Automation in Immunohematology

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Introduction

- In recent times, there have been rapid technological advances in the field of Transfusion Medicine with an increasing emphasis on quality and safety of blood components
- The conventional pre-transfusion testing techniques in immunohematology are quite cumbersome and not amenable to automation
- The introduction of newer techniques such as
  - Column Agglutination Technique (CAT)
  - Solid Phase Red Cell Adherence Assay (SPRCA)
  - Erythrocyte Magnetic Technique (EMT)
- have tried to overcome these short comings of conventional techniques and bring about an improvement in the quality of testing and the reproducibility of results

Past, Present and Future…

Advantages of Automation in immunohematology

- Reducing human errors while performing tests and subjective variations during interpretation of results
- Preventing transcription errors during documentation of results
- Improving objectivity, reproducibility, and storage and retrieval of results
- Improving traceability of all variables during testing including, samples, reagents and operating staff
- It reduces manual input and therefore results in manpower economy
- High throughput devices with lesser turnaround time improve the quality of services in large tertiary care settings

Different Automated Immunohematology platforms in India

1. Bio-Rad (Switzerland) – IH-500/IH-1000
2. DIAGAST (France) – QWALYS / ONYX
3. Ortho-Clinical Diagnostics (Johnson & Johnson, USA) – Vision / Vision max/ Autovue Innova
4. IMMUCOR (USA) - NEO Iris/ Echo Lumena
5. GRIFOLS (Singapore) – WADIANA / ERYTRA

Conventional Tube Testing (CTT)

- Conventional Tube Testing (CTT) is still considered as the Gold Standard technique

- It has following unique advantages-
  - Amenable to all modifications of RBC and serum during testing
  - ABO–Rh typing may be done in the shortest possible time

Conventional Tube Testing (CTT): Disadvantages

- Large sample volume required
- Procedure in cumbersome and time consuming
- The results are variable and may depend on the expertise of the personal performing the test
- Sensitivity for clinically significant antibodies is less than other techniques

Advantages and disadvantages summary of CTT

Test methods	Advantages	Disadvantages
Saline-CTT	• No additives • Reduced cost • Avoids reactivity with auto Abs • Ability to assess multiple phases of reactivity	• Long incubation • Least sensitive • Requires highly trained staff • Most procedural steps • Fewer method-dependent Abs detected
LISS-CTT	• Reduced cost • Avoids reactivity with auto Abs • Shortest incubation time • Increased Ab uptake • Most common tube method • Ability to assess multiple phases of reactivity	• Inability to be automated • Requires highly trained staff • Many procedural steps • Fewer method-dependent Abs detected
PEG-CTT	• Reduced cost • Decreased incubation time • Increased Ab uptake • Enhances most Abs • Ability to assess multiple phases of reactivity (not 37°C)	• Requires highly trained staff • Many procedural steps • Detects more unwanted Abs • Inability to be automated • Fewer method-dependent Abs detected

Gel Technology

- Innovative approach to red cell serology
- Invented by Dr. Yves Lapierre of France in 1988
- Developed to minimize problems associated with conventional techniques of blood grouping
- Addresses the issues of standardization and documentation with very high sensitivity, specificity and efficiency

Principle of Gel Technology

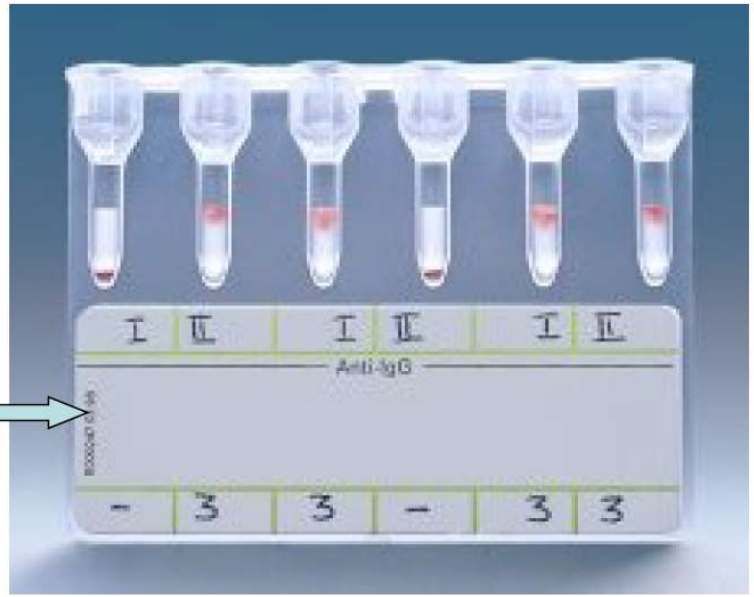
- Controlled centrifugation of RBCs through a gel column
- Serum and cell reaction takes place in a microtube
- Six microtubes in a plastic card – easy handling
- Microtube consists of a reaction chamber that narrows to become a column with a conical bottom
- Reaction chamber is designed to allow prior incubation of test serum and RBCs
- Each column contains Sephadex gel suspended in a buffer solution
- Depending on the configuration of card , the gel is premixed with antisera/AHG/other reagents

Add Reactants
Serum/plasma/
red cells

Principle
Reaction
Chamber

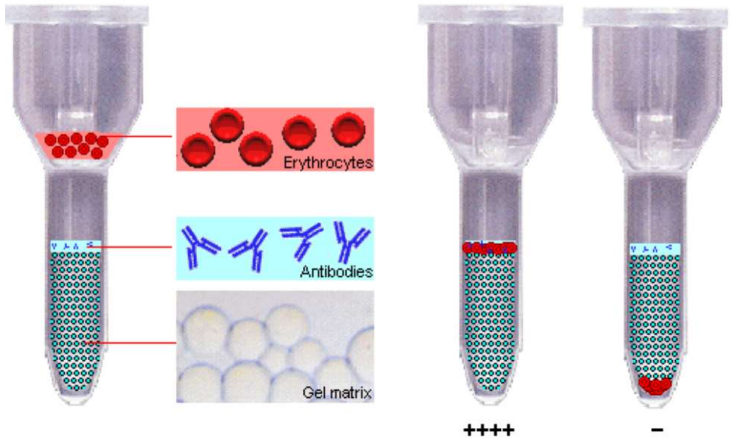
Gel and
Reagent

6 Microtubes in Plastic Card



Principle of Gel Technology

- Sephadex gel matrix acts as a sieve
  - Large agglutinates remain on or near the top of gel interface
  - Smaller agglutinates pass partway through gel , depending on size
  - Unagglutinated cells pass to base of microtube to form a button
- Cells are always first then serum
- Grading of reaction depending on the distribution of RBCs throughout the column



Uses of Gel Technology

- Any immunohaematology test that has haemagglutination as its end point:
  - ABO-Rh typing, typing for other blood group systems
  - Antibody screening and identification
  - Compatibility testing – crossmatching
  - DAT/IAT, other Coombs phase test
  - Antibody classification- IgG, IgM, IgA, complement…
  - Specialized hematological tests: PNH, Sickle cell anaemia

Advantages of Gel Technology

- Improved sensitivity and specificity
- Easy to use, simple to read
- No wash phase in IAT
- Minimal training required
- Reliable, reproducible results
- Easy storage and long shelf life of reagents
- Easy disposal of biodegradable cards
- Widest range of reagents and instrumentation
- More sensitive DAT method
- No need for check cells
- Enhanced anti-D detection
- Ability to be automated

Disadvantages of Gel Technology

- Following equipments are required:
  1. Special centrifuge to accommodate the microtube cards
  2. Special incubators to incubate the microtube cards
  3. Pipette to dispense 25 µl of serum
  4. Pipette to dispense 50 µl red cell suspension
- It is expensive
- Warm auto Abs enhanced
- Mixed-cell agglutination with cold Abs
- Increased chances of detected unwanted Abs
- Preparation of 0.8% suspension is cumbersome
- Rouleaux and fibrin clots may be reported as positive
- Need to maintain backup method

Glass Bead Technology

- Described in 1993 by Reis K.J.

Principle:

- Test is performed in a microcolumn prefilled with glass microbeads in suspension of antihuman globulin serum, any diagnostic reagent or neutral isotonic solution
- Detection of sensitized red cells is based on the sieving effect of glass microbeads
- Red cells & serum are incubated at the upper part of a column over the glass microbeads suspension
- These microbeads are calibrated & during centrifugation, they retain the agglutinates & the unsensitized cells sediment at the bottom

Advantages of Glass Bead Technology

- Minimum incubation time of 10 minutes for antibody screening or cross matching
- Biphasic centrifugation time is only 5 min
- In AHG test there is no need to wash cells
- No tube shaking or re-suspension of cell button leading to variation in reading & grading the agglutination
- Provision of centrifuge calibrated to spin at optimal speed for fixed & correct time reduce error during this phase
- More objective, consistent & reproducible interpretation of results

Disadvantages of Glass Bead Technology

- Special centrifuge to accommodate glass beads cassettes
- Special incubators to incubate the glass beads cassettes
- Pipettes to dispense 10 µl, 40 µl, 50 µl
- Expensive

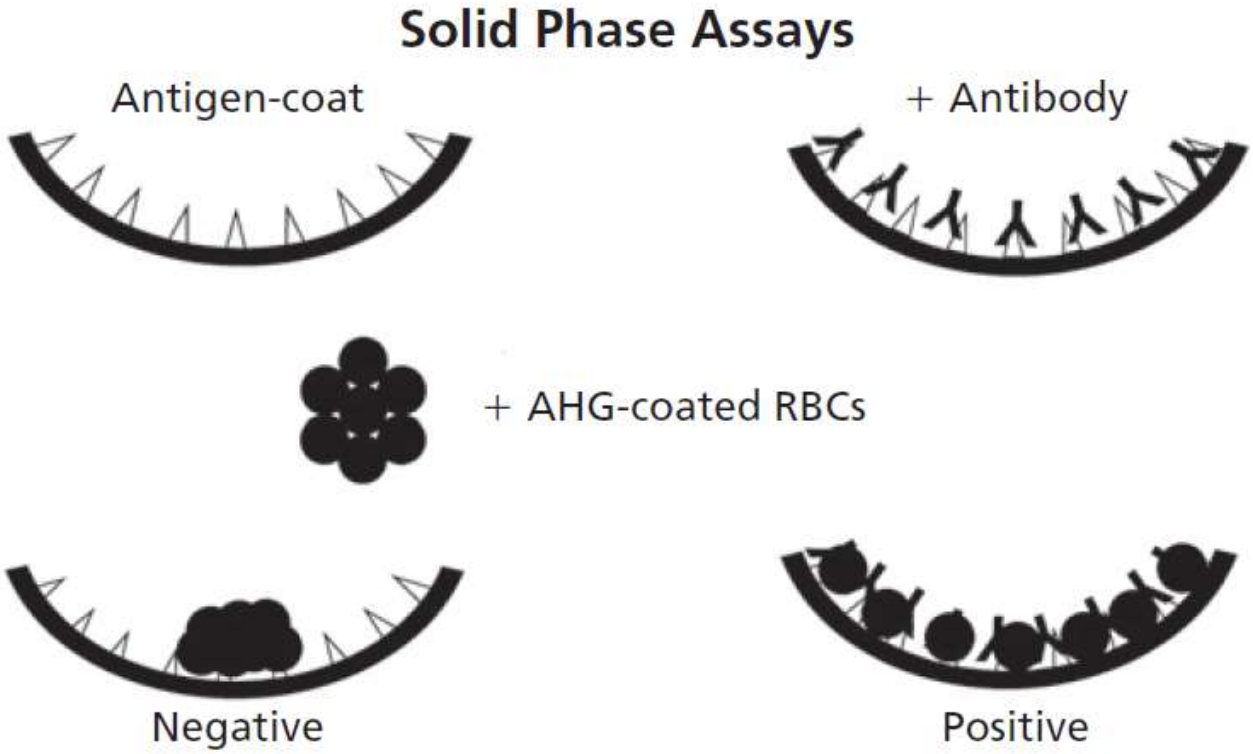
Solid Phase Red Cell Adherence Assay

- One of the components of an antigen–antibody reaction is immobilized onto a solid medium and after reaction with a free antigen/antibody the end point of the reaction is indicated by use of red cells, which may be a part of the antigen–antibody reaction or may be added as indicator cells
- In forward grouping U shaped micro plate wells are coated with Anti-A antiserum, Anti-B antiserum, and Anti-D antiserum, A drop of 0.5% bromelin-treated red cells are added to the well
- On centrifugation antigen positive cells spread out while antigen negative cells form a button at the bottom of the well

Solid Phase Red Cell Adherence Assay

- In case of reverse grouping a monolayer of RBC membrane is attached to the bottom of the well and plasma to be tested is added after incubation for 5 min, the excess plasma is blotted and anti-IgG bound indicator red cells are added to give a visible reaction
- SPRCA may be adapted to other red cell serology tests such as antibody screening, identification, and cross matching
- It may also be adapted to platelet serology

Solid Phase Red Cell Adherence Assay



SPRCA: Advantages

- Increased sensitivity and specificity
- Stable end point observations hence easier staff training
- Reduced number of steps in the procedure- Reduced technologist's hands-on-time
- Less waste volume
- Adaptation to automation to eliminate transcription errors
- Other applications in Red Cells Serology, eg platelet serology
- No need for check cells
- Small test volume
- Enhanced anti-D
- Improved sensitivity in testing IgG antibodies
- Good at detecting weaker expressions of blood group antigens
- Suitable for lipemic / haemolysed samples

SPRCA: Disadvantages

- Increased sensitivity to detect warm reactive autoantibody
- Increased sensitivity for all Abs
- RBC modification such as enzyme treatment not possible
- Does not detect IgM antibodies
- Low specificity may cause unnecessary delay due to further work up of the sample
- Higher cost of consumables per test
- Additional capital cost for automation

DIAGAST-Erythrocytes Magnetic Technology

Principle: Based on magnetization of RBCs in magnetizing solution

- Hemagglutination method is used in combination with a magnetic field
- Bromelin, a proteolytic enzyme, induces a marked decrease in the electronegative charge on the surface of RBCs enabling their agglutination by normally non-agglutinating antibodies in saline medium
- When subjected to a magnetic field, the magnetized RBCs migrate & form a pellet at the bottom of the well
- After shaking, free RBCs are resuspended
- Presence of agglutination in the centre shows a positive reaction while spread out RBCs are seen in a negative reaction

Antibody Screening

Positive reaction Negative reaction

Grouping and phenotyping

Negative reaction Positive reaction

Advantages of EMT

- Quality: Clear-cut reactions to get an unequivocal interpretation of results
- Comfort: Centrifugation step is completely eliminated during the analytical process

Disadvantages of EMT

- Stages in the procedure are to be implemented in succession without interruption
- Only suitably trained personnel should run the tests
- Reactions are to be read at the latest within 2 minutes of the last shaking
- Error in reading lipaemic and fibrinic samples

Comparison of technologies used for immunohematology tests

Technology	Column Agglutination Technology	Solid Phase Red Cell Adherence Assay	Erythro-Magnetic Technology	Conventional Tube Testing (CTT)
Number of steps required	8-12	13-15	8 to 14	14-19
Washing step	Omitted	One washing step	Omitted	Multiple washing steps
Advantages	Small sample volume Uniformity of testing in repeat testing Clear and easily readable results Results may be stored for 24hrs Sensitivity for clinically significant antibodies (CSAs) better than CTT	Small sample volume Easy handling of large batches Improved sensitivity in testing IgG antibodies. Good at detecting weaker expressions of blood group antigens Suitable for lipaemic / haemolysed samples	Small sample volume Highly suitable for automation as there are no washing / centrifugation steps Suitable for ABO/D grouping and K typing Sensitivity for CSAs comparable to CAT	Amenable to all modifications of RBC and serum during testing ABO –Rh typing may be done in the shortest possible time

Comparison of technologies used for immunohematology tests

Technology	Column Agglutination Technology	Solid Phase Red Cell Adherence Assay	Erythro-Magnetic Technology	Conventional Tube Testing (CTT)
Disadvantages	More suited to batch testing in terms of time efficiency Preparation of 0.8% suspension is cumbersome It takes a minimum of 20 min to do an ABO/D grouping. Rouleaux and fibrin clots may be reported as positive	Technique takes time to learn RBC modification such as enzyme treatment not possible. Low specificity may cause unnecessary delay due to further work up of the sample. Does not detect IgM antibodies	Does not detect IgM antibodies Manual method is labour intensive and requires comprehensive training Error in reading lipaemic and fibrinic samples Does not detect IgM antibodies	Large sample volume required Procedure in cumbersome and time consuming The results are variable and may depend on the expertise of the staff doing the test. Sensitivity for CSAs less than other techniques
Antibody Screening				
Sensitivity for clinically significant antibodies	90 – 94%	Aprox. 97%	83.3-90.4%	Subjective Aprox. 43% (LISS-IAT)
Specificity	94.4%	94.3%	98.2% (R2)	98.6%

What to consider in Automation

Work Station
Technology on which automation is based
Type of model
Dimensions (cm)
Tests which can be done
Minimum Sample volume Required
Sample tube requirements
Number of samples that may loaded at a time
Throughput (Samples/Hour)
Stat Function (Priority Sample)
Loading options
Shelf life of reagents used (Shortest expiry reagent)

Fully Automated Immunohematology Workstations

	All the systems support more than one type of bar-coding and all have bi-directional interfacing with Hospital Information Systems
	The presently available systems have password protected levels of access
	In process control/ activity logging (use audit)
	Verification of Reagent- lot no. /expiry
	Integrated quality control management
	Liquid level detection
	Clot Detection
	Positive sample and reagent identification
	Archiving of results

Software Advances

Intuitive Irregular Antibody Identification Software

- Uses unique algorithm that classifies antibodies by probabilities
- Gives prompts for additional tests to be done
- Open system and may even use expired reagents
- Reference guide is integrated into software

Remote Validation of results

- Validation of results at remote location through web interface.
- Thus physical presence of expert not required in blood bank for routine/complex interpretations/validation

Benefits of Automation

- Increased productivity
- Vein to Vein traceability
- Barcode reading
- Enhanced quality assurance
- Controlled and standardised process
- HIS interface reduces potential for transcription errors
- Reduced stress leads to fewer errors, staff satisfaction
- More flexibility with staff scheduling

Correlation between Inter-operator variation between five different operators – Manual CAT

Blood group	A		B		O				All Blood Groups				Overall	
Antibody	ANTI B		ANTI A		ANTI A		ANTI B		Anti A		Anti B			
	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG
Cronbach's Alpha	0.944	0.936	0.953	0.931	0.657	0.934	0.865	0.776	0.690	0.938	0.889	0.866	0.766	0.931
Kendall's W	0.957	0.954	0.952	0.987	0.930	0.988	0.733	0.763	0.932	0.985	0.841	0.881	0.877	0.934

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Correlation between repeatability evaluated by repeating five samples by auto-CAT and man-CAT.

Blood group	A		B		O				All Blood Groups				Overall	
Antibody	ANTI B		ANTI A		ANTI A		ANTI B		Anti A		Anti B			
	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG
Intra class correlation coefficient of auto-CAT	0.981	0.976	0.999	0.999	0.999	0.955	0.963	0.968	0.999	0.966	0.976	0.966	0.976	0.980
Intra class correlation coefficient for man-CAT	0.946	0.925	0.985	0.986	0.980	0.984	0.956	0.905	0.979	0.985	0.946	0.905	0.944	0.966

Meenu Bajpai, Abhay Gupta – Under Study

Challenges for Automation

- Alteration of the workflow
- Changes of current procedures, habits
- Changes of the needed know-how in your laboratories
- Staff need to be trained
- Understanding the software
- Hospital Information System/laboratory Information System Interfacing
- Learning new technologies
- Back-Up for the Automated Equipment
- Validation of Equipment and Continuous Quality Assurance
- Cost

Approach to Automation Selection

- Assess the important reasons for going for an automation
- Make sure the automation justifies the need
- Have a hands on trial prior to taking a decision or at least a demonstration
- Assess your current workflow and ensure automation benefits TAT
- Ensure that the automation would be able to meet your demand for at least the next 5 years

Thank You