Laboratory guidance for the measurement of Factor VIII Inhibitor

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Risk Inhibitors in haemophilia A

Overall: 20 – 30%

Non-severe: 3 – 14%

Severe: > 40%

Chambost, H. (2010) Haemophilia; 16, suppl.2: 10 – 15

DiMichelle, D.M. (2013) Pediatr Blood Cancer; 60:S30–S33

Risk Inhibitors in haemophilia B

Overall: 1.5 - 3%

Severe: 9 – 23%

DiMichele, D.M. (2005) BJH; 138: 305 - 315

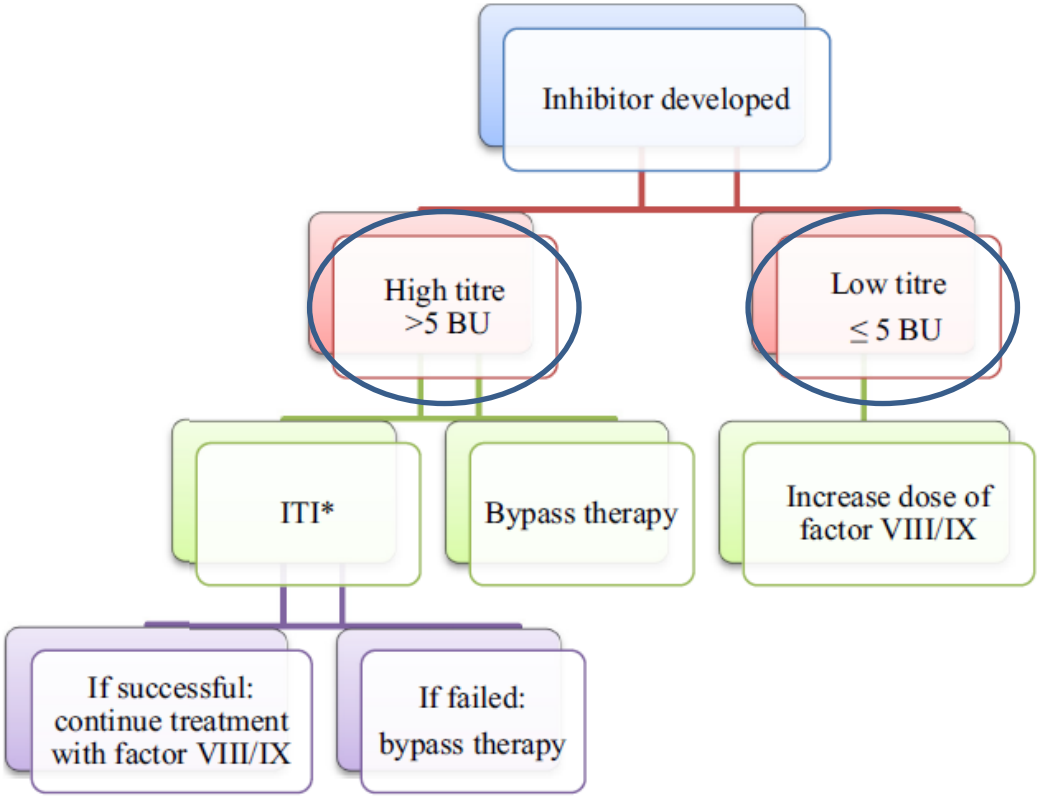
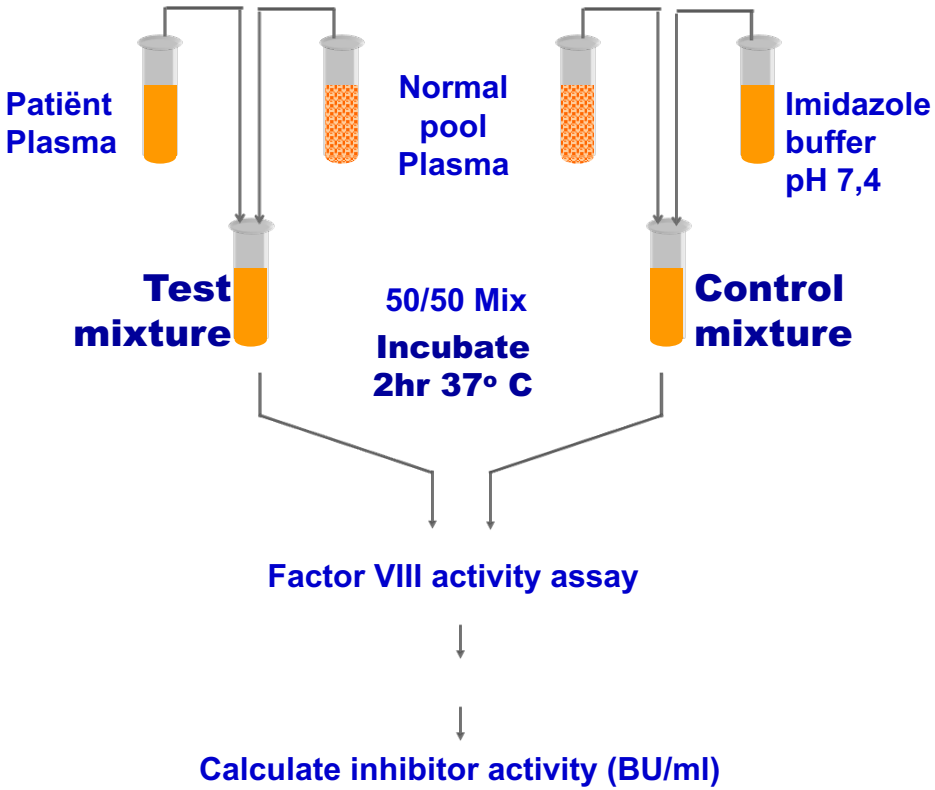


Fig. 2 *Haemophilia inhibitor treatment algorithm. *ITI is the gold standard of treatment for inhibitor eradication. ITI, immune tolerance induction; BU, Bethesda unit.*

Osooli, M. *et al* (2015) J Int Med; 277: 1 - 15

Factor VIII Inhibitor Testing

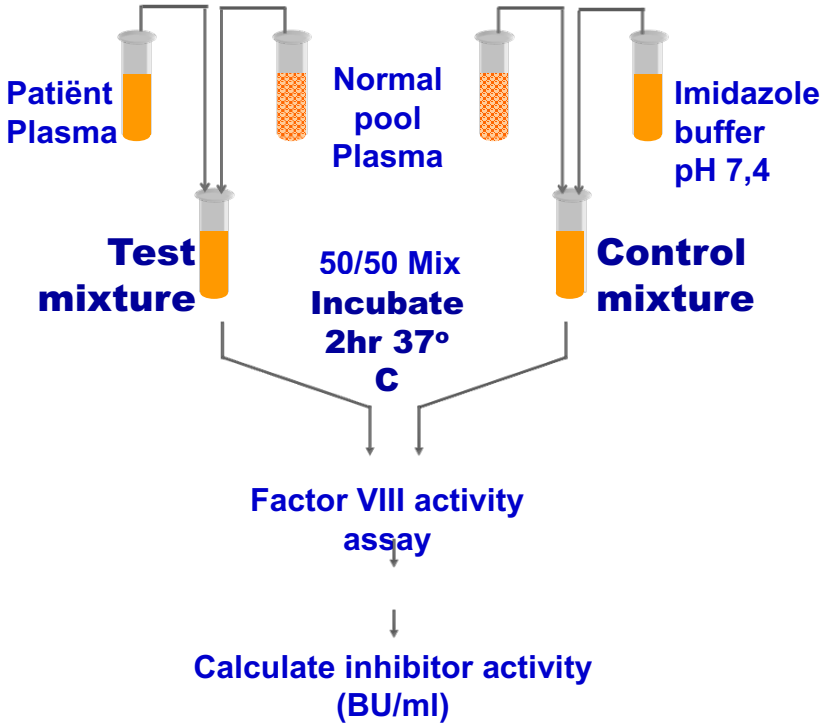
BETHESDA ASSAY



Kasper *et al* (1975) Thromb Diath Haemorrh; 34: 869 - 72

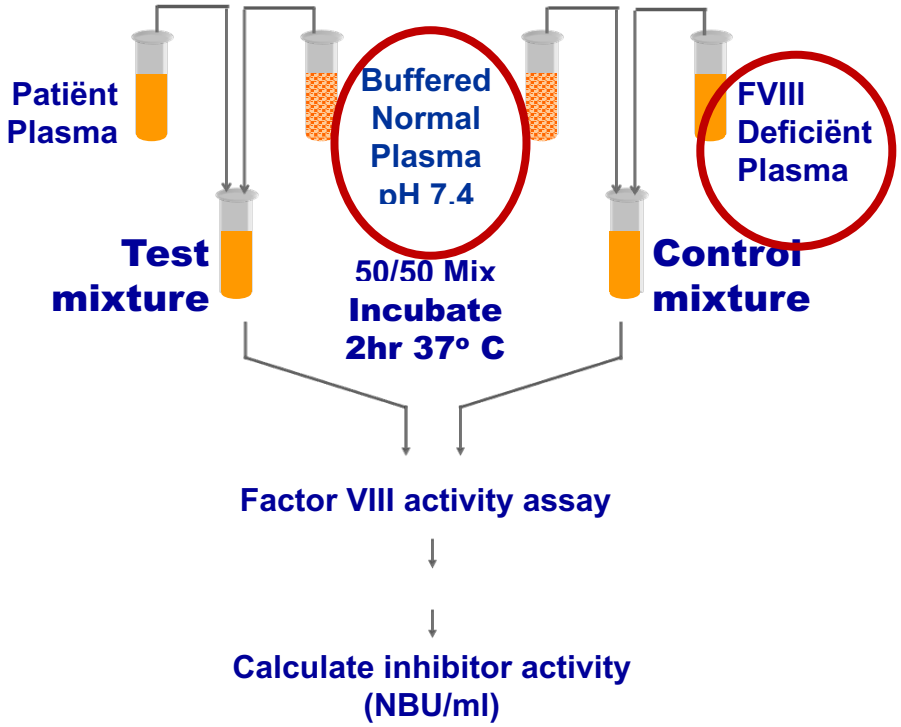
Factor VIII Inhibitor Testing

BETHESDA ASSAY



Kasper *et al* (1975) Thromb Diath Haemorrh; 34: 869 - 72

NIJMEGEN ASSAY



Vebruggen *et al* (1995) Thromb Haemost; 73: 247 - 51

ICSH guideline initiatives in Haemostasis

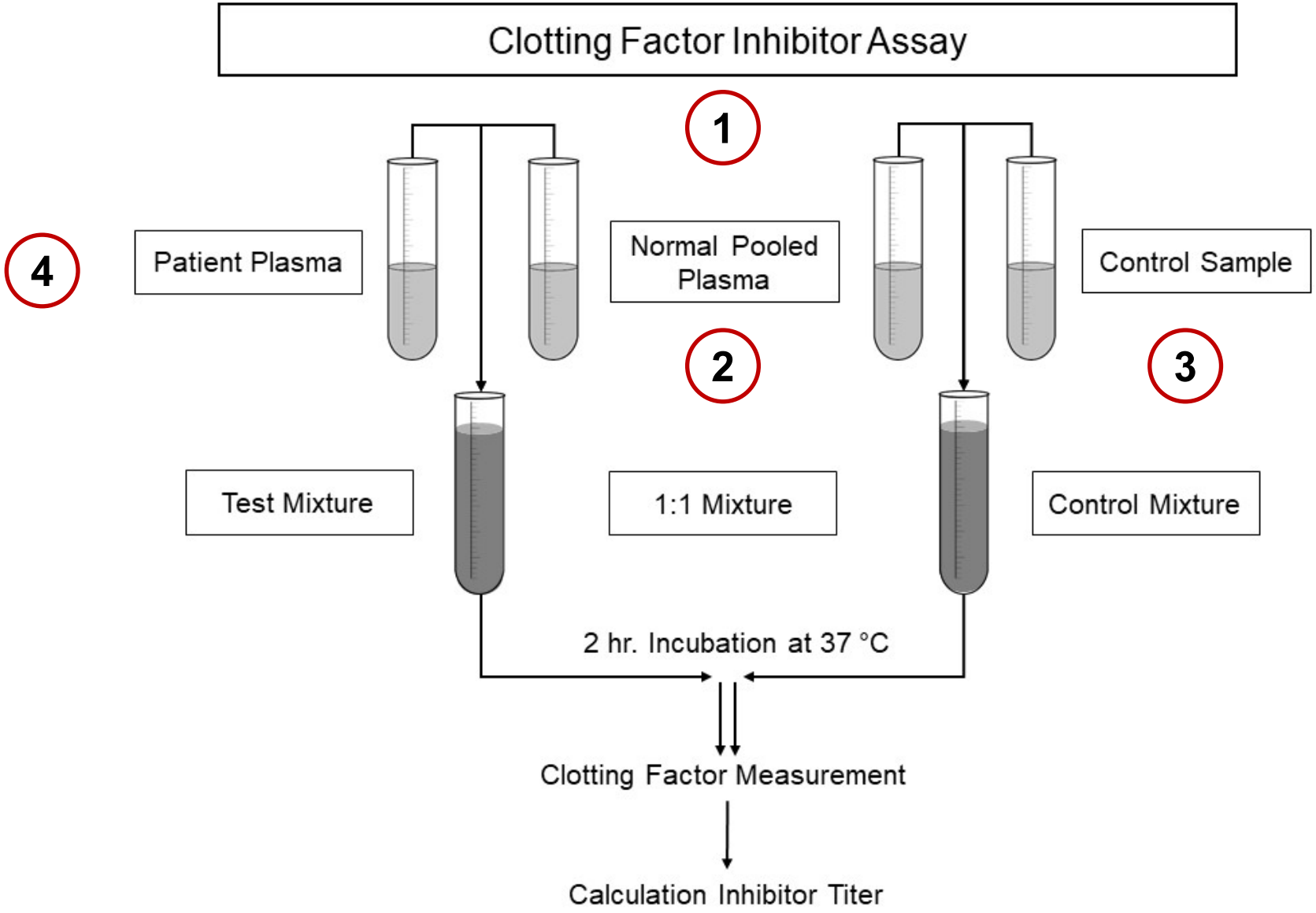
Guideline for laboratory testing of FVIII and FIX inhibitors

Guideline group

Piet Meijer (NL) – chair
Flora Peyvandi (Italy)
Rajiv Pruthi (USA)
Guy Young (USA)
Silmara Montalvão (Brazil)
Clarence Lam (Hong Kong)

Guideline issues

- Clinical context
- Pre-analytical issues
- Assay principle
- Buffering normal plasma
- Control Mixture
- Factor deficient plasma
- Factor concentration in normal plasma
- Measurement of FVIII/FIX
- Calculation of test results
- Quality control


Clotting Factor Inhibitor Assay
1
4
Patient Plasma
Normal Pooled Plasma
Control Sample
2
3
Test Mixture
1:1 Mixture
Control Mixture
2 hr. Incubation at 37 °C
Clotting Factor Measurement
Calculation Inhibitor Titer

1 Pre-heating

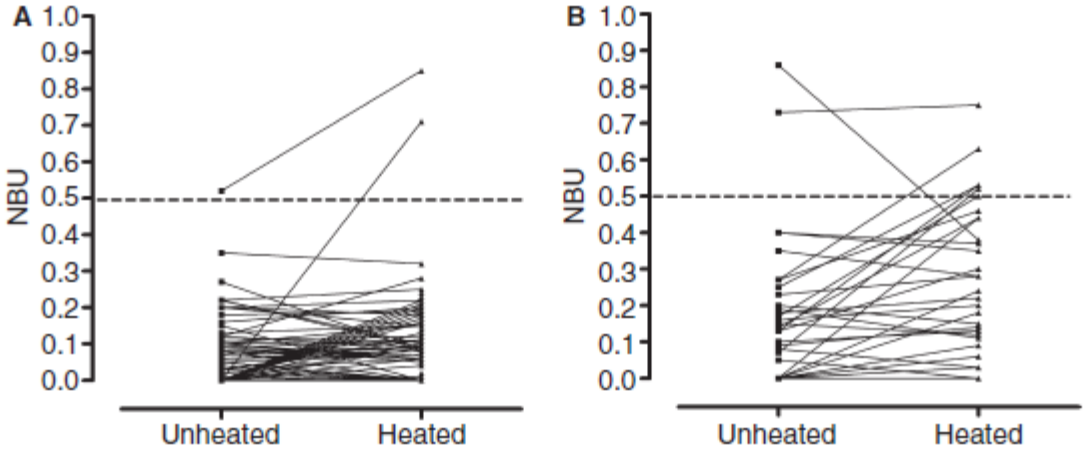


Fig. 1. Change in factor VIII inhibitor titer with heat treatment. Results are shown before rounding to one decimal place. (A) Patients with a negative history of inhibitor. (B) Patients with a positive history of inhibitor.

Miller *et al* (2012) J Thromb Haemost; 10: 1055 - 61

1 Pre-heating

Table 1
FVIII coagulation activity (FVIII:C) and FVIII antigen (FVIII:Ag) in samples from group II and group III patients that became positive in Nijmegen–Bethesda assay after heat treatment.

Patient		FVIII:C (IU/dL)	FVIII:Ag (ng/dL)	Inhibitor titer (BU)	
	Reference range	60–120	64–189[a]	0.6	
	Detection limit	0.2	0.8	0	
				Non-heated sample	Heated sample
Group II patients with history of inhibitor not recently exposed to FVIII					
1		0.8	3.25	0	0.95
2		0.1	4.46	0	0.9
3		0.1	1.42	0	3.31
4		0.1	1.43	0	3.41
5		0.3	1.50	0.2	2.05
6		0.3	1.53	0.54	6.46
Group III patients undergoing ITI treatment					
ITI-2		1.6	2.11	0.19	2.47
ITI-1		1.8	20.55	0.24	3.62
ITI-4		0.2	7.42	0.28	0.87
ITI-4		0.2	6.00	0.29	1.8
ITI-2		0.5	1.64	0.39	3.69

Montalvão *et al* (2015) Thromb Res; 6: 1280 - 84

2 + 3 Buffering + Control Mixture

B

N

Table 1 pH values and remaining FVIII:C activities in test- and control mixtures of the original and modified Bethesda assay

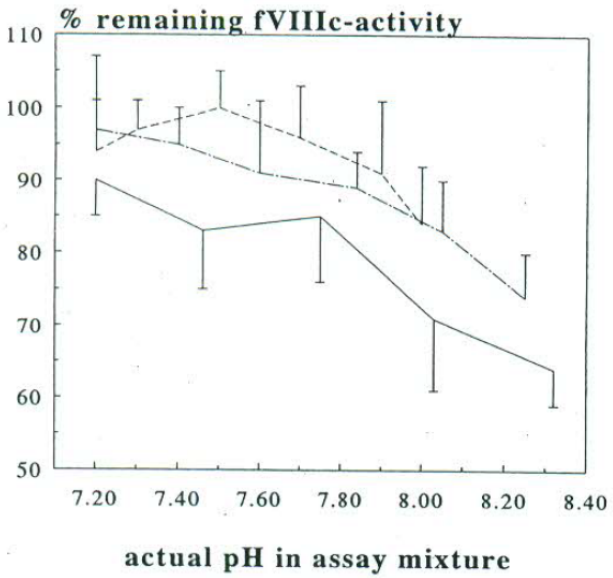
Assay mixtures	pH before incubation	pH after 2 h incubation	Remaining FVIII:C activity (%)
Non-buffered N-pool and haemophilic plasma (inhibitor free) 1:1	7.7 ± 0.1	8.3 ± 0.1	68 ± 6
Non-buffered N-pool and 0.1 M imidazole buffer pH 7.4 1:1	7.6 ± 0.1	7.8 ± 0.1	83 ± 6
0.1 M imidazole buffered N-pool pH 7.4 and haemophilic plasma 1:1	7.4 ± 0.1	7.4 ± 0.1	95 ± 5
0.1 M imidazole buffered N-pool and immunodepleted factor VIII deficient plasma 1:1	7.4 ± 0.1	7.4 ± 0.1	97 ± 4
0.1 M imidazole buffered N-pool and 0.9% saline 1:1	7.4 ± 0.1	7.4 ± 0.1	83 ± 8

All data are the means of 5 determinations ± the standard deviation.

Verbruggen *et al* (1995) Thromb Haemost; 73: 247 - 51

2 + 3 Buffering + Control Mixture

Effect Buffering NPP



Imm. depl. plasma

Haem. plasma

Saline

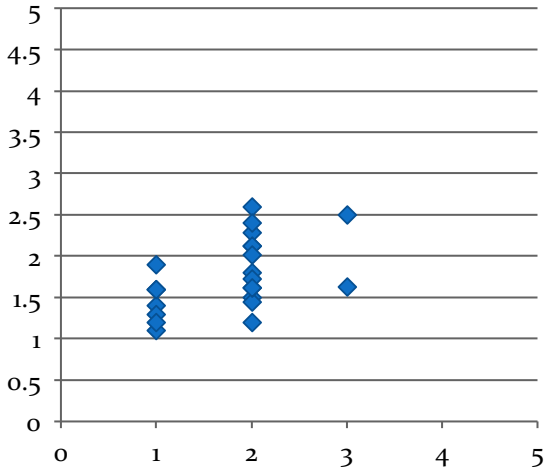
Verbruggen *et al* (1995) Thromb Haemost; 73: 247 - 51

4 Effect Pre-dilution

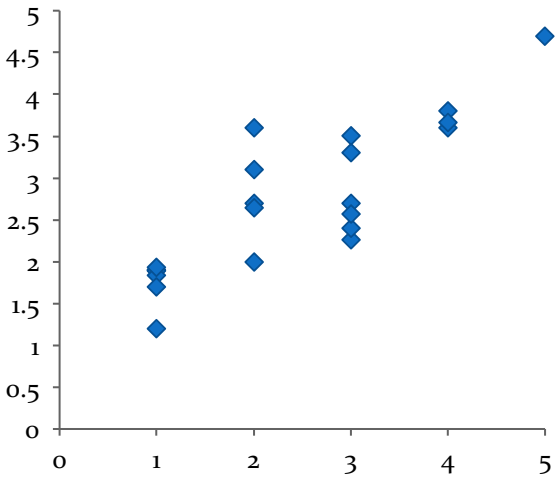
	diluted		undiluted	
	mean	CV (%)	mean	CV (%)
sample 1	3.99	27.9	1.78	9.5
sample 2	1.88	28.2	0.83	23.9
sample 3	0.94	58.0	1.00	19.0
sample 4	0.34	141.4	0.54	24.0
sample 5	2.55	35.3	1.82	17.1

4 Effect Pre-dilution

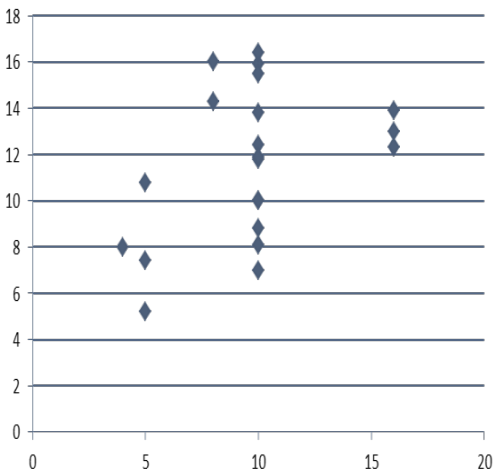
Sample 5 (VC 22%)



Sample 1 (VC 31%)



Sample 6 (VC 27%)



Appendix 1

Procedure for Standardization of Factor VIII inhibitor assay

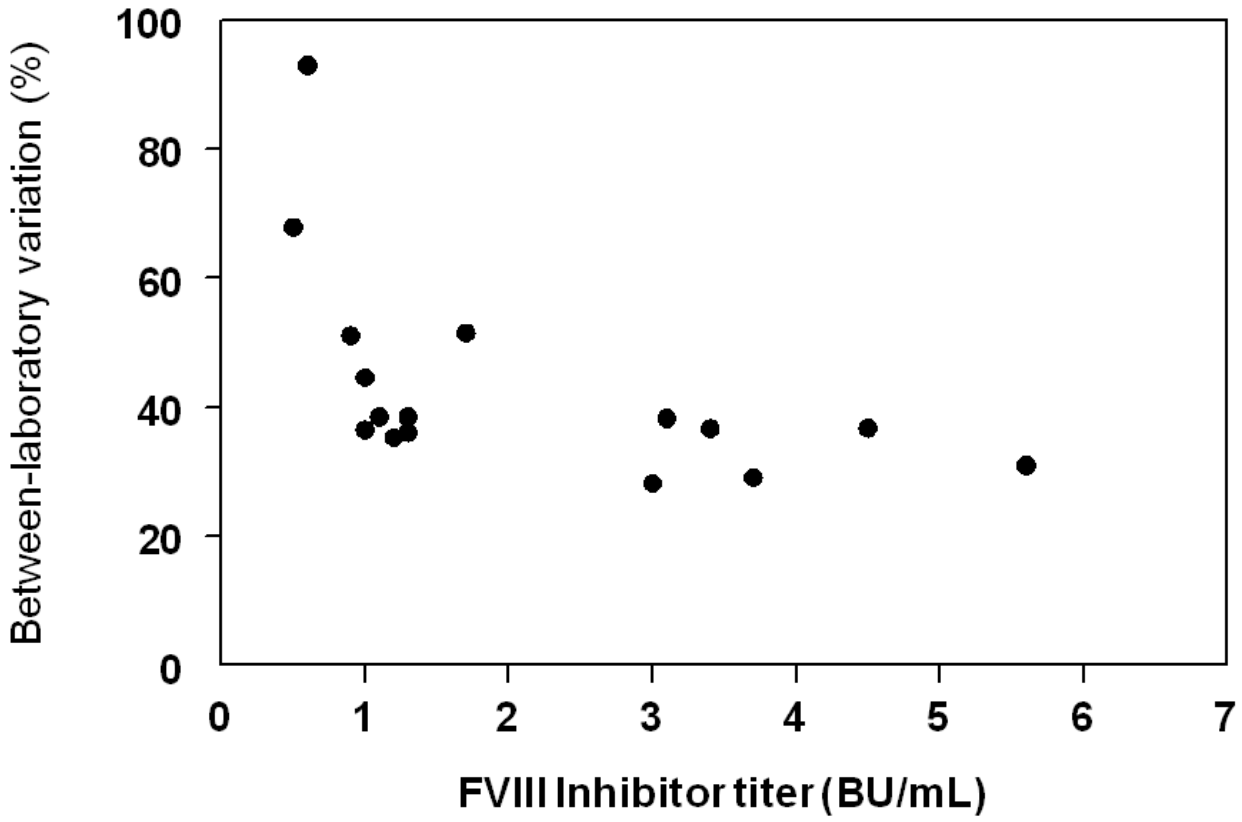
Inhibitor samples

- The samples have to be assayed undiluted or, in case of titers >2BU/mL, in a dilution as low as possible. In the diagnostic practice it renders the next combination of measuring ranges and dilution factor:

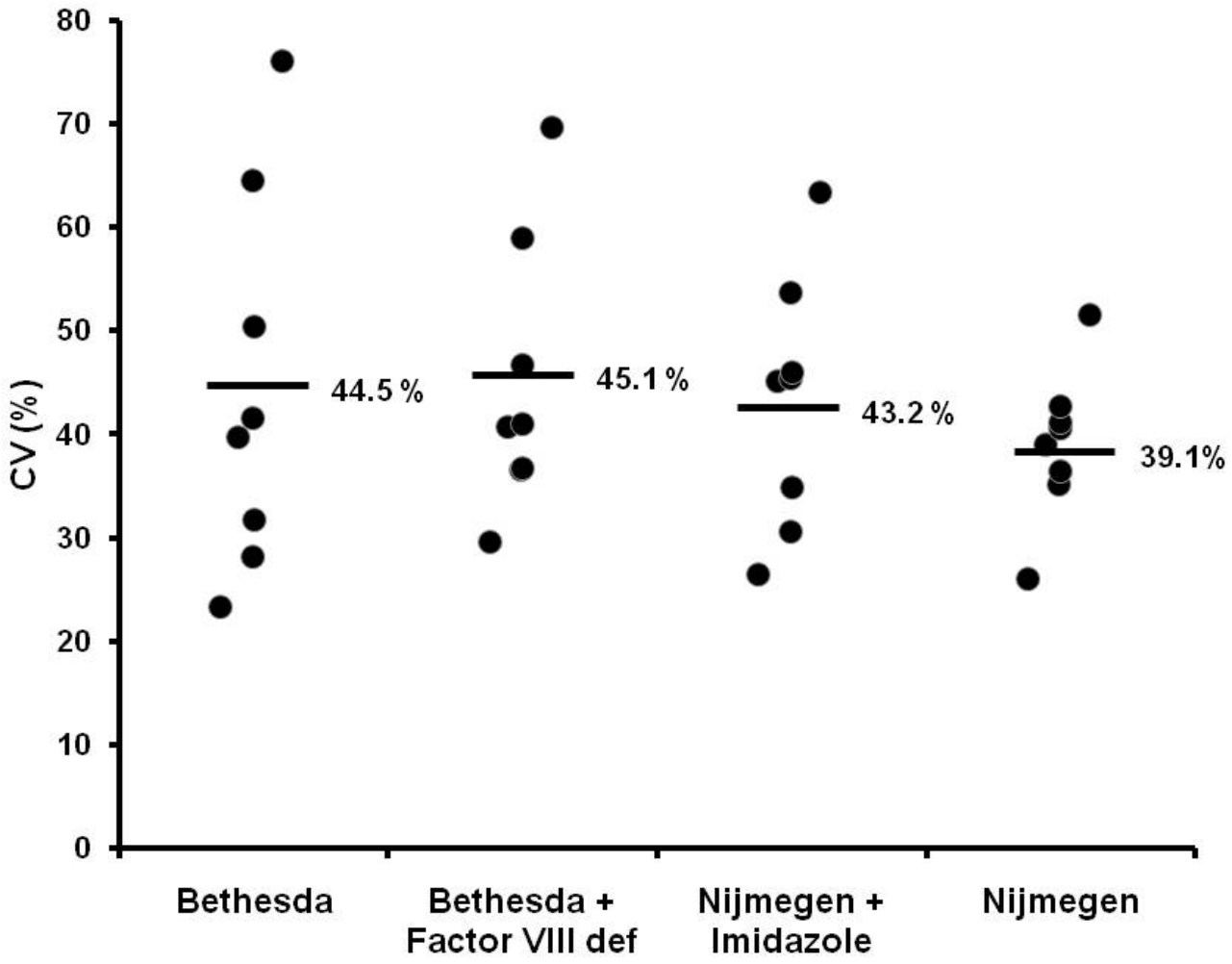
Inhibitor range (BU/ml)	Corresponding dilution factor
0-2.0	0
2.1-6.0	3 (= 1+2)
6.1-20.0	10 (= 1+9)
20.1-60.0	30 (=1+29)

- Dilutions have to be prepared with factor VIII deficient plasma.

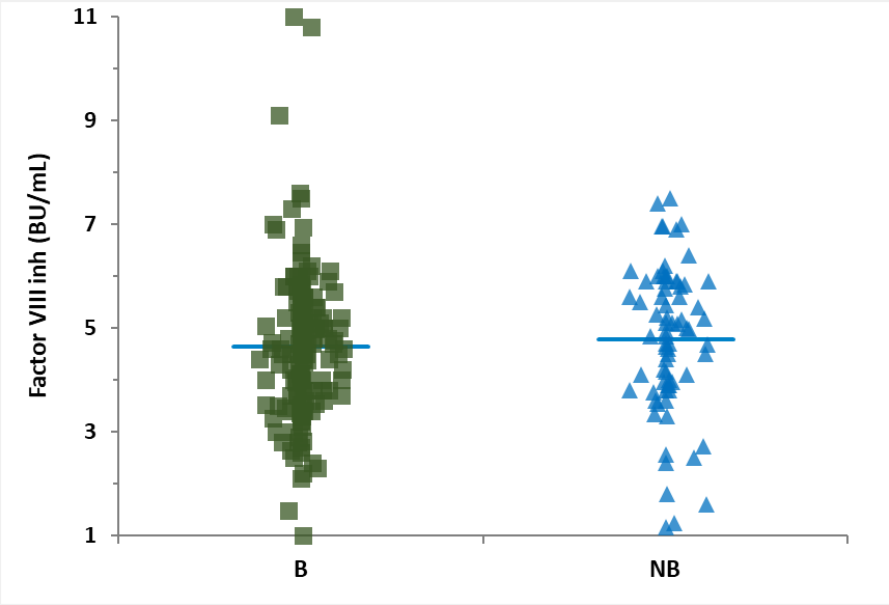
Factor VIII Inhibitor Testing

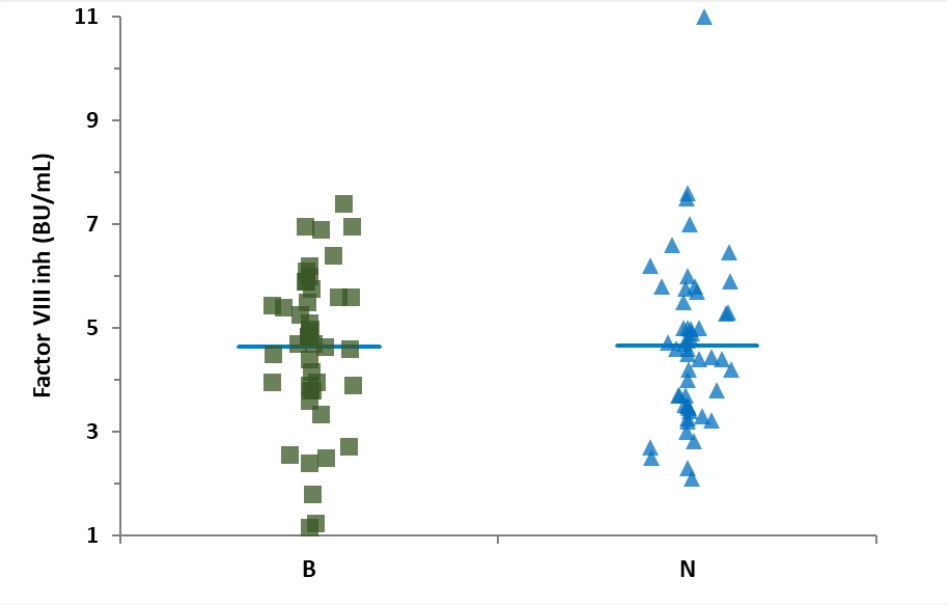


Factor VIII Inhibitor Testing



Factor VIII Inhibitor Testing





4.6	4.8	4.6	4.7
30.4%	29.2%	32.6%	34.0%

Is standardisation for Factor Inhibitor assays feasible?

Workshop Results

Table 2 Mean and coefficient of variation of the inhibitor results of the six inhibitor-positive samples in the two preworkshop surveys, in 51 respectively. the selected 15 laboratories, and in the four sessions of the workshop. The inhibitor activities are expressed in U mL^{-1} and the coefficient of variation (between brackets) as percentage

	Preworkshop survey		Workshop results			
Sample no. (nominal inhibitor activity)	51 laboratories	15 laboratories selected for the workshop	Session 1	Session 2	Session 3	Session 4
1 (1.6 U mL^{-1})	2.32 (35.8)	2.69 (42.6)	2.97 (39.0)	3.40 (40.7)	1.94 (9.6)	1.93 (7.6)
2 (0.8 U mL^{-1})	0.79 (49.0)	1.02 (30.6)	1.33 (69.1)	1.18 (51.4)	0.90 (14.1)	0.94 (5.2)
3 (1.4 U mL^{-1})	0.97 (41.2)	1.16 (38.6)	1.17 (30.2)	0.98 (34.6)	1.18 (12.5)	1.16 (6.4)
4 (0.7 U mL^{-1})	0.44 (69.5)	0.59 (68.7)	0.61 (45.2)	0.50 (42.8)	0.58 (14.7)	0.50 (13.4)
5 (2.0 U mL^{-1})	1.74 (36.0)	1.74 (37.1)	2.34 (41.0)	2.31 (35.7)	2.32 (12.8)	2.22 (12.0)
6 (15.0 U mL^{-1})	11.0 (35.8)	11.5 (44.3)	14.9 (41.3)	17.0 (38.1)	15.5 (19.1)	14.6 (5.8)
Mean CV	46.8	43.6	44.3	40.6	13.8	8.4

Verbruggen *et al* (2011) J Thromb Haemost; 9: 2003 - 8

	Pre-Workshop Survey (2009)		Workshop (2009)	results	Post-workshop survey (2010)	Standardized final survey 2012
Sample no. and nominal inhibitor activity	51 Laboratories	15 laboratories selected for the workshop	First Session	Last Session	13 Laboratories	22/51 Laboratories
1 1.6 BU/ml	2.3 (36%)	2.7 (43%)	3.0 (39%)	1.9 (8 %)	2.9 (41%)	2.7 (31%)
2 0.8 BU/ml	0.8 (49%)	1.0 (31%)	1.3 (69%)	0.9 (5%)	1.1 (88%)	0.7 (17%)
3 1.4 BU/ml	1.0 (41%)	1.2 (39%)	1.2 (30%)	1.2 (6%)	1.1 (31%)	1.0 (23%)
4 0.7 BU/ml	0.4 (70%)	0.6 (69%)	0.6 (45%)	0.5 (13%)	0.6 (61%)	0.5 (30%)
5 1.9 BU/ml	1.7 (36%)	1.7 (37%)	2.3 (41%)	2.2 (12%)	1.9 (31%)	1.8 (22%)
6 15.4 BU/ml	11.0 (36%)	11.5 (44%)	14.9 (41%)	14.6 (6%)	12.0 (36%)	12.4 (27%)
Mean CV	45%	44%	44%	8%	48%	25%

Concluding remarks

- Up to now there is still a lack in the standardisation of Factor Inhibitor testing.
- The current variability between laboratories may have impact on Haemophilia Inhibitor Treatment Algorithm.
- It has been shown that a decrease of the between-laboratory variation to approx. 15 – 20% is feasible.
- With the development of a laboratory guideline for the measurement of Factor VIII Inhibitor we hope to improve standardisation.