

Assay of Factor VIII:C and other
factors :
How can they be improved?

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Are FVIII assays affected by the Blood Sample collection system?

- 1 stage FVIII assay in 32 subjects
- Results equivalent in 8 different tubes (BD, Greiner, Sarstedt)
- Maximum difference in mean FVIII – 7%

(Munro et al 2003)

Stability of Frozen samples for FVIII assay (1 stage)

(Woodhams et al 2001)

	Frozen/stored – 74°C	Frozen/Stored –24°C
< 5% change	6 months	3 months
< 10% change	18 months	3 months

FVIII:C assay types

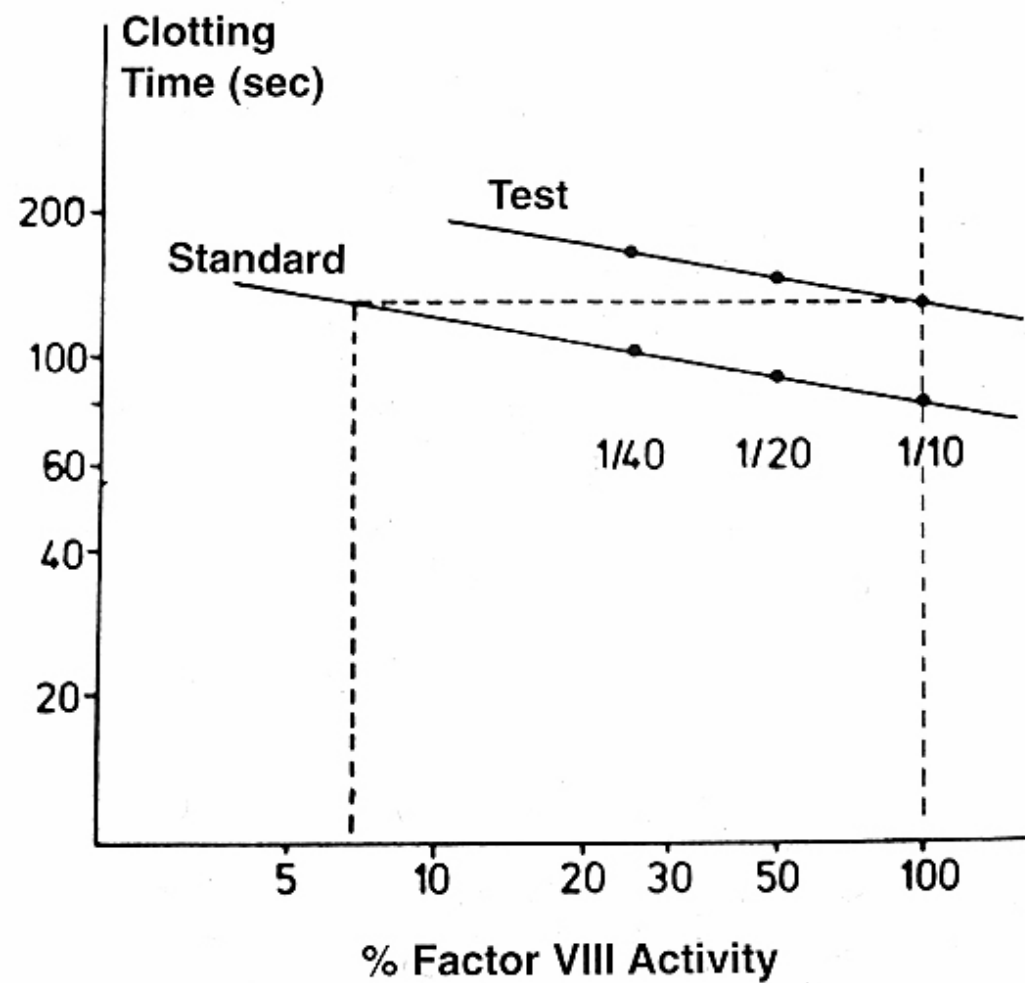
One-stage FVIII assay (*Langdell 1953*)

Two-stage FVIII assay (*Biggs 1955*)

Chromogenic FVIII assay (*Seghatchian &
Miller –Anderson 1978*)

One stage Assay - based on APTT

- Factor VIII deficient plasma has grossly prolonged APTT
- Add different concentrations of FVIII and the APTT corrects (shortens) more as more FVIII is added
- Different dilutions of a standard/reference plasma with known FVIII are added
- APTT performed on each mix
- Concentration plotted against clotting time to produce a calibration curve



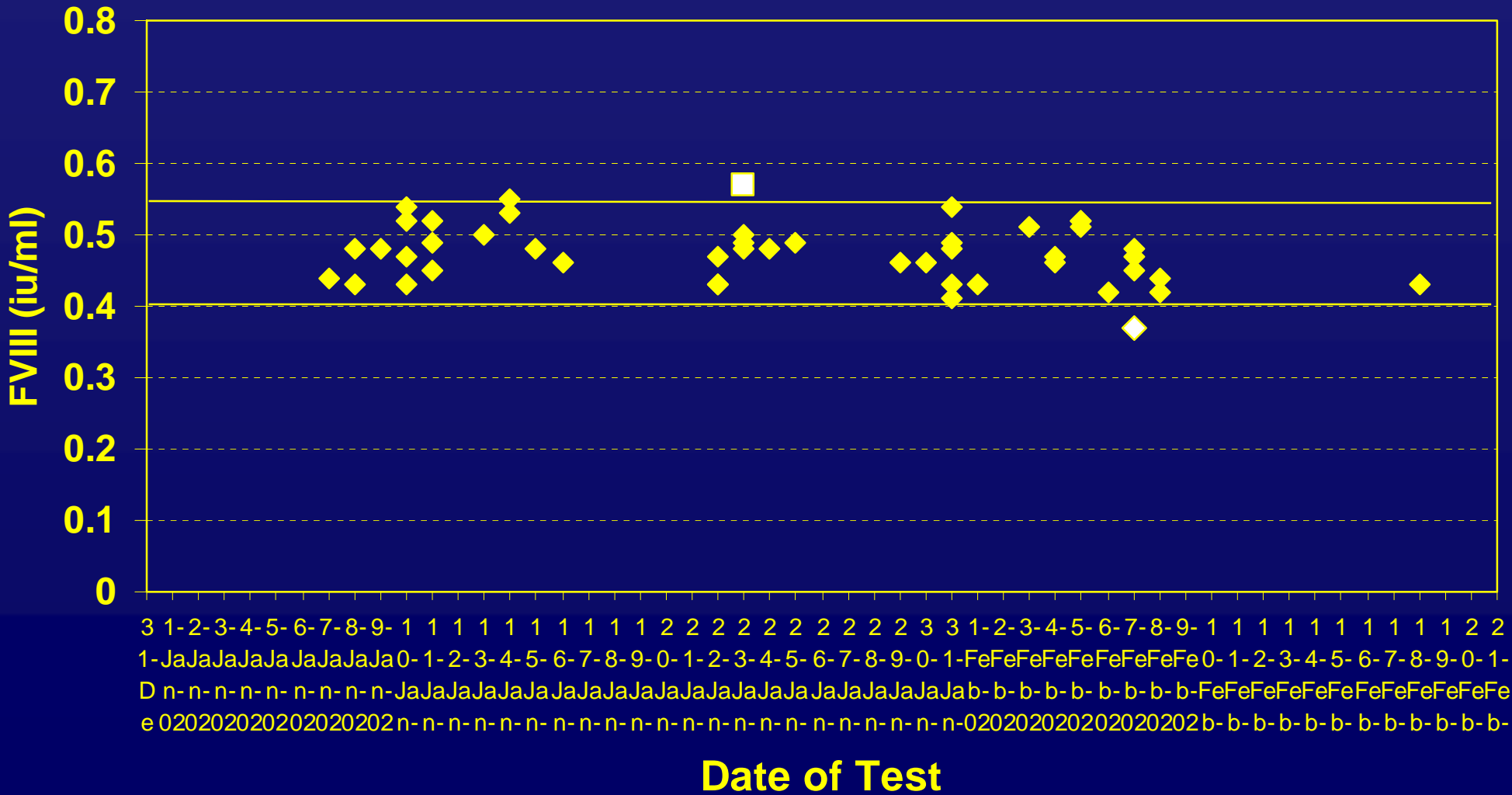
One-stage Factor VIII:C Assays

Important Components

- **Reference / calibration plasma**
 - traceable to WHO reference plasma
- **APTT reagent**
 - contact activator
 - phospholipid profile
- **Factor deficient plasma**
 - VWF content may be relevant
 - normal levels of other factors

Internal Quality Control results for FVIII:C Assay

Target range is mean +/- 2sd of 20 determinations



FVIII IQC out of target range?

- Suspend new patient testing and reporting of results since last QC result within limits.
- Replace QC material and retest. **Still out?**
- Replace reagents and retest. **Still out?**
- Suspend method and switch to backup

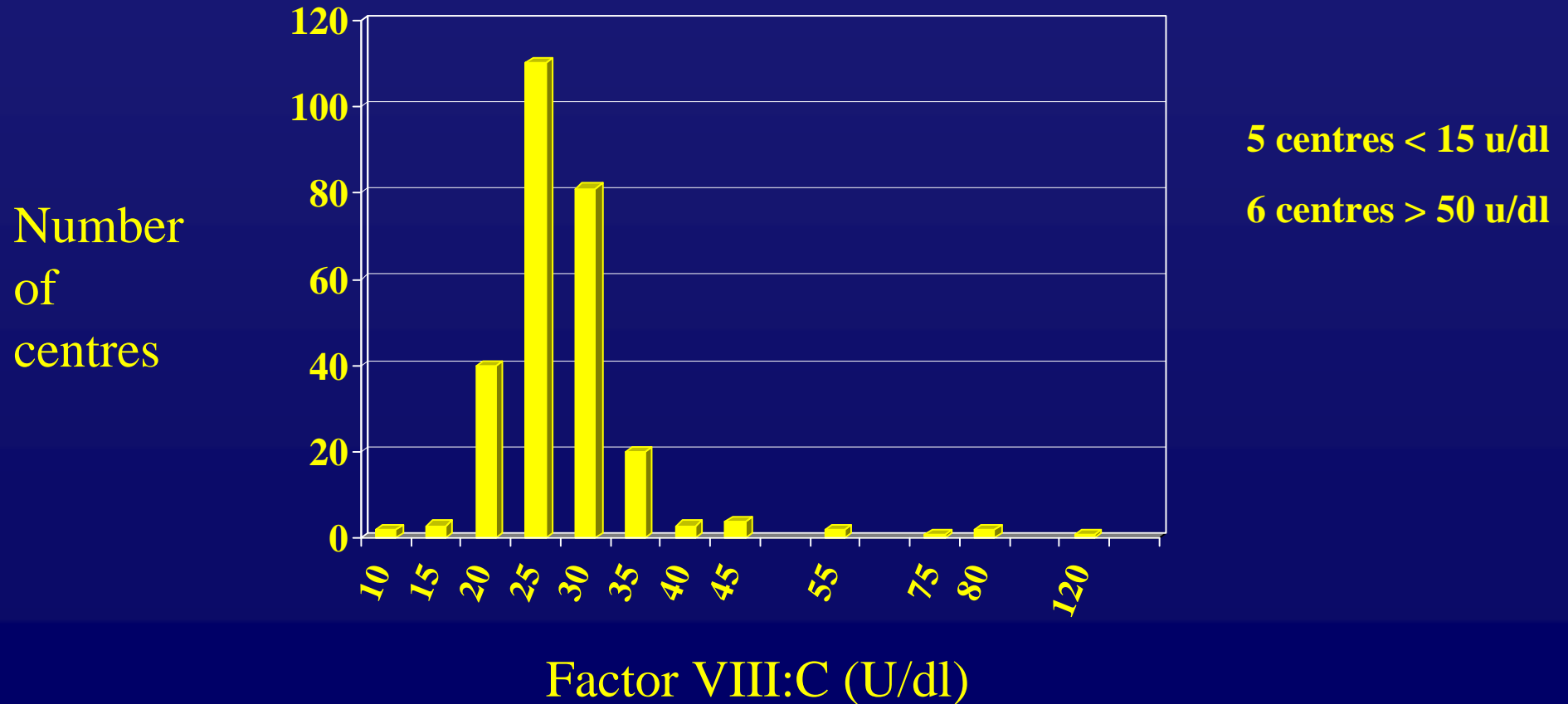
Factor VIII:C Assays

UK NEQAS Participants (2002)

- **No. of participants** **n=296**
- **One-stage** **n=288**
- **Two-stage** **n=3**
- **Chromogenic** **n=5**

Factor VIII:C results in Different centres

256 centres



Factor VIII:C Assay Results in Different centres

UK NEQAS

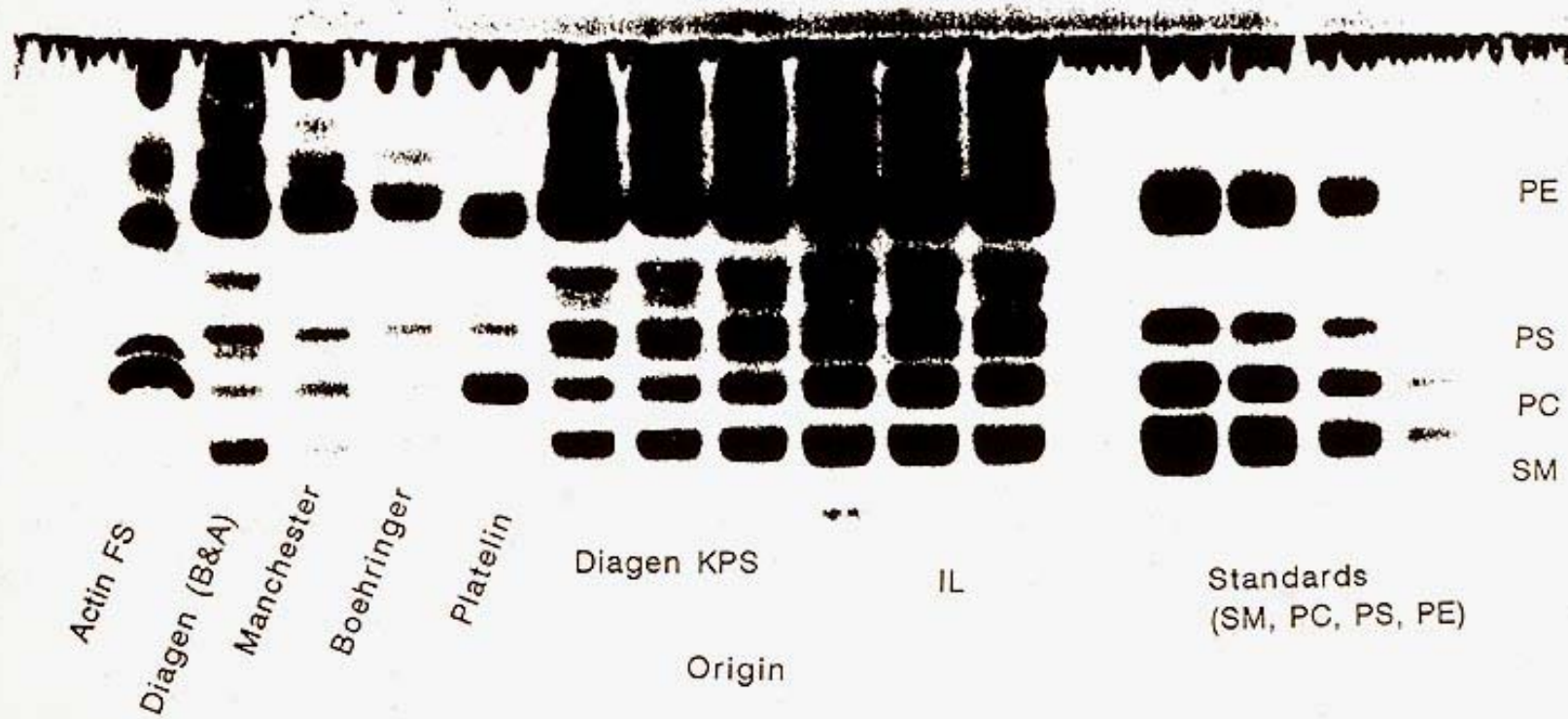
Median U/dl	Range U/dl	CV (%)
7.4	0.8-23	38
27	9 -119	34
151	85-294	20

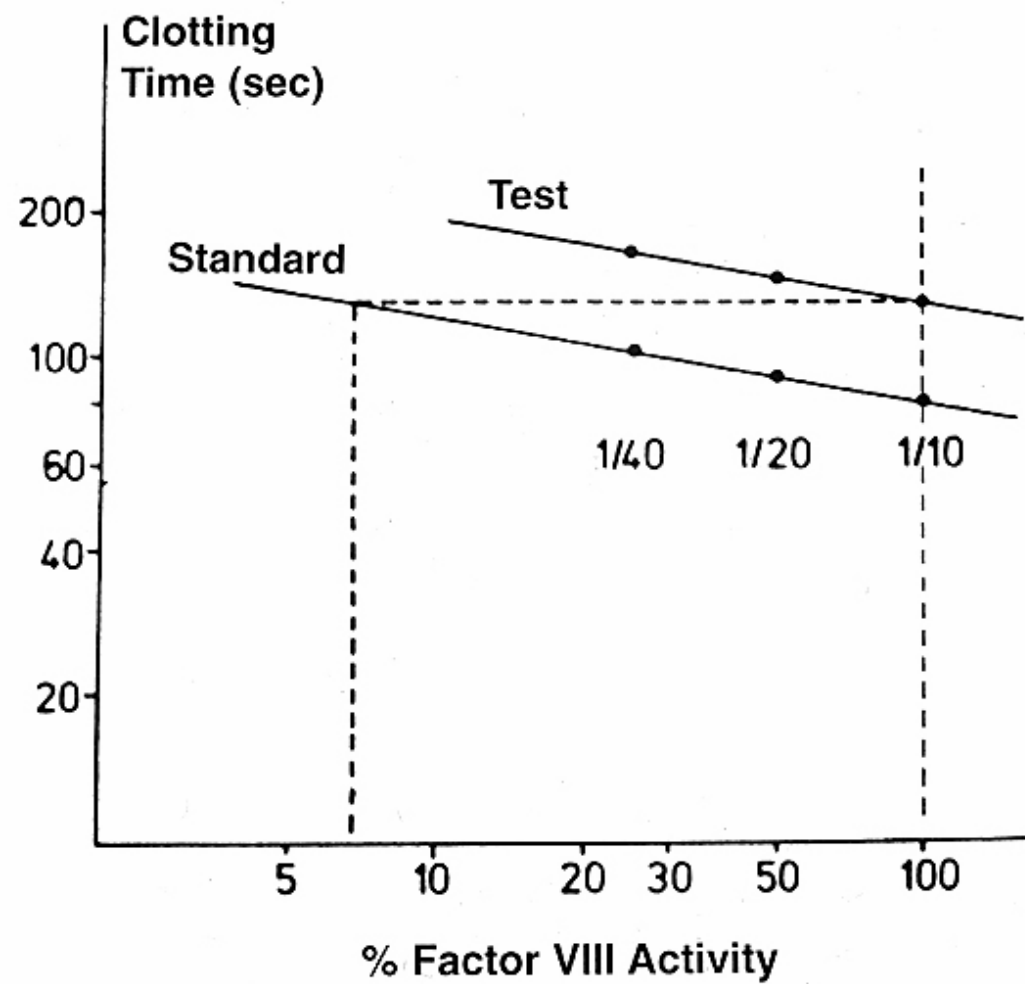
One-stage Factor VIII:C Assays

UK NEQAS Participants

- **29** **APTT reagents**
- **22** **Substrate plasma**
- **18** **Reference plasmas**
- **26** **Coagulometers**

Phospholipid quantitation by HPTLC/Laser densitometry





FVIII assay design

200 centres UK NEQAS 1999

- 25% of centres employ a stored calibration curve
- 35% use a single test dilution
- 22% use 2 test dilutions, 40% of centres employ 3 or more test dilution

Factor assays - Why 3 test dilutions?

FIX supplementary exercise 2003

Test dilutions	n	Mean FIX U/dl	CV %
1	22	6.3	54%
2	17	6.5	29%
3	42	6.0	23%
ANOVA		ns	P = 0.03

FIX assay design

100 UK Haemophilia centres UK NEQAS 2003

- 25% used single test dilution, 20% used 2 dilutions,
- 55% used 3 test dilutions
- 32% used a stored calibration curve

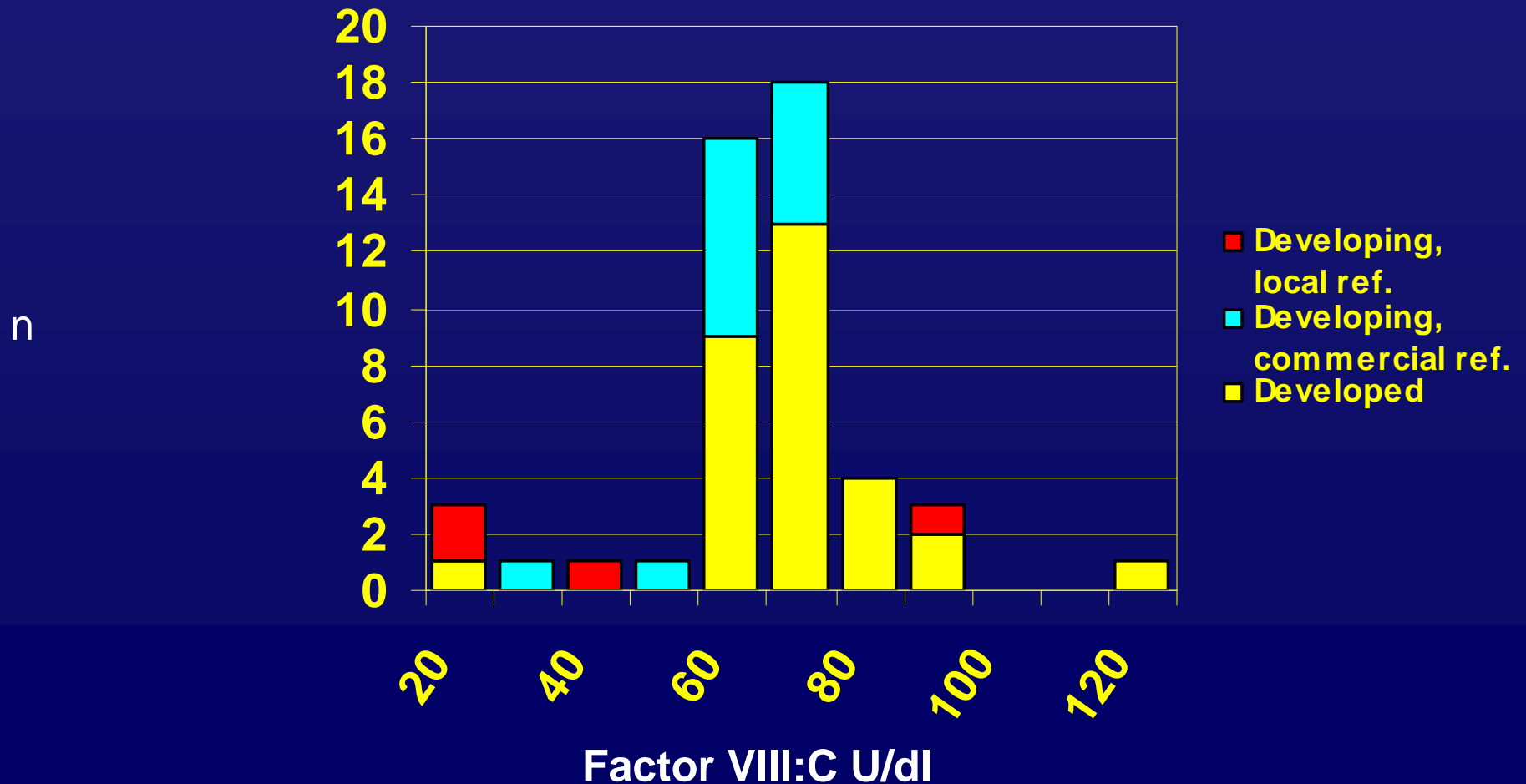
Factor VIII:C – NEQAS survey

Commercial reference plasmas (n>10)

Source	n	Median (u/dl)
1	46	86
2	81	76
3	86	76
4	14	72
5	12	73
6	10	75
All	299	77

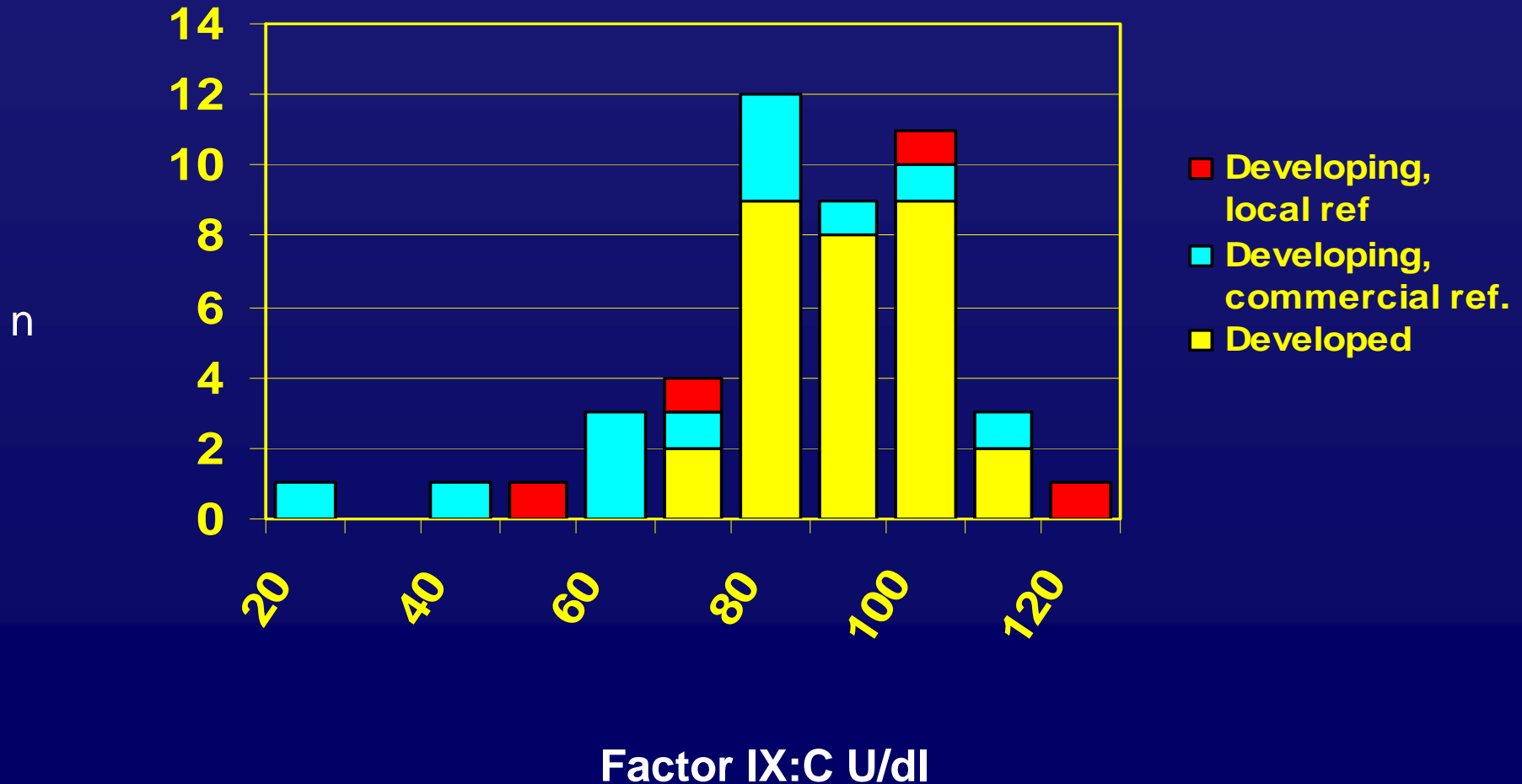
WFH Survey 4, 2005: FVIII:C (UK NEQAS median 75.0 IU/dl)

Developing Country results by source of reference plasma



WFH Survey 4, 2005: FIX:C (UK NEQAS median 95.3 IU/dl)

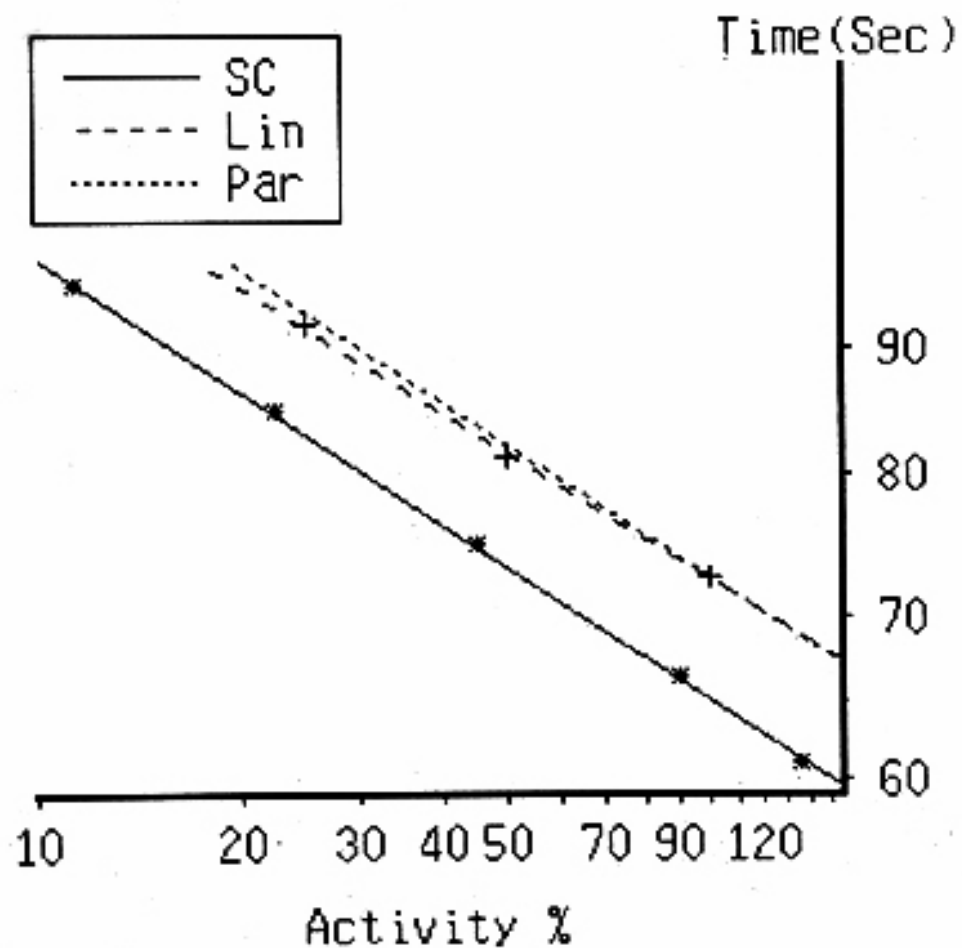
Developing Country results by source of reference plasma



Normal 1 stage FVIII in Haemophilia A (50 families)

patient	1 stage IU/dl	2 stage (clotting) IU/dl
TB	58	14
ER	71	12
RS	89	22
RM	60	28
AH	43	28
RC	40	18
SS	43	14
DT	43	16

Multi Dilution Analysis SYSMEX CA6000



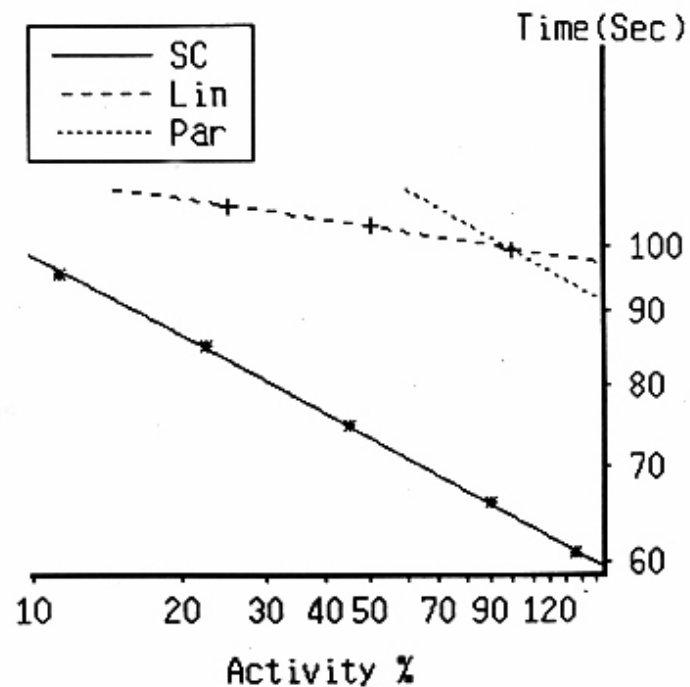
Multi Dilution Analysis SYSMEX CA6000

Factor VIII MDA		
ID No 8020		[0003-01]
24/ 9/97	9:27	37.2°C

MDA Ratio	Clot time	Activity %
1/ 1	72.6 sec	52.6 %
1/ 2	81.4 sec	55.9 %
1/ 4	92.0 sec	56.9 %
		Mean 55.1 %
SCr= -1.000	Test r= -1.000	

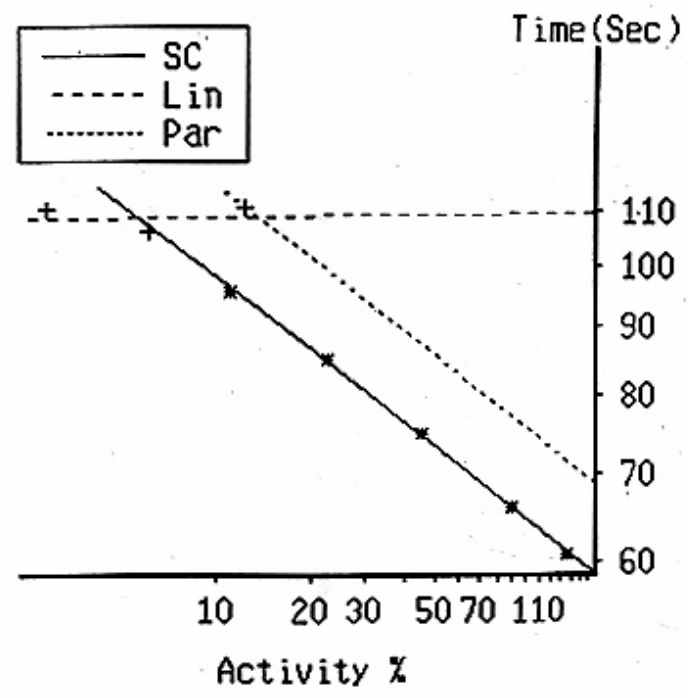
Factor VIII MDA
 ID No 7004 [0004-04]
 29/ 9/97 16:03 37.1°C

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 VIII MDA
 ID No 7004 [0006-01]
 29/ 9/97 16:43 37.3°C

MDA Ratio	Clot time	Activity %
1/ 8	110.0 sec	42.6 %
1/16	105.6 sec	105.9 %
1/32	109.6 sec	175.5 %
		Mean 108.0 %
SCr= -1.000		Test r= 0.071



Valid factor assays in the presence of Strong Lupus anticoagulant

	DRVVT	Corrected ratio	FIX Synthasil	FIX AFS
1	1.72	1.17	>25 u/dl	93 u/dl
2	1.73	1.29	>31	99
3	1.45	1.14	>40	131
4	1.51	1.01	>19	129

Clotting factor assays

Important features

- Aim to compare test and standard under identical conditions
- Wide range of dilutions covering standard curve
- Test clotting times within range of standard curve
- Standard analysed a beginning and end of assay run
- Include IQC plasma

NCCLS/CLSI recommendations (1997)

Factor assays

- Deficient plasma < 1 U/dl and > 50 U/dl for other factors
- Normal and abnormal QC with each run
- Reference plasma calibrated against WHO IS
- ≥ 3 dilutions for test and standard
- Standard curve with each run
- Local reference range

UK HCDO - Rare bleeding disorders working party

(Bolton-Maggs et al 2004)

- Deficient plasma < 1 U/dl and normal levels of other factors
- QC with each run
- Reference plasma calibrated against WHO IS
- At least 3 dilutions for test and standard
- Standard curve with each run
- Local reference range
- Participate in accredited EQA

Factor VIII:C and IX assays

- Clinically significant variation between results in different centres.
- Reference plasma labelling effects, but standardisation of reference plasmas has improved.
- Assay design not well standardised.