters having Grade C (Class 10,000) environment as given in the Table below.
(2) The filling and sealing shall be carried out under aseptic conditions in centrally air-conditioned areas fitted with HEPA Filters having Grade A or, as the case may be, grade B (Class 100) environment given in the said Table.

TABLE
AIR **CLASSIFICATION SYSTEM FOR MANUFACTURE OF STERILE PRODUCTS.**

Maximum number of particles permitted per m^3

GRADE	**Maximum Number of particles permitted per m^3 0.5-5 micron**	**Maximum number of viable micro-organism permitted Less than 5 PERm^3**
A (Class 100) (Laminar-Airflow workstation)	3500	None Less than 1
B (Class 100)	3500	None Less than 5
C (Class 10000)	3,50,000	2000 Less than 100

(3) The physical and chemical operations used for the manufacture of plasma fractionation shall maintain high yield of safe and effective protein.
(4) The fractionation procedure used shall give a good yield of products meeting the in-house quality requirements as approved by the Licensing Authority and Central Licence Approving Authority reducing the risk of microbiological contamination and protein denaturation to the minimum.
(5) The procedure adopted shall not affect the antibody activity and biological half-life or biological characteristics of the products.

E. VIRAL INACTIVATION PROCESS :

The procedure used by the licensee to inactivate the pathogenic organisms such as enveloped and non-enveloped virus, especially infectivity from HIV I & HIV II, Hepatitis B surface antigens and Hepatitis C virus antibody the viral inactivation and validation methods adopted by the licensee, shall be submitted for approval to the Licensing Authority and Central Licence Approving Authority

NOTES:

(1) No preservative (except stabiliser to prevent -protein denaturation such as glycine, sodium chloride or sodium caprylate) shall be added to Albumin,Plasma Protein Fraction, Intravenous Immunoglobulins or Coagulation Factor Concentrates without the prior approval of Licensing Authority and Central Licence Approving Authority.

(2) The licensee shall ensure that the said stabilisers do not have deleterial effect on the final product in the quantity present so as not to cause any untoward or adverse reaction in human beings.

F. QUALITY CONTROL:

Separate facilities shall be provided for Quality Control such as Hematological, Bio-chemical, Physico-chemical**,** Microbiological, Pyrogens. Instrumental and Safety testing. The Quality Control Department shall have inter alia the following principal duties, namely.—

(1) To prepare detailed instructions, in writing for carrying out test and analysis.

(2) To approve or reject raw material, components, containers, closures, in-process materials, packaging material, labeling and finished products.

(3) To release or reject batch of finished products which are ready for distribution.

(4) To evaluate the adequacy of the conditions under which raw materials, semi-finished products and finished products are stored.

(5) To evaluate the quality and stability of finished products and when necessary of raw materials and semi-finished products.

(6) To review production records to ensure that no errors have occurred or if errors have occurred that they have been fully investigated.

(7) To approve or reject all procedures or specifications impacting on the identity, strength, quality and purity of the product

(8) To establish shelf-life and storage requirements on the basis of stability tests related to storage conditions.

(9) To establish and when necessary revise, control procedures and specifications.

(10) To review complaints, recalls, returned or salvaged products and investigations conducted thereunder for each product

(11) To review Master Formula Records/Cards periodically.

G. TESTING OF BLOOD PRODUCTS :

The products manufactured shall conform to the standards specified in the Indian Pharmacopoeia and where standard of any product is not specified in the Pharmacopoeia, the standard for such product shall conform to the standard specified in the United States Pharmacopoeia or the British Pharmacopoeia. The final products shall be tested for freedom from HIV 1 and HIV II antibodies, Hepatitis B surface antigen and Hepatitis C virus antibody.

H. STORAGE OF FINISHED PRODUCT :

(i) The final products shall be stored between 2°C - 8°C, unless otherwise specified by the Central Licence Approving Authority.

(iii) The shelf-life assigned to the products by the licensee shall be submitted for approval to the Licensing Authority and Central Licence Approving Authority.

I. LABELLING

The products manufactured shall be labeled as specified in the Indian Pharmacopoeia, the British Pharmacopoeia or the United States Pharmacopoeia which shall be in addition to any other .requirement stated under Part IX or Part X of these rules The labels shall indicate the results of tests for Hepatitis B surface antigen and Hepatitis C virus antibody, freedom from HIV I and HIV II antibodies.

J. RECORDS:

The licensee shall maintain records as per Schedule U and also comply with Batch manufacturing records as specified in Paragraph 9 of Part I of Schedule M and any other requirement as may be directed by Licensing Authority and Central Licence Approving Authority.

K. MASTER FORMULA RECORDS :

The licensee shall maintain Master Formula Records relating to all manufacturing and quality control procedures for each product, which shall be prepared and endorsed by the competent Technical Staff, i.e., Head of the manufacturing unit. The Master Formula Records shall contain:-

(i) the patent or proprietary name of the product alongwith the generic name, if any, strength and the dosage form;

(ii) a description or identification of the final containers, packaging materials, labels and closures to be used;

(iii) the identity, quantity and quality of each raw material to be used irrespective of whether or not it appears in the finished product. The permissible overage that may be included in a formulated batch shall be indicated;

(iv) a description of all vessels and equipments and the sizes used in the process;

(v) manufacturing and control instructions along with parameters for critical steps such as mixing, drying, blending, sieving and sterilising the product;
(vi) the theoretical yield to be expected from the formulation at different stages of manufacture and permissible yield limits;

(vi) detailed instructions on precautions to be taken in the manufacture and storage of drugs and of semi-finished products; and

(vii) the requirements in-process quality control tests and analysis to be carried out during each stage of manufacture including the designation of persons or departments responsible for the execution of such tests and analysis.

II. REQUIREMENTS FOR MANUFACTURE OF BLOOD PRODUCTS FROM BULK FINISHED PRODUCTS

Where the blood products, such as Albumin, Plasma Protein Fraction, Immunoglobulins and Coagulation Factor Concentrates are manufactured through the manufacturing activities of filling and sealing the blood products from bulk powder or solution or both, the requirements as they apply to the manufacture of blood products from whole blood shall apply mutatis mutandis to such manufacture of blood products, unless other requirements have been approved by the Central licence Approving Authority.

Guidelines for approval of blood and or its components Storage Centres run by First Referral Unit, Community Health Centre, Primary Health Centre or any hospital:

Ministry of Health & Family Welfare (Deptt. of Health) vide notification No. GSR 909(E) dated 20th December, 2001 exempted blood storage centres run by First Referral Unit (FRU), Community Health Centre, Public Health Center (PHC) or any hospital from the purview of obtaining licence for operation. This notification has been inserted under Schedule K of Drugs & Cosmetics rules, 1945 under serial no- 5B. The main aim of this notification is to make abundant availability of whole human blood or its components to the said hospitals without taking licence. However, this exemption is applicable to those centers which are transfusing blood and/or its components less than 2000 units per annum.

In order to ensure the safety and quality of blood and/or its components to be stored in such blood storage centres, the following conditions are applicable before getting exemption from the purview of taking of a licence from the respective State Drugs Controllers:-

(1) The First Referral Unit, Community Health Centre, Primary Health Centre and/ or any Hospital shall be approved by the State / Union Territory Licensing Authority after satisfying the conditions and facilities through inspection.

(2) The captive consumption of Whole Human blood I.P. or its components in the First Referral Unit, Community Health Centre, Primary Health Centre and / or any Hospital shall not be more than 2000 units annually.

(3) The Whole Human Blood and /or its components shall be procured only from Government Blood Banks and/or Indian Red Cross Society Blood Bank and/ or Regional Blood Transfusion Centres duly licensed.

(4) The approval shall be valid for a period of two years from the date of issue unless sooner suspended or cancelled and First Referral Unit, Community Health Centre, Primary Health Centre or the Hospital shall apply for renewal to the State Licensing Authority three months prior to the date of expiry of the approval.

(5) The First Referral Unit, Community Health Centre, Primary Health Centre and / or any Hospital shall have the following technical staff for storage of blood or its components:-

(a) A trained Medical Officer for proper procurement, storage and cross matching of blood and / or its components. He/she shall also be responsible for identifying haemolysed blood and ensure non-supply of date expired blood or its components.

(b) A blood bank Technician with the qualification and experience as specified in Part XII B of Schedule F or an experienced laboratory technician trained in blood grouping and cross matching.

(2) The First Referral Unit. Community Health Centre, Primary Health Centre and Hospital shall have an area of 10 Sq. Metres. It shall be well lighted, clean and preferably air-. conditioned. Blood bank refrigerator of appropriate capacity fitted with alarm device and temperature indicator with regular temperature monitoring shall be provided to store blood

units between 2°C to 8°C and if the components are proposed to be stored, specified equipments as specified in Part XII B of Schedule F shall also be provided.

(3) The First Referral Unit, Community Health Centre, Primary Health Centre and Hospital shall maintain records and registers including details of procurements of Whole Human Blood I.P. and/or blood components, as required under Part XII B of Schedule F.

(4) The First Referral Unit, Community Health Centre, Primary Health Centre and Hospital shall store samples of donors blood as well as patients sera for a period of seven days after transfusion."

Requirements for exempting the First Referral Unit, Community Health Center, Primary Health Center or any hospital as Storage Center of blood and or its components:

1. The applicant shall be First Referral Unit, Community Health Centre, Primary Health Centre or any Hospital.
2. The applicant shall furnish an undertaking to the licensing authority that the captive consumption of Whole Human Blood or Components shall not be more than 2000 units annually.
3. The applicant shall enclose list of equipment needed for storage viz blood bank refrigerator with alarm system & temperature indicator. A separate list of equipments for blood components would be enclosed if proposed to be stored.
4. The applicant shall furnish the following:
   a Name of the medical officer responsible for conducting operation of blood storage center.
   b Arrested certified copies of MBBS or MD qualification
   c Name, certified copies of qualification and experience of the blood bank technician.
   d Name. attested certified copies of qualification and experience of the blood bank technicians, having no DMT qualification.
5. The applicant shall furnish the source of procurement of Whole Human Blood/Blood Components namely the name and address of the Blood Banks.

a. The source of procurement of blood/ components shall be from licensed Blood Banks run by Govt. Hospitals/ Indian Red Cross Society / Regional Blood Transfusion Centres only.

b A letter of consent from the above Blood Banks who intend to supply Whole Human Blood/ Blood Components to the Blood Storage Centres shall be furnished along with the application.

c The applicant shall submit the plan of the premises. A minimum area of 10 sq. meter is essential for the Blood Storage Centre.

d In order to satisfy the conditions and facilities, an inspection of the proposed Blood Storage Centre may be carried out by the respective State Drug Control Department.

e The Inspection team shall also inspect the Blood Banks who have given consent letters for surely of Whole Human Blood/Components. The inspection team may verify whether the Blood Banks have sufficient quantity of blood units to be supplied to the Blood Storage Centres and also verify the mode of transport or containers used for supply of blood units /

components to ensure that the proper storage conditions is maintained as per the pharmacopeia. The Blood Bank shall label the blood units/ components as per the Drugs & Cosmetics Rules, 1945.

9. The Blood Banks who intend to supply the blood units / components shall test the following mandatory tests before supplying to Blood Storage Centres.,

a Blood grouping
b Antibody testing
c Haemoglobin contents
d HIV I & II antibodies
e Hepatitis B Surface antigen
f Hepatitis C antibody
g Malarial parasite
h Syphillis orVDRL

The label of the tested blood unit shall contain the above particulars with date of testing before supplying to Blood Storage Centres.

The Blood Bank shall maintain a separate register for supply of blood units/ components to Blood Storage Centres with all necessary details.

10. The validity of approval shall be for a period of 2 years from the date of issue of the approval.
11. The State Licensing Authority shall forward the approved Blood Storage Centres to the concerned Zonal Officer immediately.
12. A format of the approval proforma is enclosed. (Appendix VII).

APPENDIX I

"Form27-C

(See rule 122-F)

Application for Grant/Renewal * of Licence for the operation of a blood bank for processing of whole blood and/or* preparation of blood components,

1. I/We _____ of M/s_____hereby apply for the grant of licence / renewal of licence number_____dated_____ to operate a Blood Bank, for processing of whole blood and/or* for preparation of its components on the premises situated at_____

2. Name(s) of the item(s):
   1.
   2.
   3.

3. The name(s), qualification and experience of component Technical Staff are as under:
   (a) Name(s) of Medical Officer.
   (b) Name(s) of Technical Supervisor.
   (c) Name(s) of Registered Nurse.
   (d) Name(s)of Blood Bank Technician.

4. The premises and plant are ready for inspection will be ready for inspection on _____

5. A licence fee of rupees _____ and an inspection fee of rupees _____has been credited to the Government under the Head of Account _____(receipt enclosed).

Signature_____

Dated_____ Name and Designation_____

* delete, whichever is not applicable.

Note 1. The application shall be accompanied by a plan of the premises, list of machinery and equipment for collection, processing, storage and testing of whole blood and its components, memorandum of association / constitution of the firm, copies of certificate relating to educational qualifications and experience of the competent technical staff and documents relating to ownership or tenancy of the premises.

Note 2. A copy of the application together with the relevant enclosures shall also be sent to the Central Licence Approving Authority and to the concerned Zonal/Sub-Zonal Officers of the Central Drugs Standard Control Organization.";

APPENDIX II

"Form 27-E

(See rule 122-F)

Application for grant/renewal*of licence to manufacture blood products for sale or distribution1. I/We_____ of M/s_____ hereby apply for the grant of licence / renewal of licence number_____ dated_____ to manufacture blood products on the premises situated at_____

2. Name(s) of item(s):
   1.
   2.
   3.
   4.
3. The name(s), qualification and experience of competent Technical Staff as under:

(a) responsible for manufacturing	(b) responsible for testing
1.	1.
2.	2.
3.	3.

4. The premises and plant are ready for inspection / will be ready for inspection on_____

1. 5. A licence fee of rupees_____.and an inspection fee of rupees _____has been credited to the Government under the Head of Account _____(receipt enclosed).

Signature_____

Dated_____ Name and Designation_____

* delete, whichever is not applicable.

NOTE 1. The application shall be accompanied by a plan of the premises, list of machinery and equipment for manufacture of blood products, memorandum of association / constitution of the firm, copies of certificate relating to educational qualifications and experience of the competent technical staff and documents relating to ownership or tenancy of the said premises.

N0TE.2. A copy of the application together with the relevant enclosures shall also be sent to the Central Licence Approving Authority and to the concerned Zonal / Sub Zonal Officers of the Central Drugs Standard Control Organisation.";

"Form28-C

(See rule 122-G)

Licence to operate a blood bank for collection, storage and processing of whole human blood and/or* its components for sale or distribution

1. Number of licence_____ date of issue_____ at the premises situated at_____
2. M/s_____ is hereby licensed to collect, store, process and distribute whole blood and / or its components.
3. Name(s) of the item(s):
   1.
   2.
   3.
4. Name(s) of competent Technical Staff:

(a) responsible for Testing	(b) responsible for manufacturing
1.	1.
2.	2.
3.	3.

5. The licence authorizes licensee to manufacture, store, sell or distribute the blood products, subject to the conditions applicable to this licence.
6. The licence shall be in force from_____to_____
7. The licence shall be subject to the conditions stated below and to such other conditions as may be specified from time to time in the Rules made under the Drugs and Cosmetics Act, 1940.

5.

Signature_____

Dated_____

Name and Designation_____
Licensing Authority_____
Central Licence Approving Authority

*delete, whichever is not applicable

CONDITIONS OF LICENCE

1. The licensee shall neither collect blood from any professional donor nor paid donor nor shall he prepare blood components from the blood collected from such a donor.
2. This licence and any certificate of renewal in force shall be displayed on the approved premises and the original shall be produced at the request of an Inspector appointed under the Drugs and Cosmetics Act, 1940.
3. Any change in the technical staff shall be forthwith reported to the Licensing Authority and / or Central Licence Approving Authority.
4. The licensee shall inform the Licensing Authority and/or Central Licence Approving

Authority in writing in the event of any change in the constitution of the firm operating; under the licence. Where any change in the constitution of the firm takes place, the current licence shall be deemed to be valid for maximum period of three months from the date on which the change has taken place unless, in the meantime, a fresh licence has been taken from the Licensing Authority and/or Central Licence Approving Authority in the name of the firm with the changed constitution."

APPENDIX IV

"Form 28-E
(See rule 122-G)

Licence to manufacture and store blood products for sale or distribution

1. Number of licence_____date of issue _____at the premises situated at _____
2. M/s_____ is hereby licensed to manufacture, store, sell or distribute the following blood products :-
3. Name(s) of the item(s):
   1. 2.
   3. 4.
   5.

4. Name(s) of competent Technical Staff:

(a) responsible for testing (b responsible for manufacturing
   1. 1.
   2. 2.
   3. 3.

5. The licence authorizes licensee to manufacture, store, sell or distribute the blood products, subject to the conditions applicable to this licence.
6. The licence shall be in force from_____to_____.
7. The licence shall be subject to the conditions stated below and to such other conditions as may be specified from time to time in the Rules made under the Drugs and Cosmetics Act, 1940.

Signature_____
Dated_____ Name and Designation_____
Licensing Authority_____
Central Licence Approving Authority

*delete, whichever is not applicable

CONDITIONS OF LICENCE

1. The licensee shall not manufacture blood products from any professional donor or paid donor.
2. This licence and any certificate of renewal in force shall be displayed on the approved premises and the original shall be produced at the request of an Inspector appointed under the Drugs and Cosmetics Act, 1940.
3. Any change in the technical staff shall be forthwith reported to the Licensing Authority and / or Central Licence Approving Authority.
4. The licensee shall inform the Licensing Authority and/ or Central Licence Approving Authority in writing in the event of any change in the constitution of the firm operating under the licence. Where any change in the constitution of the firm takes place, the current licence shall be deemed to be valid for maximum period of three months from the date on which the change has taken place unless, in the meantime, a fresh licence has been taken from the Licensing Authority and/or Central Licence Approving Authority in the name of the firm with the changed constitution.

APPENDIX V

"Form26-G

(See Rule 122-F)

Certificate of renewal of licence to operate a blood bank for processing of whole human blood and/or* for preparation for sale or distribution of its components

1. Certified that licence number_____ granted on_____ to M/s_____for the operation of a Blood Bank for processing of whole blood and / or for preparation of its components at the premises situated at_____is hereby renewed with effect from_____ to_____.

2. Name (s) of Items :
   1.
   2.
   3.
3. Name(s) of competent Technical Staff:
   1. 2.
   3. 4.
   5. 6.

Signature_____

Dated_____ Name and Designation_____

Licensing Authority_____

Central Licence Approving Authority

*delete, whichever is not applicable

APPENDIX VI

"Form 26-1
(See **rule** 122-1)

Certificate of renewal of licence for manufacture of **blood products**

1. Certified that licence number _____granted on _____ to M/s_____ for manufacture of blood products at the premises situated at_____ is hereby renewed with effect from _____to_____.

2. Name(s) of item(s):
   1.
   2.
   3.

3. Names of competent Technical Staff:

(a) responsible for testing	(b) responsible for manufacturing
1.	1.
2.	2.
3.	3.
4.	4.

Signature_____

Name and Designation_____

Licensing Authority

Central Licence Approving Authority

Dated:

APPENDIX VII

Certificate of approval to storage center for storage of whole human blood and*/or its components

No. _____ Date of Issue_____

M/s_____ is hereby approved to store the following items on the premises situated at _____ under the supervision of the following technical staff:

1. Names of theapproved medical officer :
2. Names of the items :
3. Name of the qualified Blood Bank Technician :
4. Name & address of the licensed Blood Bank from whom theblood units wouldbe procured. :
5. Theapproval shall be inforce from to

Signature_____

Name and Designation_____

Licensing Authority_____

Date

* *Delete whichever is not applicable.*

CONDITIONS

The blood storage center shall comply with the conditions as stipulated under item 5B of Schedule K of the Drugs and Cosmetics Rules which also includes as under:-

1. The captive conception of Whole Human Blood or its components in the above said center shall not be more than 2000 units annually.
2. In the event of any change in the technical staff shall be forthwith reported to the licensing authority.
3. In the event of anychange in the name of the licensed blood bank from whom the blood units are procured, the same shall be intimated to thelicensing authority for approval.
4. The center shall apply for renewal of the approval to the licensing authority three months prior to the date of expiry of the approval.
5. The center shall maintain records and registers including the detials of procurement of blood*/ its components
6. The center shall store samples of donors' as well as patients'sera for a period of 7 days after transfusion.

GLOSSARY

Absorption: Removal of an unwanted antibody from a serum; often used interchangeably with absorption.
Acquired antigen: Antigen not genetically determined and sometimes transient.
Adsorption: Providing an antibody with its corresponding antigen under optimal conditions so that the antibody will attach to the antigen, thereby removing the antibody from the serum; often used interchangeably with absorption.
Agglutinin (normal or **typical)**: An agglutinin which is regularly present in the serum of an individual lacking corresponding antigen on red cells.
Agglutinin {atypical): An agglutinin irregularly present in the serum of an individual lacking corresponding antigen i.g. anti-D present in Rh(D) negative person.
Agglutinin (cold): An antibody which react at cold temperature, whose potency decreases with rise in temperature and at 35°C is very week or absent.
Agglutinin (auto) :An agglutinin which reacts with the red cells of the individual in whose serum it is found; it reacts with the red cells of most other individual also.
Allele: One of two or more different genes that may occupy a specific locus on a chromosome.
Amorph: A gene that does not appear to produce a detectable antigen; a silent gene, such as *JK, Lu, O.*
Anamnestic response: An accentuated antibody response following a secondary exposure to an antigen. Antibody levels from the initial exposure are not detectable in the patient's serum until the secondary exposures, when a rapid rise in antibody liter is observed.
Antenatal: Occurring before birth.
And-A lectin: A reagent anti- A, serum produced from the seeds of the plant Dolichos biflours; reacts with A. cells but not with A subgroup cells such as A_2, A_3, and so on; reacts weakly with A_{int} cells.
Anti-B lectin: A reagent anti-B serum produced from the seeds of the plant Bandeiraea simplicifolia.
Antigenic determinant(Epitope) : The particular site on an antigen molecule which combines with the corresponding antibody.
Antihuman globulin test or Antiglobulin Test (AGT): Test to ascertain the presence or absence of red cell coating by immunoglobulin G (IgG) or complement, or both; *Direct antihuman globulin test (DAT):* Used to detect in vivo cell sensitization. *Indirect antihuman globulin test (IAT):* Used to detect antigen – antibody reactions that occur in vitro.
Antihuman serum: An antibody prepared in rabbits or other suitable animals that is directed against human immunoglobulin or complement, or both; used to perform the antihuman globulin or coombs' test. The serum may be either polyspecific (anti - IgG plus anti - complement) or monospecific (anti IgG or anti - complement).
Anti - M lectin: A reagent anti - M serum produced from the plant Iberis amara.
Anti - N lectin: A reagent anti - N serum produced from the plant Vicia graminea.
Apheresis: A method of blood collection in which whole blood is withdrawn , a desired component separated and retained, and the remainder of the blood returned to the donor. See also Plateletpheresis and Plasmapheresis.
Aplasia: Failure of an organ or tissue to develop normally.
Arachis hypogoea: A peanut lectin used to differentiate T polyagglutination from Tn polyagglutination.
Autoabsorption: A procedure to remove a patient's antibody using the patient's own cells.
Autologous control: Testing the patient's serum with his or her own cells in an effort to detect autoantibody activity
Bilirubin: The orange-yellow pigment in bile carried to the liver by the blood; produced from hemoglobin of red blood cells by reticuloendothelial cells in bone marrow, spleen, and elsewhere. *Direct biliubin:* The conjugated water soluble form of bilirubin. *Indirect bilirubin:* The unconjugated water-insoluble form of bilirubin.
Blood group-specific substances : Soluble antigens present in fluids' that can be used to neutralize their corresponding antibodies: systems that demonstrate blood group-specifiic substances include ABO, Lewis, and P blood group systems.

Blood Filters: (1) Standard blood filters (first-generation) - have screen filters with a pore size of 170 – 200 microns. These filters are used for blood/component transfusion and have ability to screen out large derbis such as fibrin and clots. **(2) Microaggregate filters (second generation) -** screen or depth type filters that remove aggregates smaller than 170 microns. Microaggregate screen filters have pore size 20 - 40 microns and effectively remove 70 - 90 percent of the leukocytes. **(3) Leukocytes reduction filters (third generation)-** adhesion that effectively remove 99.0 to 99.9 percent of the leukocytes.

Bombay: Phenotype occurring in individuals who possess normal A or B genes but are unable to express them because they lack the gene necessary for production of H antigen, the required precursor for A and B. These persons often have potent anti -H in their serum, which reacts with all cells except other Bombays. Also known

Bromelin: A proteolytic enzyme obtained from the pineapple.
Buffy coat: Light stratum of a blood seen when the blood is centrifuged or allowed to stand in a test tube. The red blood cells settle to the bottom, and between the plasma and the red blood cells is a light-colored layer that contains mostly white blood cells and platelets.
Chimera: Genetic Chimera: (1) An individual who possesses a mixed cell population genetically derived from the. distinct zygotic linkages e.g. the vascular anastomosis between twins during embryonic life. (2) Dispermic chimerism: fertilization of one egg with two sperms may also cause chimera.
Chimera Artificial: Blood transfusion, bone marrow transplant, and fetal/maternal bleed may result in artificial chimera.
Chloroquine diphosphate: Substance that dissociates IgG antibody from red cells with little or no damage to the red cell membrane.
Chromosome: The structures within a nucleus that contain a linear thread of DNA, which transmits genetic information. Genes are arranged along the strand of DAN and constitute portions of the DNA.
Colony-forming unit committed to erythropoiesis (CFU-E): A progenitor cell that is committed to forming cells of the red blood cell series.
Colony-forming unit-culture (CFU-C); Generation of stem cells using tissue culture methods. Current synonym is CFU-GM. which is a colony - forming unit committed to the production of myeloid cells (granulocytes and monocytes).
Cord cells: Fetal cells obtained from the umbilical cord at birth; may be contaminated with Wharton's jelly. Coumarin (Coumadin): A commonly employed anticoagulant that acts as a vitamin K antagonist that prolongs the prothrombin time.
Cryoprecipitate: A concentrated source of coagulation factor VIII prepared from a single unit of donor blood; it also contains fibrinogen, factor XIII, and von Willebrand factor.
Cryopreservation: Preservation by freezing at very low temperatures.
Cytopheresis: A procedure performed using a machine by which one can selectively remove a particular cell rype normally found in peripheral blood of a patient or donor.
23 -Diphosphoglycerate (2,3-DPG): An organic phosphate in red blood cells that alters the affinity of hemoglobin for oxygen. Blood cells stored in a blood bank lose 2,9-DPG. But once infused, the substance is re-synthesized or reactivated.
Disseminated intravascular coagulation (DIC); Clinical condition of altered blood coagulation secondary to a variety of diseases.
Dithiothreitol (DTT): A sulfhydryl compound used to disrupt the disulfide bonds of immunoglobulin IgM, yielding monomeric units rather than the typical pentameric molecule.
Dominant: A trait or characteristic that will be expressed in the offspring even though it is only carried on one of the homologous chromosomes.
Eletrophoresis: The movement of charged particles through a medium (paper, agar gel) in the presence of an electrical field: useful in the separation and analysis of proteins.
Elution: A process whereby cells that are coated with antibody are treated in such a manner as to disrupt the bonds between the antigen and antibody. The freed antibody is collected in an inert diluent such as saline or 6

percent albumin. This antibody serum then can be tested to identify its specificity using routine methods. The mechanism to free the antibody may be physical (heating, shaking) or chemical (ether, acid), and the harvested antibody containing fluid is called an eluate.

Enzyme treatment: A procedure in which red blood cells are incubated with an enzyme solution that cleaves some of the membrane glycoproteins, then washed free of the enzyme, and used in serologic testing. Enzyme treatment cleaves some antigens and exposes other.

Epitope: The portion of the antigen molecule that is directly involved in the interaction with the antibody; the antigenic determinant.

Erythroblast: Any form of nucleated red corpuscles, containing hemoglobin, which are not normally seen in the circulating blood.

Ethylenediaminetetraacetic acid (EDTA): An anticoagulant useful in hematologic testing and preferable when direct antihuman globulin testing is indicated.

Febrile reaction: A transfusion reaction caused by leukoagglutinins that is characterized by fever; usually observed in multiply transfused of multiparous patients.

Fibrinogen: A protein produced in the liver that circulates in plasma. In the presence of thrombin, an enzyme produced by the activation of the clotting mechanism, fibrinogen is cleaved into fibrin, which is an insoluble protein that is responsible for clot formation.

Fibrinolysin: The substance that has the ability to dissolve fibrin; also called plasmin.

Fresh frozen plasma (FFP): A frozen plasma product (from a single donor) that contains all clotting factors, especially the labile factors V and VIII; useful for clotting factor deficiencies other than hemophilia A, von **Willebrand*s** diseases, and hypofibrinogenemia.

G6PD (glucose -6-phosphate dehydrogenase): A liver enzyme used to monitor liver function.

Gel test: A blood group serology test method that uses a microtube containing gel (incorporating antisera or antiglobulin sera) that acts as a reaction vessel for agglutination.

Gene: A unit of inheritance within a chromosome.

Genotype: An individual's actual genetic makeup.

Goodpasture syndrome: A disease entity that represents a rapidly progressive glomerulonephritis associated with pulmonary lesions. Usually the patients possess an antibody to the basement membrane of the renal glomeruli.

Graft - versus -host (GVH) disease: A disorder resulting from engraftment of donor tissue into an immuno-compromised host..

Hemolysin: An antibody that activates complement leading to cells lysis.

Hemolysis: Disruption of the red cell membrane and the subsequent release of hemoglobin into the suspending medium or plasma.

Hemolytic disease of the newborn (HDN): A disease characterized by anemia, jaundice, enlargement of the liver and spleen, and generalized edema (hydrops fetalis) that is caused by maternal IgG antibodies crossing the placenta and attacking fetal red cells when there is a fetomatenal blood group incompatibility (usually ABO or Rh antibodies). Synonym is erythroblastosis fetalis.

Hemolytic transfusion reaction (HTR): A reaction from red cell destruction caused by patient's antibody (ies) directed to donor red cell antigen(s).

Hemophilia A: A hereditary disorder characterized by greatly prolonged coagulation time. The blood fails to clot and bleeding occurs; caused by inheritance of a factor VIII deficiency, it occurs almost exclusively in males.

Hemophilia B: "Christmas disease", which is hemophilia like disease caused by a lack of factor IX.

Hepatitis B Immunoglobulin (HBIg): An immune serum given to individuals exposed to the hepatitis B virus.

Heterozygous : Possessing different alleles at a given locus.

HLA: Human leukocyte antigen.

Homozygote: An individual developing from gametes with similar alleles and thus possessing like pairs of genes for a given hereditary characteristic.

Hybridoma: A hybrid (cross) between a plasmacytoma cell and a spleen (o antibody - producing) cell that produces a monoclonal antibody, resulting in a malignant cell line thai can grow indefinitely in culture and can

produce high quantities of antibody. This antibody is monoclonal because only one antibody-producing cell combined with the plasmacytoma cell is present.

Hydroxyethyl starch (HES): A red cell sedimenting agent used to facilitate leukocyte withdrawal during sukapheresis.

Hypogammaglobulinemia: Decreased levels of gamma globulins seen in some disease states.

Hypovolemia: Diminished blood volume.

Hypoxia: Deficiency of oxygen.

Icterus: A condition characterized by yellowish skin, whites of the eyes, mucous membranes, and body fluids caused by increased circulating bilirubin resulting from excessive hemolysis or from liver damage due to hepatitis. <w> is jaundice.

Idiopathic: Pertaining to conditions without clear pathogenesis, or disease without recognizable cause, a contaneous origin.

Idiopathic thrombocytopenic purpura (ITP): Bleeding owing to a decreased number of platelets; the etiology is unknown, with most evidence pointing to platelet auto-antibodies.

Immune response: The reactions of the body to substances that are foreign or are interpreted as being foreign. fell-mediated or cellular immunity pertains to tissue destruction mediated by T cells, such as graft rejection and persensitivity reactions. Humoral immunity pertains to cell destruction response during the early period of the action.

Immunodediciency: A decrease from the normal concentration of immunoglobulins in serum.

Incubation: In vitro combination of antigen and antibody under certain conditions of time and temperature to allow antigen - antibody complexes to occur.

Rernicterus: A form of icterus neonatourm occurring in infants, developing at 2 to 8 days of life; prognosis poor untreated.

Leukemia: Malignant proliferation of leukocyte, which spill into the blood, yielding an elevated leuckocyte <w>.

Leukoagglutinins: Antibodies to white blood ceils.

Low ionic strength solution (LISS): A type of potentiating medium in use for serologic testing. Reducing the strength of the red cell suspending medium increases the affinity of the antigen for its corresponding antibody such that sensitivity can be increased and incubation time decreased.

Lymphocyte: A type of white blood cell involved in the immune response. Lymphocytes are normally total 20 to -■ percent of total white cells. T lymphocytes mature during passage through the thymus or after interaction with :nic hormones; these cells function both in cellular and humoral immunity. Subset includes helper T cells (Th), :ch enhance B-cell antibody production, and suppressor T cells (Ts), which inhibit B-cell antibody production. lymphocyte cells are not processed by the thymus. Through morphologic and functional differentiation, they future into plasma cells that secrete immunoglobulin.

Macrophage: End-stage development for the blood monocyte; these cells can ingest (phagocytose) a variety of substances for subsequent digestion or storage and are located in a number of sites in the body (e.g. spleen, liver, -~g) existing as free mobile cells or as fixed cells. Functions include elimination of senescent blood cells and anticipation in the immune response.

Mercaptoethanol (2-ME): A sulfhydryl compound used to disrupt the disulfide bonds of immunoglobulin -’ I. yielding monomric units rather than the typical pentameric units.

Mixed field: A type of agglutination pattern in which numerous small clumps of cells exist amid a sea of free

Monoclonal: Antibody derived from a single ancestral antibody- producing parent cells.

Multiple myeloma: A neoplastic proliferation of plasma cells, which is characterized by very high immunoglobulin - -els of monoclonal origin.

Muraminidase: An enzyme that cleaves sialic acid from the red cell membrane.

Muturalization: Inactivating an antibody by react ins it with an antigen (blood group substance) against which is -;al.

Neutrophil: A leukocyte that ingests bacteria and small particles and plays a role in combating infection.
Oliguria: Diminished amount of urine formation.
Panagglutinin: An antibody capable of agglutinating all red blood cells tested, including the patient's own cells.
Fancytopenia: A reduction in all cellular elements of the blood, including red cells, white cells, and platelets.
Papain: A proteolytic enzyme derived from papaya.
Paroxysmal nocturnal hemoglobinuria (PNH): An intrinsic defect in the red .blood cells membrane rendering it more susceptible to hemolysins in an acid environment; characterized by hemoglobin in the urine following periods of sleeps.
Phenotype: The outward expression of genes (e.g. a blood type). On red blood cells, serologically demonstrable antigens constitute the phenotype.
Phlebotomy: The procedure used to draw blood from a person.
Phototherapy: Exposure to sunlight or artificial light for therapeutic purpose.
Plasma: The liquid portion of whole blood containing water, electrolytes, glucose, fats, proteins, and gases Plasma contains all the clotting factors necessary for coagulation, but in an inactive form. Once coagulation occurs, the fluid is converted to serum.
Plasmapheresis: A procedure using a machine to remove only plasma from a donor or patient.
Platelet: A round or oval disc, 2 to 4 μm in diameter, that is derived from the cytoplasm of the megakaryocyte, a large cell in the bone morrow. Platelets play an important role in blood coagulation, hemostasis, and blood thrombus formation. When a small vessel is injured, platelets adhere to each other and to the edges of the injury, forming a "plug" that covers the area and stops the bleeding.
Plateletpheresis: A procedure using a machine to remove only platelets from a donor or patient.
Platelet refractoriness: Failure to yield an increase in recipient's platelet count on transfusion of suitably preserved platelets. HLA alloimmunization is a common cause.
Polyagglutination: A state in which an individual's red cells are agglutinated by all sera regardless of blood type.
Polyagglutinins: Naturally occurring immunoglobulin antibodies that are found in most normal human adult sera.
Polycythemia vera: A chronic life shortening myeloproliferative disorder involving all bone marrow elements, characterized by an increase in red blood cell mass and hemoglobin concentration.
Polymerase chain reaction (PCR): An in vitro method of amplification of a specific DNA segment.
Polyspecific Cooombs' sera: A reagent that contains antihuman globulin sera against immunoglobulin IgG and C3d & C3b.
Prothrombin complex: A concentrate of coagulation factors II, VII, IX, and X in lyophilized form.
Quality assurance (QA): A set of planned actions to provide confidence that systems and elements that influence the quality of the product or service are working as expected individually and collectively.
Radioimmunoassay (RIA): A very sensitive method for determination of substance present in low concentrations in serum or plasma buy using specific antibodies and radioactively labeled or tagged substances.
Recessive: A type of gene that, in the presence of its dominatnt allele, does not express itself; expression occurs when it is inherited in the homozygous state.
Reticuloendothelial system (RES): The fixed phagocytic cells of the body , such a the macrophage, having the ability to ingest particulate matter.
RH immunoglobulin (RHIg): A concentrated, purified anti-Rh (D) prepared from human serum (of immunized donors) which is given to Rh(D)-negative mothers after they have given birth to a RH(D)-positive baby or after abortion or miscarriage. It acts to prevent the mother from becoming immunized to Rh(D)-positive fetal cells that may have entered her circulation and thereby prevents formation of anti-Rh (D) by the mother.
Rouleaux: Coin like stacking of red blood cells in the presence of plasma expanders or abnormal plasma proteins.
Saline anti-D: A synonym for low protein anti-Rh(D) reagents.

Secretor: An individual who is capable or secreting soluble glycoprotein ABH- soluble substances into saliva and other body fluids.
Shelf life: The amount of time blood or blood products may be stored upon collection.
Sialic acid: A group of sugars found on the red cell membrane attached to a protein backbone; the major source of the membrane's net negative charge.
Sickle trait: Blood that is heterozygous for the gene-coding for the abnormal hemoglobin of sickle cell anemia.
Single - donor platelets; Platelets collected from a single donor by apheresis.
Specificity: The affinity of an antibody and the antigen against which it is directed.
Stem **cell:** An unspecialized cell, capable of self-renewal, that gives rise to a group of differential cells such as the hematopoietic cells.
Stroma: The red cell mmbrane that is left after hemolysis has occurred.
Thalassemia Major: The homozygous form of deficient beta-chain synthesis, which is very severe and present itself during childhood. Prognosis varies; however, the younger the child at diseases onset., the less favorable the outcome.
Thrombin: An enzyme that converts fibrinogen to fibrin so that a soluble clot can be formed.
Thrombocytopenia: A reduction in the platelet count below the normal level, which is associated with spontaneous Hemorrhage.
Thrombotic thrombocytopenic purpura (TTP): A coagulation disorder characterized by (1) increased bleeding owing **to** a decreased number of platelets, (2) hemolytic anemia, (3) renal failure, and (4) changing neurologic signs. **The** characteristic morphologic lesion is thrombotic occlusion of small arteries or capillaries in various organs.
Vasovagal syncope: Syncope resulting from hypotension caused by emotional stress, pain, acute blood loss, rear, or rapid rising from a recumbent position.
Venipuncture: Puncture of a vein for any purpose.
Viability: Ability of a cell to live or to survive for a reasonably normal lifespan.
von Willebrand factor : Coagulation factor VIII.
von **Willebrand**'s **disease:** A congenital bleeding disorder.
WAIHA: Warm autoimmune hemolytic anemia. A hermolytic anemia caused by the patient's antuantibody that reacts at 37°C
Wharton;s Jelly: A gelatinous intercellular substance consisting of primitive connective tissue of the umbilical cord.
Window period: that period between infection and the ability to detect disease through laboratory testing.
Zeta potential: The difference in charge density between the inner and outer layers of the ionic cloud that surrounds red cells in an electrolyte solution.
ZZAP: A mixture of cysteine-activated papain and di-thiothreitol. Red cells treated with ZZAP have reduced IgG and are enzyme pretreated

REFERENCES

Akerblom, O., Hogman, C.F. Frozen Blood, A method of low glycerol, liquid nitrogen allowing different postthaw deglcerolization procedure, Transfusion, volume 14 number 3,1974,

Archer, Gordon and Parker Grace, A Guide to blood transgusion, 4th Edn., 1982, Australian Red Cross Society (NSW Division)

Bensinger WI, Weaver CH, Appelbaum FR, et al. Transplantation of allogeneic peripheral blood stem cells mobilized by recombinant human granulocyte colony-stimulating factor. Blood 1995:85:1655-1658.

Bensinger WI, Rowley S, Appelbaum FR, et al. CD34 selected allogeneic perioheral blood stem cell (PBSC) transplantation in older patients with advanced hematologic malignancies. (Blood 86:97a, 1995 (Abstr.).

Bensinger WI, Singer J, Applebaum FR,et al. Autologous transplantation with peripheral blood mononuclear cells collected after administration of recombinant granulocyte stimulating factor. Blood 1993:81:3158-3163.

Bhatia, H.M., Procedurec in Blood Banking and Immunohematology, 1977.

Bhende, Y.M., Despande, C.K., Bhatia, H.M., Sanger, Ruth, Race, .R.R. et al. (1952) A new blood group character related to the ABO system. Lancent, 1,903.

Boorman, K.E., Dodd, B.E., Uncon, P.J. Blood Group Serology, 6th Edn., 1988. Churchill Livingstone.

Branko Brozovic and Milica Brozovic, Manual of Clinical blood Transfusion, Churchill Livingstone, U.K. 1986.

Bowman, J.M., Chown, B., Lews, Marion and Pollack, Janet (1978) Rh-immunization during pregnancy, antenatal prophylaxis, Canad, Med. Assoc.J. 118,623.

Branch, D.R. and Petz, L.D. (1982) A new reagent (ZZAP) having multiple applications in immunohematology, Amer. J. Cin, Path. 78,161-167.

Bruce M, O' Neill CM, Bamardo MC, Krausa P, et al. Phototyping: comprehensive DNA typing for HLA-A, B, C, DRB1, DRB9, DRB4, DRB5 & DQB1 by PCR with 144 primer mixes utilizing sequence-specific promers (PCR-SSP). Tissue Antigens, 1995: 46: 355-67.

Dacie, Sir John V, Lewis, S.M., Practicle Hematiology, 6th Edn., 1984, Churchill Livingstone.

DiPersio J, Martin B, Abboud C, et al.: Allogeneic BMT using bone marrow and CD34- seleted mobilized PBSC: comparison to BM alone and mobilized PBSC alone. Blood 84 (10): 91a, 1994 (abstr.).

Dodd RY, The risk of transusion-transmitted infection. N. Engl J. Med. 1992:327:419-2.

Edwards, J.M., Moulds, J.J. and Judd. W.J. (1982) Chloroquine dissociation of antigen-antibody complex. A new technique for typing red blood cells with a positive direct antiglobulin test, Transfusion. 22,59-61.

Gianni AM, Siena S, Bregni M, et al. Granulocyte-macrophase colony-stimulating factor to harvest circulating hematopoietic stem cells for autotransplantation. Lancent 1989:2:580-585.

Heaton, A., Miripol, J., Aster, R. et al. (1984) Use of Adsol preservative solution for prolonged storage of low viscosity As-1 red blood cells, Brit. Heamto. 57,467-468.

Hogman, C.F., Akerblom, O., Hedlund, K., et al. (1983) Red cells suspension in SAGM medium, Vox Sang. 45, 217-223.

Huestis, D.W., Bove, J.R. and Busch. S. (1981) Haemapheresis in Practicle Blood Transfusion, 3rd Edn. Little Brown Boston.

Issit, P.D. (1985) Applied Blood Serology, 3rd Edn. Montogomercy Scientific publication. Miame, Floridia.

Issaragrisil S, Visuthisakchai S, Suvaue V, et al. Brief reprt: Transplantation of cord blood stem cells into a patient with severe thalassemia. New Eng J Med 332:367, 1995.

Klein J: Blood cells and their origin: Immunology. Boston, Blackwell Scientific, 1990.

Kessinger A, Armitage JO, Landmark JD et al. Autologous peripheral hematopoietic stem cell transplantation restores hematopoietic function following marrow ablative therapy. Blood 71:723-72"'. 1988.

Lane, T.A., Anderson, K.C., Goodnough, L.T. et al., Leukocyte reduction in blood component therapy. Ann Intern Md. 199; 117:151-62.

Levy, J.A., Human Immunodefigiency Viruses and the Pathogenesis of AIDS, JAMA: 1989: 261: 2907-3006.

Low, B. (1995) A practical method using papain and incomplete Rh-antbodies in routine Rh blood-grouping. Vox Sang. (O.S.), 5, 94.

Low, B. and Messeter, L. (1974) Antiglobulin test in low-ionic strength salt solution for rapid antibody screening and cross-match ing Vox. Sang. 26, 53.

Mollison PL., Engelfriet, C.P., Contreras Marcela, Blood Transfusion in Clinical Medicine, 9th Edn., 1993.

Mehra NK, Verduijin W, Taneja V, Drabbles J, Singh SP, Giphart MJ. Analysis of HLA-DR2-associated polymorphism by oligonucleotide hybridization in an Asian Indian population. Hum Immunol. 1991; 32:246-53.

Mehra NK, Bouwens AG, Naipal A, Rajalingam R, Grubic Z, Taneja V, et al. Asian Indian HLA-DR2-, DH4-, and DR52-related DR-DQ genotypes analysed by polymerase chain reaction based nonradioactive oligo nucleotide typing. Unique haplotypes and a novel DR4 subtype. Hum Immunol. 1994;39; 202-10.

Mehra NK, Jaini R, Rajalingam R, Balamurugan A, Kaur G. Molecular diversity of HLA-A*02 in Asian Indians: predominance of A*0211. Tissue Antigens. 2001:57:502-7.

Meryman, H.T., and Homblower, M. (1972) A method of freezing and washing red blood cells using of high glycerol concentration, Transfusion, 12,145.

Moore, H.C and Mollison, P.L. (1976) Use of low-ionic strength medium in manual tests for antibody detection. Transfusion, 15,266.

Mourant, A.E. (1949) Rh phenotypes and Fisher's motation. Nature (lond), 163, 913.

Rajalingam R, Mehra NK, Jain RC, Myneedu VP, Pandey JN. Polymerase chain reaction-based sequence-specific oligonucleotide hybridization analysis of HLA class II antigens in pulmonary tuberculosis: relevance to chemotherapy and disease severity. J Infect Dis. 1996: 173;669-76.

Petz, Lawrence, D. and Swisher, Scott, N. Clinical Practice of Transfusion Medicine, Sec. Edn. Chuchill Livingstone, 1989.

Opelz G. Analysis of the 'NIMA effect' in renal transplantation. Collaborative Transplant Study. Clin Transpl. 1990;63-7.

Race, R.R. and Sanger, Ruth (1975) Blood Groups in Man, 6th Edn., Blankwell Scientific Publication, Oxford.

Robinson J., Malik A, Partha P, Bodmer JG, Marsh SG. IMGT/HLA database-a sequence database for the human major histocompatibility complex. Tissue Antigens. 2000:55:280-7.

Rowley SD, Bensinger WI, Gooley TA, et al. The effect of cell concentration of bone marrow and peripheral blood stem cell cryopreservation. Blood 1994;83:2731-2736.

Saran, R.K. and Makroo, R.N., Transfusion Medicine, Technical Manual, Directorate General of Health Services, Ministry of Health and Family Welfare, Govt. of India (1991).

Sarin, S.K. and Hess, G., Transfusion Associated Hepatitis. (1998).

Simon, E.R. (1977) Adenine in Blood Banking, Transfusion, Vol. 17, number 4.

Standards of Blood Banks and Transfusion Services, Directorate General of Health.

Services, Ministry of Health Services & Family Welfare. Govt. of India (1987) Standards for Blood Bank and Transfusion services, 20lh Edn. Ammerican Association of Blood Banks, USA (2000).

Stratus, R.G. Transfusion therapy in neonates. Am. J. Dis Child 1991; 145: 904-11. Technical Manual, 13th Edn. American Association of Blood Banks, USA (2000).

The Technical Manual of the Canadian Red Cross, Blood Transfusion, B.P.L. Moore et al. 1972.

Terasaki PI and McClelland JD. Microdroplet assay of human serum cytotoxins. Nature 1964: 204: 998-1000.

Valeri, C.R. (1975) Simplification of methods for adding and removing glcerol during freeze-preservation of human red blood cells with the high or glycerol methods: biochemical mdification prior to freezing. Transfusion, 15,195.

WHO (1977) Twenty-eight Report of WHO Expert Committee on Biological Standardization. Technical Report Series, 610.

Wick, M.R., Moore, S. and Taswell, H.F. (1985) Non-a, Non-B Hepatitis associated with blood transfusion, Transfusion, 25,93-101.

Wiener, A.S. (1951) The Rh-Hr blood types; serology, genetics and nomenclature, Trans. N.Y. Acad. Sci, 13, 198.

Weiner, A.S., Unger, L.J., Cohen, L. and Feldman, J. (1956) Type-specific cold auto-antibodies as a cause of acquired hemolytic anemia and hemolytic transfusion rections: biologic test with bovine red cells. Amn. Intern. Med. 44, 221.

Wright, G. and Sanderon, J.M. (1974) A.C.D. and C.P.D. blood preservation, Lancet ii, 173.

ERRATA

Page No.	**Written as**	**Read as**
40	cytokins	cytokines
41	Interferon-y	Interferon-y
46	Fb	Fab
140	HBsAg	HBV
140	P.vivus	P.vivax
153	alkaline phosphate	alkaline phosphatase
167	50-60 mm	50-60 nm
244	hemolytic urinary syndrome	hemolytic uremic syndrome
246	Paroxysmal nocturnal anemia	Paroxysmal nocturnal hemoglobinuria