DIAGNOSIS OF HAEMOPHILIA

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INTRODUCTION

- Different bleeding disorders either inherited or acquired present with similar clinical features as seen in haemophilia.
- Thus, a correct diagnosis of haemophilia is essential to ensure that a patient gets appropriate treatment and management.
- The accurate diagnosis of haemophilia is based on;

    1. Family history of haemophilia.
    2. Clinical features/findings.
    3. Laboratory testing.

PRINCIPLES OF HAEMOPHILIA DIAGNOSIS

- Understanding the clinical features of haemophilia.
- Use of laboratory screening tests.
- Confirmation of diagnosis.

HISTORY OF HAEMOPHILIA DIAGNOSIS

- Then Rabbi and physician Maimonides in the XII century noted that the mothers were the carriers.
- In 1800 John Otto a physician in Philadelphia wrote a description of the disease where he clearly appreciated the cardinal features.
- "The disease of Kings".
- End of 19th century- crude assays, clotting time of plasma of haemophiliacs compared with non-bleeders.
- 1947-defect is single deficient plasma protein.
- Pavlosky: Mixing 2 sources of this plasmas- correction/Multiple types.
- Currently, automated factor assays and genetic testing.

CLINICAL DIAGNOSIS

HAEMOPHILIA SHOULD BE SUSPECTED IN PATIENTS WITH HISTORY OF

- Easy bruising in early childhood.
- Spontaneous bleeding (apparently no reasons previously) in joint ,muscles and soft tissue.
- Excessive bleeding after trauma or surgery.
- Family history of a bleeding disorder.

TABLE 45.1 CLINICAL DISTINCTION BETWEEN DISORDERS OF VESSELS OR PLATELETS AND DISORDERS OF BLOOD COAGULATION

Finding	Disorders of Coagulation	Disorders of Platelets or Vessels
Petechiae	Rare	Characteristic
Deep dissecting hematomas	Characteristic	Rare
Superficial ecchymoses	Common; usually large and solitary	Characteristic; usually small and multiple
Hemarthrosis	Characteristic	Rare
Delayed bleeding	Common	Rare
Bleeding from superficial cuts and scratches	Minimal	Persistent; often profuse
Sex of patient	80%-90% of inherited forms occur only in male patients	Relatively more common in females
Positive family history	Common	Rare (except von Willebrand disease and hereditary hemorrhagic telangiectasia)

BASELINE INVESTIGATIONS

TABLE 3-1: INTERPRETATION OF SCREENING TESTS

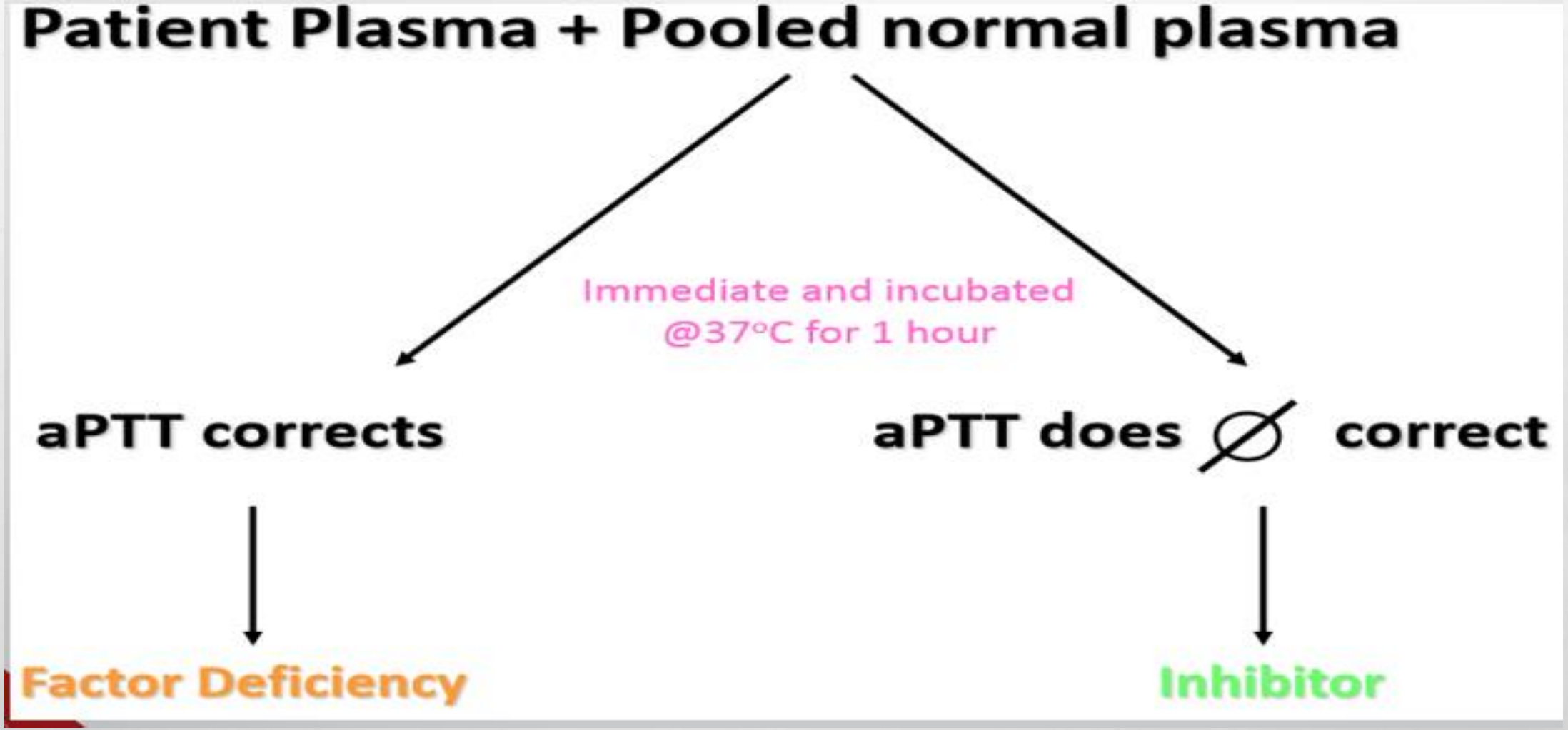
POSSIBLE DIAGNOSIS	PT	APTT*	BT	PLATELET COUNT
Normal	Normal	Normal	Normal	Normal
Hemophilia A or B**	Normal	Prolonged*	Normal	Normal
VWD	Normal	Normal or prolonged*	Normal or prolonged	Normal or reduced
Platelet defect	Normal	Normal	Normal or prolonged	Normal or reduced

* Results of APTT measurements are highly dependent on the laboratory method used for analysis.
** The same pattern can occur in the presence of FXI, FXII, prekallikrein, or high molecular weight kininogen deficiencies.

MIXING STUDIES

APTT – when prolonged, do correction studies.

- If corrected with factor IX – haemophilia B
- If corrected with factor VIII – haemophilia A


Patient Plasma + Pooled normal plasma
Immediate and incubated
@37°C for 1 hour
aPTT corrects
aPTT does ∅ correct
Factor Deficiency
Inhibitor

SPECIFIC/DEFINTIVE TESTS

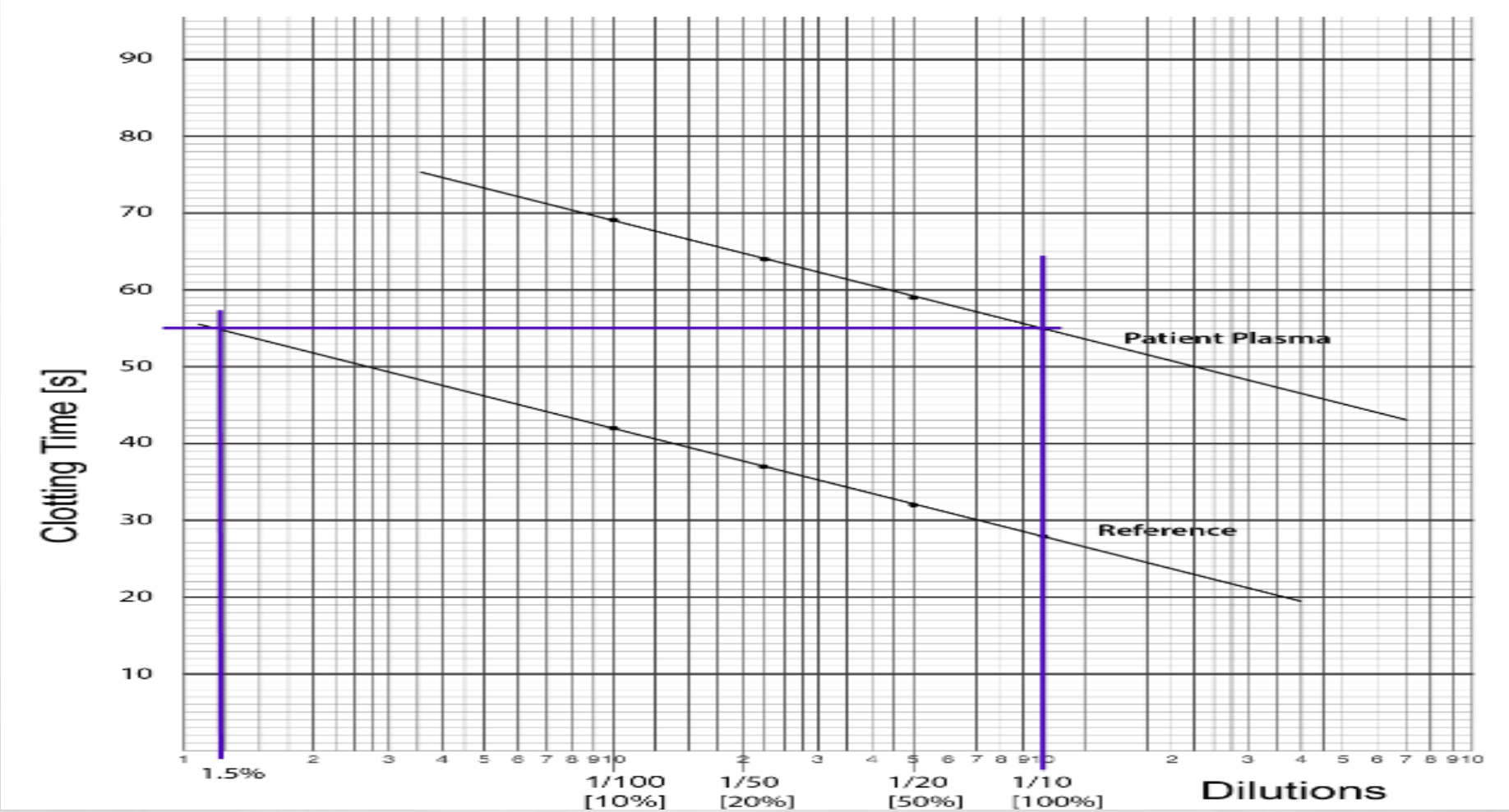
Factor VIII & IX Assays

2 methods

- Clot-based assay; 1- stage or 2- stage assays
- Chromogenic assay.

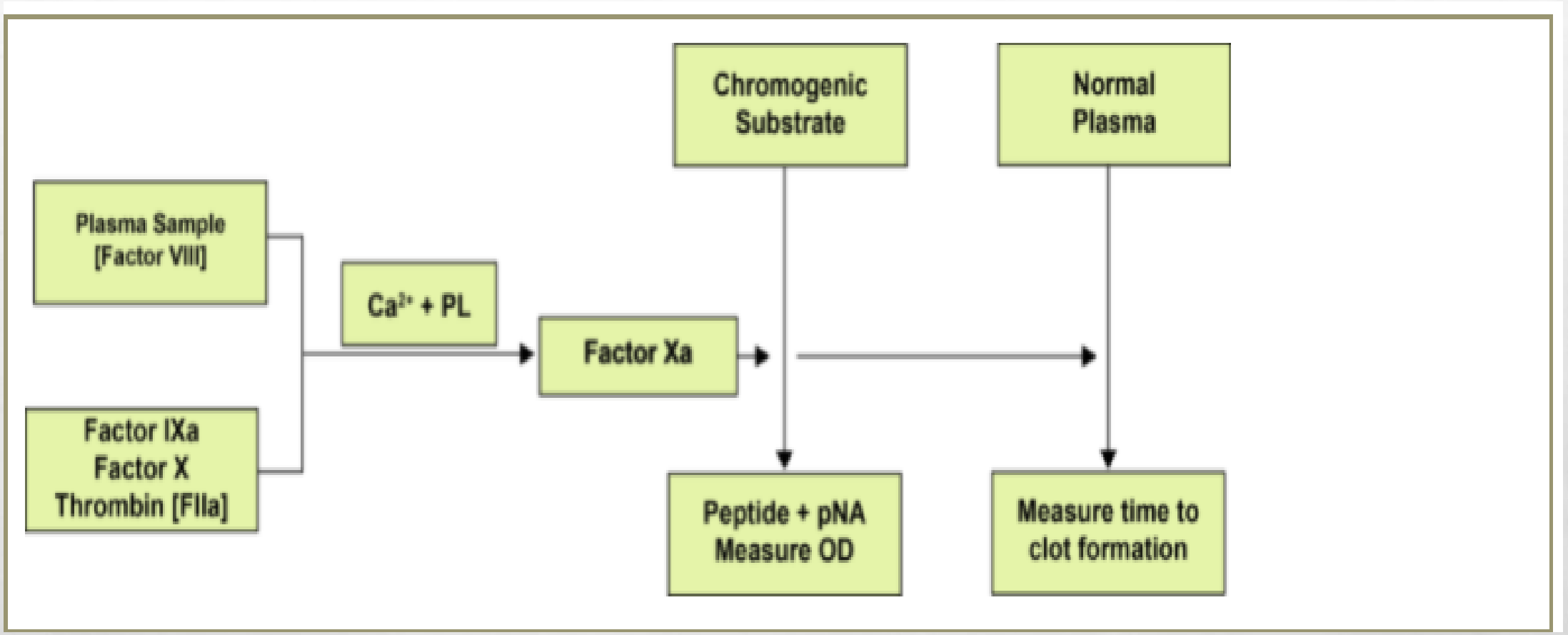
Raw Data

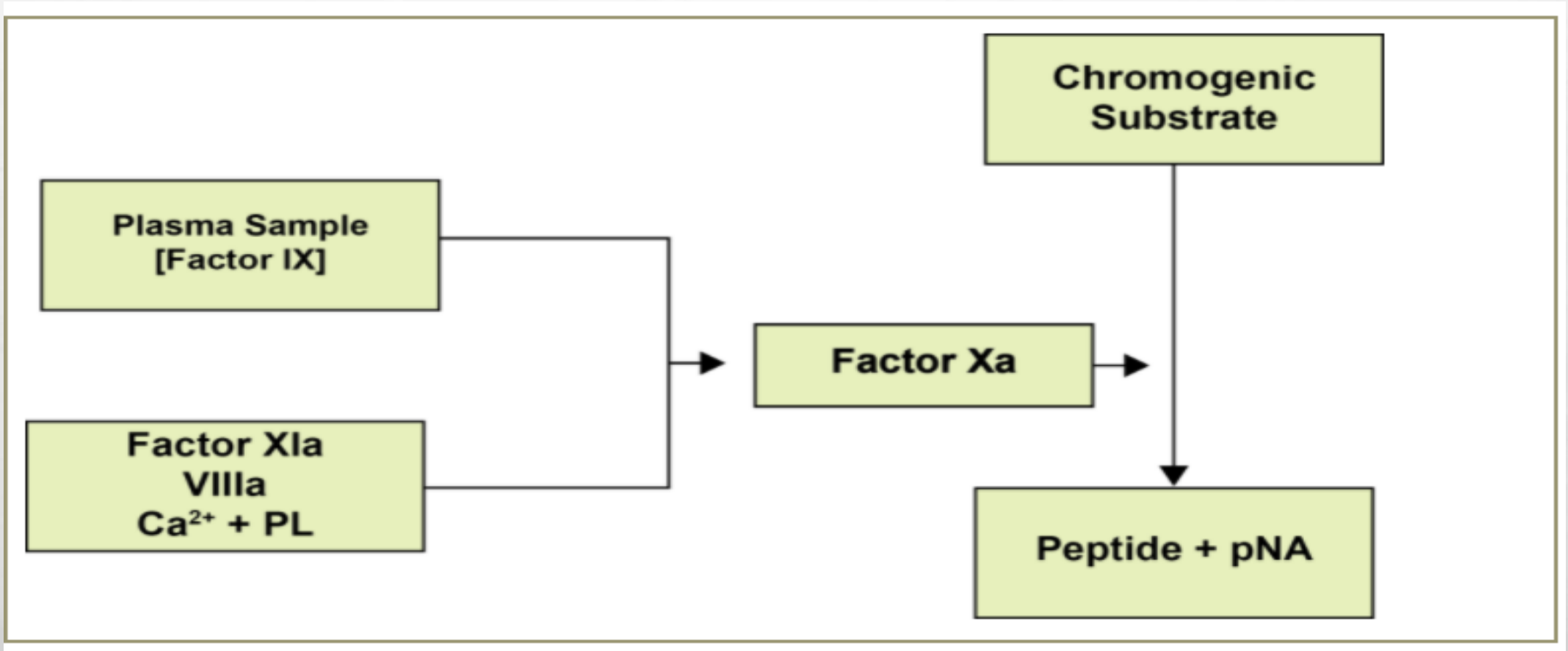
Sample	Dilutions			
	1/10 [100% FVIII Activity]	1/20 [50% FVIII Activity]	1/50 [20% FVIII Activity]	1/100 [10% FVIII Activity]
APTT [s] Reference Plasma [FVIII:C = 95 IU/dL]	28s	32s	38s	42s
APTT [s] Patient Plasma	55s	59s	73s	80s


Clotting Time [s]
90
80
70
60
50
40
30
20
10
Patient Plasma
Reference
1.5%
1/100
[10%]
1/50
[20%]
1/20
[50%]
1/10
[100%]
Dilutions

CHROMOGENIC METHOD

Component	Explanation
Reagent cocktail for generating FXa	Contains FIXa, FX in excess, Thrombin [Factor IIa], a source of calcium ions and phospholipid
Chromogenic substrate	A substance cleaved by FXa to produce a colour change. May also contain a Thrombin inhibitor to stop the FXa generation when the chromogen is added
Patient plasma	Platelet poor plasma


Chromogenic Substrate
Normal Plasma
Plasma Sample [Factor VIII]
Ca^{2+} + PL
Factor Xa
Factor IXa
Factor X
Thrombin [FIIa]
Peptide + pNA
Measure OD
Measure time to clot formation


Chromogenic Substrate
Plasma Sample [Factor IX]
Factor XIa VIIIa Ca^{2+} + PL
Factor Xa
Peptide + pNA

GENETIC TESTING

- **Prenatal diagnosis**

  – genetic consultation

  – choronic villi sampling, amniocentesis, cord blood sampling

- **Mutation screening**

  - screen for intron 22 inversion - only in patients with severe haemophilia A

If negative for that, there is the need for DNA sequencing

- In mild to moderate hemophilia A, full sequencing of the FVIII gene.

- In hemophilia B, perform full sequencing of FIX gene.

CARRIER STATE DETECTION

Clinical method

- A woman is a definite carrier if;

(i) her father has haemophilia.

(ii) she has one son with haemophilia and a 1st degree male relative with haemophilia.

(iii) she has two sons with haemophilia.

- A possible carrier if;

(i) she has one or more maternal relatives with haemophilia.

(ii) she has one son with haemophilia & no other affected relatives.

Phenotypic method:

– FVIII:C assay.

Genotypic method

– restriction endonuclease analysis.

– oligonucleotide probe analysis.

OTHER INVESTIGATIONS

- X-rays of joints
- CT Scans
- MRI

CONCLUSION

- Haemophilia is one of the commonest inherited bleeding disorders that can lead to numerous morbidities and in severe cases can lead to death.
- an accurate and definitive diagnosis will ensure that patients are appropriately treated to prevent these morbidities and complications associated with haemophilia, thus improving the quality of life of haemophiliacs.

THANK YOU

REFERENCES

- Guidelines for the Treatment of Haemophilia- World Federation of Haemophila 2005 (wfh.org)
- Rhona M, Maclean and Michael Makris Haemophilia A and B in Practical Haemostasis and Thrombosis 3rd edition .
- Rajiv K. Pruthi. Haemophilia: A Practical Approach to Genetic Testing. mayo clin proc. 2005;80(11):1485-1499