

Practical Issues in Laboratory Detection of Inhibitors

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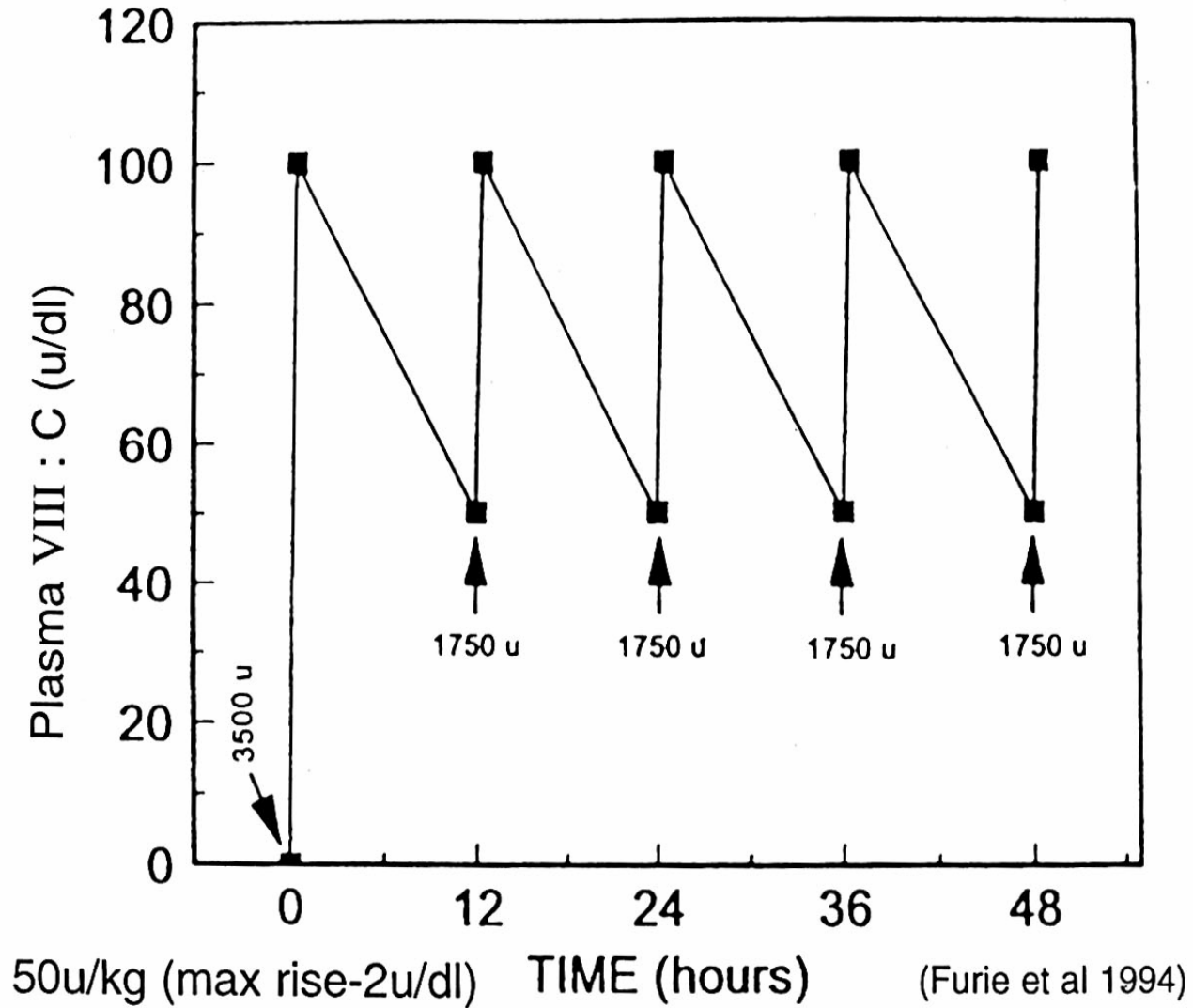
Inhibitors and haemophilia:

- Occurs in around 10-15% of patients with haemophilia A
- Rare in haemophilia B (?1%)
- Tend to develop early in life (10 Exposure days)
- Product purity does not influence incidence of inhibitor development
- Can rarely develop in mild haemophilia
- Major risk factor is underlying genotype

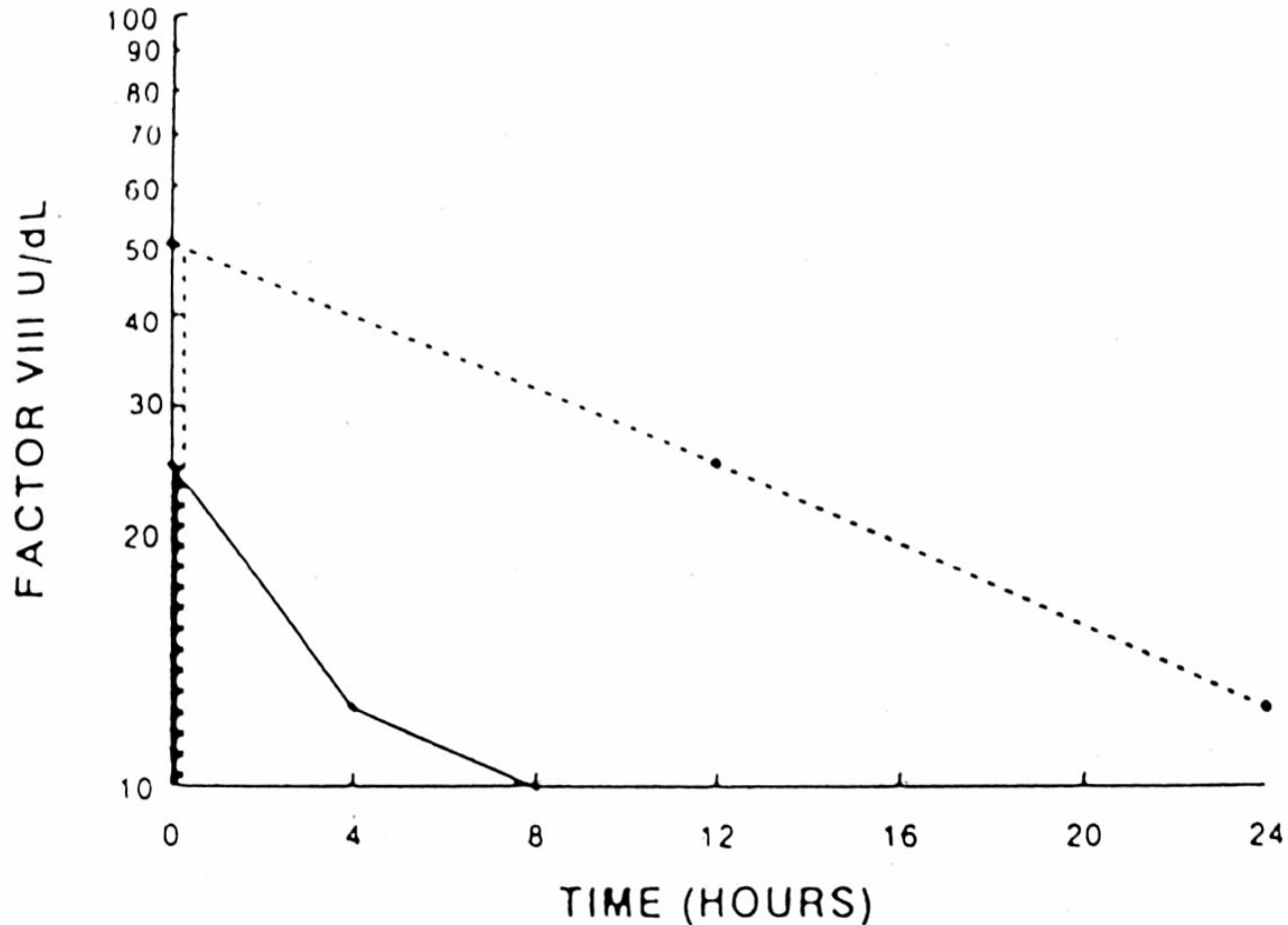
Evidence of Inhibitor development

- Altered clinical picture in the patient.
- Lower than expected recovery of infused FVIII
- Shorter half life of infused FVIII
- In severe Haem A (<1 IU/dl) - Inhibitor patients typically have longer APTT than non inhibitor patients

PHARMACOKINETICS OF FVIII INFUSION



HALF-LIFE AND RECOVERY OF VIII : C IN THE PRESENCE OF INHIBITOR

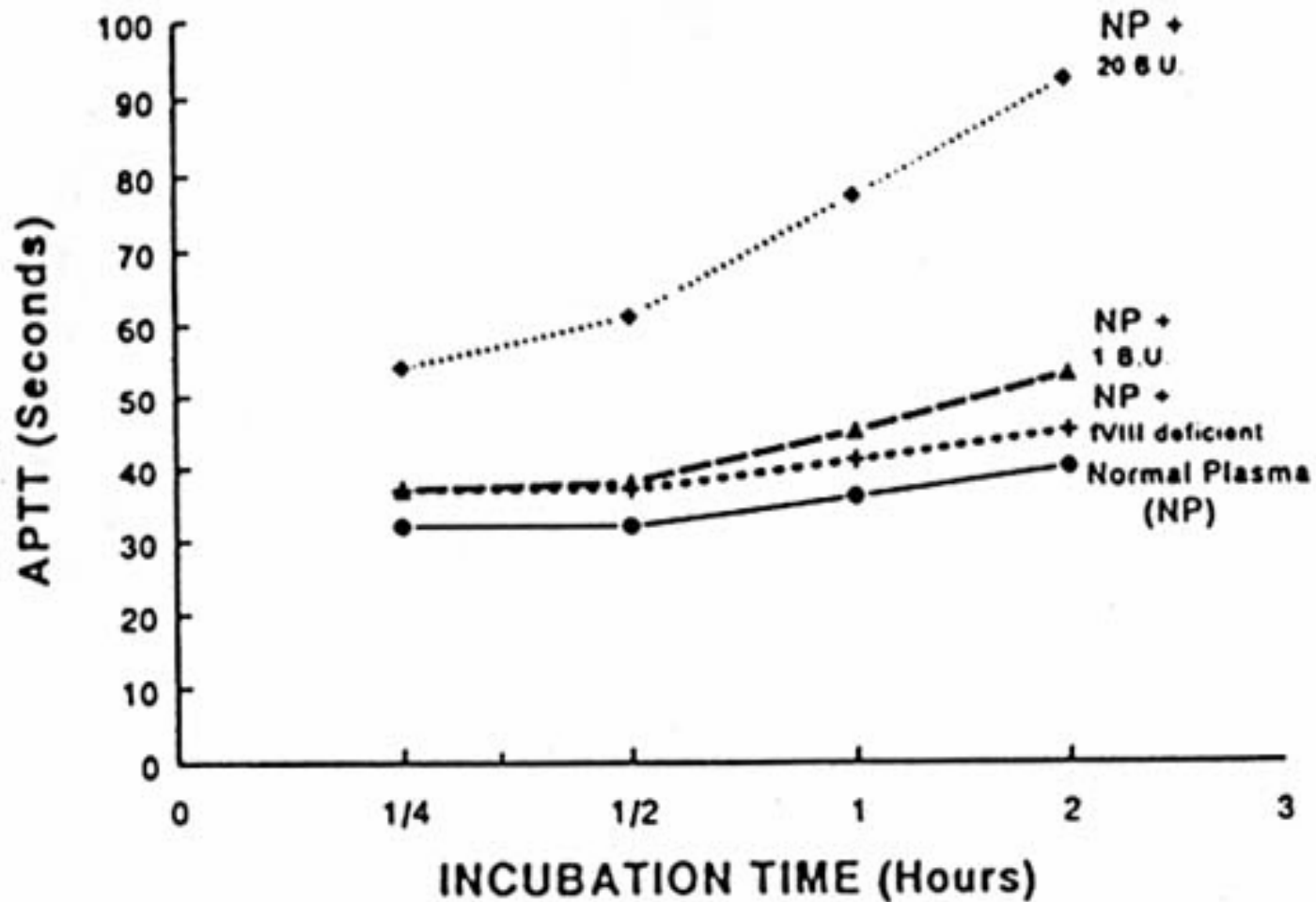


$t_{1/2}$ - 4h recovery $1/2$ of expected

(Goldsmith 1994)

APTT AS A SCREEN FOR INHIBITORS

Incubated mixture of test and normal plasma



(Goldsmith 1994)

Screening for inhibitors: UK guidelines

British Journal of Haematology 11: 78-90 (2000)

Screen for inhibitors:

- every 5th exposure day, or every 3 months, until 20th exposure then
- Every 6-12 months thereafter, and
- Prior to any surgical procedure
- If clinical response to factor is unexpectedly poor

Acquired Haemophilia A

- Rare - approximately 1 per million per year
- 40-50% associated with other conditions
- Post partum, malignancy, autoimmune diseases, RA, SLE
- Bleeding pattern differs from congenital haemophilia A – skin (bruising), muscles, haematuria, haematemesis, melaena (Not Haemarthrosis)

Initial Diagnosis

- Prolonged APTT – can be longer than severe congenital haemophilia A
- APTT on mixture of patient/normal plasma (20%, 50%) is intermediate between test and normal value (compare with deficiency states and lupus antibodies)
- Prothrombin Time , Thrombin Time normal

Differential diagnosis from Lupus anticoagulant

- Prolongation of APTT is extreme with all reagents, including lupus insensitive reagents such as Actin FS
- APTT on a 50:50 mix prolongs over 1- 2 hours
- DRVVT normal or borderline
- Inhibition similar in all 1 stage intrinsic assays
- Specific anti FVIII shows less interference in FIX and XI assays than FVIII:C (often undetectable)
- Beware patients with combinations of antibodies

Correction tests APTT

8 different Acquired Haem A

(Normal <37sec)

Bethesda	APTT (sec)	+ 20% np	+50% np
1.1 u/ml	83	52	38
1.0 u/ml	210	137	77
2.0 u/ml	82	43	34
6.6 u/ml	107	51	37
8.4 u/ml	150	55	39
21 u/ml	145	62	48
23 u/ml	123	127	55
120 u/ml	69	50	38

Factor VIII:C assays in Acquired Haemophilia

- One stage Factor VIII:C assay often < 1 U/dl
- Some activity may be detectable and in this case non parallel assays occur
- Two stage clotting assay sometimes gives higher results

Classical Bethesda assay

- Pooled normal plasma containing FVIII
- Incubated for 2 hours at 37°C
- Some factor VIII activity is lost
- Control mixture of PNP + Buffer
- Does FVIII activity loss from test plasma/PNP exceed loss from buffer/PNP? (control)

Classical Bethesda

Problems

- May not be reproducible for low results
- False positive results may occur as a result of loss of FVIII activity not related to inhibitor activity
- Nijmegen modification
(Thromb Haem 1995; 73: 247-51)

Nijmegen modification

- Buffer the FVIII in the pooled normal plasma (0.1M imidazole to pH 7.4).
(Note that commercially available lyophilised PNP may be buffered differently)
- Control mixture constructed with FVIII deficient plasma (with VWF) in place of buffer
(note that 4g% albumen may be a possible replacement for FVIII deficient)

FVIII Inhibitor method

- WFH Laboratory manual
- Downloadable (free !)
- Website : www.wfh.org
 - WFH library/Publications
 - Other publications
 - Diagnosis of Haemophilia and other bleeding disorders. A laboratory manual
S Kitchen & A McCraw

Bethesda units

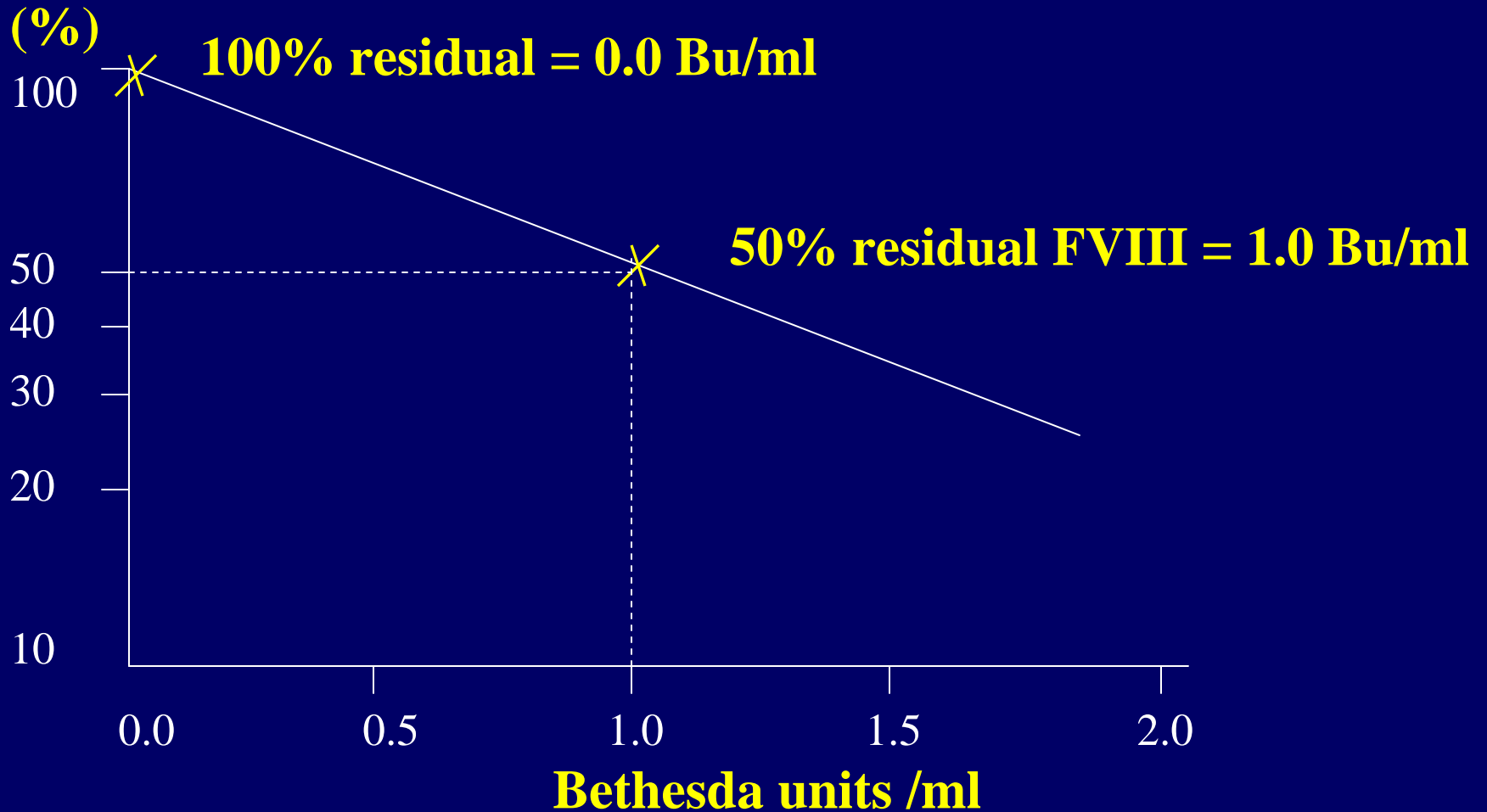
- 1 unit/ml inhibitor destroys 50% of the added FVIII in 2 hrs at 37°C
- Graph relating inhibitor concentration to residual FVIII constructed from 2 points
 - 100% residual FVIII = 0 u/ml inhibitor
 - 50% residual FVIII = 1.0 u/ml inhibitor

Graph for Bethesda Assay

- Inhibitor concentration on horizontal axis using linear scale
- Residual FVIII plotted on vertical axis on a log scale

Bethesda Inhibitor graph

**Residual
FVIII
activity**

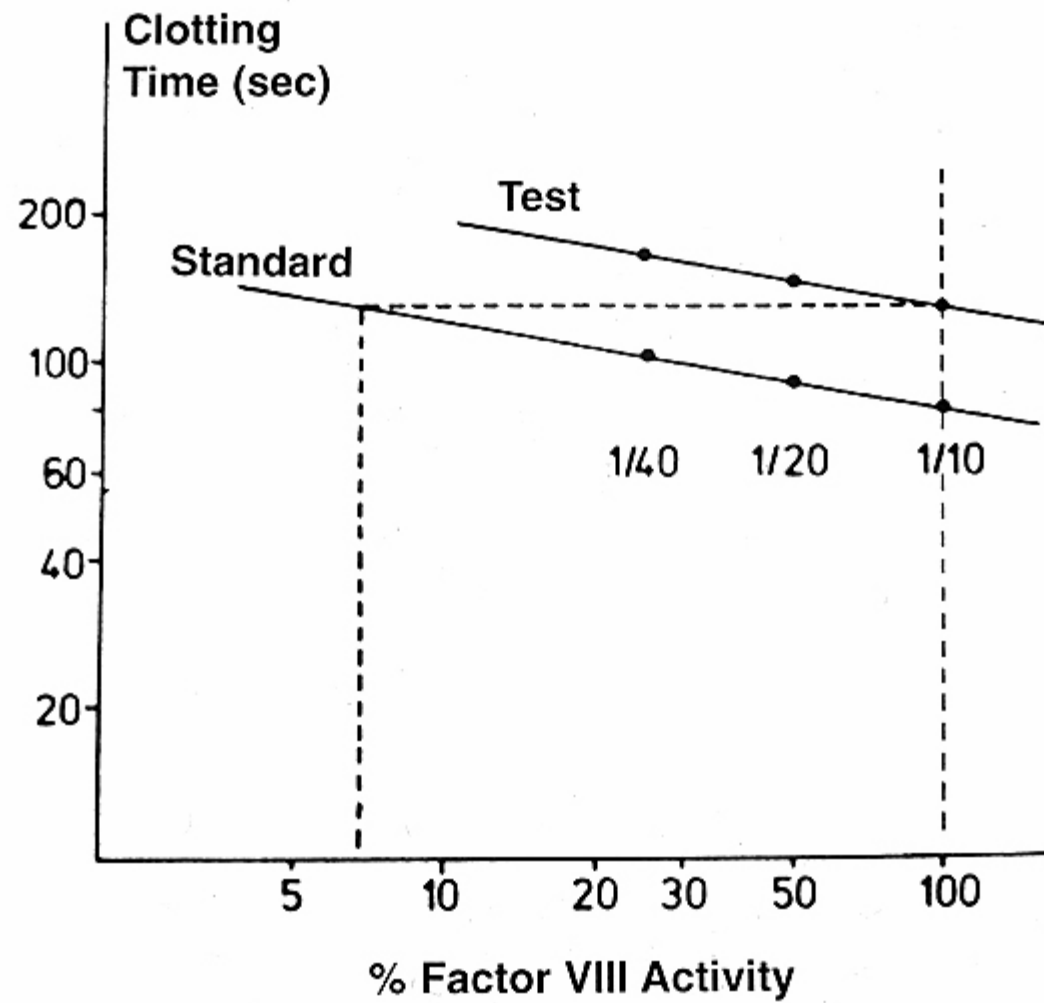


Inhibitor assays – congenital Haem A

Pre dilution of test plasma	Residual FVIII:C (%)	Inhibitor estimate
1 in 4	33%	6.4 u/ml
1 in 8	61%	5.6 u/ml
1 in 16	75%	6.3 u/ml

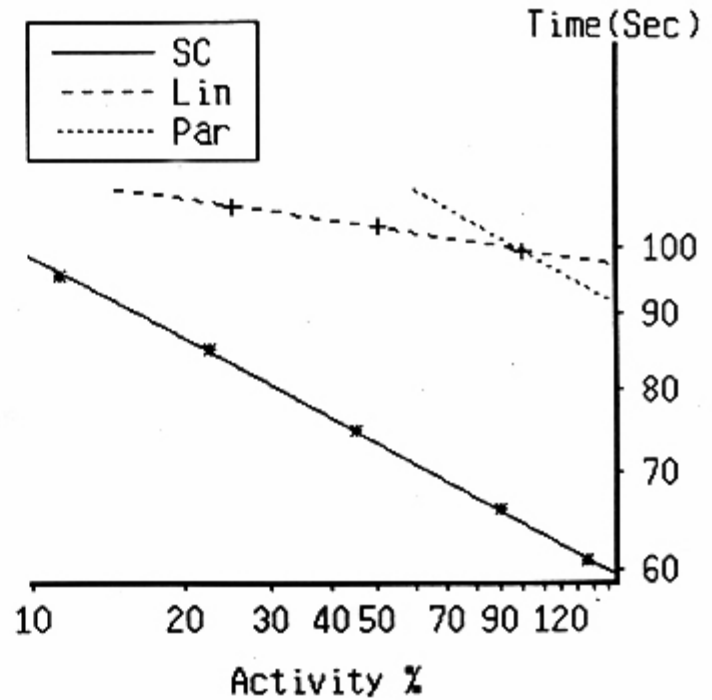
Acquired Haemophilia -Bethesda assays often non linear

Pre dilution of test plasma	Residual FVIII:C (%)	Inhibitor estimate
neat	16%	-
1 in 2	20%	-
1 in 5	21%	-
1 in 10	44%	11 u/ml
1 in 100	71%	50 u/ml



Factor VIII MDA
 ID No 7004 [0004-04]
 29/ 9/97 16:03 37.1°C

MDA Ratio	Clot time	Activity %
1/ 1	99.0 sec	9.5 %
1/ 2	103.0 sec	15.3 %
1/ 4	106.0 sec	26.1 %
		Mean 17.0 %
SCr= -1.000		Test r= -0.996



Factor IX and XI assays in presence of anti FVIII:C (11 u/ml)

Dilution	Factor IX:C	Factor XI:C
1 in 10	25 u/dl	17 u/dl
1 in 20	61 u/dl	39 u/dl
1 in 40	88 u/dl	50 u/dl
1 in 80	92 u/dl	86 u/dl
1 in 160	108 u/dl	165 u/dl

Acquired Haem A

Errors in diagnosis

(Kazmi, Savage et al 1998)

test	Case 1	Case 2
APTT ratio	2.9	2.1
APTT 50/50	2.6	2.8
FVIII:C	< 1 u/dl	< 1 u/dl
FIX	<1 u/dl	< 1 u/dl

Acquired Haem A

Errors in diagnosis

(Kazmi, Savage et al 1998)

Test	Case 1	Case 2
ELISA FVIII inhib	Postive++	Neg
DRVVT	1.1	1.7
FVIII Bethesda	105 u/ml	28 u/ml
FIX Bethesda		71 u/ml
High diln FIX assay	81 u/ml	<1 u/ml
Chromogenic FVIII	<1	209 u/ml
	Acquired Haem A	Potent Anti phospholipid

Diagnosis of Acquired haemophilia A

- Screening tests and mixing experiments
- APTT on incubated mixtures?
- Factor VIII:C, IX and XI assays with multiple test dilutions
- Exclude Antiphospholipid/Lupus antibodies
- Specific Inhibitor assays with multiple pre dilutions
- Keep an open mind and get the full picture!!