Inhibitor Work Up in Hemophilia

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Case - History

- 13 years Male with long history of haemarthrosis causing fixed flexion deformities on the right knee. He had h/o muscle bleed and bleeding after trauma.

- Most of the bleed is covered with CFC replacement therapy. But this has not happened since last two months.

- He has an maternal uncle who died very young after prolonged bleeding episode.

Laboratory Findings

- BT : 2 min
- Platelet count : 345000/cumm
- PT : 12.2 Sec (Control : 11.2 Sec)
- aPTT : 88.4 Sec (Control: 27.8 Sec)
- FVIII level : < 1%
- Mixing study with PNP : 85.3 Sec after 2 hours incubation.

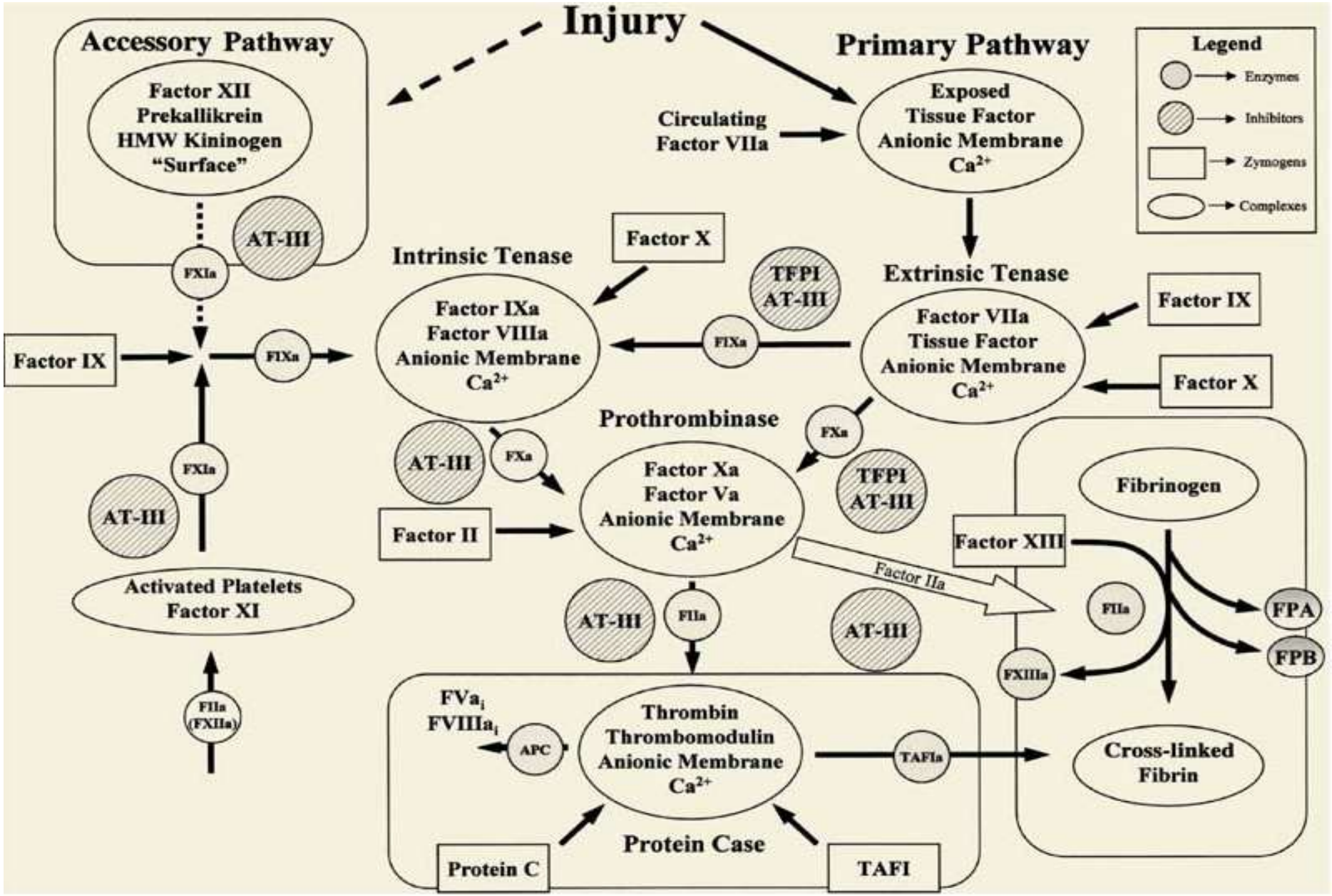
What is Hemophilia ?

- Hemophilia is an X-linked congenital bleeding disorder caused by a deficiency of function of the coagulation Factor VIII (Hemophilia A) or Factor IX (Hemophilia B).

- The deficiency is the result of the mutations of the respective clotting factor gene.

- Estimated frequency : approximately 1 in 10000 live births.

Overview of Hemostasis



Relationship of Bleeding Severity to Clotting Factor Level

Severity	Clotting Factor Level	Bleeding Episodes
Severe	< 1 IU/dl or 1% of normal	Spontaneous bleeding into joints or muscles, predominantly in the absence of identifiable hemostatic challenge
Moderate	1-5 IU/dl or 1-5% of normal	Occasional sponataneous bleeding; prolonged bleeding with minor trauma or surgery.
Mild	5-40 IU/dl or 5-<40 % of normal	Severe bleeding with major trauma or surgery; spontaneous bleeding is rare

Laboratory Diagnosis

- Sample collection.

1. Screening test :

- Platelet count
- Bleeding time (Selected situation)
- Prothrombin time
- Activated partial thromboplastin time
- Platelet functions screening tests

2. Confirmation by Factor assay.

Therapy of Hemophilia

- Comprehensive care – Multidisciplinary in nature.

- Paediatrician/Haematologist, Nurse, Musculoskeletal expert, physiotherapist, psychosocial expert, laboratory specialist.

- Prophylaxis vs On demand (Episodic) Factor replacement therapy.

What is Inhibitors ?

- “Inhibitors’’ in hemophilia refer to IgG antibodies that neutralize clotting factors.

- Inhibitors to FVIII and FIX are considered to be the most serious treatment related complication.

- More common in severe hemophilia.

Frequency of Inhibitors

- The incidence of inhibitors in severe hemophilia A : approx. 30 %, of which 79% occur within first 20 exposures and remaining 21% within first 75 exposures of FVIII/FIX containing products.

- The incidence of inhibitors in mild and moderate hemophilia A : 5 to 10%.

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Original Article

"Prevalence of Inhibitors in Hemophilia Patients and its Clinical Implications": A Study of 276 Patients in Western India

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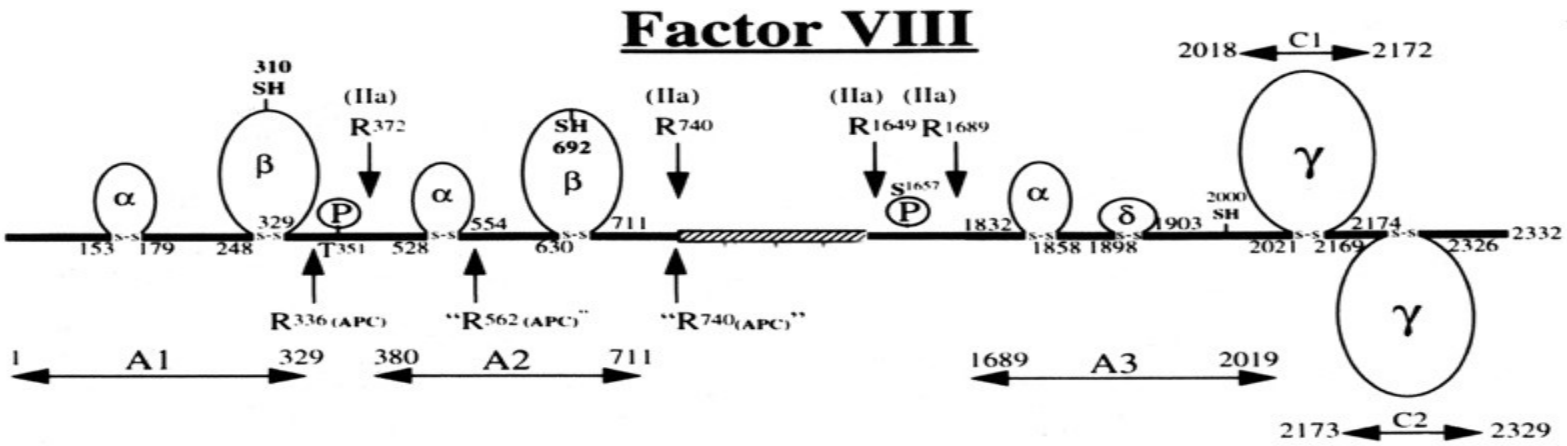
ABSTRACT

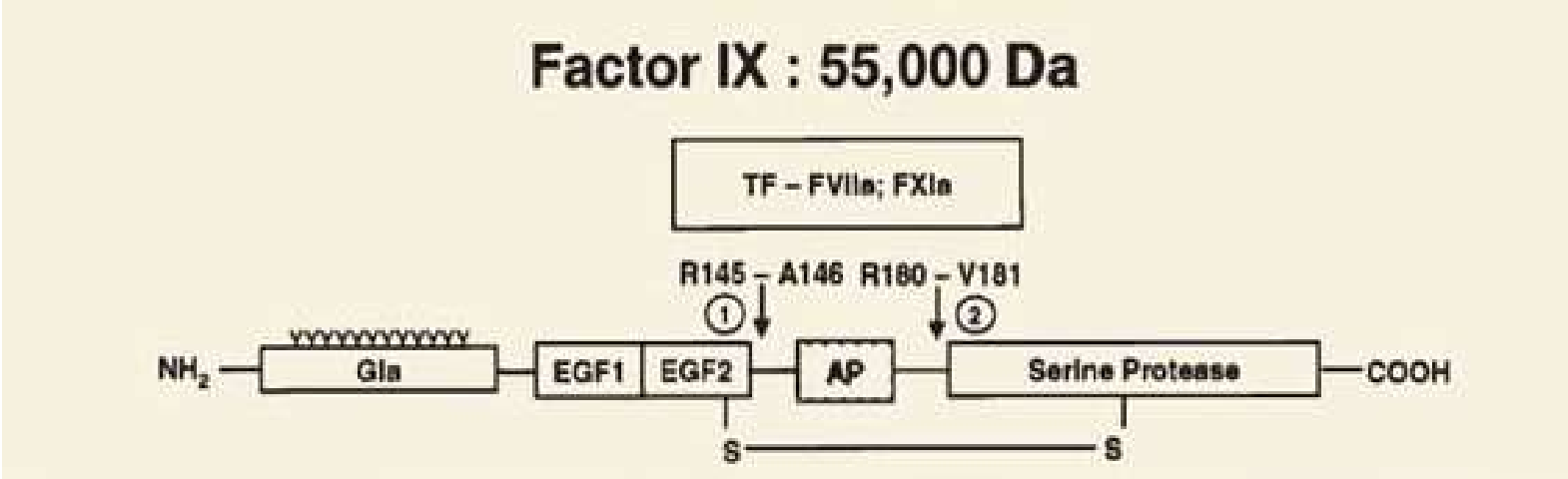
Introduction: Hemophilia is an X-linked congenital bleeding disorder caused by a deficiency of coagulation factor VIII (FVIII) in hemophilia A (HA) or factor IX (FIX) in hemophilia B (HB). Accurate diagnosis of hemophilia by factor assay to demonstrate deficiency of FVIII or FIX is essential for appropriate management. Inhibitor development results in partial or complete lack of the efficacy of replacement

- Results

Severity	Total No.of Patients	Inhibitor Positive	% Positive
Hemophilia A			
Mild	33	00	00
Moderate	27	02	7.40
Severe	183	48	26.22
Hemophilia B			
Mild	00	00	00
Moderate	11	00	00
Severe	17	02	11.76

Biochemical Structure of FVIII and IX





Factor VIII Inhibitor Kinetics

Type	Kinetics	Inhibition of FVIII	Seen with
Type I	Simple – First order	Complete	Alloantibodies arising in a person with Hemo A treated with FVIII concentrate and who make an antibody to the foreign protein
Type II	Complex- Second order	Incomplete	Autoantibodies seen in acquired Hemo A

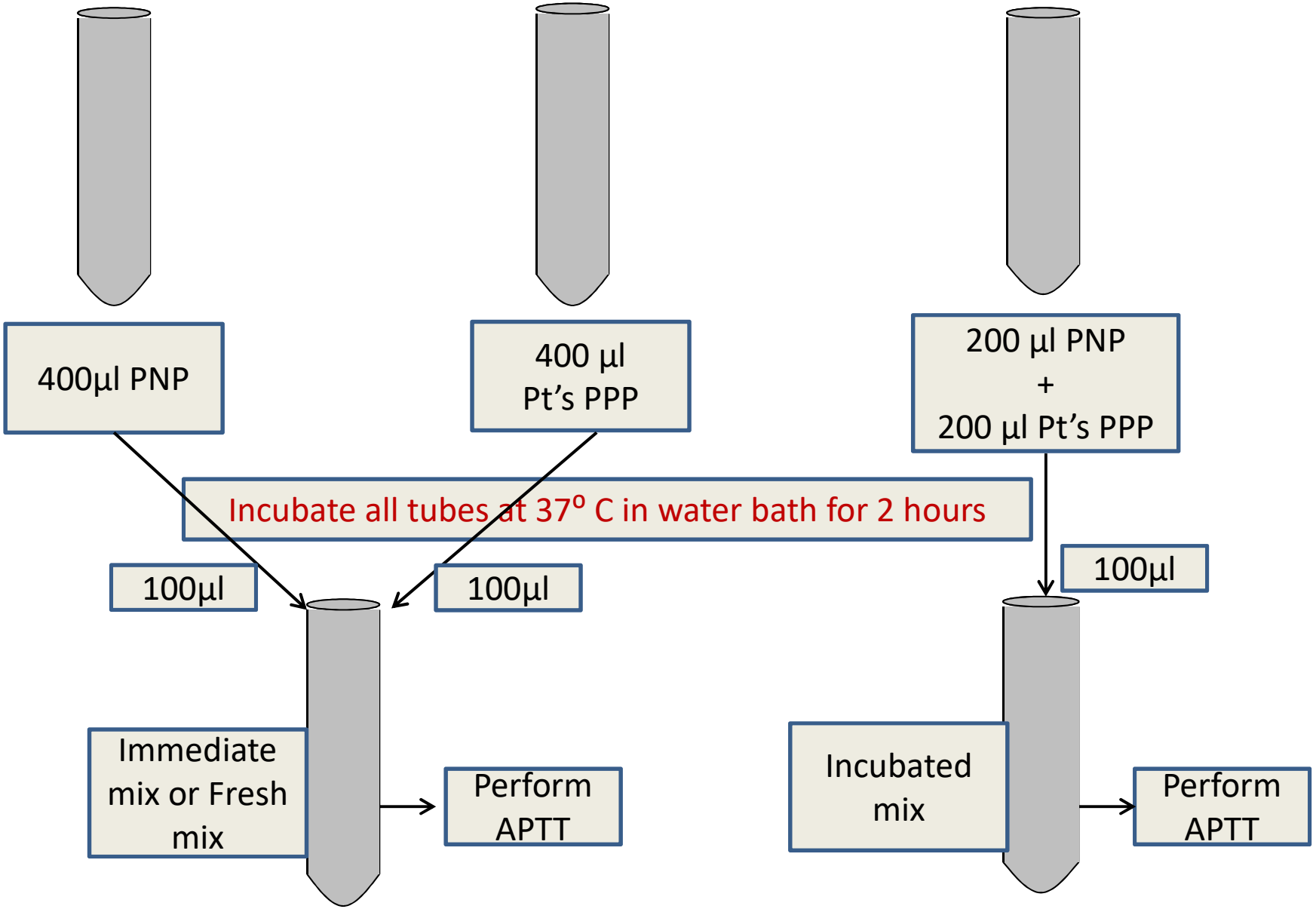
Genetic and Environmental Risk Factors

- Type of hemophilia.
- Family history and race.
- Type of mutation.
- Polymorphic immune regulatory gene-HLA.
- High intensity factor exposure(CFC).
- CFC type.
- Patient’s age at first exposure of CFC

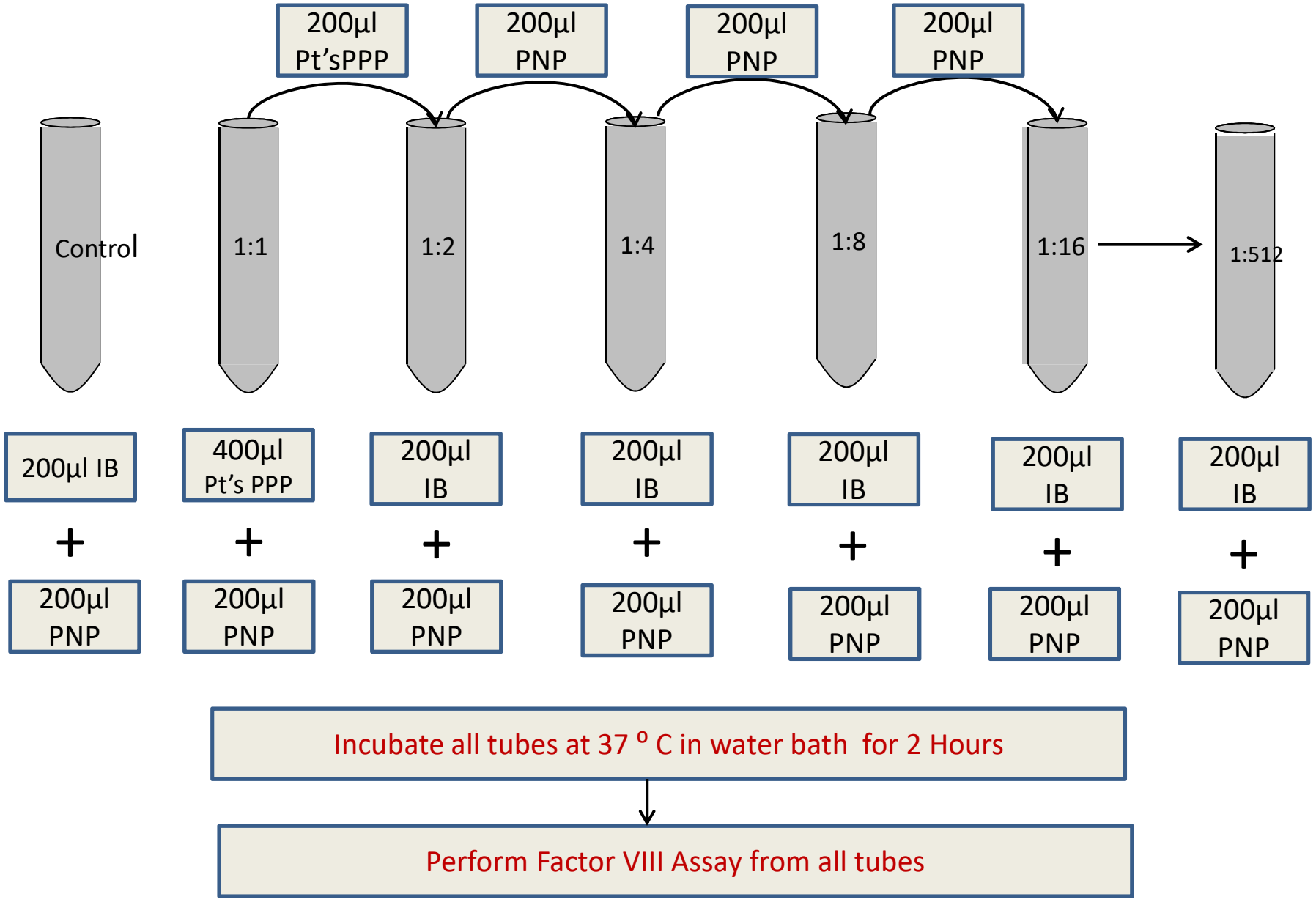
Indications for Inhibitor Testing

- After initial exposure.
- After intensive factor exposure.
- Recurrent bleeds or target joint bleeds, despite adequate CFC replacement therapy.
- Failure to respond to adequate CFC replacement therapy.
- Lower than expected factor recovery or half life after CFC replacement therapy.
- Before surgery.
- Suboptimal post-operative response to CFC-replacement therapy.

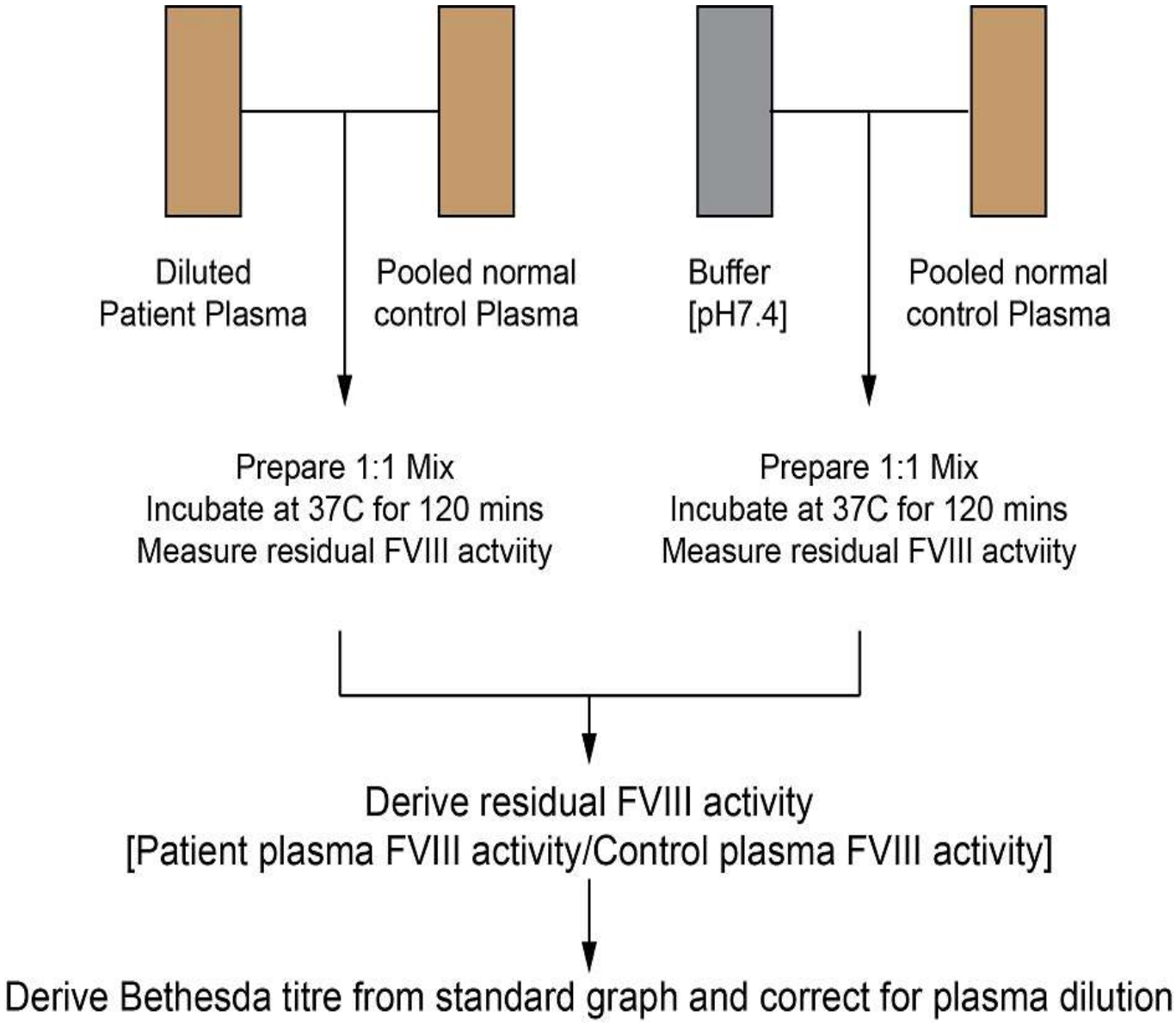
Inhibitor Screening



Method of Bethesda Assay



Bethesda Assay



Definition of Bethesda Unit

- Bethesda unit (BU) is defined as the amount of an inhibitor that will neutralise 50 % of 1 unit of FVIII:C in normal plasma after 120 minutes incubation at 37^{o}C.

- The definition of a positive inhibitor is a Bethesda titer of >0.6 BU for FVIII and ≥0.3 BU for FIX.
- A low-responding inhibitor : <5.0 BU
- A high-responding inhibitor : ≥5.0 BU

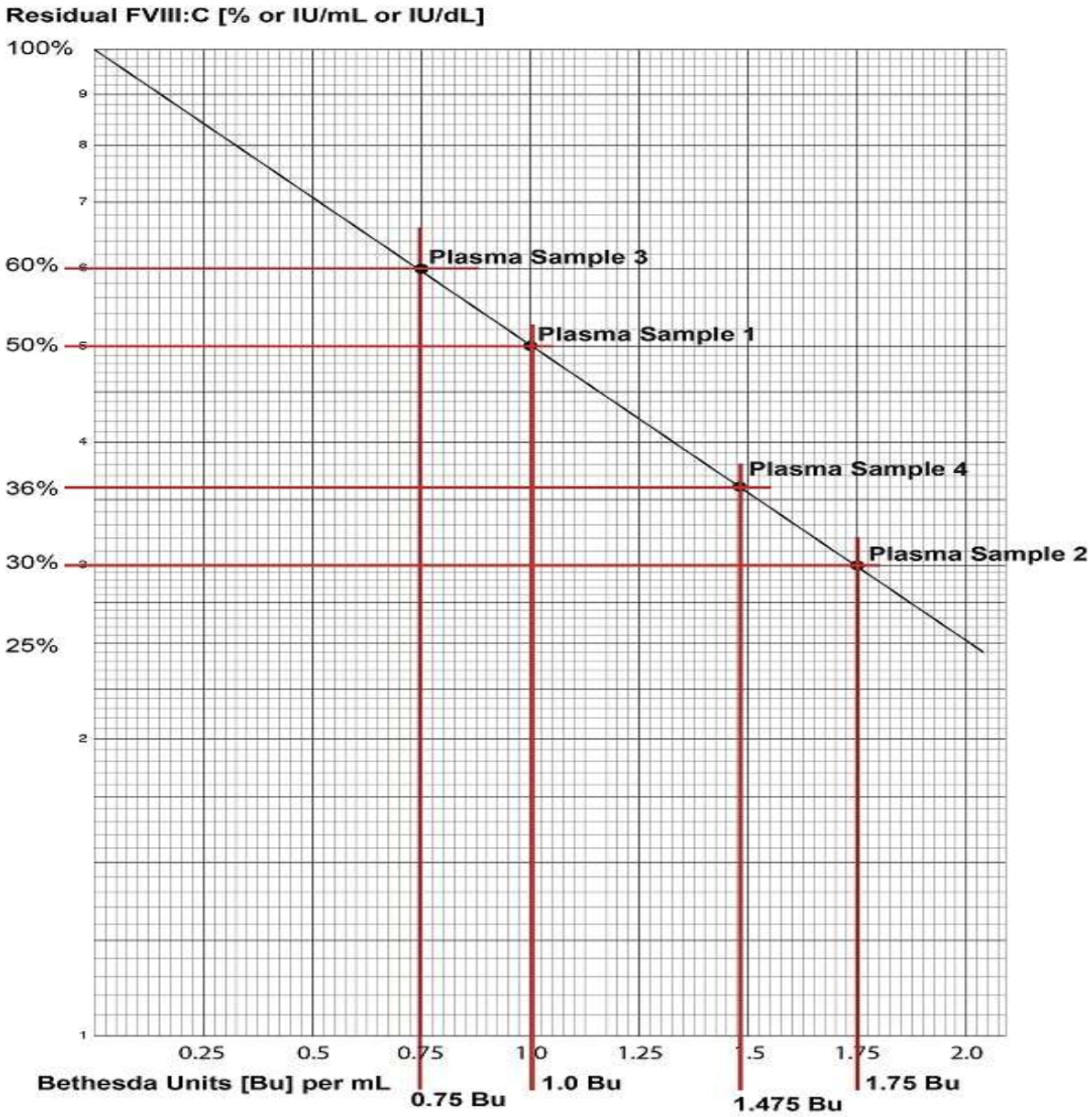
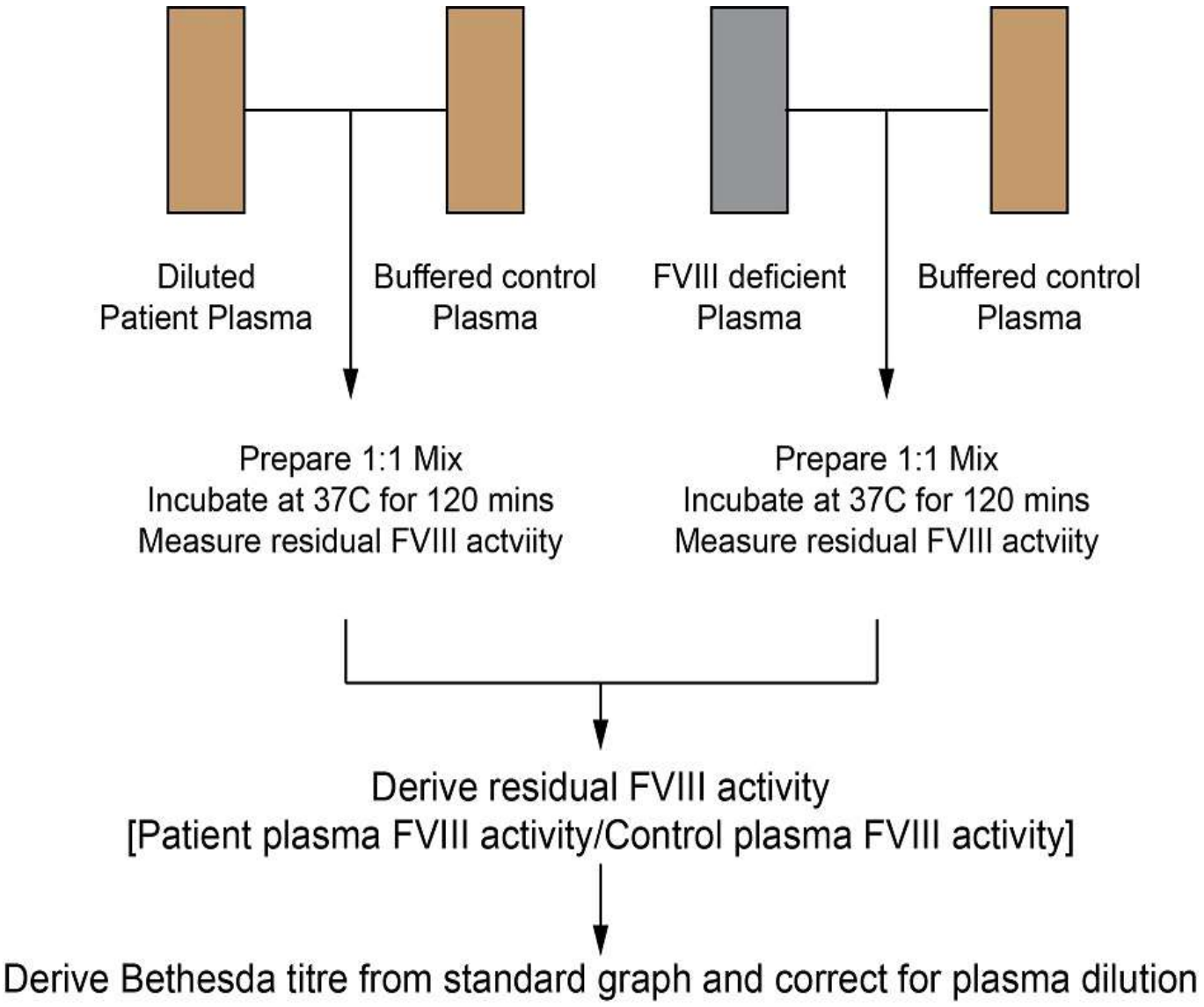

Residual FVIII:C [% or IU/mL or IU/dL]
100%
60%
50%
36%
30%
25%
Plasma Sample 3
Plasma Sample 1
Plasma Sample 4
Plasma Sample 2
0.25
0.5
0.75
1.0
1.25
1.5
1.75
2.0
Bethesda Units [Bu] per mL
0.75 Bu
1.0 Bu
1.475 Bu
1.75 Bu

Chart showing Residual Factor activity and Multiplication Factor

Residual FVIII %	Factor	Residual FVIII %	Factor	Residual FVIII %	Factor
97	0.05	61	0.7	40	1.35
93	0.1	59	0.75	38	1.4
90	0.15	57	0.80	37	1.45
87	0.2	55	0.85	35	1.5
84	0.25	53	0.9	34	1.55
81	0.3	51	0.95	33	1.6
78	0.35	50	1	32	1.65
75	0.4	48	1.05	30	1.7
73	0.45	46	1.1	29	1.75
70	0.5	45	1.15	28	1.8
68	0.55	43	1.2	27	1.85
66	0.6	42	1.25	26	1.9
64	0.65	41	1.3	25	2

Nijmegen Modification Assay



Other Method for Inhibitor Assay

- Modifying the Nijmegen Assay to detect very low titre inhibitors.
- The Bethesda Assay : Heat treatment.
- Factor VIII Inhibitor detection by ELISA.
- The Bethesda Assay : Chromogenic Assay.

Therapeutic option for FVIII inhibitor patient

- **Low-responding inhibitors** : FVIII CFC replacement therapy.
- Dose : Body wt(kg) x 80 x [1-Hct] x antibody titer BU.
- Additional 50 IU/kg above the calculated loading dose is added to achieve a measurable FVIII activity.
- **High responding inhibitors** :
- Bypass agent : rFVIIa or aPCC or porcine FVIII
- FVIII mimic bispecific monoclonal antibody : Emicizumab.
- Immune tolerance induction.

Therapeutic option for FIX inhibitor patient

- **Low responding inhibitor** : FIX CFC replacement therapy.
- **High responding inhibitor** : Bypassing agent rFVII.
- Allergic reactions and anaphylaxix may occur in upto 50 % of Hemophilia B patients with inhibitor.

Summary

- Inhibitor development is one of the most serious treatment related complication in Hemophilia.
- Inhibitor screening and quantitative estimation of inhibitor is necessary to decide appropriate therapy and decrease morbidity and mortality of patients.
- Technical knowledge and expertise in coagulation laboratory testing, use of correct equipment and reagents and quality assurance are key factors for inhibitor work up.

Thank You