Internal Quality Control in the Haemostasis laboratory

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The Philadelphia Inquirer

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Sixers Make Trades, Lose MacCulloch

■ The Sixers traded Tyronn Hill and Jumaine Jones to the Cleveland Cavaliers for Matt Harpring, Cedric Henderson and Robert Traylor. The Sixers also made a trade with the Boston Celtics and announced they would lose Todd MacCulloch to free agency. Sports, C1.

Votes show Bush's skill in making the big deal

The President can claim two victories in the House this week. But more challenges await after his vacation.

By Steven Thomma and Ken Hutcheson

WASHINGTON — After a last-minute burst of legislative wheeling and dealing, President Bush heads home to his Texas ranch this weekend for vacation, confident that he can play the inside game of Washington politics but still facing a nation skeptical of his policies.

Analysis

"I was very bitter and shocked. ... I was hysterical."

— Carmella Vitello, whose husband's death has been linked to an error at St. Agnes

Carmella Vitello, at her lawyer's Center City office, says her family doctor and St. Agnes Medical Center's president visited her South Philadelphia home after her husband's death to tell her about the hospital lab's error.

St. Agnes efforts only add to anguish after lab error

1st Union wins bid to acquire Wachovia

A rival, SunTrust Banks, gave up after Wachovia's shareholders approved a $11.3 billion deal with the Phila. area's largest ban

By Paul Sonnell

WINSTON-SALEM, N.C. — Mo of fighting over ownership of Wa via Corp. ended abruptly yesterda its shareholders approved a $11.3 last merger with First Union Co creating rival SunTrust Banks Inc. extends defeat.

Just as swiftly, the focus of F Union chairman Ken Thompson Wachovia chairman L.M. "Bud" Ba It has now shifted from simply ch ing the way for the merger to figu out what will happen down the ro

The new bank — which will re the Wachovia name and be base Charlotte, N.C. — will be the sixth Fourth-largest, with $321 billion in sets, 19 million customers on the E Coast, and 90,000 employees.

In the Philadelphia area, First U has 3,200 workers in the city and 9 outside more in the suburbs, Read Wilmington, and the Trenton area claims about one-quarter of local b deposits and up to half of local b ness loans, mostly because so much o nearest rivals, PNC Financial Serv Corp. and Mellon Bank Corp.

Why do we need Quality control?

- Philadelphia Enquirer Aug 4 2001
- Lab used an insensitive “chemical” for 7 weeks believing it to be sensitive.
- Patient questioned escalating coumadin dose
- Patient with “INR” 2.6 - bleeding from gums
- INR result at another site 5.7

International Normalised Ratio

$$INR = \left[\frac{\text{Test PT}}{\text{MNPT}} \right]^{ISI}$$

Why do we need Quality control?

- PT of 29 sec tested with ISI 1.8
- PT ratio 2.9 (INR 2.9)
- INR calculated used ISI 1.0 – INR 2.9
- ISI of 1.8 should have been used
- Overdose of patients
- Amongst 932 patients - 5 deaths linked

Quality Assurance

measures taken to ensure the reliability of laboratory sampling, testing and reporting

IQC

ensures precision and consistency of results for reporting

EQA

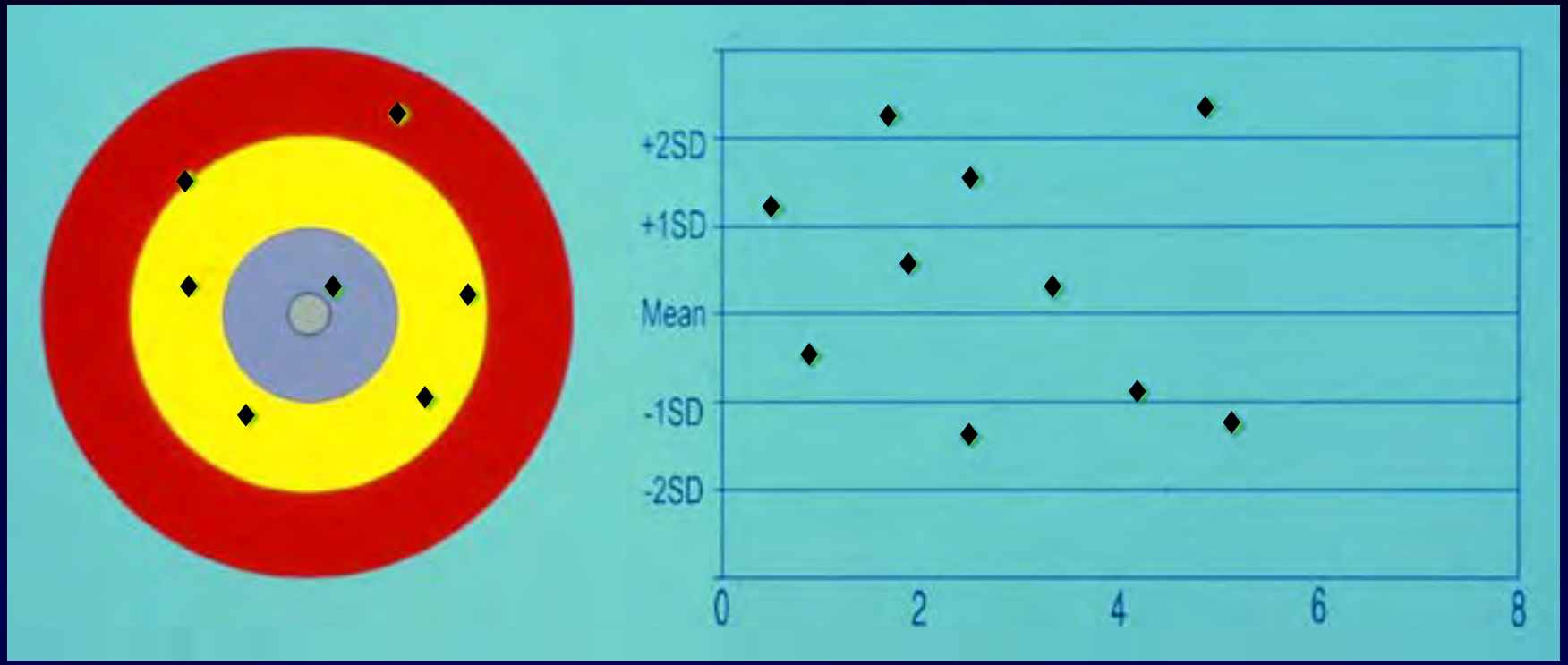
retrospective analysis comparing results between laboratories and between methods

IQC and EQA: Precision and Accuracy

- IQC is required to ensure results are precise. Consistent over time (from day to day etc)

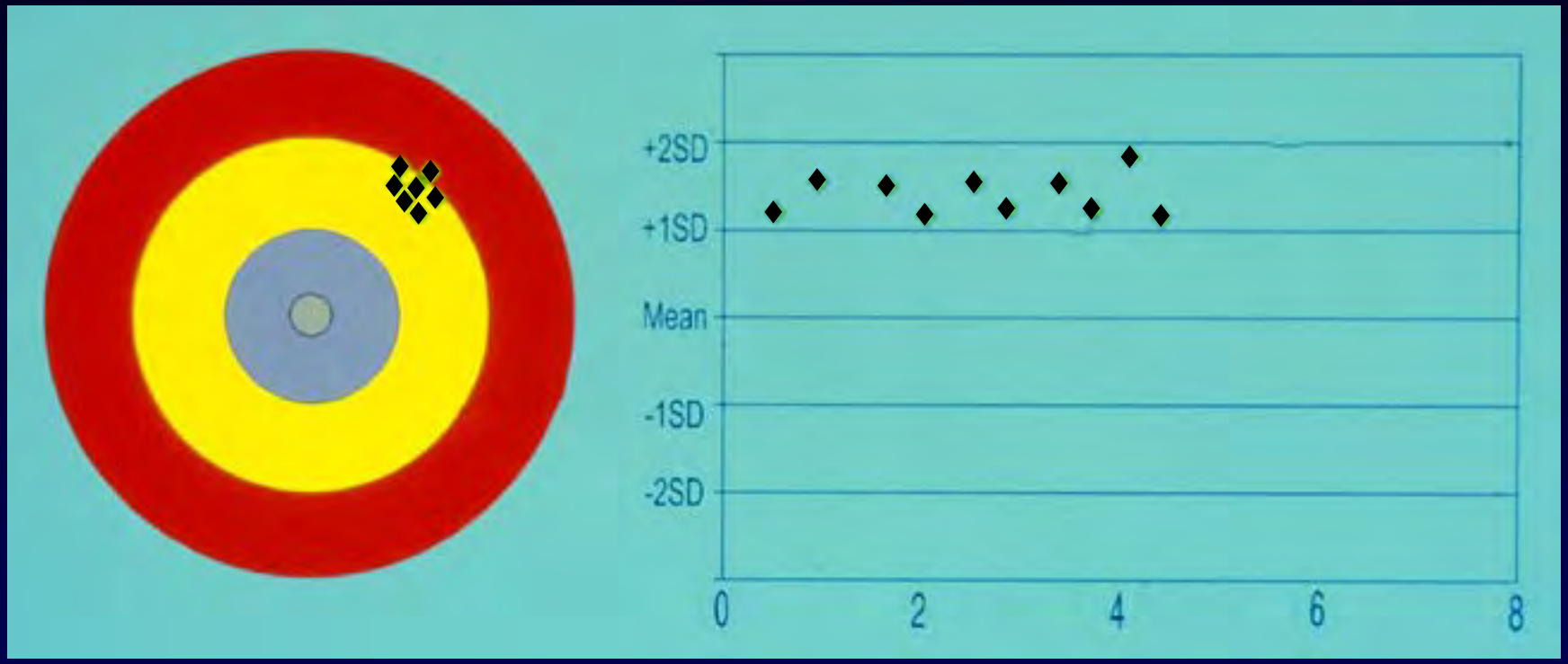
- EQA is required to confirm that results are accurate. Results are in agreement with those in other centres.

Inaccurate and imprecise



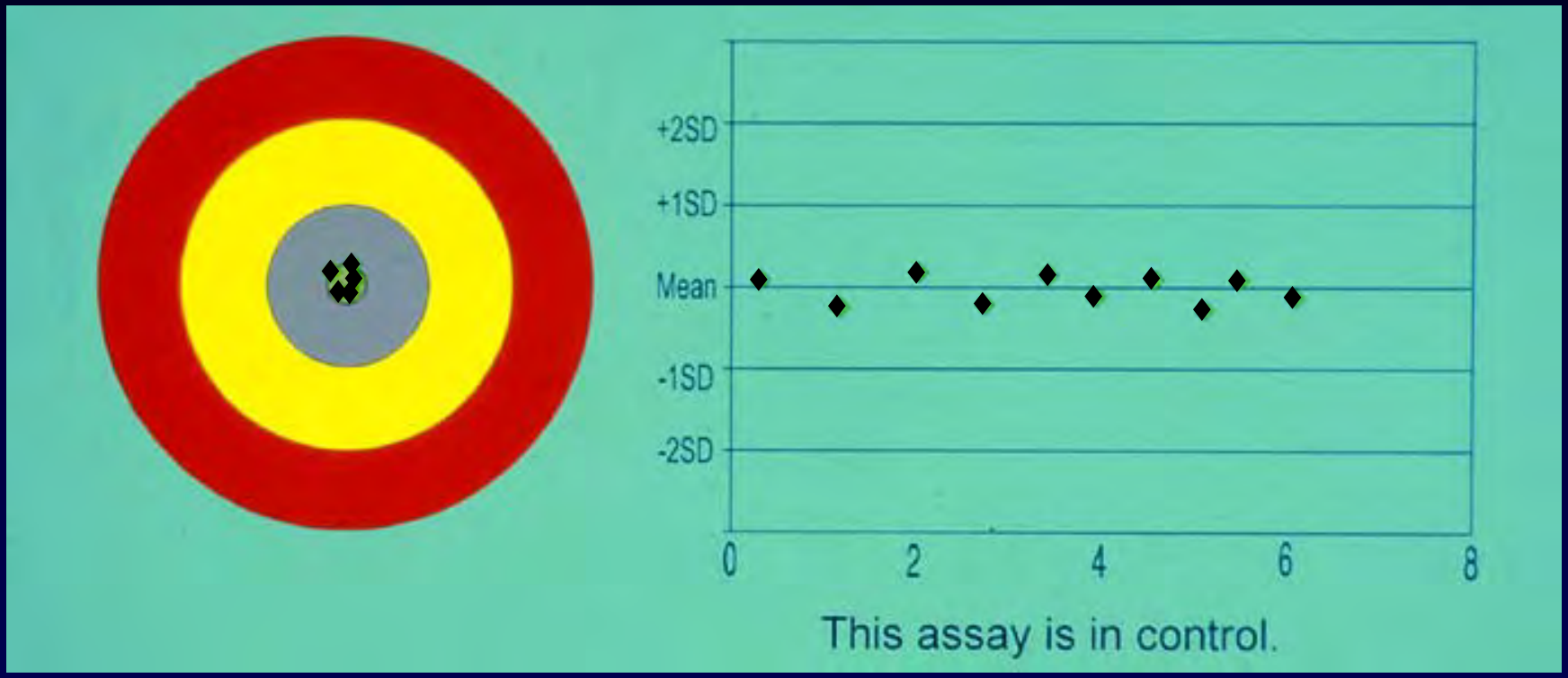
This assay is inaccurate and imprecise

Inaccurate but precise



This assay is inaccurate but precise
The assay is said to have a positive 'BIAS'

Accurate and precise



The assay producing the results shown is both accurate (i.e. no positive or negative bias) and precise (i.e. very little scatter of results about the mean value)

Quality control materials

Quality Control Material

- A substance used in routine practice for checking the concurrent performance of an analytical process
- It must be similar in properties to and be analysed along with the patient specimens

IQC materials

- Similar in properties to test sample
- All vials or aliquots identical
- Stable over period of use (lyophilised,frozen)

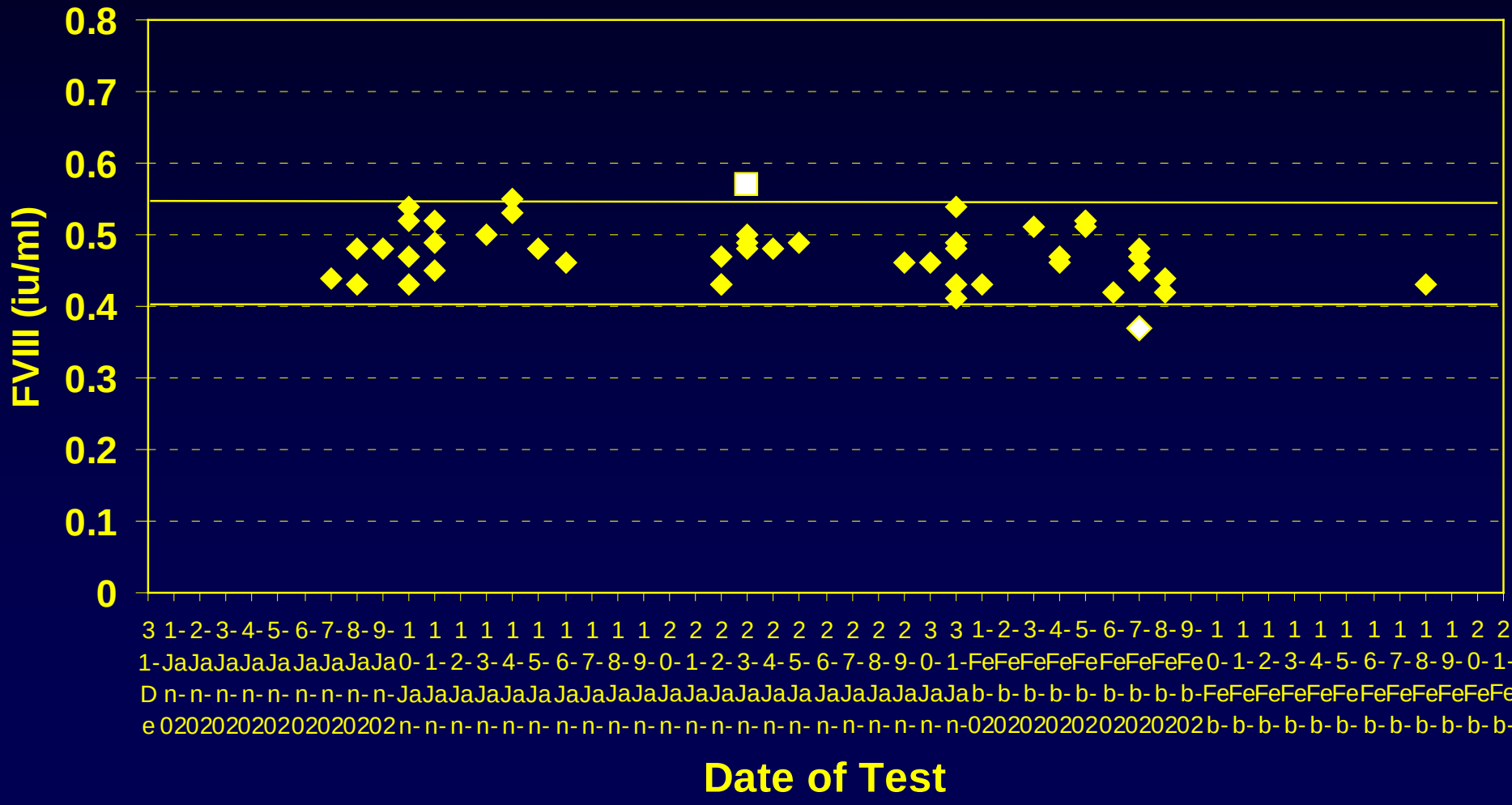

UK NEQAS
OD COAGUL
IDUAL PLASMA DONAT
EN TESTED AND FOUND
GATIVE FOR HB$_s$ A$_g$ A
ANTIBODY.

IQC for Coagulation Tests

- Display target values
- Maintain a cumulative record
- Keep a written procedure for intervention with record of actions taken

Internal Quality Control results for FVIII:C Assay

Target range is mean ± 2sd of 20 determinations



IQC out of target range?

- Suspend new patient testing and reporting of results since last QC result within limits.
- Re-test to exclude analytical error. **Still out?**
- Replace QC material and retest. **Still out?**
- Replace reagents and retest. **Still out?**
- Suspend method and switch to backup, and contact higher authority

Internal Quality Control

Point of Care INR testing

On-Board QC

- Built into test strips
- Currently
  - -CUC XS and CUC XS Plus
  - -Protime
  - -INRatio
- Useful for strip integrity
- Not all show result and/or range
- Can not give information on strip calibration

Useful but need other form of QC.

IQC Material

The CoaguChek, CoaguChek S and CoaguChek XS Plus devices use lyophilised plasma as IQC material.

IQC Material

Some devices have IQC material containing lyophilised red blood cells (Hemochron and Protime)

IQC information to record

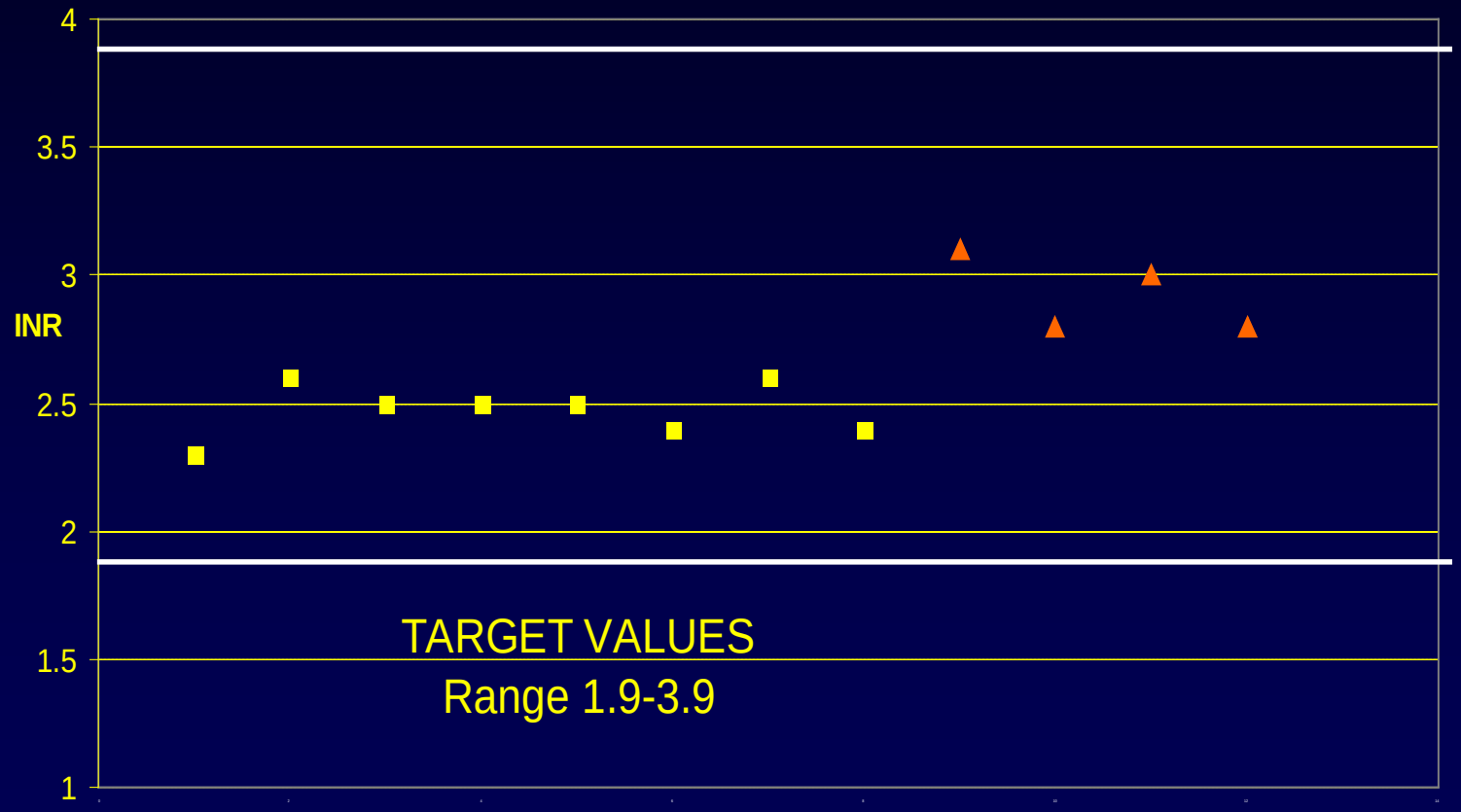
It is essential to keep good records of IQC testing

- Date of test
- Batch of IQC used
- Range for IQC batch
- Batch of test strips used
- Operator ID

When to test IQC?

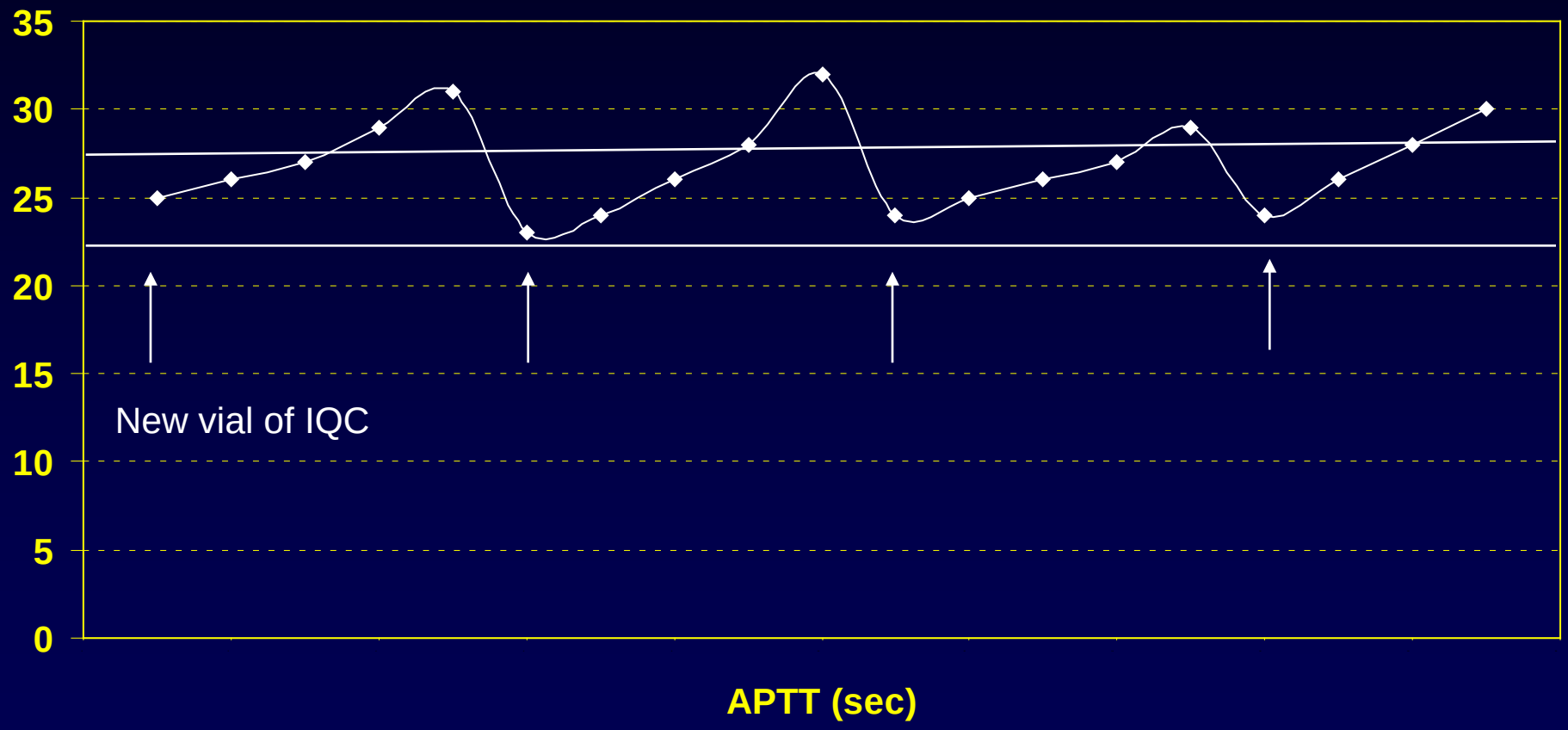
- When starting a new batch of test strips
- Any unexpected high or low results
- At least one per clinic (depending on clinic size)

Single lot of IQC shift following change in lot number of test strip

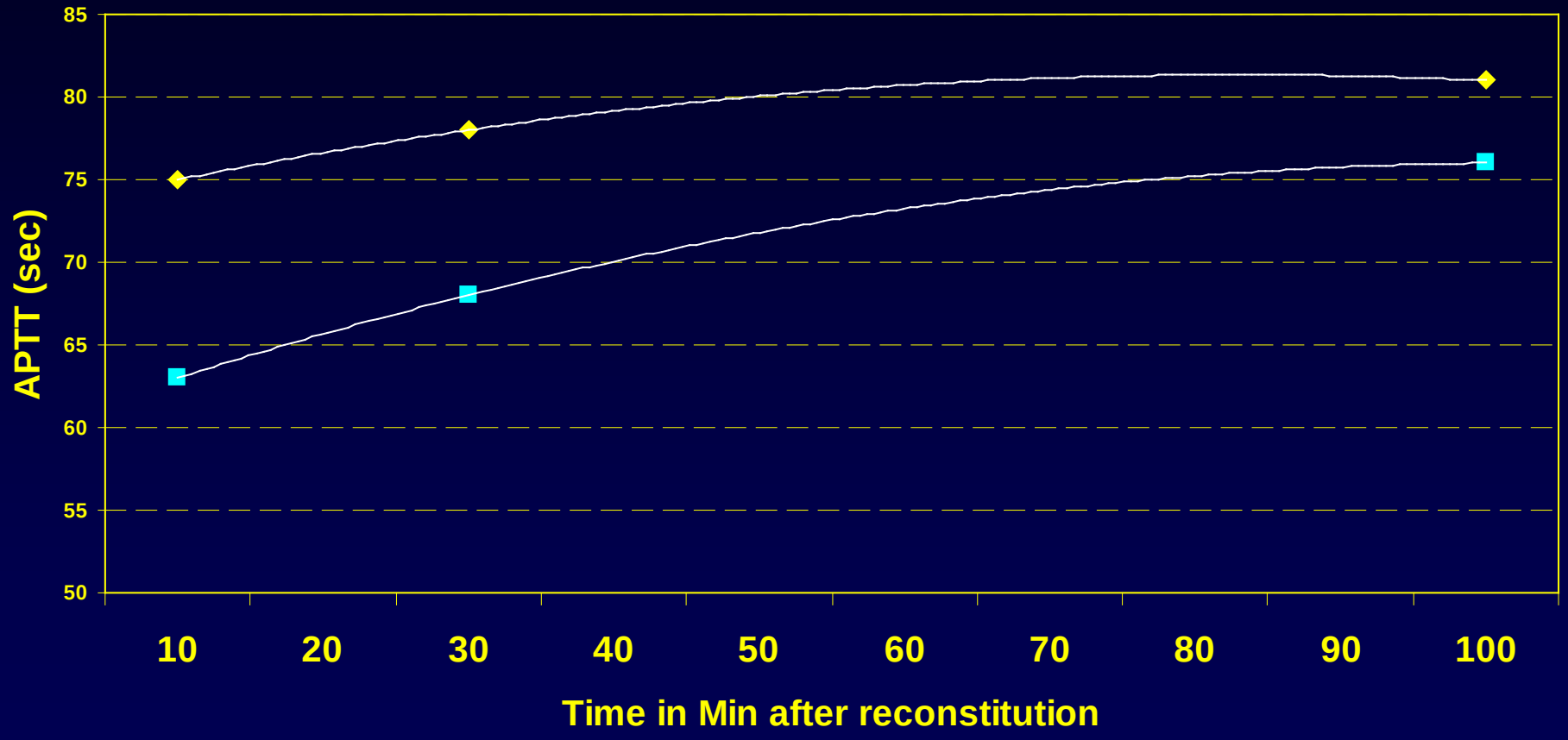


POC INR with excessively wide target range and showing a shift

IQC chart APTT- Unstable IQC sample

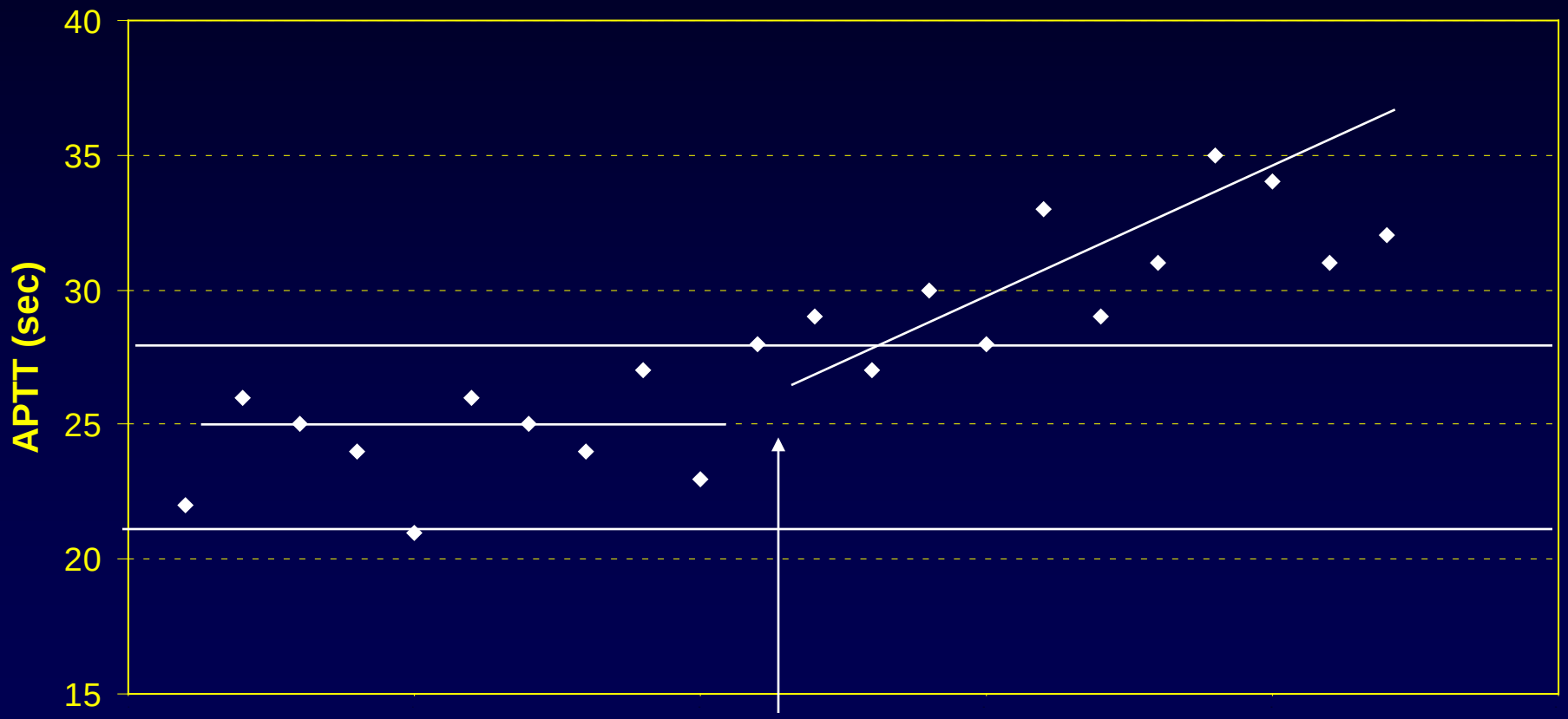


Stability of APTT on 2 lyophilised plasmas after reconstituion



pH 8.6 and 8.9 indicates in adequate buffering

IQC _APTT results showing a trend



Trend to higher results as a gradual change in Material, reagent or analyser

Troubleshooting IQC
Why 2 levels?

PT 1	PT 2	APTT 1	APTT 2	problem
out	in	out	in	QC 1 material
In	Out	In	out	QC 2 material
out	out	in	in	PT reagent
In	In	Out	Out	APTT reagent
Out	Out	Out	Out	Instrument or common reagent

UK NATIONAL EXTERNAL QUALITY ASSESSMENT SCHEME (NEQAS) for BLOOD COAGULATION

www.ukneqasbc.org

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UK NEQAS for Blood Coagulation: Surveys

Participation available in the following programmes:

- Blood Coagulation: Level 1
  Level 2
- Point of Care / Near Patient Testing (POCT/NPT)
- Homocysteine Assay
- Factor V Leiden / Molecular Genetics of Thrombophilia
- Haemophilia Molecular Genetics

UK NEQAS for Blood Coagulation: Registrations

- 1020 participants registered main prog
- 645 (63%) in UK NHS and private labs
- 16 (2%) manufacturers of reagents/ instruments
- 359 (35%) outside UK in 30 countries

Additionally:

- 3400 participants in NPT/POCT programme

UK NEQAS for Blood Coagulation: Assistance to the participant

- Professional Advice
- Technical Advice
- Additional samples for ‘troubleshooting’
- Information resource

UK NEQAS for Blood Coagulation: Test Registrations; Level 1

- Prothrombin Time (PT)/INR (Quick and/or capillary methods)
- PT (diagnostic)
- Activated Partial Thromboplastin Time (APTT)
- Heparin Dosage Assessment (HDA)
- Heparin Assay (HA)
- Thrombin Time (TT)
- Fibrinogen evaluation
- Fibrin(ogen) Degradation Products (FDP)/ D-Dimer
- Lupus anticoagulant

UK NEQAS for Blood Coagulation: Test Registrations; Level 2 Assays

- Factor II assay
- Factor V assay
- Factor VII assay
- Factor VIII:C assay
- Factor IX:C assay
- Factor X assay
- Factor XI assay
- Factor XII assay
- Factor XIII screen
- Quantitative VIII inhibitor
- Von Willebrand factor antigen assay
- Von Willebrand factor RCo (activity) assay

UK NEQAS for Blood Coagulation: Test Registrations; Level 2 Thrombophilia

- Antithrombin antigen assay
- Antithrombin activity assay
- Protein C antigen assay
- Protein C activity assay
- Protein S total antigen assay
- Protein S free antigen assay
- Protein S activity assay
- Plasminogen assay
- Activated Protein C resistance assay

The Importance of EQA

- EQA - retrospective analysis comparing results between laboratories and between methods

- EQA is required to confirm that results are accurate and are in agreement with those of other centres

EQA can identify:

- problems a laboratory has with a particular test
- problems with a particular method
- problems with reference plasmas
- problems in diagnosis or interpretation of results

Principles of EQA

- **Lyophilised samples distributed to participants**
- **Participants instructed to perform specific tests** (usual method)
- **Results returned to EQA centre for analysis**
- **Individual laboratory report issued**
- **Overall survey review published**


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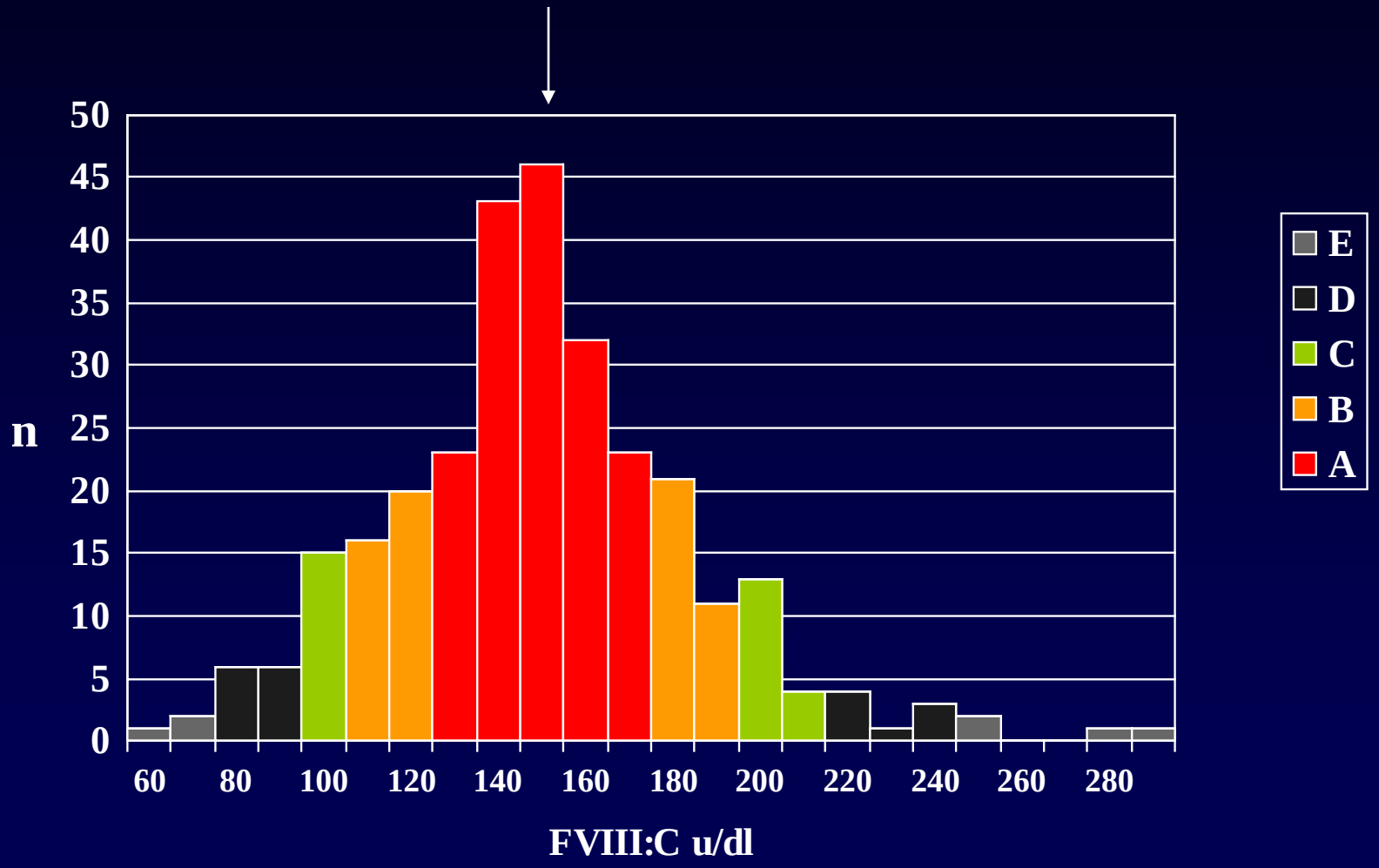
ANTIBODY.

EQA Performance Analysis:
Outwith Consensus

- **Screen tests** (PT; APTT)
  - **>15% deviation from median result**

- **Assays** (FVIII:C, FIX:C)
  - **Ranked Grading Analysis** (A-E)
  - **Two consecutive low grades**

Target Values



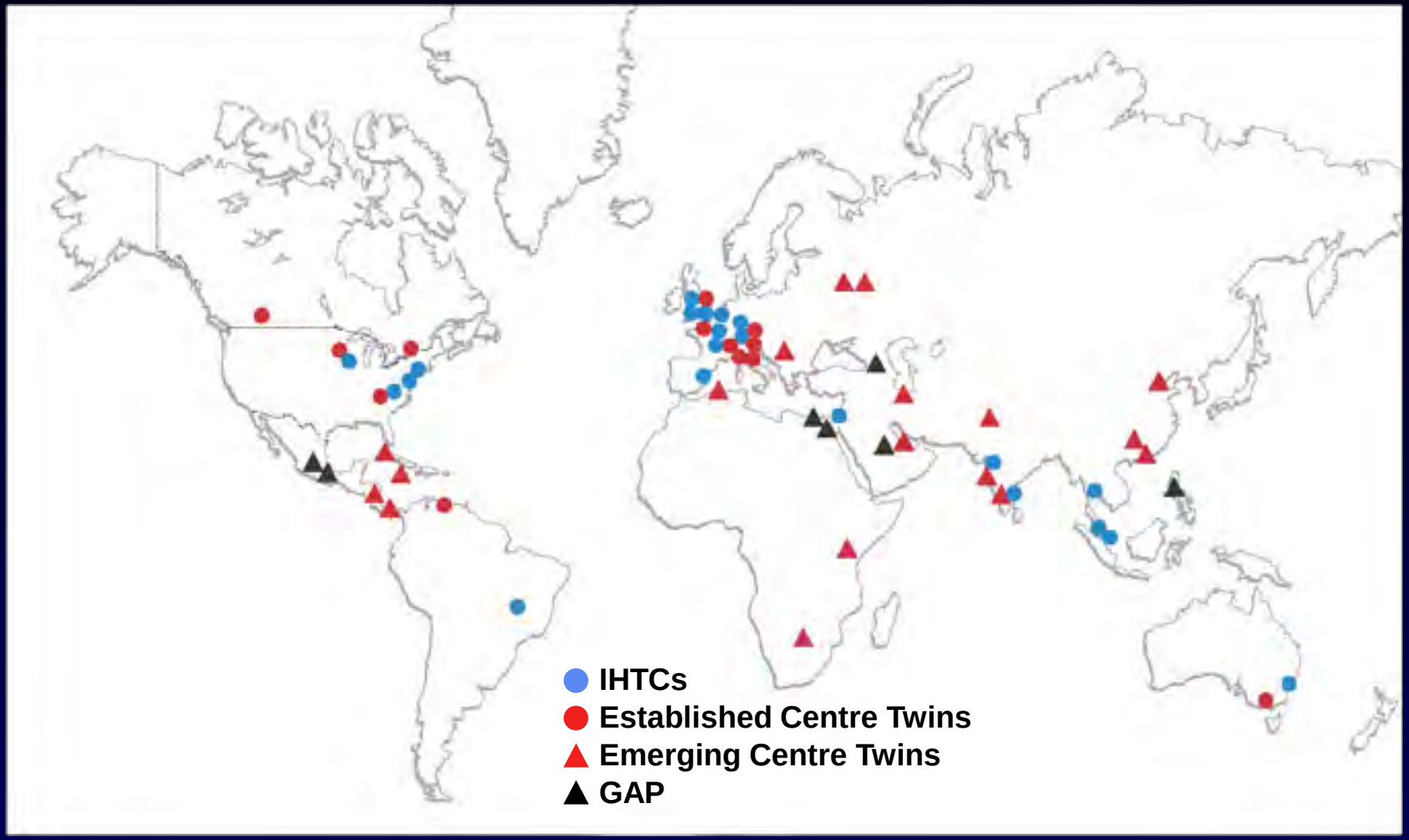
Performance:
Persistently outwith consensus

- **>15% from reagent group / overall median for screening tests on 3 consecutive occasions**

- **Three consecutive low grades for assays**
  - **eg E/E/E, D/D/D, E/C/E, D/E/E**

UKNEQAS
(Blood Coagulation)

- **Persistently ‘outwith consensus’**
- **Communication from Director**



WFH IEQAS programme

Participation in WFH EQA can improve laboratory performance

	Factor VIII		Factor IX	
	Local result	median	Local result	Median
Survey 1 2003	79 U/dl	24 U/dl	36 U/dl	91U/dl

Participation in WFH EQA can improve laboratory performance

	Factor VIII		Factor IX	
	Local result	Median	Local result	Median
Survey 1 2003	79 U/dl	24 U/dl	36 U/dl	91U/dl
Survey 12 2008	15 U/dl	21 U/dl	56 U/dl	50 U/dl

Inter- laboratory variation FVIII:C results 2004-9

Survey	Median	Established Centre CV	Emerging centre CV
3	15 IU/dl	27%	30%
4	75 IU/dl	21%	32%
5	14 IU/dl	30%	137%
6	52 IU/dl	14%	26%
13	31IU/dl	18%	25%
17	34 IU/dl	14%	42%

Solving EQA problems
Extraction from Database

survey	sample	APTT reagent median	Local result	dev	Local interpretation
177	FVIII:C 35 IU/dl	1.29	1.08	-16%	Normal
178	FXI 35 U/dl	1.30	1.10	-12%	Normal
179	Normal	1.01	0.85	-15%	Normal
180	FXII 16 U/dl	1.18	0.99	-23%	-

Solving EQA problems Extraction from Database

- Centre contacted programme staff to discuss
- Reference range in use locally – 28 to 40 sec
- 14 other users of same reagent/instrument
- Mean normal range 25 to 33.5 sec
- Mean normal value locally therefore 34 versus 29 elsewhere
- Accurate local APTT would give a low ratio (test/mid normal)

Solving EQA problems Extraction from Database

New normal range introduced

- Following survey (181)
  - Local result 1.17
  - Reagent median 1.17
  - Deviation 0%!
- Local patient results accurate but wrong reference range led to problems of interpretation and missed diagnoses

Improving performance
VWF Ag results

Survey	Local result	median	% deviation
10	29 IU/dl	36 IU/dl	20%

Improving performance
VWF Ag results

Survey	Local result	median	% deviation
10	29 IU/dl	36 IU/dl	20%
11	7 IU/dl	11 IU/dl	36%

Improving performance
VWF Ag results

Survey	Local result	median	% deviation
10	29 IU/dl	36 IU/dl	20%
11	7 IU/dl	11 IU/dl	36%
12	41 IU/dl	55 IU/dl	25%

EQA problem solving
Repeat samples and SSC reference plasma

- Repeat samples – similar results
- SSC reference plasma available via EQAS for trouble shooting
- Local lab checked commercial reference plasma against SSC standard
- Local standard reading low by 27%
- Local WFH EQA results low by 19 - 35% (mean 27%!!)

EQA problem solving
Repeat samples and SSC reference plasma

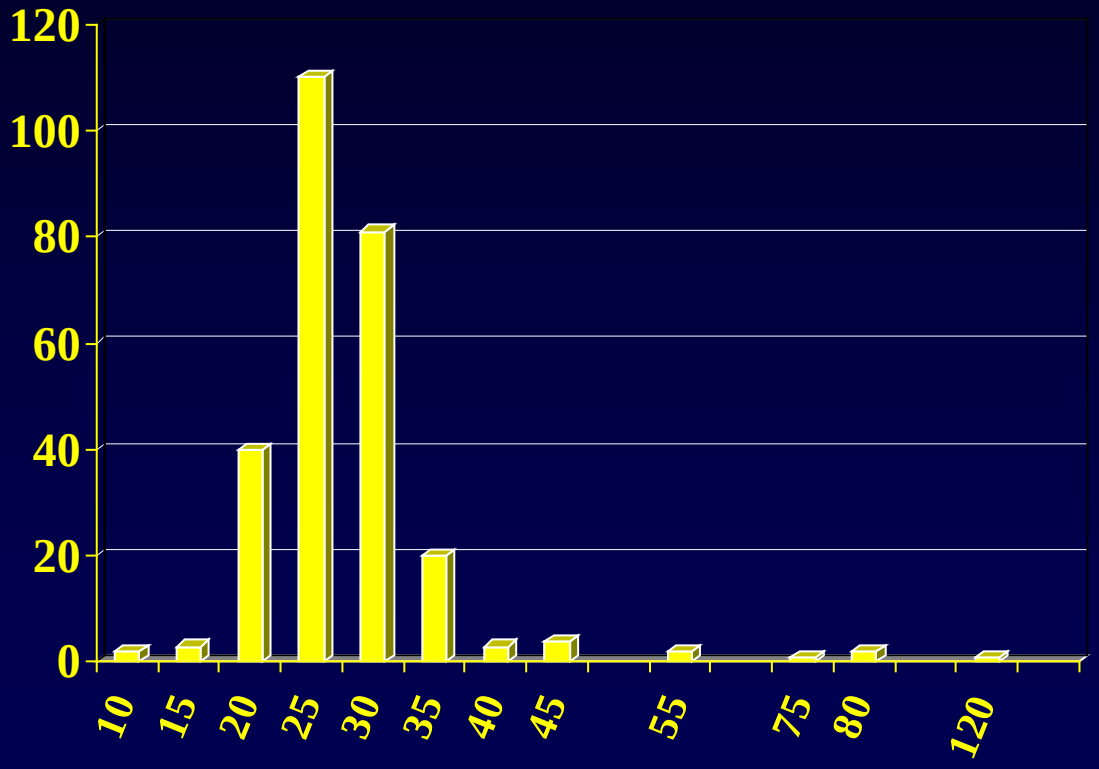
- Repeat samples – similar results
- SSC reference plasma available via EQAS for trouble shooting
- Local lab checked commercial reference plasma against SSC standard
- Local standard reading low by 27%
- Local WFH EQA results low by 19 - 35% (mean 27%!!)

EQA problem solving

- Changed reference plasma source
- Next survey
  - local result 20 IU/dl
  - Median 23 IU/dl
- Problem solved !

Factor VIII:C results in Different centres

256 centres (UK NEQAS 1999)



5 centres ≤ 15 u/dl

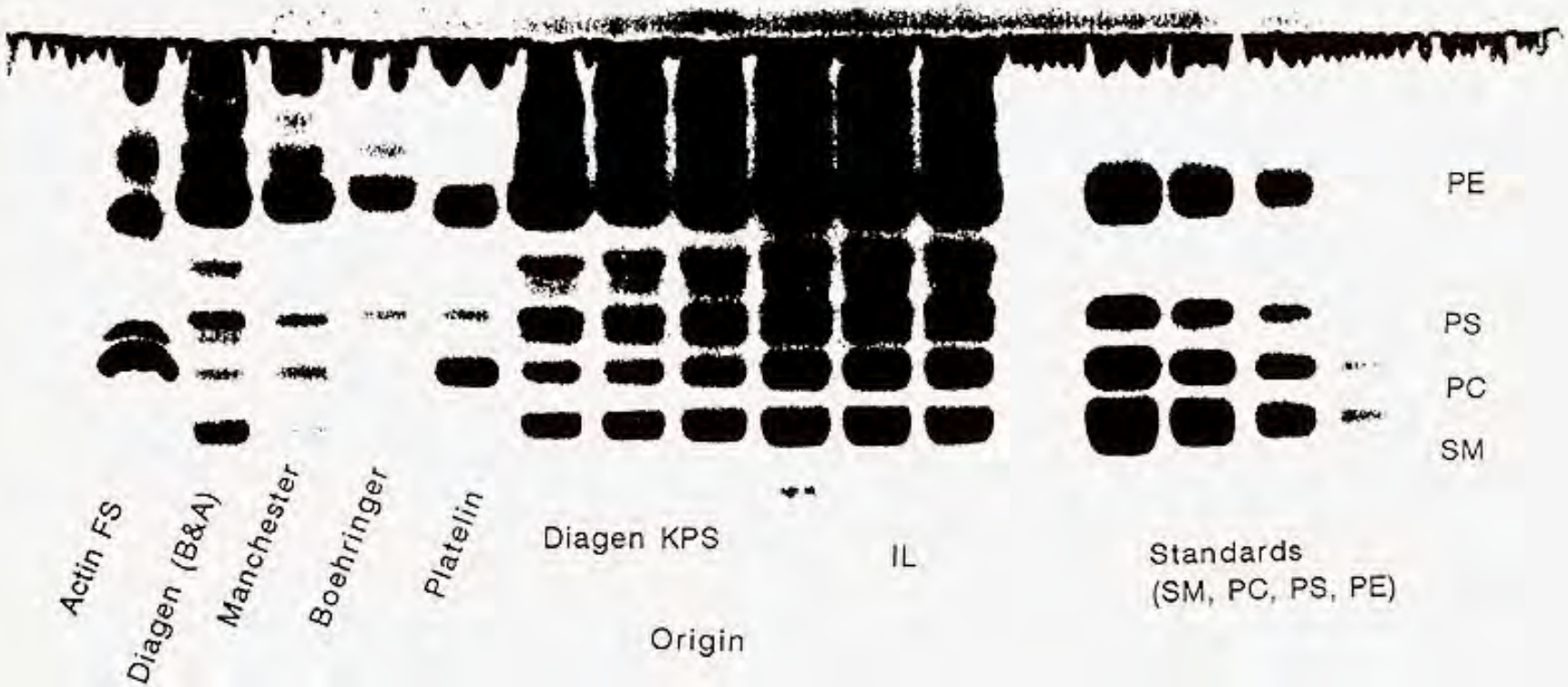
6 centres ≥ 50 u/dl

One-stage Factor VIII:C Assays

UK NEQAS Participants (2002)

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

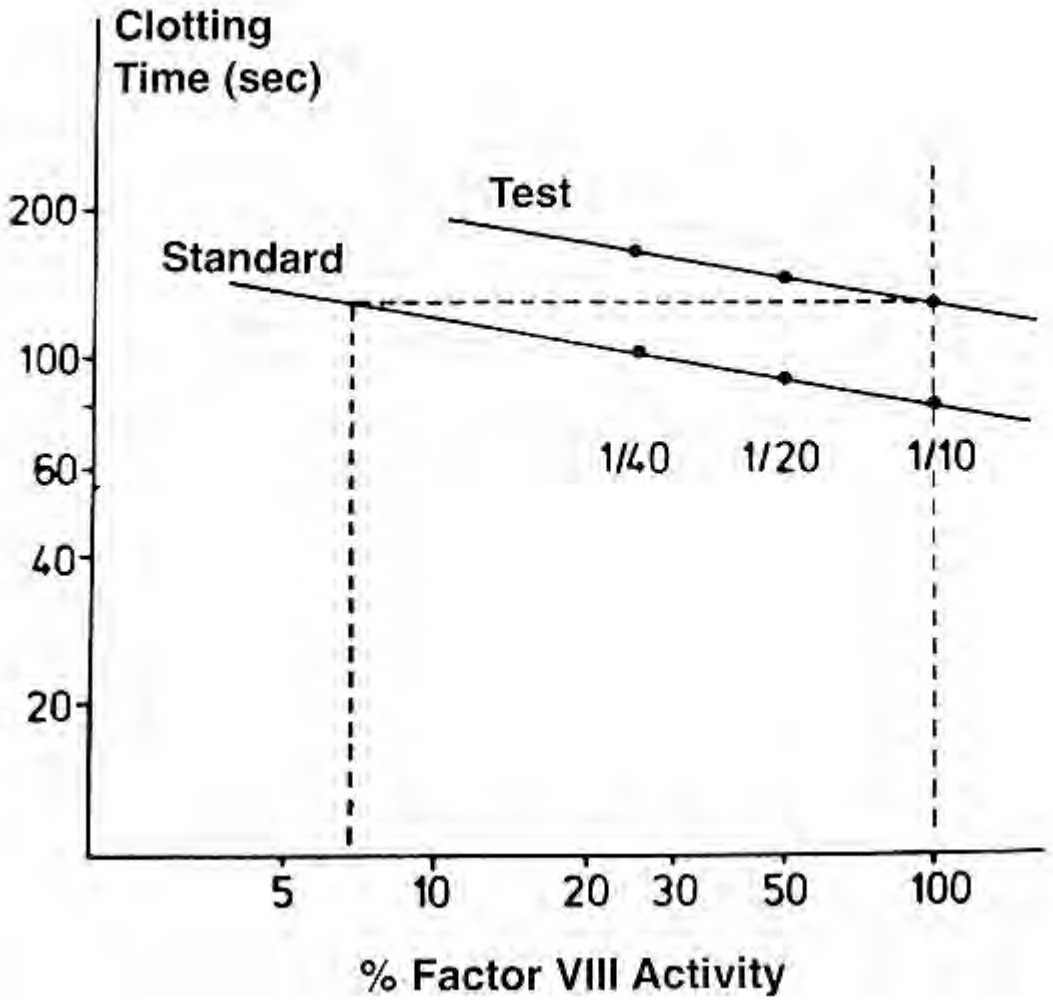
Phospholipid quantitation by HPTLC/Laser densitometry



Factor VIII:C – test sample from 2002

Commercial reference plasmas (n>10)

Reference plasma	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


Clotting
Time (sec)
200
100
60
40
20
Test
Standard
1/40
1/20
1/10
5
10
20
30
50
100
% Factor VIII Activity

Factor assay design

	1999	2003	2009
n	200	90	160
centres	UK/overseas	UK Haem centres	UK/overseas
factor	VIII:C	IX	VIII:C
Single test dilution	25%	25%	33%
Stored calibration curve	33%	32%	49%

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

Factor assays - Why 3 test dilutions?

FVIII:C 2009

Test dilutions	n	Mean FVIII:C IU/dl	CV %
1	39	6.6	96%
2	18	5.8	28%
3	49	6.1	44% (29%)*

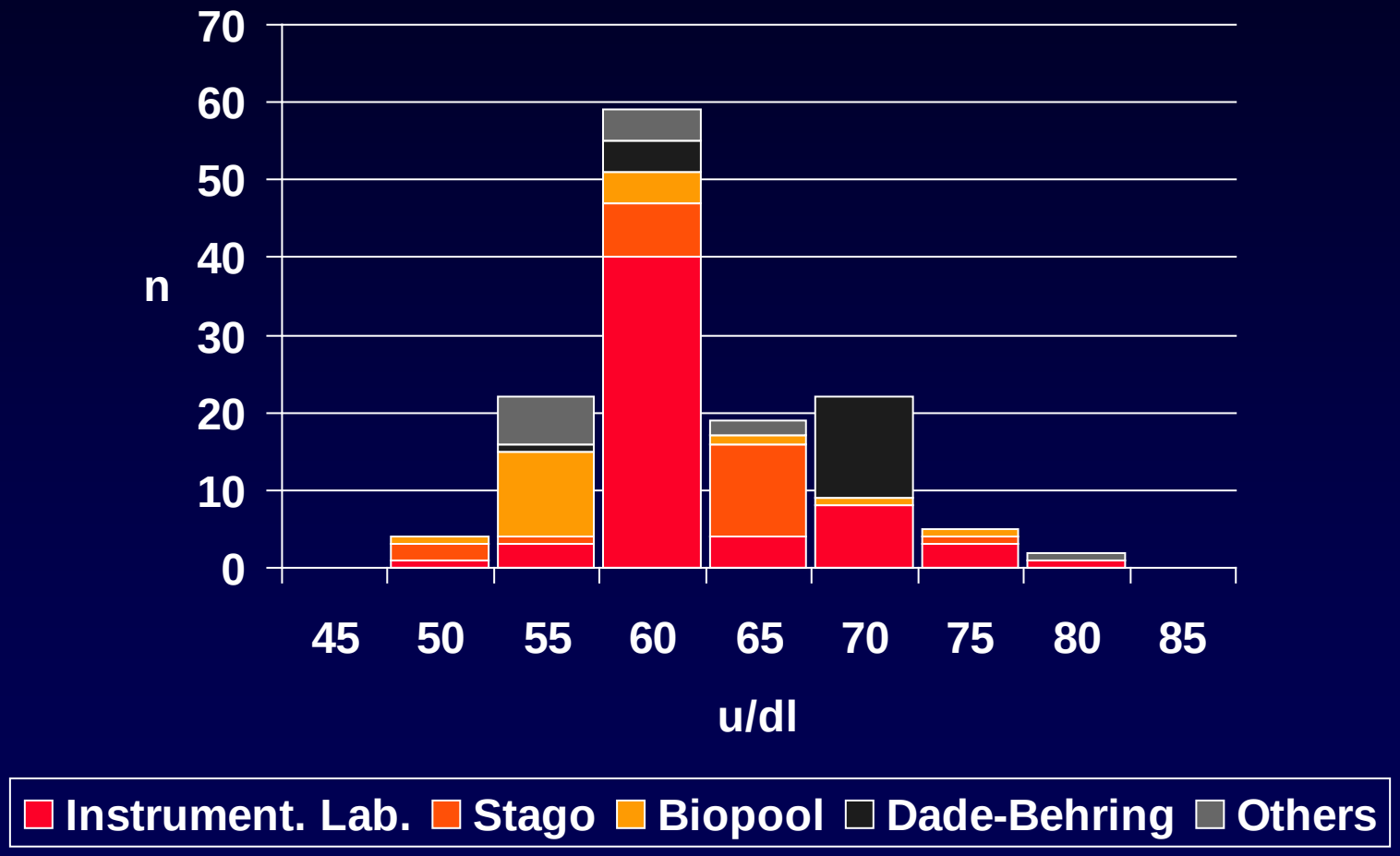
Factor VIII:C

Commercial deficient plasmas (S149 2005)

Source	n	Median (u/dl)
A	32	13.0
B	82	15.0
C	7	30.0
D	47	15.0
E	84	17.0
F	18	12.6
All	327	15.0

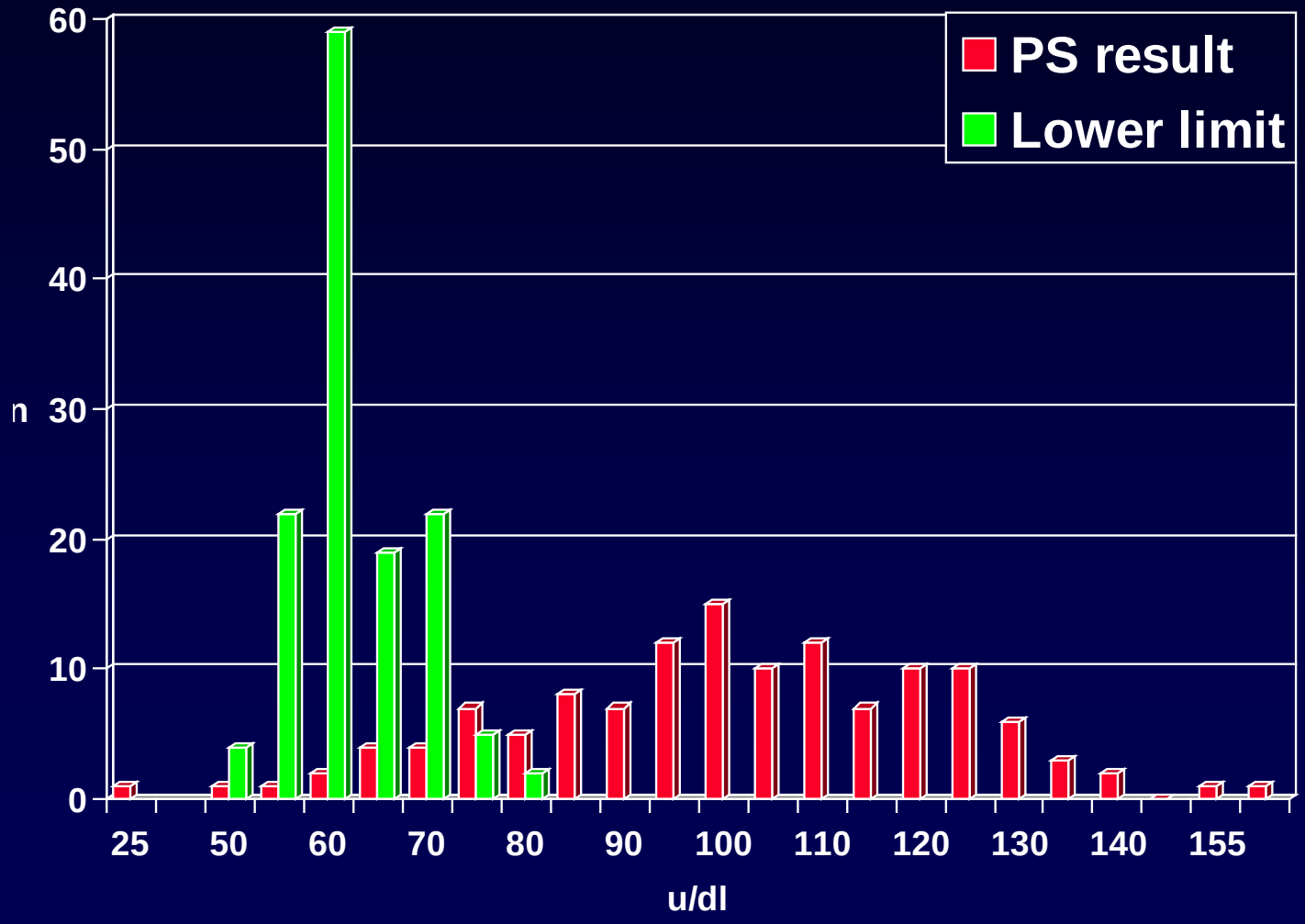
C – FVIII< 1 U/dl, FV = 3 U/dl, other factors normal

Lower Limit of Reference Range
(PS activity)



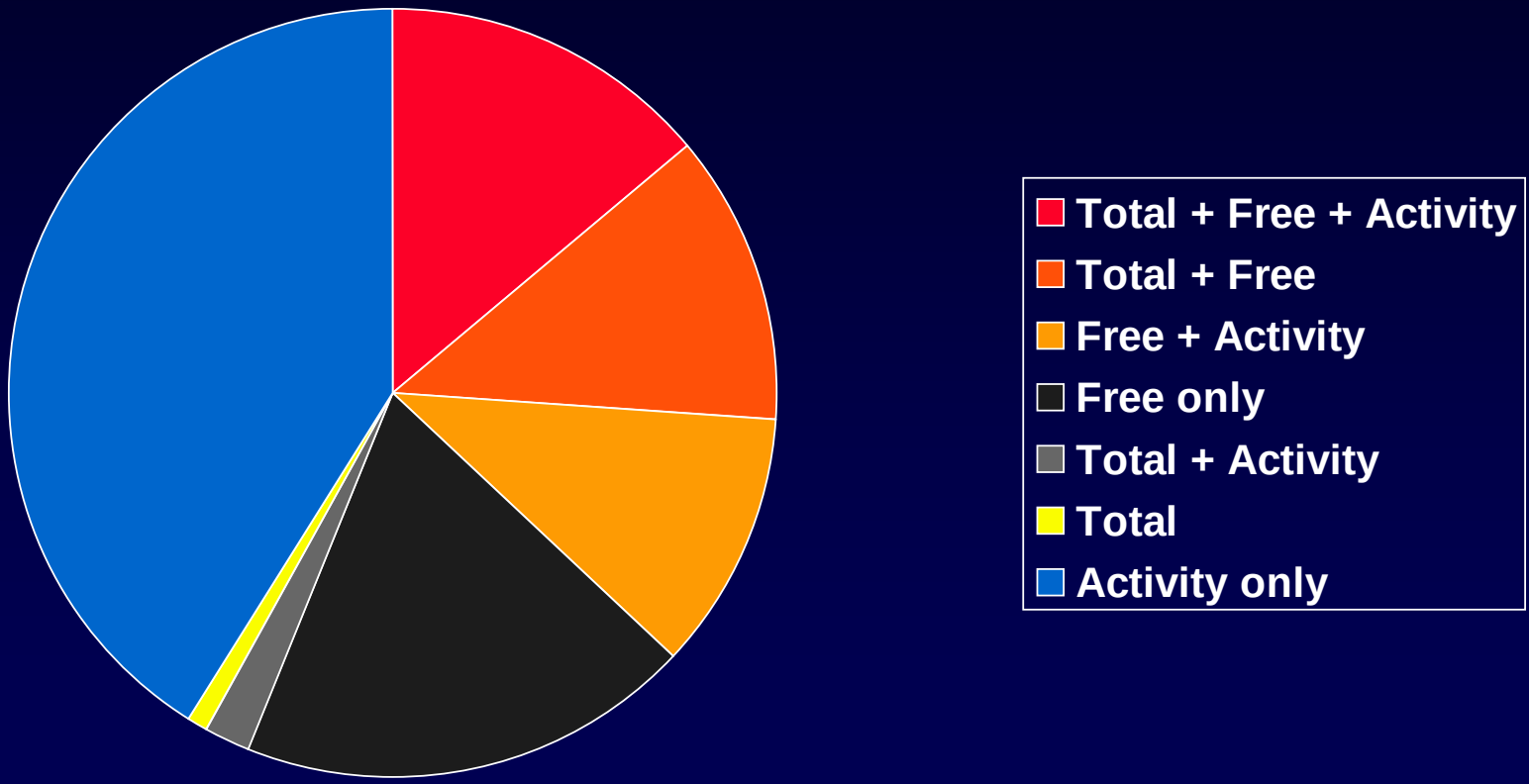
PS Activity:

Interpretation is influenced by lower limit of reference range

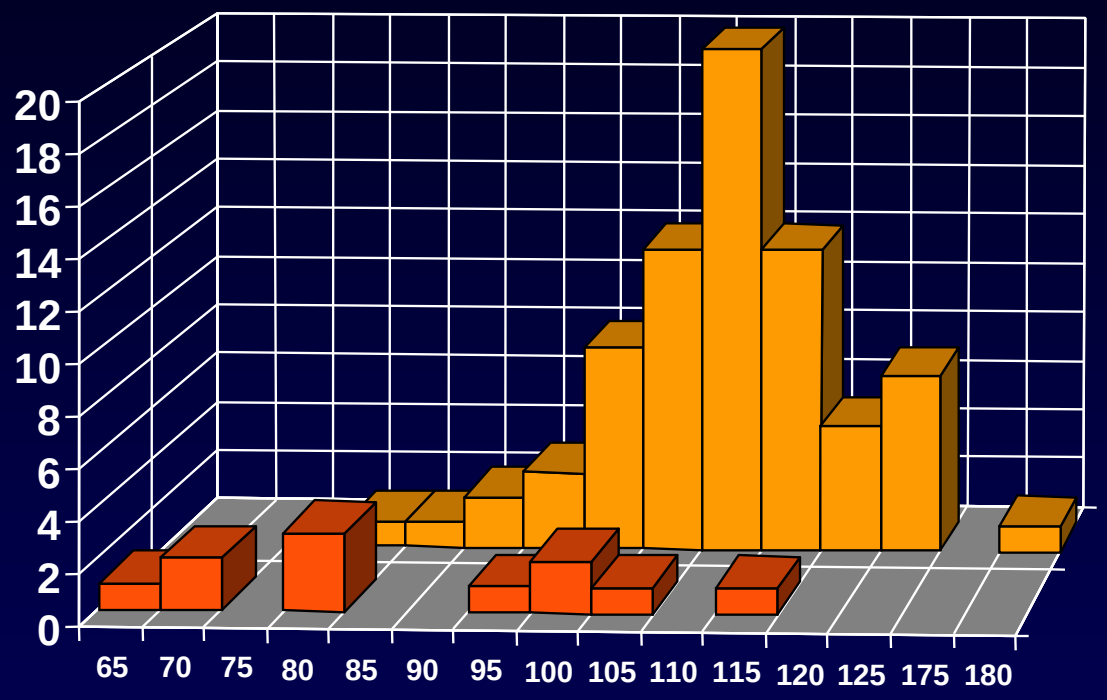


UK NEQAS Thrombophilia Testing:

Protein S Assays employed by participants



PS activity assays



Kit A: n = 72, median = 109.0u/dl

Kit B: n = 11, median = 82.0u/dl

P<0.0001

Familial Thrombophilia Testing
Protein S Assay Kits

Manufacturers of Kits A and B quote same reference range!

They are clearly different

PS activity

PS reference ranges by method (n=20)

	Bovine TPN	***Factor Va***	***Factor Xa***
In-house reference range	>66.6u/dl	>66.8u/d	>74.2u/dl
Manufacturers reference range	>61.9u/dl	>65.0u/d	>55.0u/dl

PS activity

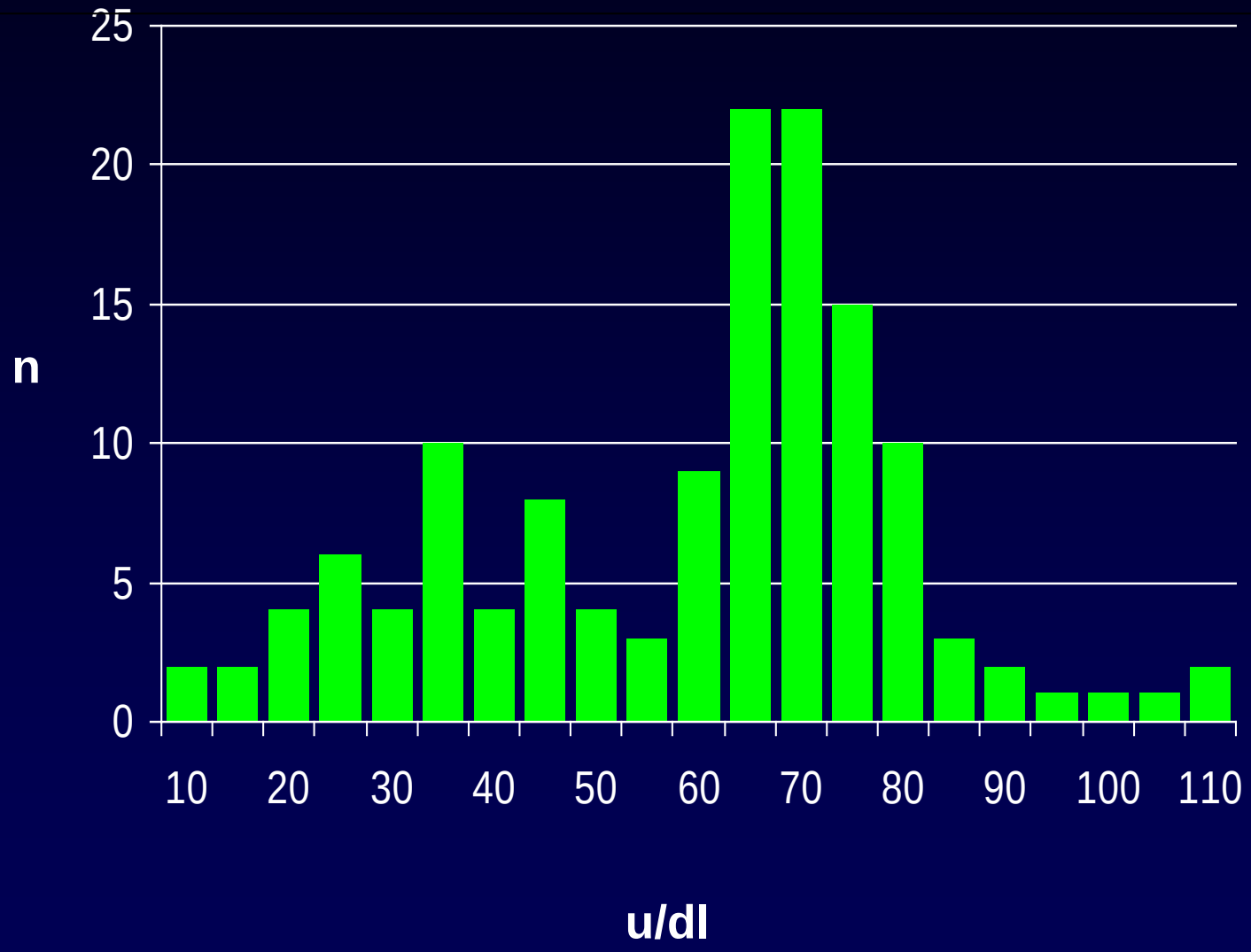
PS reference ranges by method (n=20)

	Bovine TPN	***Factor Va***	***Factor Xa***
In-house reference range	>66.6u/dl	>66.8u/d	>74.2u/dl
Manufacturers reference range	>61.9u/dl	>65.0u/d	>55.0u/dl

Sensitivity to genetically confirmed PS deficiency (n=23)

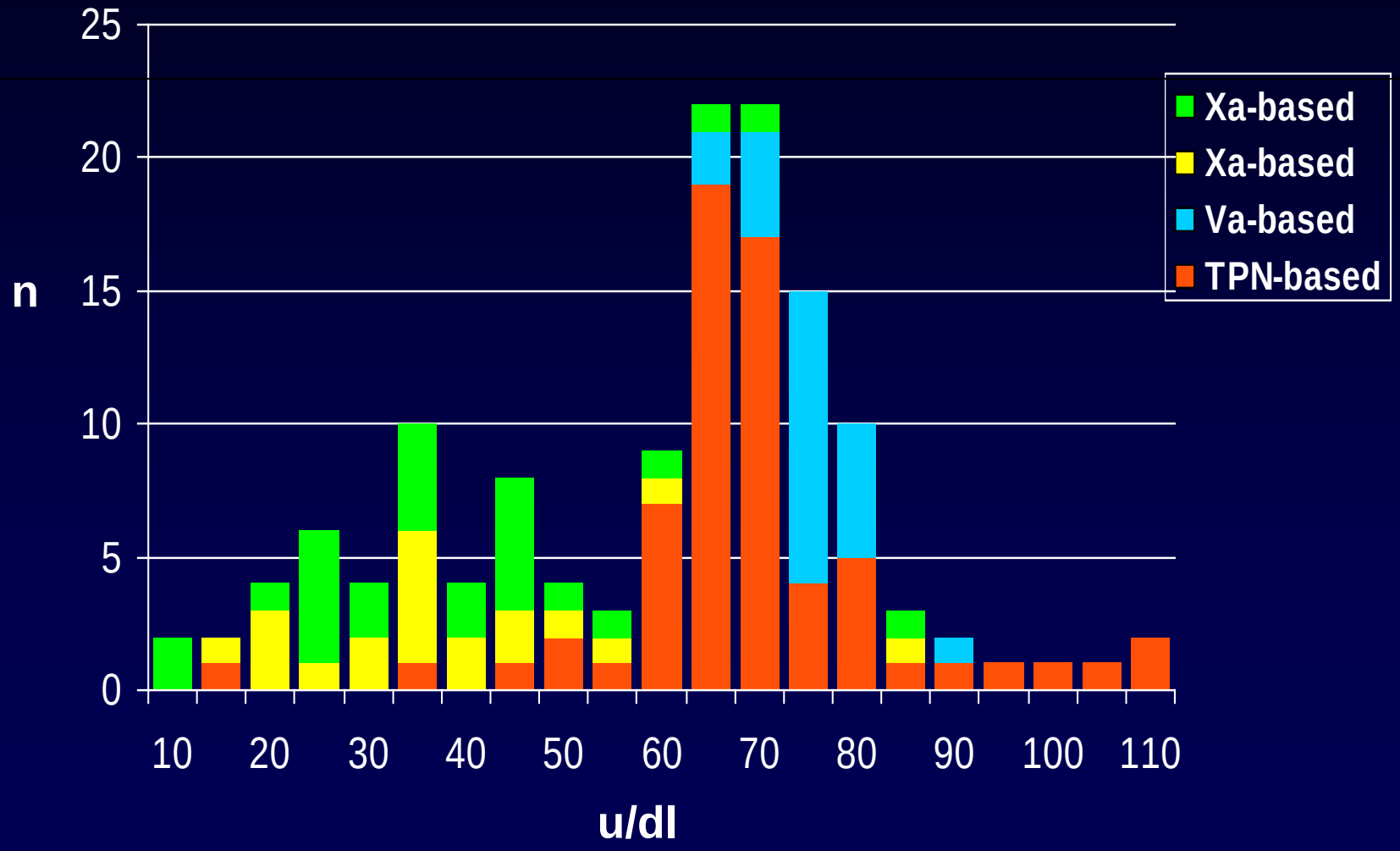
	Bovine TPN	***Factor Va***	***Factor Xa***
Using in-house range	100%	100%	100%
Using manufacturers range	100%	100%	87%

Protein S activity



Protein S activity

Donor homozygous for FV Leiden

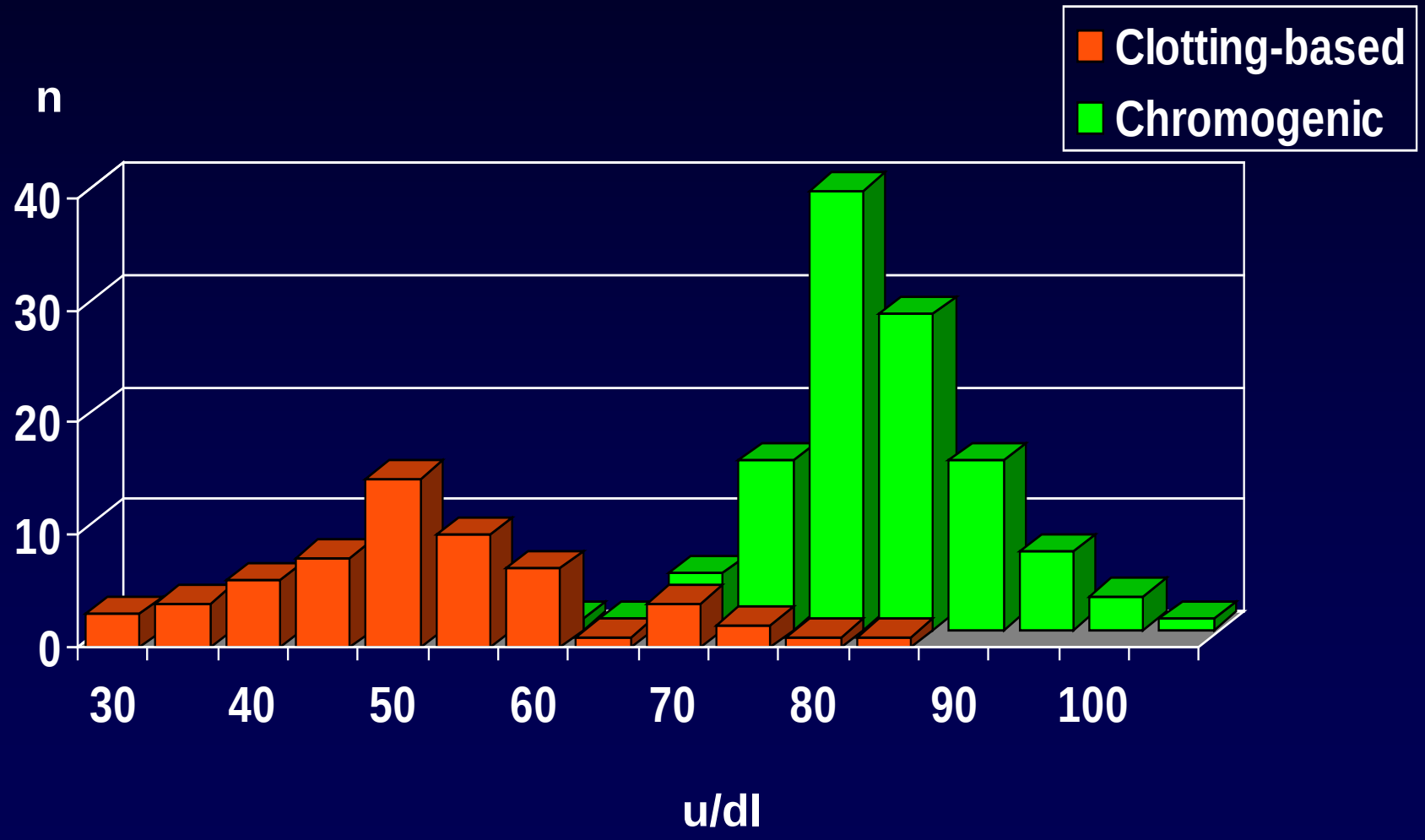


Familial Thrombophilia Testing: Problems of interpretation

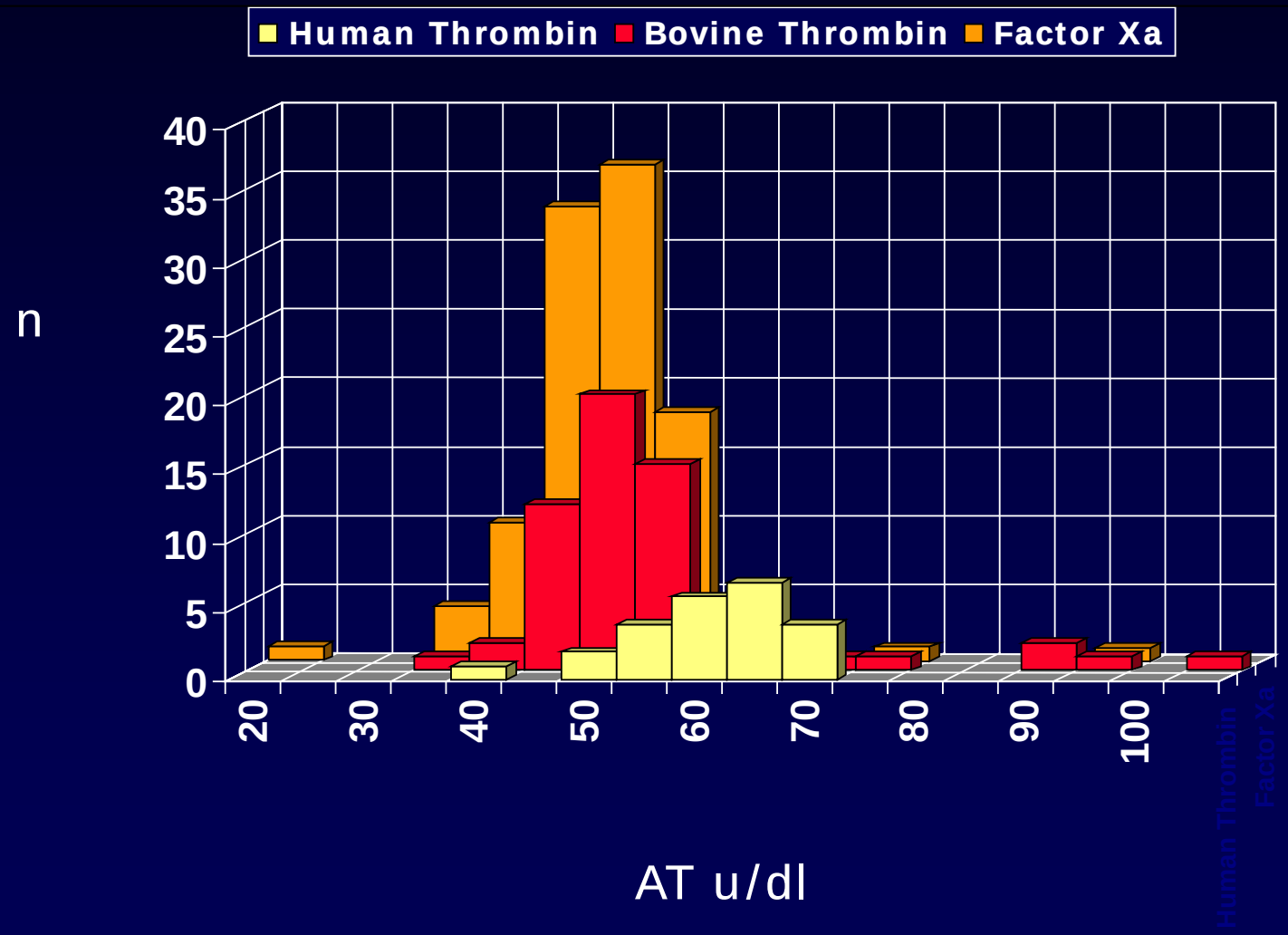
Factor V Leiden reduces PS activity assays and PC clotting assays!

Protein C activity

Donor homozygous for FV Leiden



Antithrombin activity assays:
Antithrombin Wobble (Thr85Lys)



Familial Thrombophilia:
Molecular Genetic Testing

- **Period of Testing** **July 99 - May 02**
- **Participating Labs** **42 - 76**
- **No. of distributed samples** **36**
- **Incorrect Results (%)**
  - **FVL heterozygous** **1.1**
  - **FVL homozygous** **2.8**
  - **P20210A** **0.9**