The ISTH and Coagulation Diagnostic Standardization and Quality

Gilbert C. White, II, MD
Executive Director, ISTH

Blood Research Institute
Milwaukee, WI

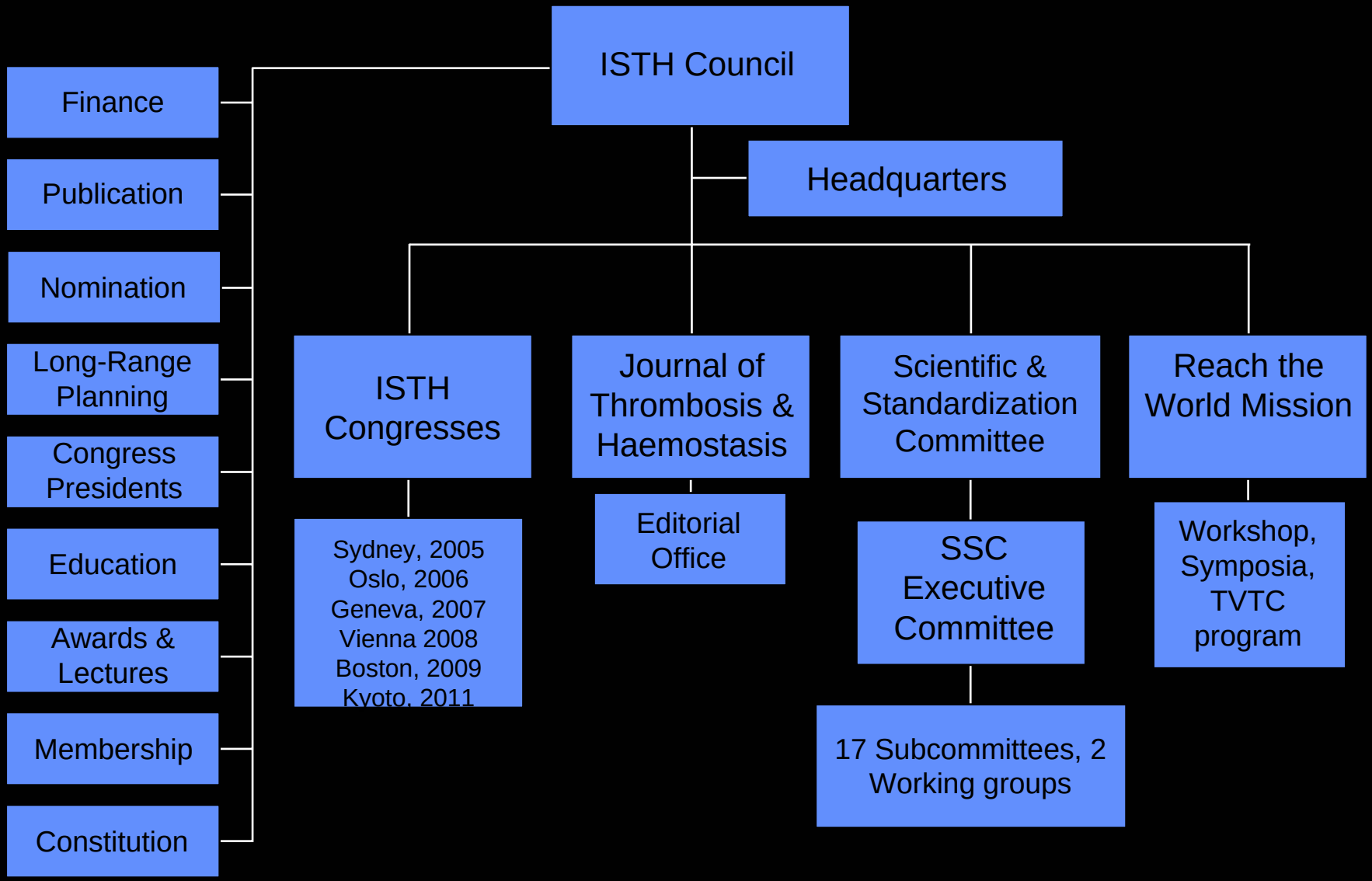
ISTH and its Mission

What is ISTH?

- The International Society on Thrombosis and Haemostasis
- Membership open to all – currently 2715 members

What is its mission?

To foster scientific interchange and interactions in the clinical and scientific fields of blood coagulation, haemostasis, thrombosis, and vascular biology through scientific meetings, workshops, and printed materials.


ISTH Council
Finance
Publication
Nomination
Long-Range Planning
Congress Presidents
Education
Awards & Lectures
Membership
Constitution
Headquarters
ISTH Congresses
Sydney, 2005
Oslo, 2006
Geneva, 2007
Vienna 2008
Boston, 2009
Kyoto, 2011
Journal of Thrombosis & Haemostasis
Editorial Office
Scientific & Standardization Committee
SSC Executive Committee
17 Subcommittees, 2 Working groups
Reach the World Mission
Workshop, Symposia, TVTC program

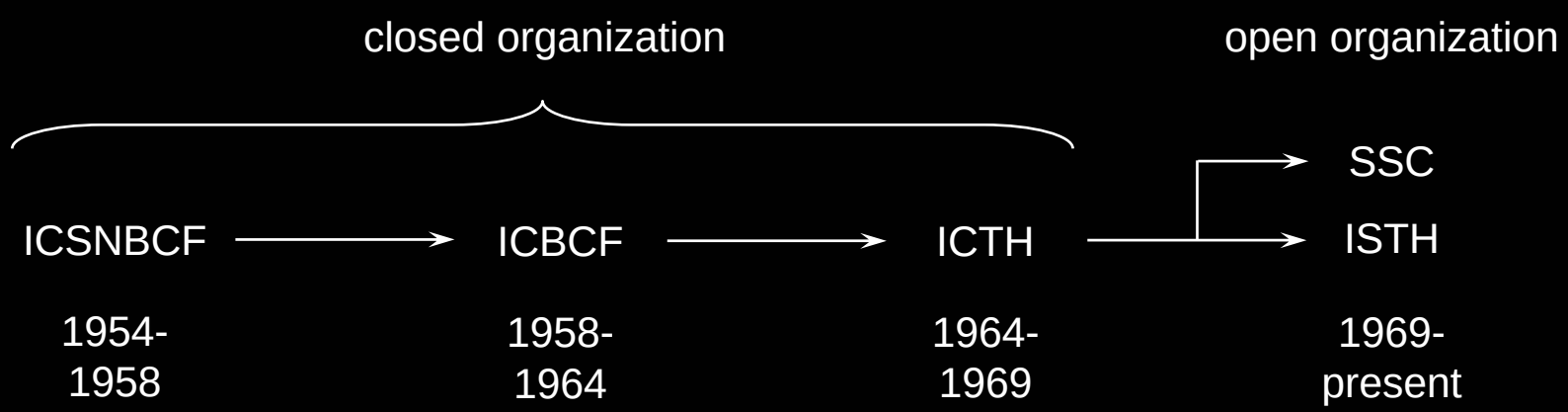
ISTH Roots Extend Back to 1954

International Committee on the Nomenclature of Blood Clotting Factors, Rome, September 1958

The original "active" members of the International Committee for the Standardization of the Nomenclature of the Blood Clotting Factors (formed in 1954) were T. Astrup, K. Brinkhous, P. De Nicola, E. Deutsch, R.B. Hunter, L.B. Jaques, H.F. Jensen, E. Jorpes, F. Koller, K. Lengenhager, R.G. Macfarlane, W. Merz, P.A. Owren, A. Pavlovsky, A.J. Quick, W.H. Seegers, J.P.Soulier, M. Verstraete, E. Wöhlisch, I.S. Wright.

Roman numerals accepted as official nomenclature
Thrombosis et Diathesis Haemorrhagica adopted as official journal

Evolution of the ISTH

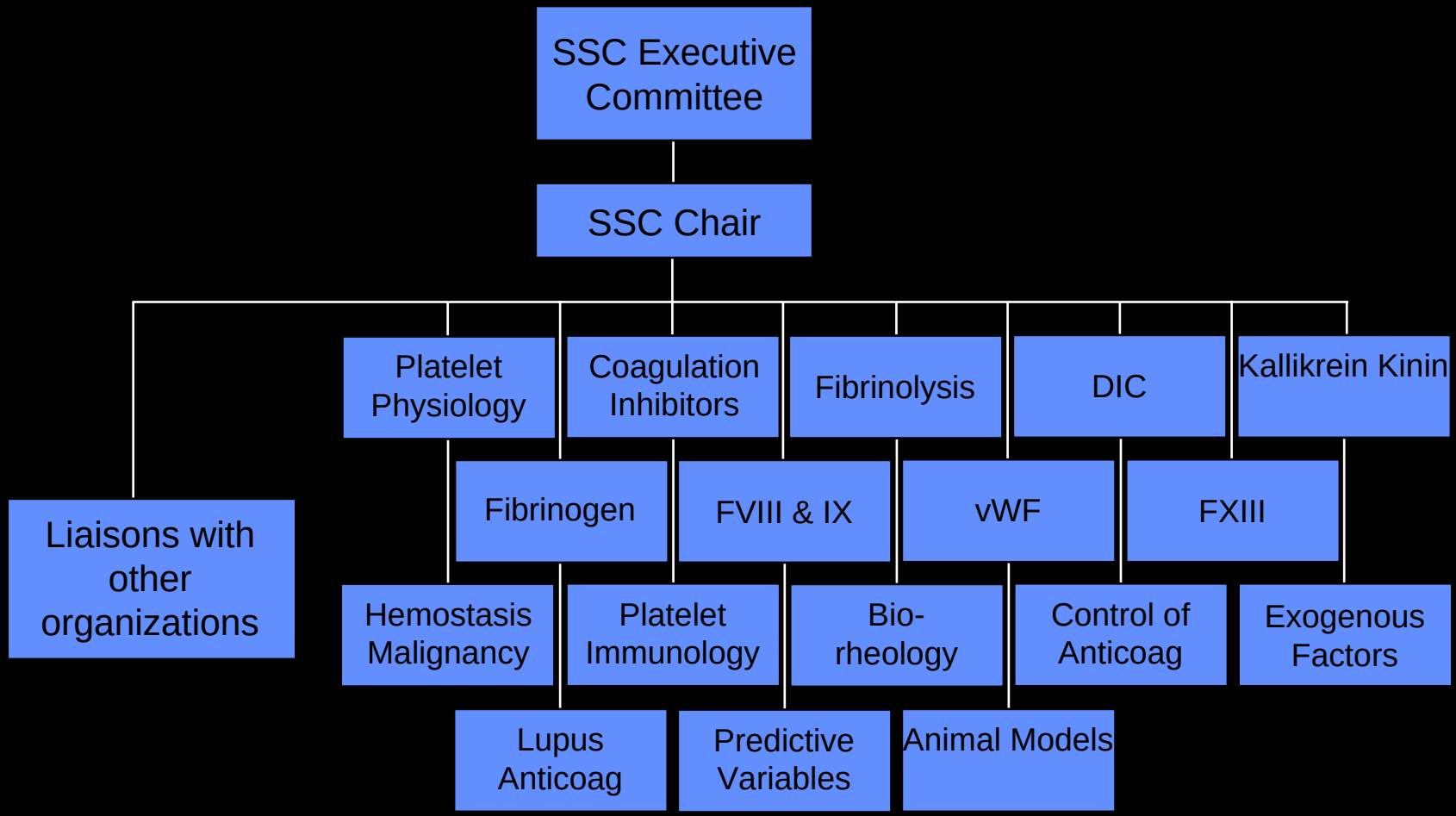


SSC Mission

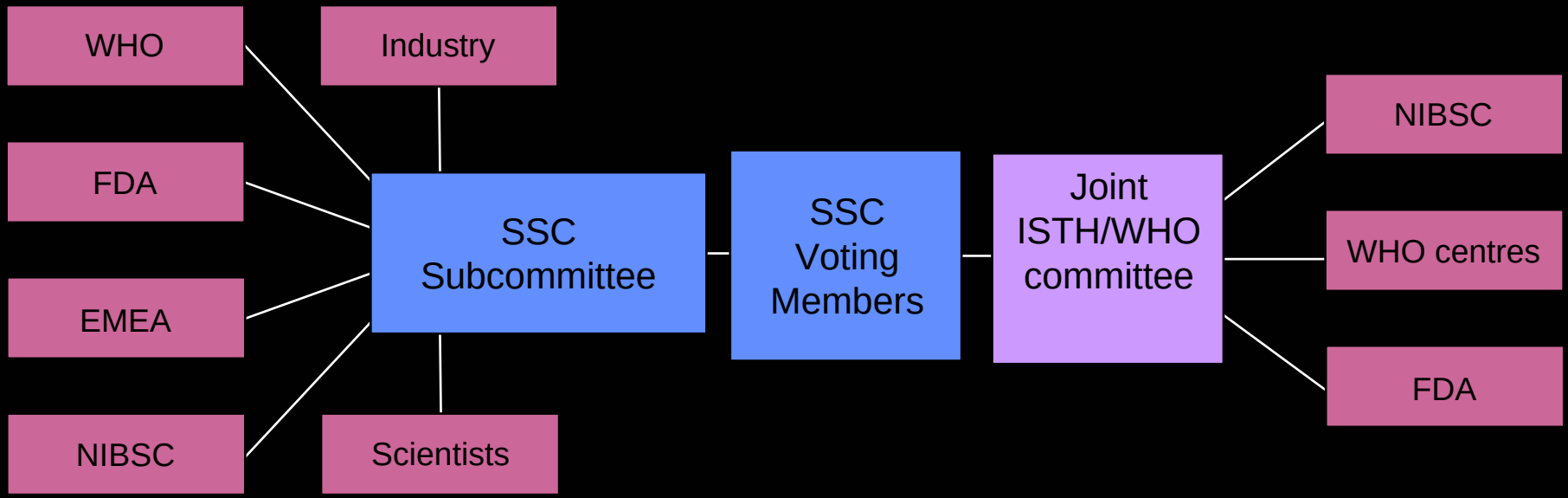
The Scientific and Standardization Committee is a permanent committee of the ISTH that accomplishes the work of the Society, initially related to the development of reference materials, standardization of methods, and nomenclature, but increasingly focused on broader scientific issues. The SSC:

- provides a forum for consideration of practical issues related to thrombosis, disorders of haemostasis and their underlying vascular biology by internationally recognized leaders in these research areas
- supports scientific subcommittees of international expert researchers working on these problems and their application to clinical issues
- promotes education and the exchange of ideas through scientific meetings and publication of official reports, recommendations and deliberations
- standardizes nomenclature and research methods as appropriate and timely
- provides expert consultation to standards-setting bodies
- liaises with other research organizations and collaborates on timely and important research matters

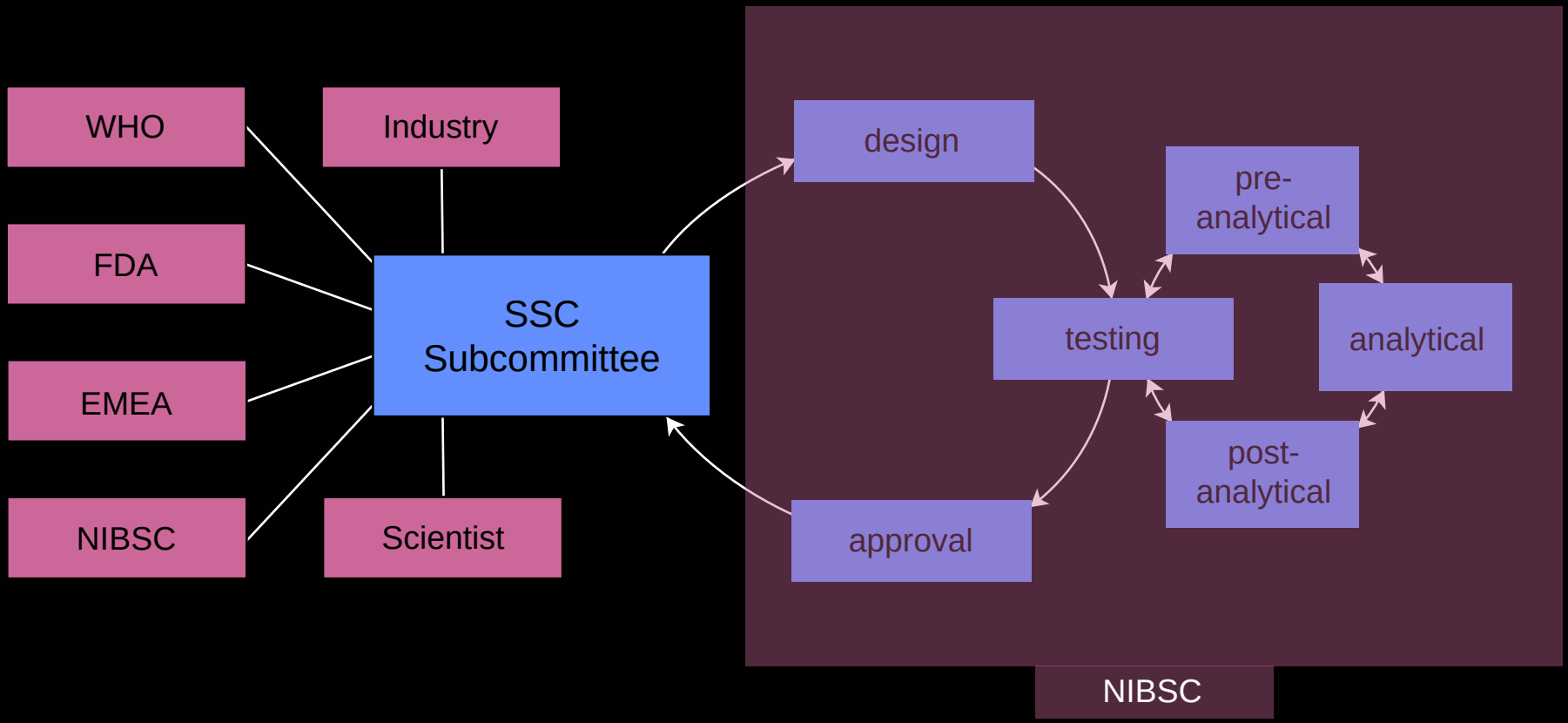
SSC Organization



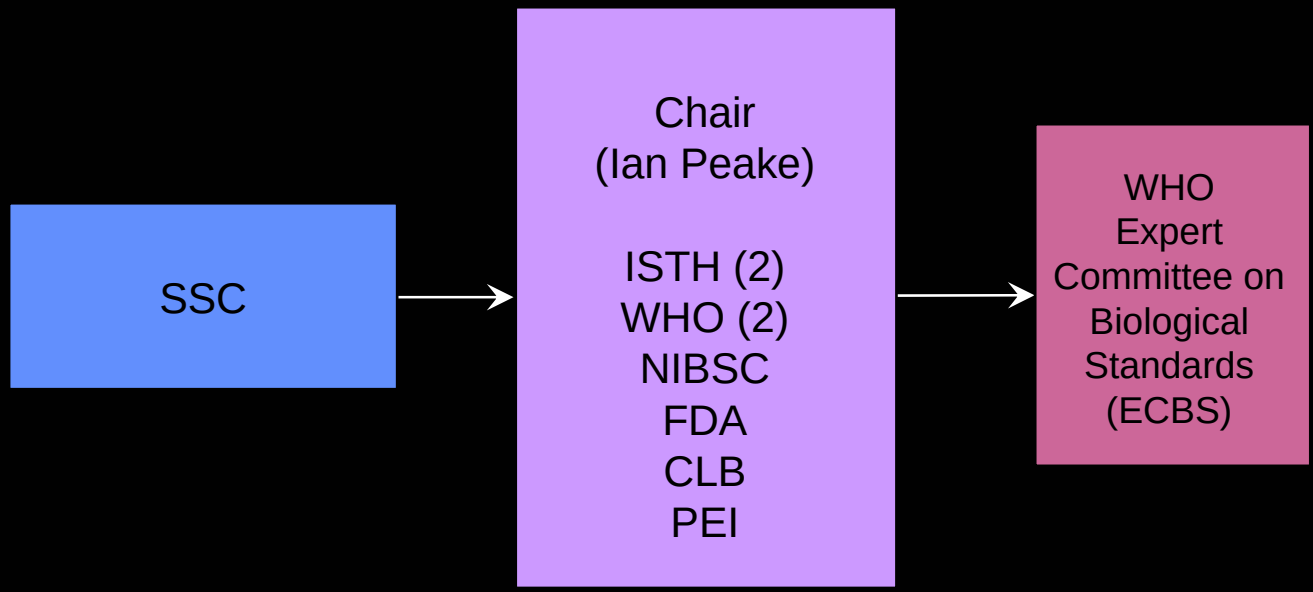
SSC Approval Process



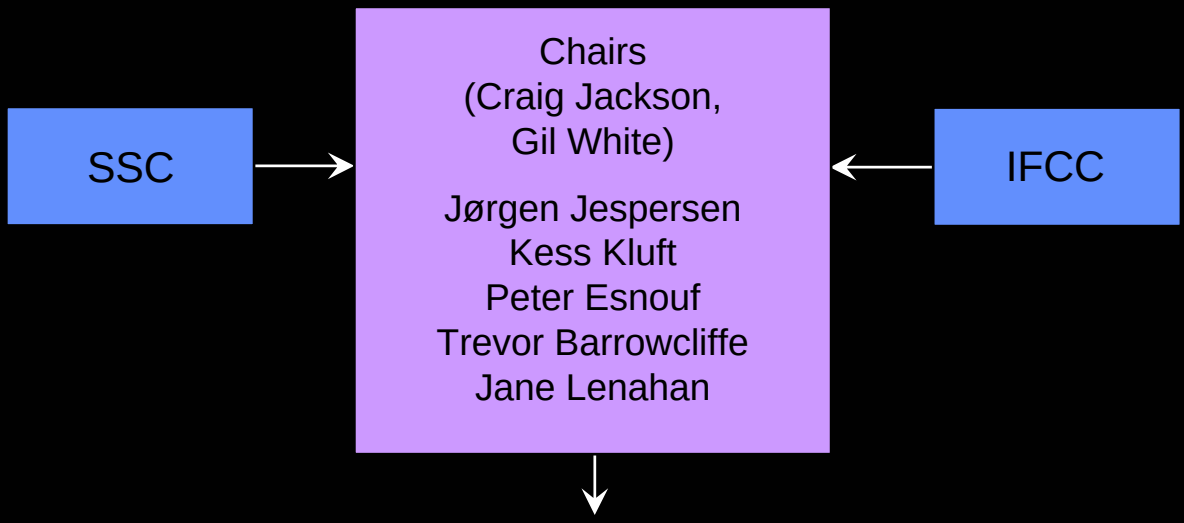
SSC Approval Process (2)



SSC Approval Process (3): Joint ISTH/WHO Committee



SSC Approval Process (4): Joint ISTH/IFCC Committee on the Standardization of Coagulation Tests (C-SCT)



First Test Method:
Antithrombin

Develop methods to achieve traceable, commutable materials and procedures for achieving comparability of test results from routine test methods.

C-SCT Recommendations

1. Approaches to the Standardization of the Coagulation factors should take into account the relationship between the structures and the functions of the individual proteins.

2. The base units for expression of amounts of protein should be moles. Conversion of moles to “traditional” units should only be done by a metrologically sound procedures.

3. Primary reference materials should be homogeneous protein preparations that have been extensively characterized by state-of-the-art methods.

4. Further characterization of the homogeneous products should be the first step toward achieving rigorous traceability between these and future reference materials.

5. Whenever possible future reference materials should be recombinant products. The exon sequence selected should be from an individual with fully functional protein, to the extent that this can be known. The recombinant product must be a chemically homogeneous protein preparation that has been extensively characterized by state-of-the-art methods.

Thrombos. Haemostas 2002

Problems with Application of Metrological Methods to the Measurement of Biological Activities of Proteins in Haemostasis & Thrombosis

1. Post-translational modifications important for function of the protein may be altered in recombinant proteins - ex. factor IX serine phosphorylation and tyrosine sulfation

2. Genes may be alternately spliced giving rise to proteins with different functions - ex. tissue factor pathway inhibitor α and β

3. Proteins may have multiple functions, but not all those functions may be detected by a given assay - ex. factor VIII role in tenase complex and association with von Willebrand factor

4. Proteins may have polymorphisms that affect function, but recombinant proteins are not polymorphic - ex. Pl^{A1} vs Pl^{A2} forms of $\alpha IIb\beta 3$ integrin

5. Multimeric forms of proteins may have different functions - ex. high vs low molecular weight multimers of vWf

Reference Measurement System for Antithrombin

- To develop a primary reference method for measurement of antithrombin with SI traceability
- To establish reference materials using primary reference method
- To establish secondary reference methods and reference materials for antithrombin type I and type II deficiencies relative to primary reference material

NIBSC

Working Group Members

- Elaine Gray
- Craig Jackson
- Steve Kitchen
- Peter Cooper
- Steffen Rosen
- Jacqueline Conard

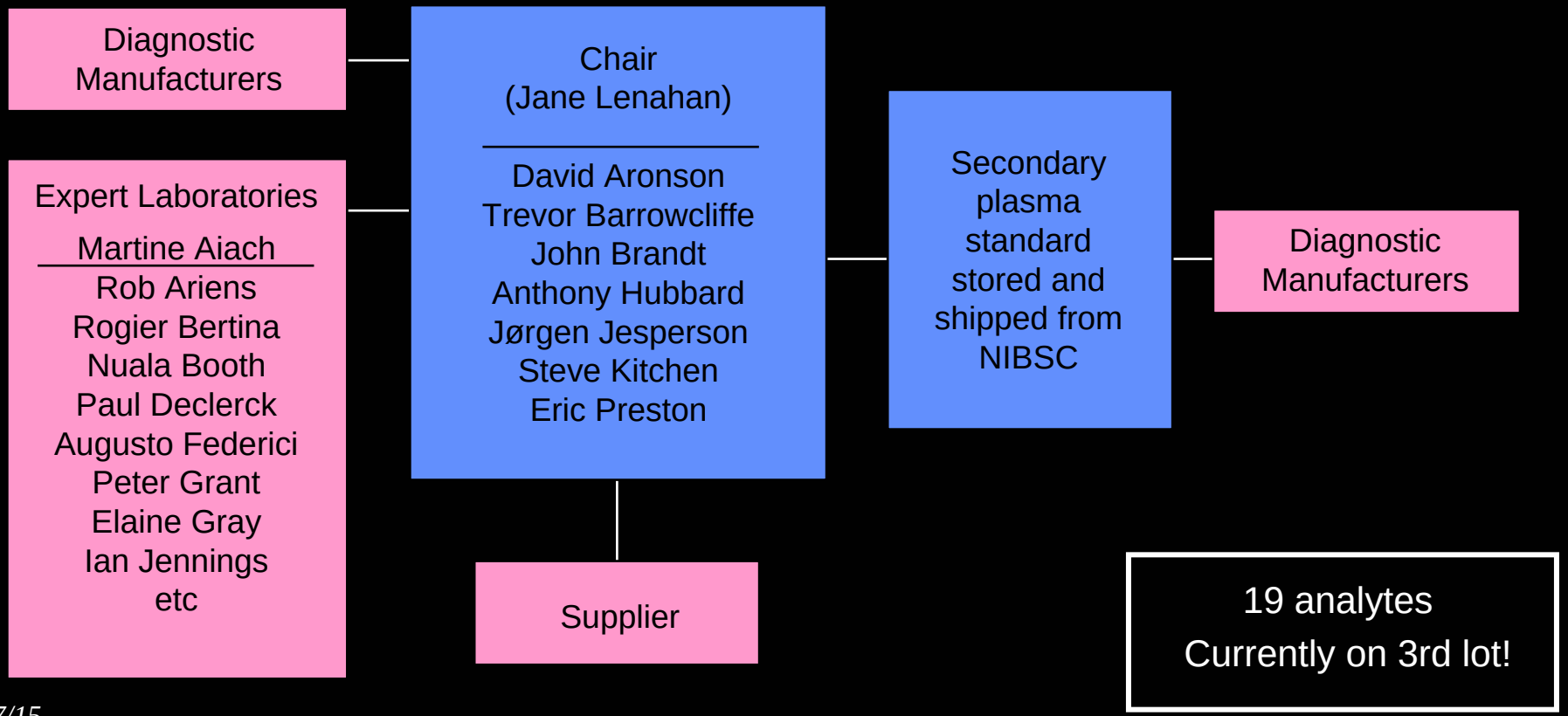
NIBSC

Plan of Action

1. To set up robust protocol for primary reference method
   - *Identifying Matrix Effects and Influence Quantities (Progressive antithrombin – NO heparin)*
2. To source critical reagents for protocol
3. To carry out pilot study on primary reference method in 5 to 6 expert laboratories

Working Group on Coagulation Standards

Collaboration between SSC and manufacturers of diagnostic reagents to develop a uniform plasma standard for use by the manufacturers in labeling their coagulation calibrators.



SSC Plasma

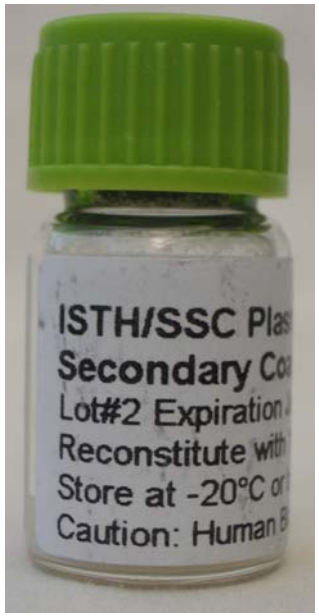
- Working Standard for diagnostic manufacturers & QA organisations
- Large batch, ~ 50,000 vials, around 5 years shelf-life, current Lot 2, about to be replaced by Lot 3
- Calibrated for 19 coagulation related parameters in collaborative studies organised by SSC
- Held and distributed by NIBSC on behalf of ISTH

SSC Plasma LOT #2

Tony Hubbard, NIBSC, Potters Bar, UK

Background

- ***Replaced lot #1 - despatch commenced in May 2001***
- ***initial stock count 46,056 vials***
- ***stored at -20 ºC***
- ***expiry end June 2006***



Summary of despatch May '01 - June '05

- *total of 101 orders and 37,855 vials despatched*
- *40 organisations in 12 countries*
- *84 orders for 100 - 200 vials*
- *10 orders for 1,000 or more vials*

STATUS OF LOT #2

STABILITY STUDIES UPDATE

Tony Hubbard and *Steve Kitchen

NIBSC, Potters Bar, UK and *Royal Hallamshire Hospital, Sheffield, UK

Introduction

- ***accelerated degradation study commenced June 1999***
- ***vials stored at elevated temperatures (4, 20, 37, 45 ^{o}C)***
- ***tested for Factor VII:C and Factor VIII:C***
- ***final testing in June 2005 after 6 years storage***

Testing methods

Parameter	NIBSC	Royal Hallamshire
FVII:C	*clotting* *ACL 3000+*	*clotting* *CA7000*
FVIII:C	*chromogenic* *ACL 3000+*	*2-stage clotting* *CA7000*

Predictions of % loss per year - Factor VII:C

Storage temp (°C)	NIBSC data		Royal Hallamshire Hospital data	
	predicted mean % loss	upper 95% conf limit	predicted mean % loss	upper 95% conf limit
-20	0.011	0.029	0.004	0.010
+4	0.315	0.631	0.183	0.330
+20	2.209	3.449	1.616	2.336
+37	13.346	15.582	12.159	14.052

Predictions of % loss per year - Factor VIII:C

Storage temp (°C)	NIBSC data		Royal Hallamshire Hospital data	
	predicted mean % loss	upper 95% conf limit	predicted mean % loss	upper 95% conf limit
-20	0.004	0.015	0.004	0.012
+4	0.193	0.489	0.167	0.342
+20	1.837	3.271	1.435	2.218
+37	14.666	17.810	10.592	13.142

Real-time comparison after 6 years: -20 °C vials as % of -70 °C vials

Assay	Factor VII:C		Factor VIII:C	
	NIBSC	RHH	NIBSC	RHH
1 2 3 4	99 99 97 101	100 101 100 102	103 95 97 102	100 107 103 103
Mean	99%	101%	99%	103%

Conclusions

- ***stability studies on Lot #2 completed after 6 years***

- ***accelerated degradation study:***
  - ***excellent fit of data to model***
  - ***predicted mean loss at -20 ^{o}C did not exceed 0.011% per year***
  - ***predicted mean loss at +37 ^{o}C did not exceed 15 % per year***
  - ***tight 95% confidence limits = robust predictions***

- ***real-time -70 vs -20 ^{o}C prediction:***
  - ***no detectable difference***

- **Lot #2 extremely stable and suitable for despatch at ambient temperature**

STATUS OF LOT #3

BACKGROUND AND CALIBRATION

Tony Hubbard, NIBSC

SSC Lot #3 Background

- ***manufactured by Technoclone, Vienna***
- ***received by NIBSC in November 2003***
- ***total of 54,800 vials***
- ***unlabelled vials in boxes of 50***
- ***stored at -20 ^{o}C***

Calibration of existing parameters (+ VWF:CB)

Parameter	WHO IS	Organiser
Factor VIII:C	5th IS FVIII/VWF, Plasma	SK
VWF:Ag		
VWF:RCo		
VWF:CB		
Factor IX	3rd IS FII,VII,IX,X, Plasma	EG/TH
Factor II		
Factor VII		
Factor X		
Protein C*	1st/2nd IS Protein C, Plasma	
Protein S	1st IS Protein S, Plasma	
Antithrombin	2nd IS Antithrombin, Plasma	
Fibrinogen	2nd IS Fibrinogen, Plasma	

*** - to be included in calibration of 2nd IS Protein C Plasma**

New parameters

Parameter	Source of calibration	Organiser
Factor V	Proposed 1st IS FV, Plasma	AH
Factor XI	Proposed 1st IS FXI, Plasma	EG
Factor XIII	1st IS FXIII, Plasma	SR
t-PA antigen	NIBSC Reagent tPA Plasma	PD/CL
PAI-1 activity	WHO 1st IS PAI-1/	
PAI-1 antigen	kit standards	

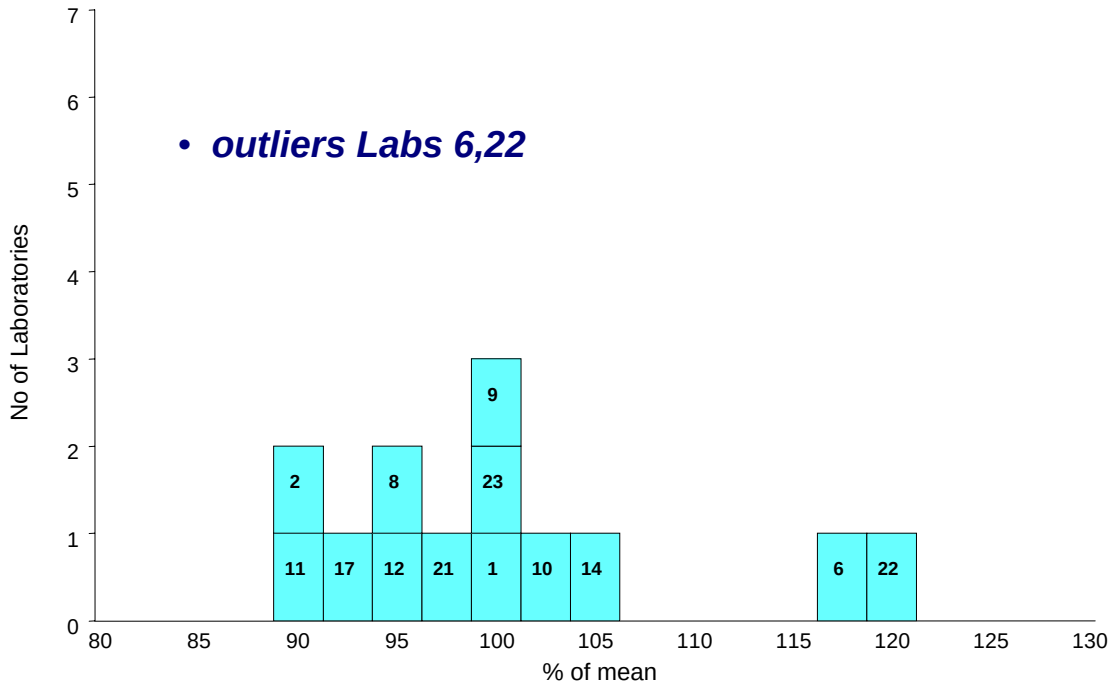
Calibration of existing parameters (+VWF:CB)

- **Participants**
  - ***pool of 30 laboratories***
  - ***17 clinical, 12 manufacturers, 1 regulatory***
  - ***12 different countries***

    Australia, Austria, Canada, Denmark, France, Germany, Italy, Netherlands, Spain, Sweden, USA, UK

SSC Lot #3 - Calibration for Fibrinogen

- ***13 laboratories***
- ***calibrated vs WHO 2nd IS Fibrinogen, Plasma***
- ***Clauss method***



SSC Lot #3 - Calibration for Fibrinogen

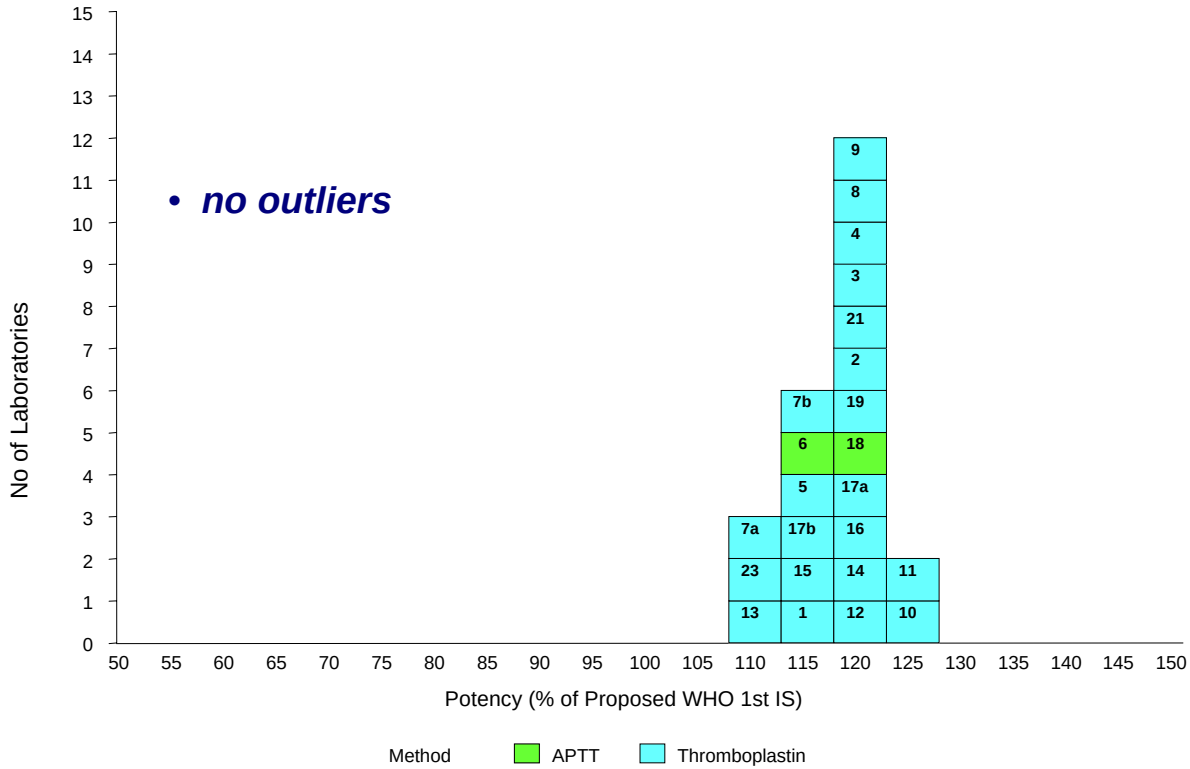
- ***Range of estimates:*** ***2.40 - 3.19 mg/ml***
- ***Combined mean (n=13):*** ***2.67 mg/ml***
  exc outliers (n=11): ***2.58 mg/ml***
- ***Inter-lab variability (GCV%):*** ***6.8 %***
  exc outliers (n=11): ***5.0%***
- ***Proposed assigned value:*** ***2.58 mg/ml***
- ***Responses from participants:*** ***8/13 - all agree***

SSC Lot #2 - Comparison of Fibrinogen

	Current study	Original Study
Mean (mg/ml)	2.55 (n=13) *(2.49 exc. outliers)*	2.43 (n=12) *(2.41 exc. outliers)*
Range (mg/ml)	2.31 - 3.00	2.03 - 2.81
"t" test	p = 0.120 *(0.087 exc outliers)*	

SSC Lot #3
Calibration for Factor V:C

- *23 estimates (21 labs in 10 different countries)*
- *calibrated vs Proposed WHO 1st IS Factor V, Plasma*
- *Clotting methods (21 Thromboplastin-based, 2 APTT-based)*



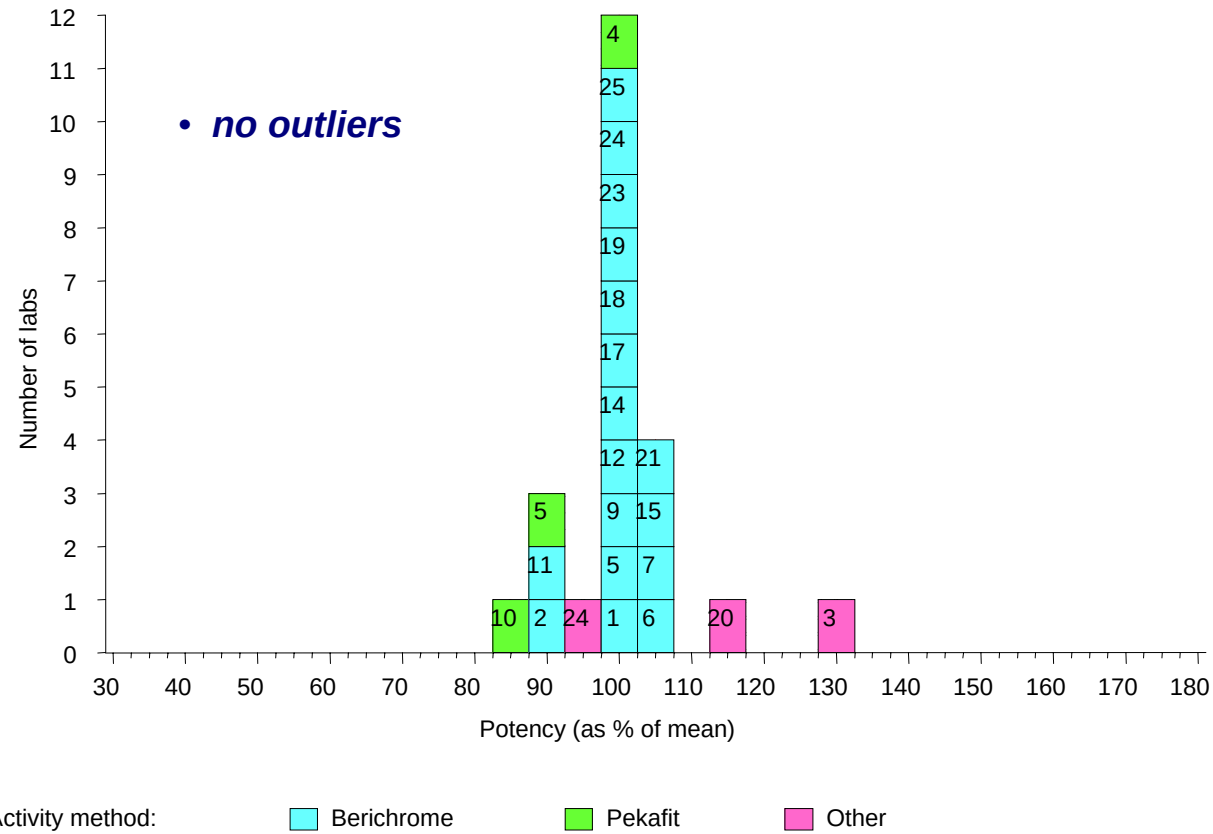
SSC Lot #3
Calibration for Factor V:C

- ***Range of estimates:*** ***110 - 124 % of Prop 1st IS***
- ***Combined mean (n=23):*** ***118 % of Prop 1st IS***
- ***Inter-lab variability (GCV%):*** ***3.55 %***
- ***Proposed assigned value:*** ****0.87 IU/ml***
- ***Responses from participants:*** ***22/22 - all agree***

*** - subject to confirmation of Proposed WHO 1st IS assigned potency of 0.74 IU/ml**

SSC Lot #3
Calibration for Factor XIII activity

- *calibrated vs WHO 1st IS Factor XIII, Plasma*
- *23 estimates (21 labs in 10 different countries)*
- *Methods (17 Berichrom, 3 Pefakit, 1 Coalink, 1 REA, 1 in house)*



SSC Lot #3
Calibration for Factor XIII activity

- ***Range of estimates:*** ***0.60 - 0.91 IU/ml***
- ***Combined mean (n=23):*** ***0.71 IU/ml***
- ***Inter-lab variability (GCV%):*** ***8.6 %***
- ***Proposed assigned value:*** ***0.71 IU/ml***
- ***Responses from participants:*** ***study approved***

SSC Lot #3 Summary of calibration

Analyte	Mean value (IU/ml)	Inter-lab variability(GCV%)	No. of estimates
Factor II	0.86	6.3 %	13
Factor V:C	0.87 *(pending WHO)*	3.55 %	23
Factor VII:C	0.87	6.8 %	11
Factor VIII:C	0.80	4.7 %	17
Factor IX	0.94	4.6 %	15
Factor X	0.86	4.1 %	13
Factor XI	*estimated completion end August 2005*		
Factor XIII	0.71	8.6 %	23
von Willebrand Factor: Antigen Ristocetin Cofactor Collagen Binding	 1.06 0.90 1.07	 5.7 % 9.7 % 11.2 %	 11 9 9
Protein C Antigen Function	*estimated completion February 2006*		
Protein S Total antigen Free antigen Function	 0.85 0.88 0.78	 8.1 % 4.5 % 4.5 %	 10 9 7
Antithrombin Antigen Function	 0.95 0.93	 2.4 % 2.8 %	 6 13
Fibrinogen	2.58 mg/ml	5.0 %	11

Conclusions

Along with its working partners, including NIBSC, WHO, FDA, and UKNEQAS, the SSC performs a vital function in coordinating scientific and standardization efforts for the coagulation community.


NIBSC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