

Antiphospholipid antibody testing:

What should be doing ?

What are we doing?

Dr Steve Kitchen
Sheffield

Lupus anticoagulant (LAC)

**Anti-phospholipid antibodies
(APA)**

(Includes but not exclusively LAC)

Lupus Anticoagulant – Some History

- 1944 Biologic False Positive Serological Test for Syphilis
- 1952 Coagulation Inhibitor in SLE (Conley & Hartmann)
- 1963 Associated with thrombosis
- 1972 'Lupus Anticoagulant' coined (Feinstein & Rapaport)
- 1983 ACA test introduced (Harris et al)
- 1990 β 2 glycoprotein 1 cofactor for ACA (Galli et al)

What is the Lupus Anticoagulant?

- Antibodies which prolong coagulation
- Many antigens are targeted
- Targets are largely /especially proteins which bind to phospholipids – Beta 2 GP1, prothrombin, etc

Lupus Anticoagulant

- Most common cause of isolated prolonged APTT
- Gender bias, ratio female to male is 3 to 1
- Associated with thrombosis
- Bleeding association only if thrombocytopaenia or very low factor II

Causes of Lupus Anticoagulant

- | Autoimmune
- | Drugs (e.g. chlorpromazine)
- | Infections may cause transient LA
- | Chronic infections may cause persistent LA
(e.g. HIV, hepatitis C and syphilis)
- | Familial APS - reported but uncommon
- | Significant numbers of healthy subjects have APA

LAC/APA

Research applications at present

Anti Annexin V

Anti Endothelial cell

Anti TFPI

Silica clotting time

Snake venom assays

FVIIa based tests

Lupus Anticoagulant Antibodies

LA is often caused by, or associated with:

- | anti cardiolipin antibodies
- | anti-beta-2 glycoprotein 1 antibodies
- | and anti-prothrombin antibodies

Why test for the Lupus Anticoagulant?

- To investigate an unexpected prolonged APTT
- To help diagnose thrombophilia
- Investigation of recurrent recurrent miscarriage
- Investigation of systemic lupus erythematosis (part of autoantibody profile)

Investigating a prolonged APTT

- Consider sampling artefacts
- Repeat samples and check clinical details
- Confirm result
- Check thrombin time (heparin)
- Tests for Lupus Anticoagulant
- Clotting factor assays (?LA insensitive reagent)

Investigations: prolonged APTT

- Samples double centrifuged to remove platelets
- Plasma separated into several aliquots
- Plasma frozen at -70°C prior to analysis
- Batching facilitates quality assurance and economic use of samples, instrument & apparatus

ISTH guidelines

(Thromb Haemost 1995; 74: 1185-1190)

1. Prolongation of at least one phospholipid dependent test (*sensitivity to APL varies!*)
2. Evidence of coagulation-inhibitory activity
3. Inhibitory activity dependent on phospholipid
4. Distinguish from other coagulopathies
5. Identify any coagulopathy that may be masked

BSH Guidelines (*BJH 2000*; 109: 704-709)

Summary of Tests:

Coagulation screen

- LA sensitive APTT (*may* be in clotting screen)
- Prolonged APTT - mixing studies with plasma/ PNP
- Second test, preferably KCT or DRVVT (with corrections)
- Assay for IgG and IgM ACA (+/- anti β 2 GP1)
- Positive results checked on samples at least 6 weeks apart to demonstrate persistent APA

Revised Sapporo Criteria for APS

(Miyakis et al JTH 2006:4;295-306)

- Mainly about classification
- Don't classify as APS if > 5 years between thrombotic event and positive lab test
- 12 weeks between symptoms and test helpful to assess relationship between test and symptom

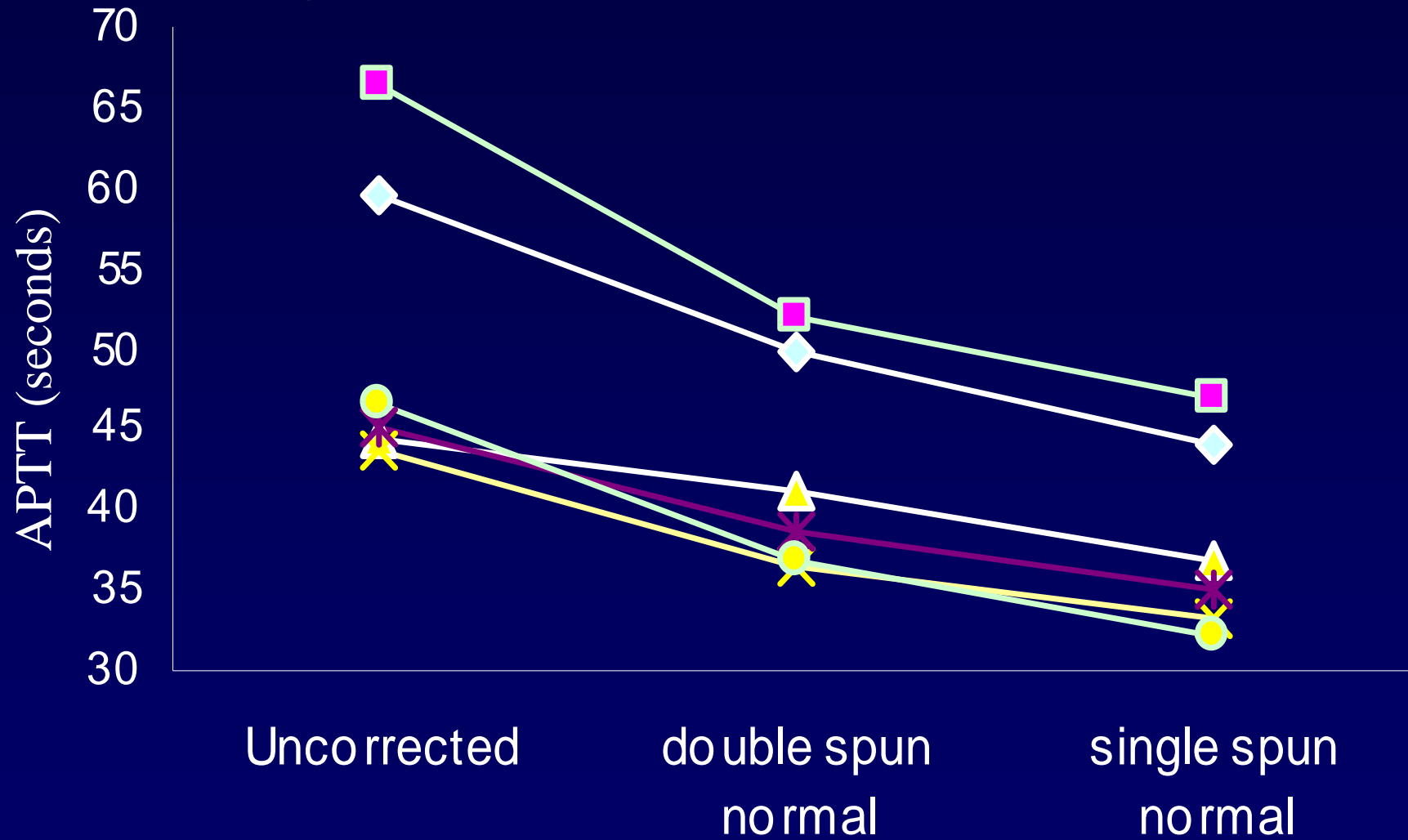
Revised Sapporo classification

Lab criteria- one or more required

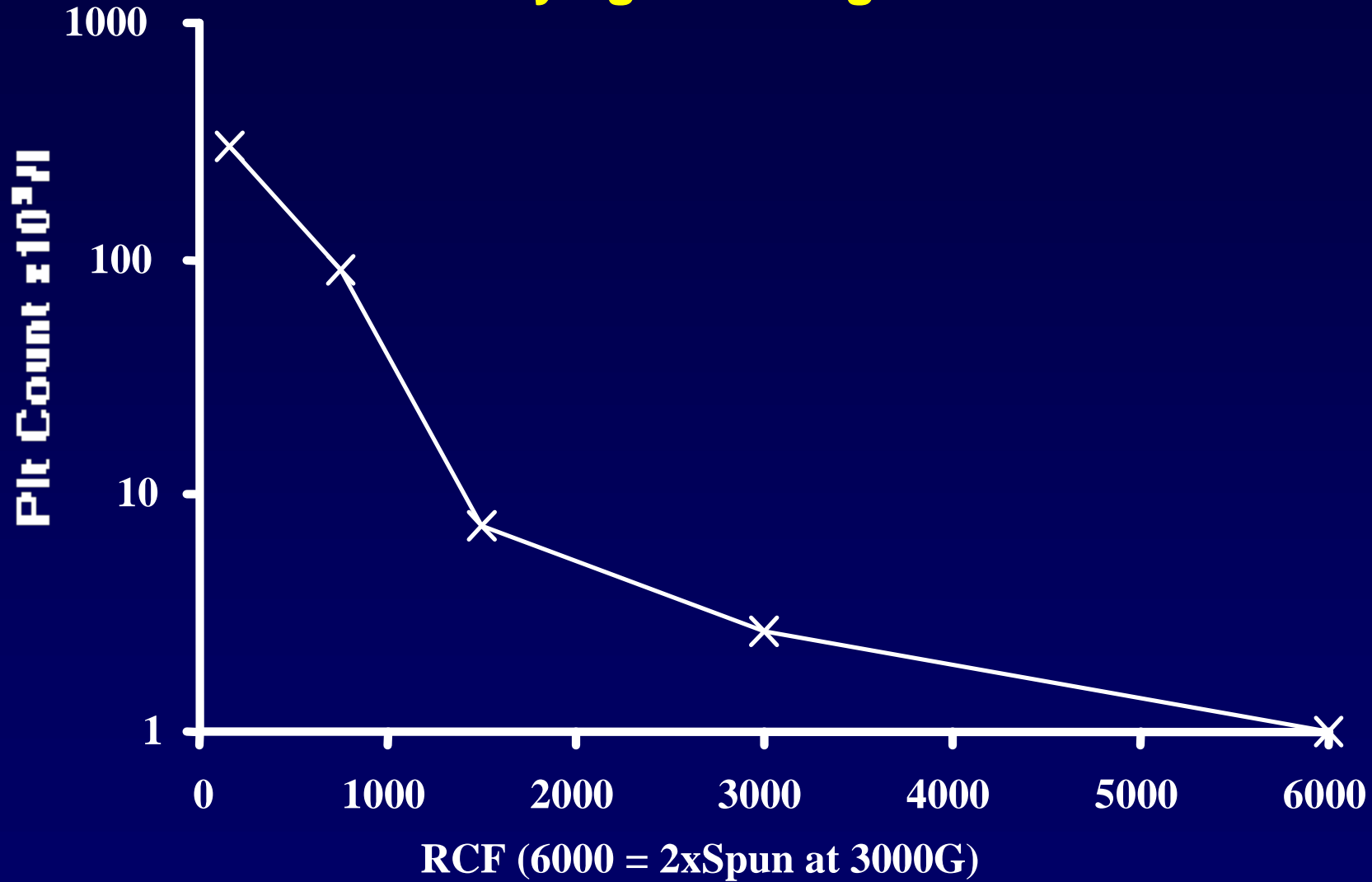
- LAC present twice 12 weeks apart (formerly 6 weeks)
- ACA IgG and or IgM > 40 units or > 99th percentile twice > 12weeks apart
- Anti Bbeta 2 GP 1 Ig G and /or IgM > 99th percentile twice 12 weeks apart

Prenalytical variables

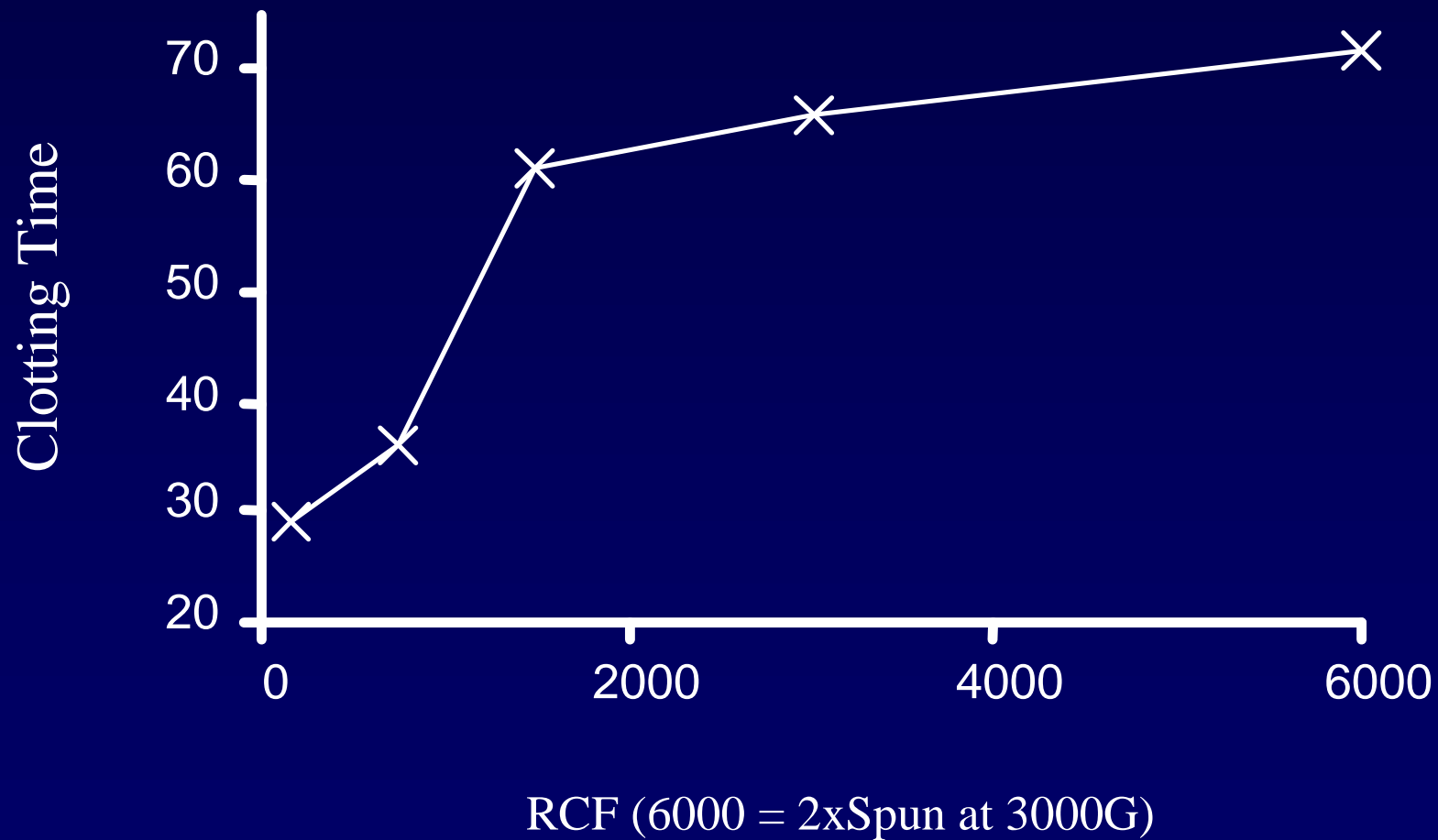
50/50 Correction of LA Positive samples using single and double-spun normal plasma



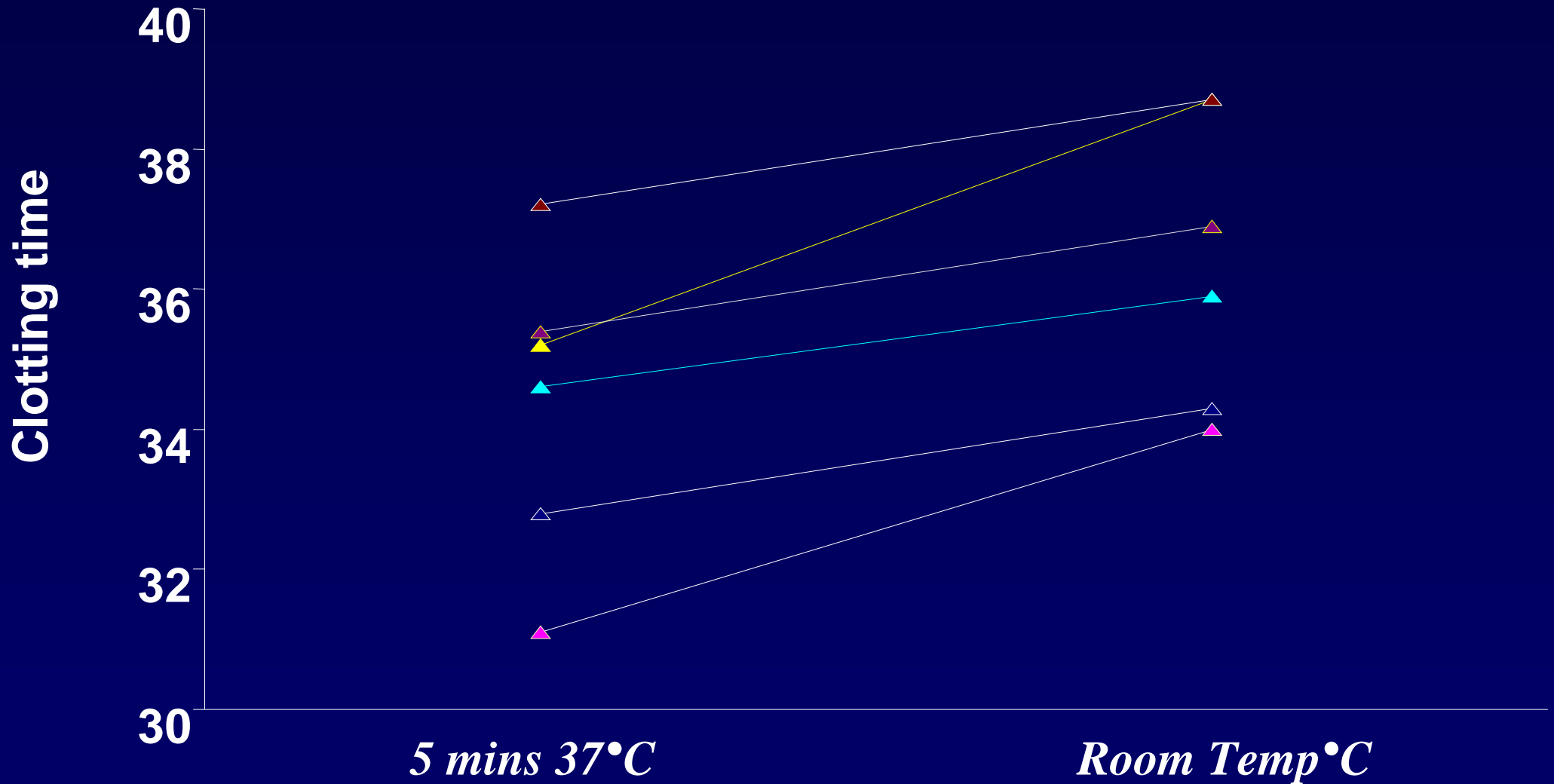
Blood Centrifugation and Platelet Count in Plasma: Varying Centrifugal Force



Blood centrifugation at Various RCF: Normal Sample - KCT



Standard thaw versus slowly thawed plasma: APTT



Testing issues

Does it matter what we test?

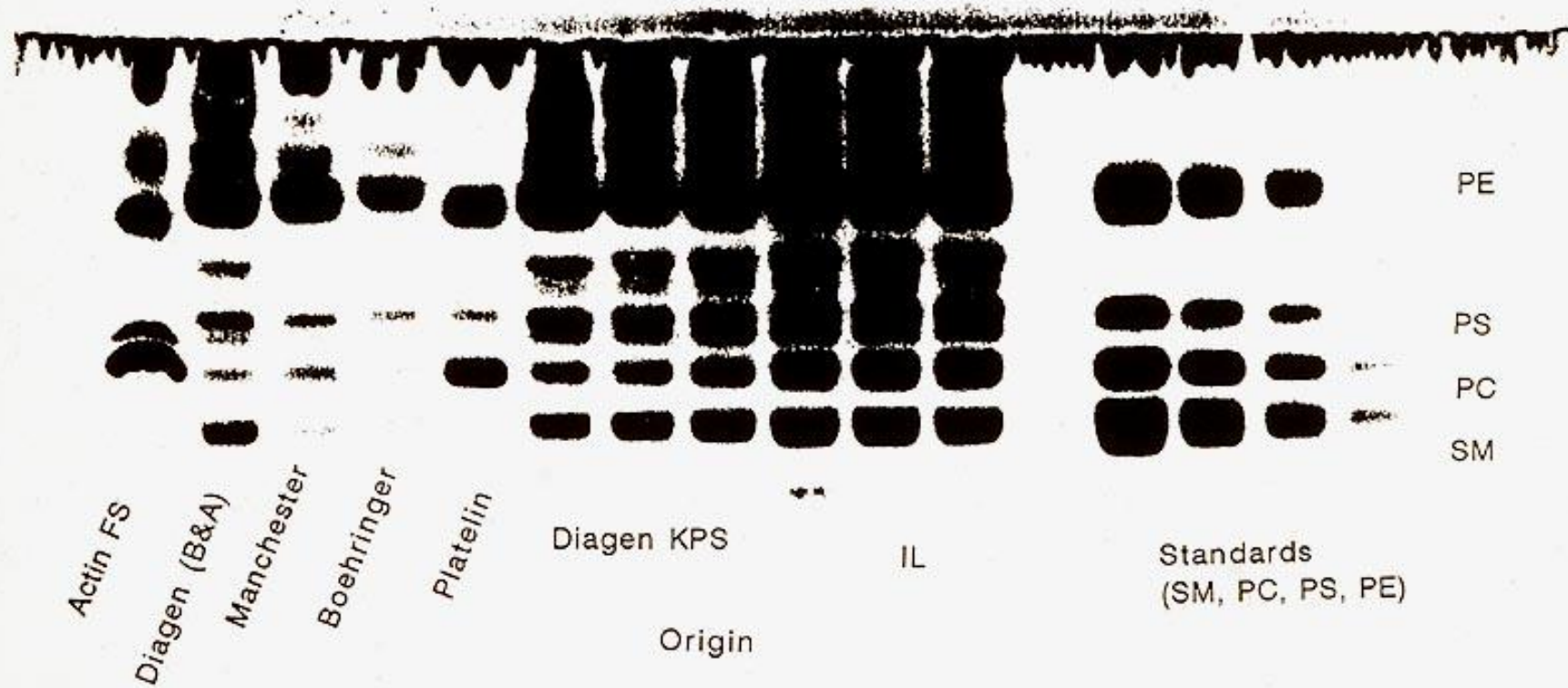
- LAC is a stronger risk factor for thrombosis than aCL (Galli et al 2003 etc)
- LAC + aCL higher risk (Finazzi et al 1996)
- Beta 2GP 1 dependant antibodies more thrombotic than aCL (de Laat, de Groot 2004)
- High avidity antibodies correlate with thrombosis (de LAat b2006)

LA sensitivity, APTT reagents

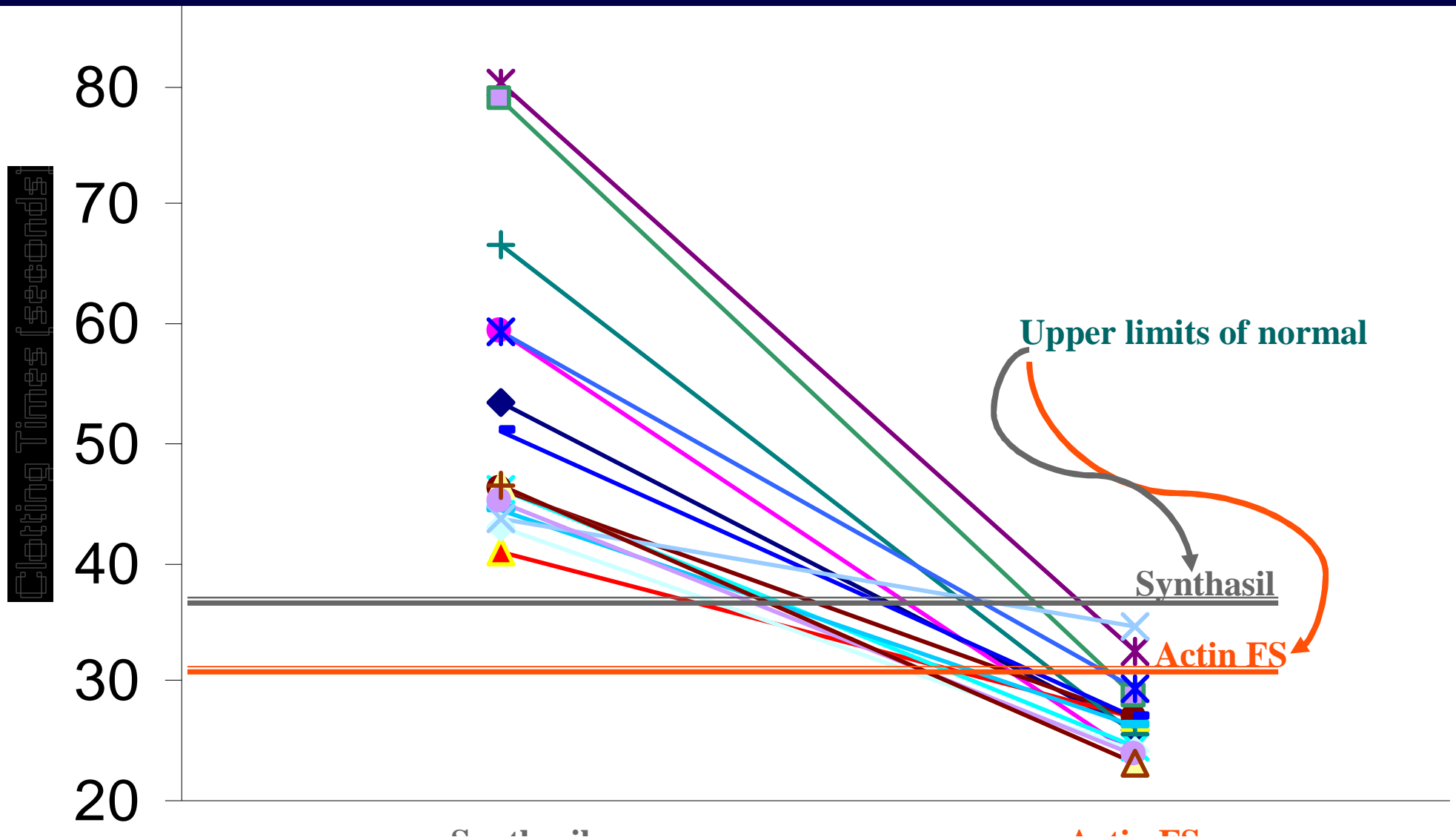
Patient with a strong LA

APTTreagent	N	S01/09
Dade-Behring Actin FS	18	1.30
Dade-Behring Actin FSL	13	2.22
Inst. Lab. (freeze-dried)	22	2.65
Inst. Lab. APTT-SP liquid	53	4.52
MDA Platelin LS	23	3.44
Organon Platelin LS	16	3.97
Stago PTT-LA	9	3.70
Overall median ratio	193	3.10

Phospholipid quantitation by HPTLC/Laser densitometry



Lupus Anticoagulant Samples: APTT Using Two APTT Reagents: (n=25)



Pattern of Results : MoAb vs Patient

Anti-B2GP1 ab	Anti-II ab	Ab mixture	LA Positive patient
Stago PTT-LA	Stago PTT-LA	Stago PTT-LA	IL SP liquid
DB Actin FS	DB Actin FSL	Synthasil	Platelin LS
DB Actin FSL	Platelin LS	DB Actin FSL	Stago PTT-LA
Synthasil	IL SP liquid	DB Actin FS	MDA Platelin LS
IL SP liquid	Synthasil	Platelin LS	IL (freeze-dried)
MDA Platelin LS	MDA Platelin LS	IL SP liquid	DB Actin FSL
IL (freeze-dried)	IL (freeze-dried)	MDA Platelin LS	Synthasil
Platelin LS	DB Actin FS	IL (freeze-dried)	DB Actin FS

Median
APTT
ratio

Pattern of Results : MoAb vs Patient

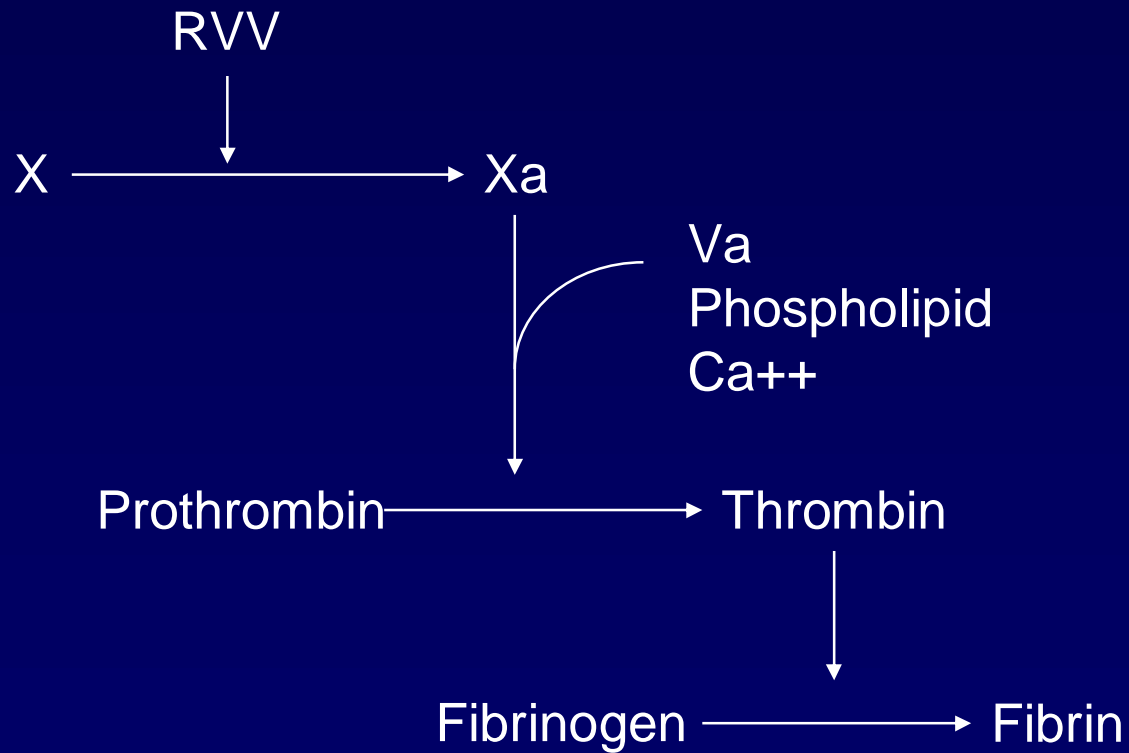
Anti-B2GP1 ab	Anti-II ab	Ab mixture	LA Positive patient
Stago PTT-LA	Stago PTT-LA	Stago PTT-LA	IL SP liquid
DB Actin FS	DB Actin FSL	Synthasil	Platelin LS
DB Actin FSL	Platelin LS	DB Actin FSL	Stago PTT-LA
Synthasil	IL SP liquid	DB Actin FS	MDA Platelin LS
IL SP liquid	Synthasil	Platelin LS	IL (freeze-dried)
MDA Platelin LS	MDA Platelin LS	IL SP liquid	DB Actin FSL
IL (freeze-dried)	IL (freeze-dried)	MDA Platelin LS	Synthasil
Platelin LS	DB Actin FS	IL (freeze-dried)	DB Actin FS

DRVVT

- Screening test of choice in most UK laboratories
- Requires particular phospholipid concentration
- Prolongation is caused by antiphospholipid antibody, anticoagulant therapy or deficiency
- Correction with excess phospholipid indicates presence of Lupus/antiphospholipid

Dilute Russell's Viper Venom Time (DRVVT)

Plasma + Weak phospholipid + Dilute RVV + $\text{CaCl}_2 \longrightarrow$ Clotting time



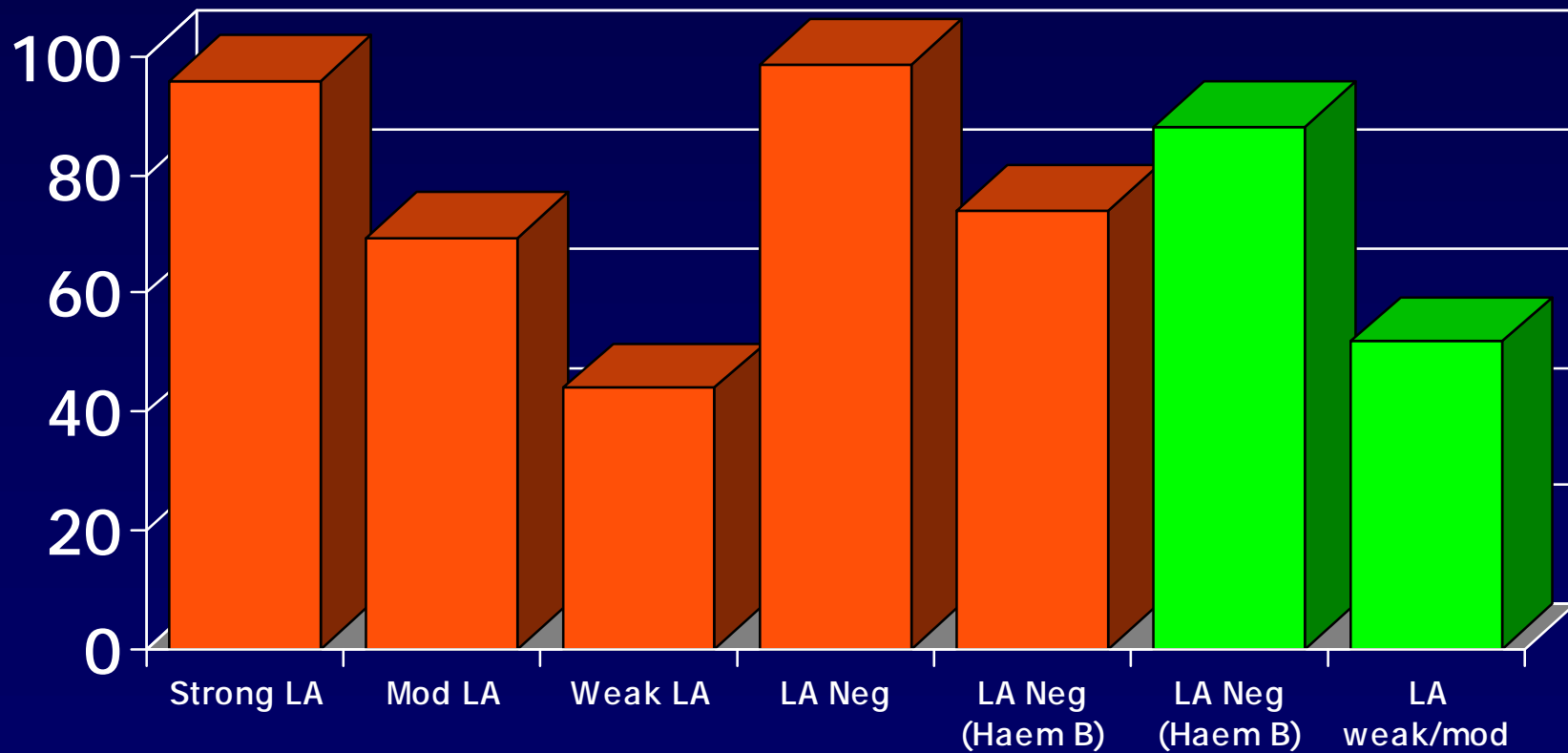
- A variety of algorithms may be used:
 - test result (secs)
 - Test/normal
 - Test/confirm
 - Normalised test/confirm
 - % correction of clotting time
 - % correction of ratio

What degree of correction is positive?

- Prolonged DRVVT calculated as test/normal which corrects to within normal range or by $> 10\%$
- Test-confirm ratio – ratio of test with dilute phospholipid : test with correction reagent outside the normal range

Correct Diagnosis of LA : Data from UK NEQAS exercises

% correct



Variation in dPT results

UK NEQAS exercise, LA positive sample

Dilution of thromboplastin	N	1:1	1:10	1:50	1:100	1:500	1:1000
Dade-Behring Innovin	12	1.56(3)	1.29 (2)	1.45(2)	1.77(2)	1.64(6)	1.68(3)
Ex-Bio	1	1.2	1.37	-	2.4	-	3.44
Helena Manchester	3	1.12(1)	-	1.06(1)	1.17(1)	1.01(2)	1.01(1)

Patterns of testing in Lupus Anticoagulant Screening

Number of centres	APTT	APTT + normal plasma	DRVVT	KCT	Others
6	ü	ü	ü	ü	ü
59	ü	ü	ü	ü	
18	ü	ü	ü		ü
77	ü	ü	ü		
1	ü	ü		ü	ü
2	ü	ü		ü	
4	ü	ü			ü
1	ü	ü			
1	ü		ü	ü	ü
8	ü		ü	ü	
2	ü		ü		ü
9	ü		ü		
1			ü	ü	ü

Compliance with guidelines : effect on sensitivity

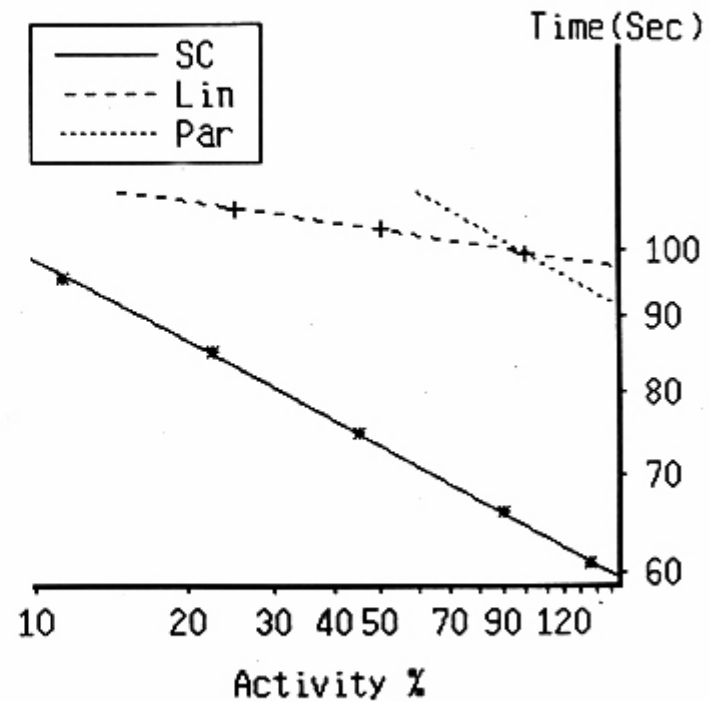
	Correct Diagnosis (n)	Incorrect Diagnosis (n)
Compliance with guidelines*	102 (84%)	19
Non-compliance with guidelines*	51 (65%)	27

* $P < 0.002$, Chi-square test

Interference in other tests

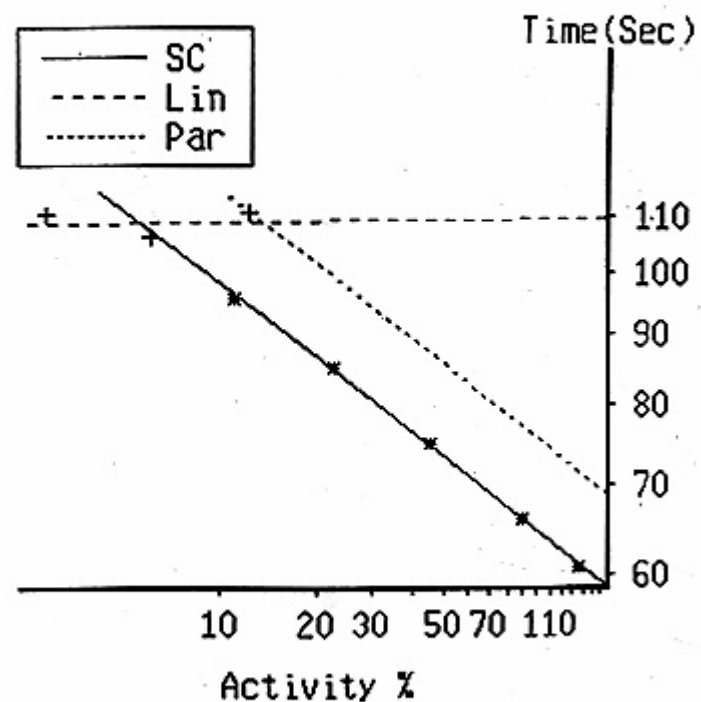
Factor VIII MDA
 ID No 7004 [0004-04]
 29/ 9/97 16:03 37.1°C

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

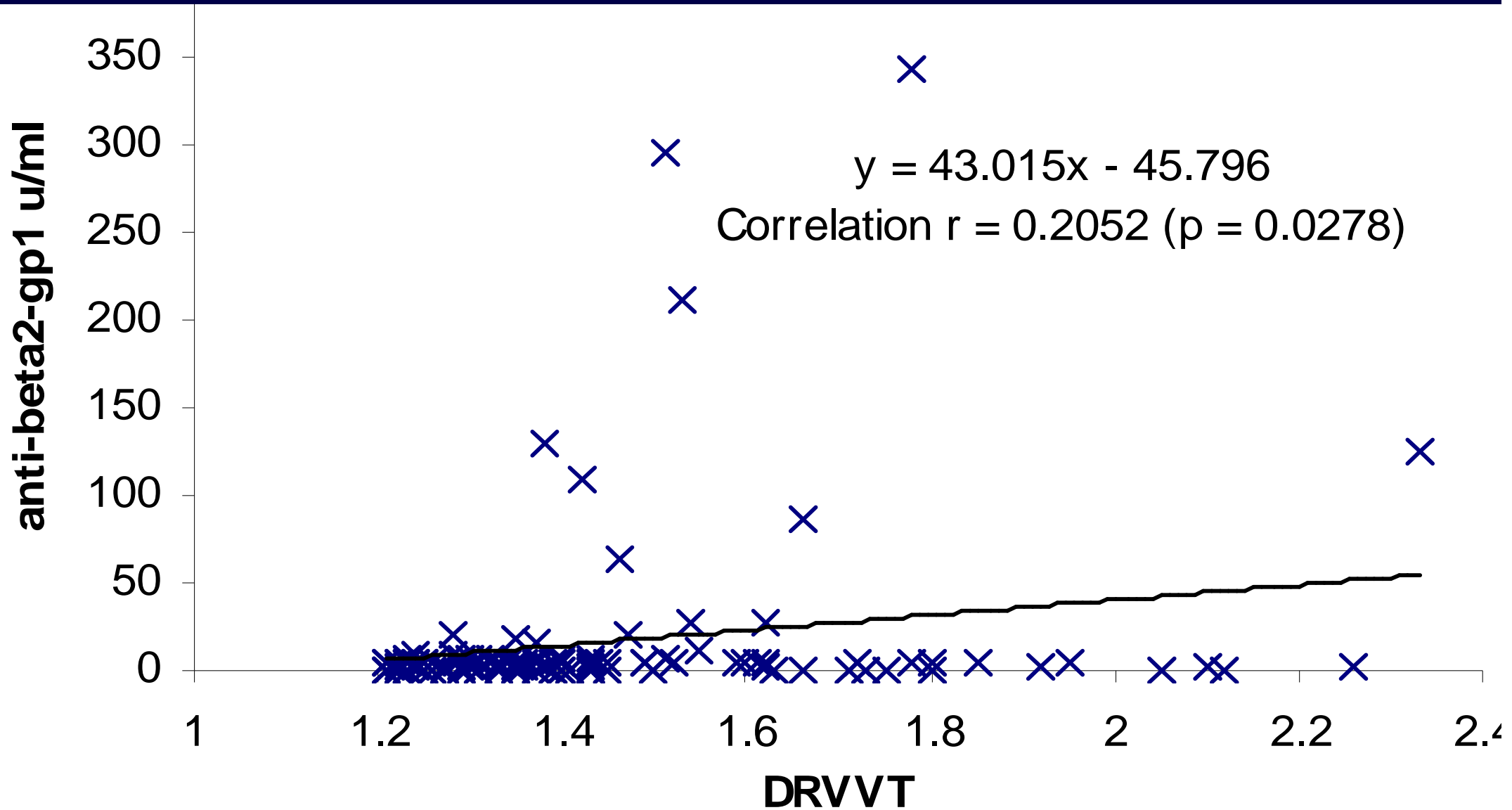


Factor VIII MDA
 ID No 7004 [0006-01]
 29/ 9/97 16:43 37.3°C

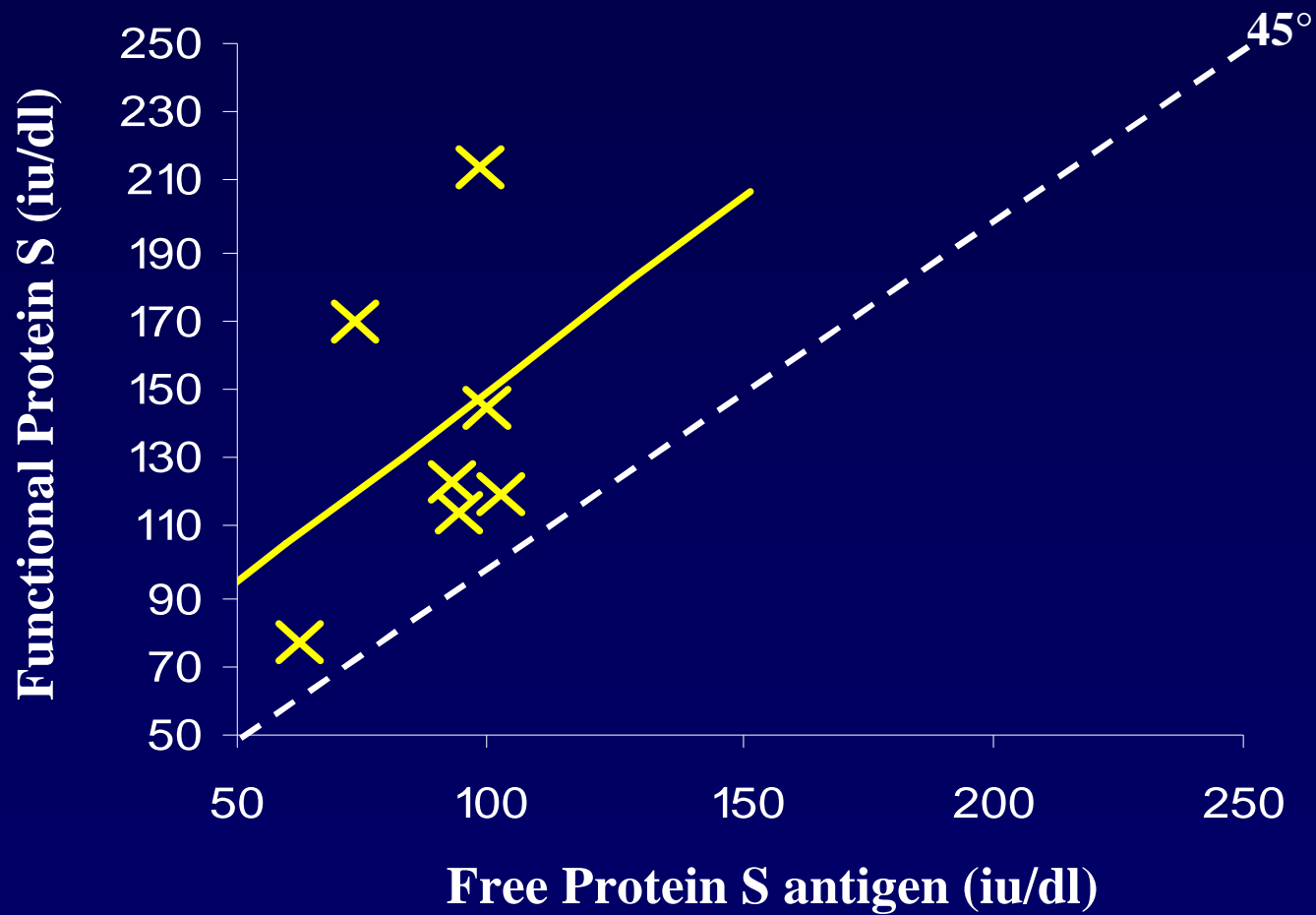
MDA Ratio	Clot time	Activity %
1/ 8	110.0 sec	42.6 %
1/16	105.6 sec	105.9 %
1/32	109.6 sec	175.5 %
		Mean 108.0 %
SCr= -1.000		Test r= 0.071



Some more patient's data: DRVVT versus anti-beta 2 – gp1 (n=114)

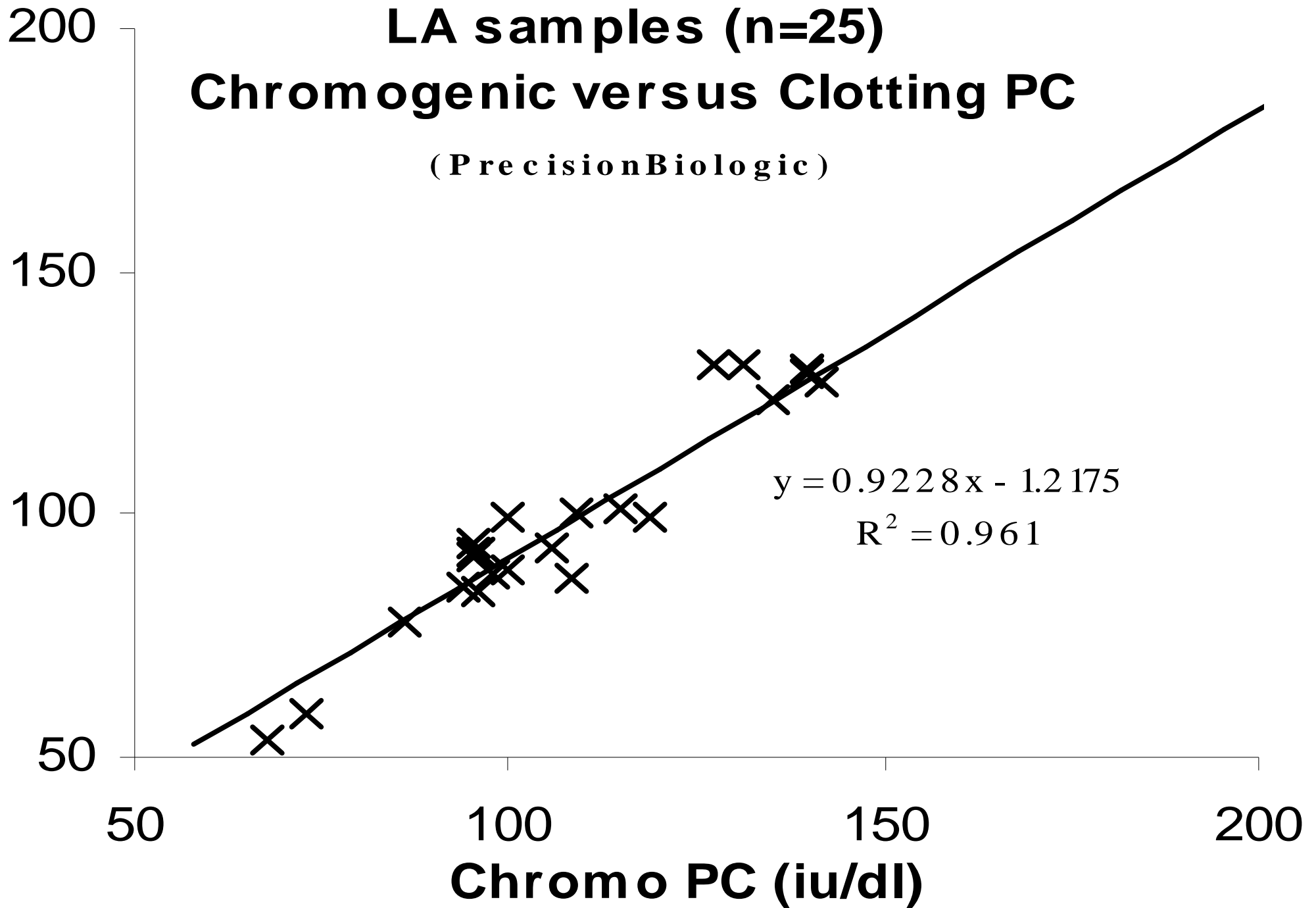


Functional versus Free Protein S Antigen in Patients with LA (n=7)



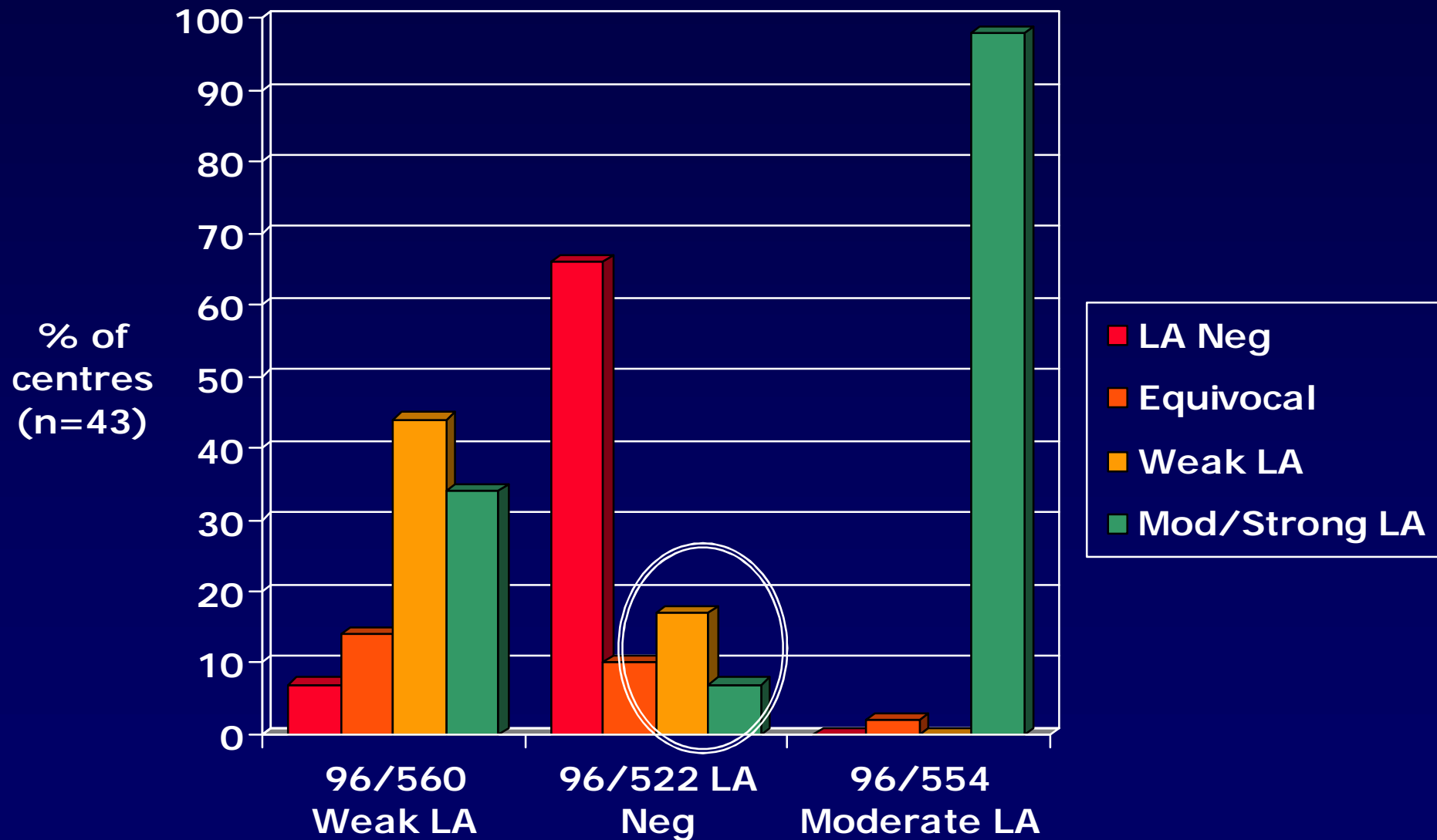
LA samples (n=25)
Chromogenic versus Clotting PC
(PrecisionBiologic)

Clotting PC (iu/dl)



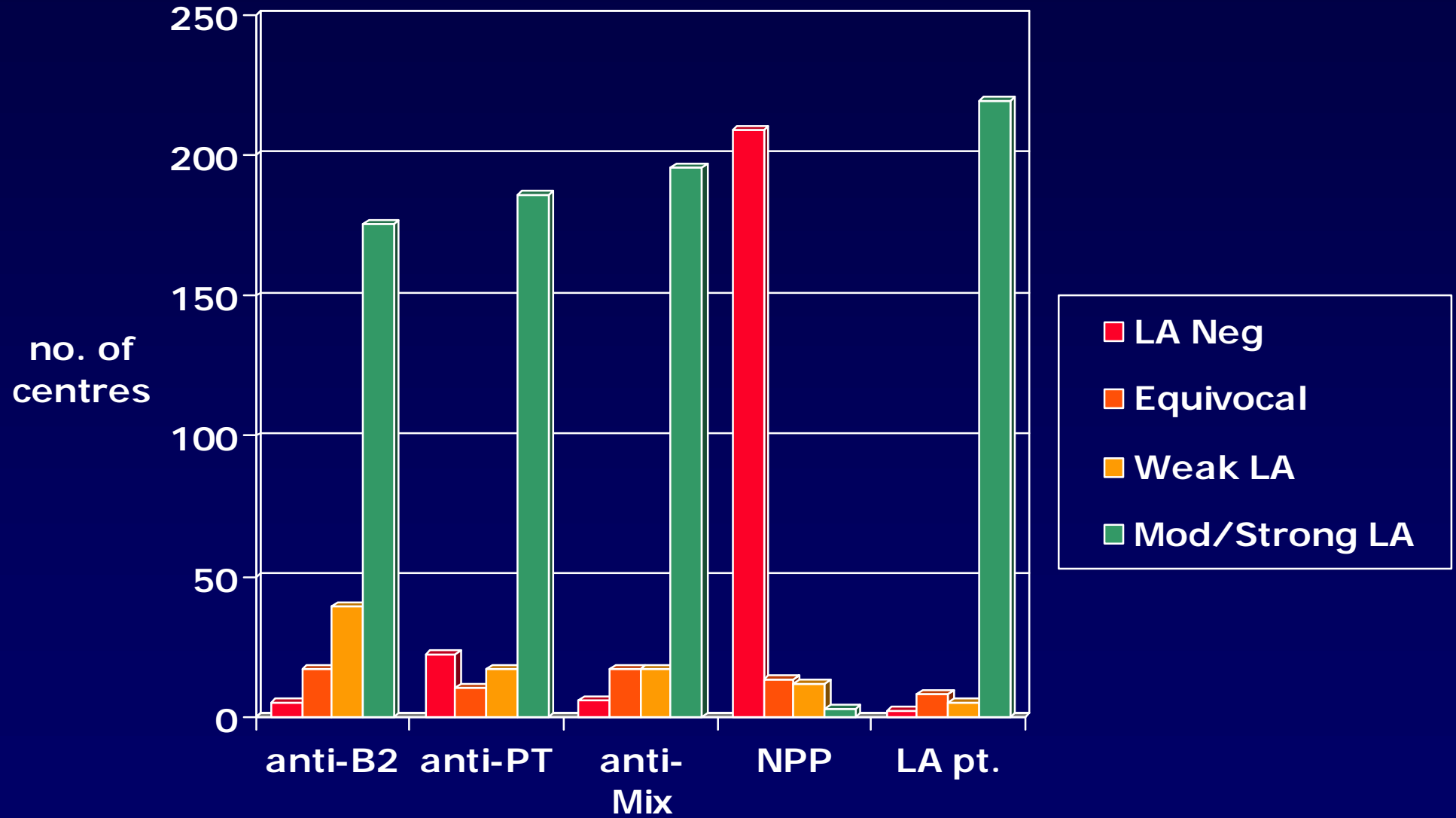
Standardisation issues

1st British Reference Plasma Panel: Interpretations by UK NEQAS participants



- NIBSC LA Negative sample 96/522: pH 8.15
- NIBSC LA Positive sample 96/560: pH 7.54
- NIBSC LA Positive sample 96/554: pH 7.46
- UK NEQAS LA Negative sample 00/46: pH 7.42
- UK NEQAS LA Negative sample S01/08: pH 7.46

Plasma Spiked with Monoclonal Ab : Interpretations



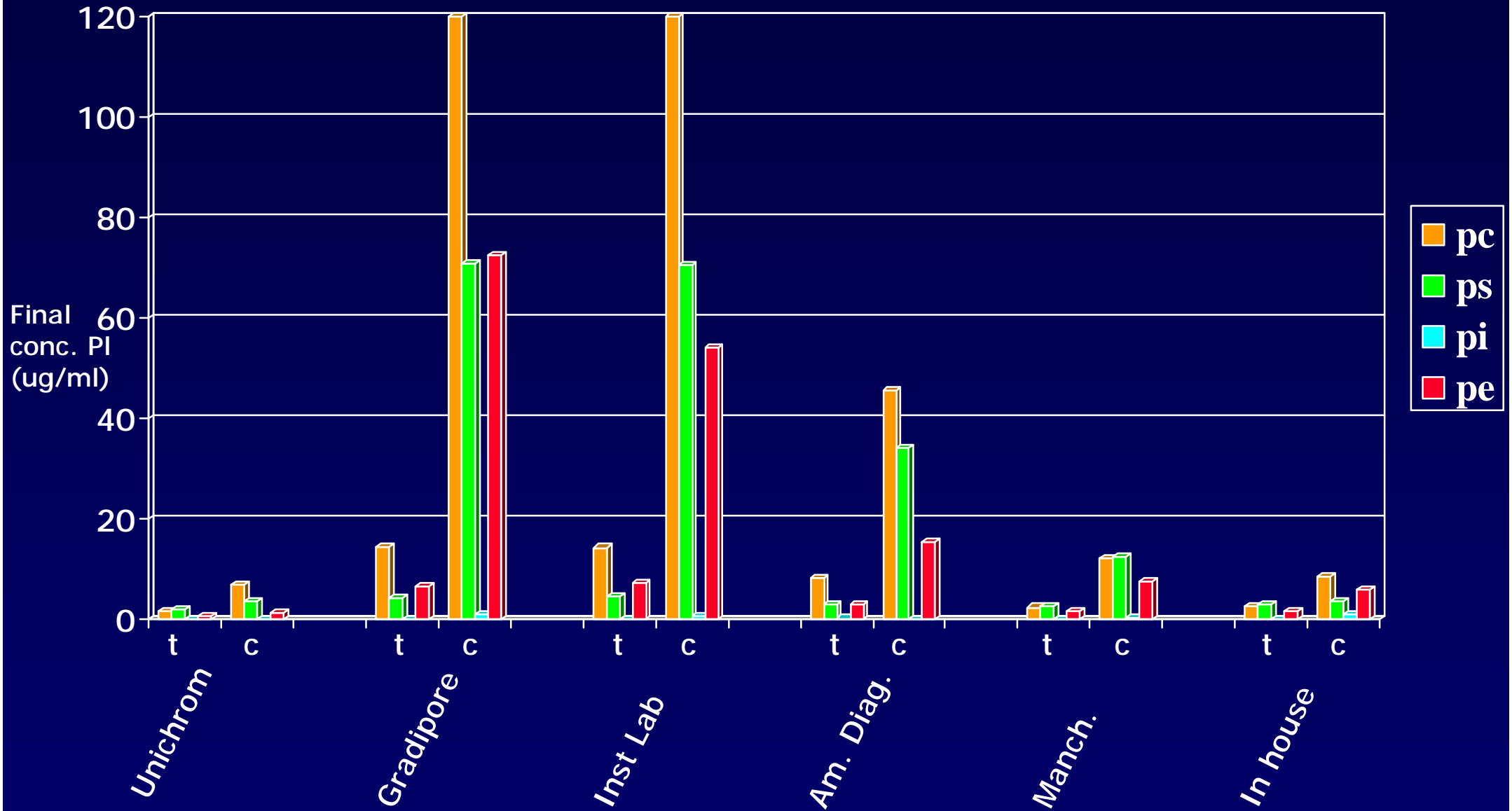
A need for standardisation?

- 11 DRVVT methods used in UK NEQAS Survey 125, November 2000.
- A variety of reporting methods may be used:
 - test result (secs)
 - Test/normal
 - Test/confirm
 - Normalised test/confirm
 - % correction of clotting time
 - % correction of ratio
- DRVVT kits are not the same !

Commercial DRVVT test systems

Kit	Origin of phospholipid	RVV form	Reagent: combined or separate	Heparin neutralising reagent
Am. Diag.	Plant	Purified	Combined	Present
Inst Lab	Vegetable	Native	Combined	Present
Manchester	Rabbit brain	Native	Multi-reagent	Not present
Unicorn	Bovine brain	Native	Multi-reagent	Not present
Gradipore	Not disclosed	Native	Combined	Present

Phospholipid content of DRVVT reagents



Use of 2 calcium concentrations (Pengo et al 2004)

- dRVVT, dPT (Recombiplastin)
- Final calcium concentration 10 or 5 mM (APTT dRVVT etc – 8 mM)
- Plasma derived purified anti beta 2GP antibodies
- LAC + anti Beta 2 neg decreased clotting times at lower final calcium
- LAC + anti beta 2 pos – increased clotting times at lower calcium concentration
- Samples in 3.8% citrate

Thanks to:

- Dr Ian Jennings
- The UK NEQAS team
- UK NEQAS participants
- Peter Cooper, Sue Cooper, Karen Goodfellow
RHH