The ISTH and Coagulation Diagnostic Standardization and Quality

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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.

Roman numerals accepted as official nomenclature
Thrombosis et Diathesis Haemorrhagica adopted as official journal
Evolution of the ISTH

Closed organization

ICSNBCF 1954-1958
ICBCF 1958-1964
ICTH 1964-1969

Primarily concerned with:
- nomenclature issues
- standardization of clotting tests
- development of standards, including thromboplastin

Open organization

SSC 1969-present
ISTH 1969-present
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 Executive Committee

SSC Chair

Liaisons with other organizations

Platelet Physiology
Coagulation Inhibitors
Fibrinolysis
DIC
Kallikrein Kinin

Fibrinogen
FVIII & IX
vWF
FXIII

Hemostasis Malignancy
Platelet Immunology
Bio-rheology
Control of Anticoag
Exogenous Factors

Lupus Anticoag
Predictive Variables
Animal Models
SSC Approval Process

- WHO
- FDA
- EMEA
- NIBSC

SSC Subcommittee
- Industry
- Scientists

SSC Voting Members

Joint ISTH/WHO committee
- NIBSC
- WHO centres
- FDA
SSC Approval Process (2)

- WHO
- FDA
- EMEA
- NIBSC

Industry
Scientist

SSC Subcommittee

- design
- testing
- approval

pre-analytical
post-analytical
analytical

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

- SSC
- Chair (Ian Peake)
  - ISTH (2)
  - WHO (2)
  - NIBSC
  - FDA
  - CLB
  - PEI
- WHO Expert Committee on Biological Standards (ECBS)
SSC Approval Process (4): Joint ISTH/IFCC Committee on the Standardization of Coagulation Tests (C-SCT)

Chairs (Craig Jackson, Gil White)
Jørgen Jespersen
Kess Kluft
Peter Esnouf
Trevor Barrowcliffe
Jane Lenahan

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

First Test Method: Antithrombin
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. PlA1 vs PlA2 forms of αIIbβ3 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
Working Group Members

- Elaine Gray
- Craig Jackson
- Steve Kitchen
- Peter Cooper
- Steffen Rosen
- Jacqueline Conard
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
Collaboration between SSC and manufacturers of diagnostic reagents to develop a uniform plasma standard for use by the manufacturers in labeling their coagulation calibrators.

- **Chair (Jane Lenahan)**
  - David Aronson
  - Trevor Barrowcliffe
  - John Brandt
  - Anthony Hubbard
  - Jørgen Jesperson
  - Steve Kitchen
  - Eric Preston

- **Diagnostic Manufacturers**
  - Chair (Jane Lenahan)
  - Jawahar Patel
  - Trevor Barrowcliffe
  - John Brandt
  - Anthony Hubbard
  - Jørgen Jesperson
  - Steve Kitchen
  - Eric Preston

- **Expert Laboratories**
  - Martine Aiach
  - Rob Ariens
  - Rogier Bertina
  - Nuala Booth
  - Paul Declerck
  - Augusto Federici
  - Peter Grant
  - Elaine Gray
  - Ian Jennings
  - etc

- **Secondary plasma standard stored and shipped from NIBSC**
  - Currently on 3rd lot!

- **Diagnostic Manufacturers**

- **Supplier**

- **19 analytes**

[Diagram of organizational structure]
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 °C
- 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 °C)
- tested for Factor VII:C and Factor VIII:C
- final testing in June 2005 after 6 years storage
## Testing methods

<table>
<thead>
<tr>
<th>Parameter</th>
<th>NIBSC</th>
<th>Royal Hallamshire</th>
</tr>
</thead>
</table>
| FVII:C    | clotting
ACL 3000+ | clotting
CA7000        |
| FVIII:C   | chromogenic
ACL 3000+ | 2-stage clotting
CA7000        |
# Predictions of % loss per year - Factor VII:C

<table>
<thead>
<tr>
<th>Storage temp (°C)</th>
<th>NIBSC data</th>
<th>Royal Hallamshire Hospital data</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>predicted mean % loss</td>
<td>upper 95% conf limit</td>
</tr>
<tr>
<td>-20</td>
<td>0.011</td>
<td>0.029</td>
</tr>
<tr>
<td>+4</td>
<td>0.315</td>
<td>0.631</td>
</tr>
<tr>
<td>+20</td>
<td>2.209</td>
<td>3.449</td>
</tr>
<tr>
<td>+37</td>
<td>13.346</td>
<td>15.582</td>
</tr>
</tbody>
</table>
### Predictions of % loss per year - Factor VIII:C

<table>
<thead>
<tr>
<th>Storage temp (°C)</th>
<th>NIBSC data</th>
<th>Royal Hallamshire Hospital data</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>predicted mean % loss</td>
<td>upper 95% conf limit</td>
</tr>
<tr>
<td>-20</td>
<td>0.004</td>
<td>0.015</td>
</tr>
<tr>
<td>+4</td>
<td>0.193</td>
<td>0.489</td>
</tr>
<tr>
<td>+20</td>
<td>1.837</td>
<td>3.271</td>
</tr>
<tr>
<td>+37</td>
<td>14.666</td>
<td>17.810</td>
</tr>
</tbody>
</table>
Real-time comparison after 6 years:
-20 °C vials as % of -70 °C vials

<table>
<thead>
<tr>
<th>Assay</th>
<th>Factor VII:C</th>
<th></th>
<th>Factor VIII:C</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NIBSC</td>
<td>RHH</td>
<td>NIBSC</td>
<td>RHH</td>
</tr>
<tr>
<td>1</td>
<td>99</td>
<td>100</td>
<td>103</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>99</td>
<td>101</td>
<td>95</td>
<td>107</td>
</tr>
<tr>
<td>3</td>
<td>97</td>
<td>100</td>
<td>97</td>
<td>103</td>
</tr>
<tr>
<td>4</td>
<td>101</td>
<td>102</td>
<td>102</td>
<td>103</td>
</tr>
<tr>
<td>Mean</td>
<td>99%</td>
<td>101%</td>
<td>99%</td>
<td>103%</td>
</tr>
</tbody>
</table>
Conclusions

- Stability studies on Lot #2 completed after 6 years

Accelerated degradation study:
  - Excellent fit of data to model
  - Predicted mean loss at -20 °C did not exceed 0.011% per year
  - Predicted mean loss at +37 °C did not exceed 15% per year
  - Tight 95% confidence limits = robust predictions

Real-time -70 vs -20 °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 °C
### Calibration of existing parameters (+ VWF:CB)

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

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

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

![Bar chart showing distribution of laboratories]
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

<table>
<thead>
<tr>
<th></th>
<th>Current study</th>
<th>Original Study</th>
</tr>
</thead>
</table>
| 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)

• no outliers
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)

• no outliers
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

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

### Additional Notes
- Estimated completion for Protein C: February 2006
- Estimated completion for von Willebrand Factor: August 2005
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.