Internal Quality Control in the Haemostasis laboratory

Dr Steve Kitchen
Sheffield Haemophilia and Thrombosis centre & UK NEQAS Blood Coagulation
Sixers Make Trades, Lose McCulloch

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"I was very bitter and shocked.... I was hysterical."
— Cornelia Vitella, whose husband's death was linked to an error at St. Agnes

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 Thomas

St. Agnes efforts only add to anguish after lab error

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1st Union wins bid to acquire Wachovia

A rival, SunTrust Banks, gave up after Wachovia's shareholders approved a $113.1 billion deal with the Phila. area's largest bank.

By Paul Newell

Winston-Salem, N.C. — More than two months after winning a legal battle, a group of Wachovia shareholders voted to approve a $113.1 billion merger with First Union.

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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.

By James Cuno Jr.

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.
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

\[ \text{INR} = \frac{\text{Test PT}}{\text{MNPT}}^{\text{ISI}} \]
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
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)
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

New vial of IQC
Stability of APTT on 2 lyophilised plasmas after reconstitution

pH 8.6 and 8.9 indicates 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?

<table>
<thead>
<tr>
<th>PT 1</th>
<th>PT 2</th>
<th>APTT 1</th>
<th>APTT 2</th>
<th>Problem</th>
</tr>
</thead>
<tbody>
<tr>
<td>out</td>
<td>in</td>
<td>out</td>
<td>in</td>
<td>QC 1 material</td>
</tr>
<tr>
<td>In</td>
<td>Out</td>
<td>In</td>
<td>out</td>
<td>QC 2 material</td>
</tr>
<tr>
<td>out</td>
<td>out</td>
<td>in</td>
<td>in</td>
<td>PT reagent</td>
</tr>
<tr>
<td>In</td>
<td>In</td>
<td>Out</td>
<td>Out</td>
<td>APTT reagent</td>
</tr>
<tr>
<td>Out</td>
<td>Out</td>
<td>Out</td>
<td>Out</td>
<td>Instrument or common reagent</td>
</tr>
</tbody>
</table>
UK NATIONAL EXTERNAL QUALITY ASSESSMENT SCHEME (NEQAS) for BLOOD COAGULATION

www.ukneqasbc.org

Dr Steve Kitchen
Sheffield Haemophilia and Thrombosis centre & UK NEQAS Blood Coagulation
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
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
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
• Persistently ‘outwith consensus’

• Communication from Director
IHTCs
Established Centre Twins
Emerging Centre Twins
GAP

WFH IEQAS programme
Participation in WFH EQA can improve laboratory performance

<table>
<thead>
<tr>
<th></th>
<th>Factor VIII</th>
<th>Factor IX</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Local result</td>
<td>median</td>
</tr>
<tr>
<td>Survey 1 2003</td>
<td>79 U/dl</td>
<td>24 U/dl</td>
</tr>
</tbody>
</table>

Mild haemophilia A misdiagnosed as normal
Normal subject misdiagnosed as haemophilia B
Participation in WFH EQA can improve laboratory performance

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<td>Median</td>
<td>Local result</td>
<td>Median</td>
</tr>
<tr>
<td>Survey 1 2003</td>
<td>79 U/dl</td>
<td>24 U/dl</td>
<td>36 U/dl</td>
<td>91 U/dl</td>
</tr>
<tr>
<td>Survey 12 2008</td>
<td>15 U/dl</td>
<td>21 U/dl</td>
<td>56 U/dl</td>
<td>50 U/dl</td>
</tr>
</tbody>
</table>
### Inter- laboratory variation
**FVIII:C results 2004-9**

<table>
<thead>
<tr>
<th>Survey</th>
<th>Median</th>
<th>Established Centre CV</th>
<th>Emerging centre CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>15 IU/dl</td>
<td>27%</td>
<td>30%</td>
</tr>
<tr>
<td>4</td>
<td>75 IU/dl</td>
<td>21%</td>
<td>32%</td>
</tr>
<tr>
<td>5</td>
<td>14 IU/dl</td>
<td>30%</td>
<td>137%</td>
</tr>
<tr>
<td>6</td>
<td>52 IU/dl</td>
<td>14%</td>
<td>26%</td>
</tr>
<tr>
<td>13</td>
<td>31 IU/dl</td>
<td>18%</td>
<td>25%</td>
</tr>
<tr>
<td>17</td>
<td>34 IU/dl</td>
<td>14%</td>
<td>42%</td>
</tr>
</tbody>
</table>
### Solving EQA problems

**Extraction from Database**

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

<table>
<thead>
<tr>
<th>Survey</th>
<th>Local result</th>
<th>median</th>
<th>% deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>29 IU/dl</td>
<td>36 IU/dl</td>
<td>20%</td>
</tr>
</tbody>
</table>
## Improving performance
### VWF Ag results

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<th>median</th>
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<tr>
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<td>29 IU/dl</td>
<td>36 IU/dl</td>
<td>20%</td>
</tr>
<tr>
<td>11</td>
<td>7 IU/dl</td>
<td>11 IU/dl</td>
<td>36%</td>
</tr>
</tbody>
</table>
## Improving performance
### VWF Ag results

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<td>36 IU/dl</td>
<td>20%</td>
</tr>
<tr>
<td>11</td>
<td>7 IU/dl</td>
<td>11 IU/dl</td>
<td>36%</td>
</tr>
<tr>
<td>12</td>
<td>41 IU/dl</td>
<td>55 IU/dl</td>
<td>25%</td>
</tr>
</tbody>
</table>
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

Number of centres

Factor VIII:C (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)

<table>
<thead>
<tr>
<th>Reference plasma</th>
<th>n</th>
<th>Median (u/dl)</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>46</td>
<td>86</td>
</tr>
<tr>
<td>2</td>
<td>81</td>
<td>76</td>
</tr>
<tr>
<td>3</td>
<td>86</td>
<td>76</td>
</tr>
<tr>
<td>4</td>
<td>14</td>
<td>72</td>
</tr>
<tr>
<td>5</td>
<td>12</td>
<td>73</td>
</tr>
<tr>
<td>6</td>
<td>10</td>
<td>75</td>
</tr>
<tr>
<td>All</td>
<td>299</td>
<td>77</td>
</tr>
</tbody>
</table>
## Factor assay design

<table>
<thead>
<tr>
<th></th>
<th>1999</th>
<th>2003</th>
<th>2009</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>n</strong></td>
<td>200</td>
<td>90</td>
<td>160</td>
</tr>
<tr>
<td><strong>centres</strong></td>
<td>UK/overseas</td>
<td>UK Haem centres</td>
<td>UK/overseas</td>
</tr>
<tr>
<td><strong>factor</strong></td>
<td>VIII:C</td>
<td>IX</td>
<td>VIII:C</td>
</tr>
<tr>
<td><strong>Single test dilution</strong></td>
<td>25%</td>
<td>25%</td>
<td>33%</td>
</tr>
<tr>
<td><strong>Stored calibration curve</strong></td>
<td>33%</td>
<td>32%</td>
<td>49%</td>
</tr>
</tbody>
</table>
Factor assays - Why 3 test dilutions?

FIX supplementary exercise 2003

<table>
<thead>
<tr>
<th>Test dilutions</th>
<th>n</th>
<th>Mean FIX U/dl</th>
<th>CV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>22</td>
<td>6.3</td>
<td>54%</td>
</tr>
<tr>
<td>2</td>
<td>17</td>
<td>6.5</td>
<td>29%</td>
</tr>
<tr>
<td>3</td>
<td>42</td>
<td>6.0</td>
<td>23%</td>
</tr>
</tbody>
</table>

ANOVA ns P = 0.03
Factor assays - Why 3 test dilutions?
FVIII:C 2009

<table>
<thead>
<tr>
<th>Test dilutions</th>
<th>n</th>
<th>Mean FVIII:C IU/dl</th>
<th>CV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>39</td>
<td>6.6</td>
<td>96%</td>
</tr>
<tr>
<td>2</td>
<td>18</td>
<td>5.8</td>
<td>28%</td>
</tr>
<tr>
<td>3</td>
<td>49</td>
<td>6.1</td>
<td>44%</td>
</tr>
</tbody>
</table>

*(29%)* Excluding one outlier
### Factor VIII:C

**Commercial deficient plasmas (S149 2005)**

<table>
<thead>
<tr>
<th>Source</th>
<th>n</th>
<th>Median (u/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>32</td>
<td>13.0</td>
</tr>
<tr>
<td>B</td>
<td>82</td>
<td>15.0</td>
</tr>
<tr>
<td>C</td>
<td>7</td>
<td>30.0</td>
</tr>
<tr>
<td>D</td>
<td>47</td>
<td>15.0</td>
</tr>
<tr>
<td>E</td>
<td>84</td>
<td>17.0</td>
</tr>
<tr>
<td>F</td>
<td>18</td>
<td>12.6</td>
</tr>
<tr>
<td>All</td>
<td>327</td>
<td>15.0</td>
</tr>
</tbody>
</table>

C – FVIII < 1 U/dl, FV = 3 U/dl, other factors normal
Lower Limit of Reference Range (PS activity)

Instrument. Lab. | Stago | Biopool | Dade-Behring | Others

<table>
<thead>
<tr>
<th>u/dl</th>
<th>45</th>
<th>50</th>
<th>55</th>
<th>60</th>
<th>65</th>
<th>70</th>
<th>75</th>
<th>80</th>
<th>85</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>50</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
PS Activity:

Interpretation is influenced by lower limit of reference range
UK NEQAS Thrombophilia Testing: Protein S Assays employed by participants

- Total + Free + Activity
- Total + Free
- Free + Activity
- Free only
- Total + Activity
- Total
- Activity only
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)

<table>
<thead>
<tr>
<th></th>
<th>Bovine TPN</th>
<th>Factor Va</th>
<th>Factor Xa</th>
</tr>
</thead>
<tbody>
<tr>
<td>In-house reference range</td>
<td>&gt;66.6u/dl</td>
<td>&gt;66.8u/d</td>
<td>&gt;74.2u/dl</td>
</tr>
<tr>
<td>Manufacturers reference</td>
<td>&gt;61.9u/dl</td>
<td>&gt;65.0u/d</td>
<td>&gt;55.0u/dl</td>
</tr>
</tbody>
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range
# PS activity

## PS reference ranges by method (n=20)

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<td>&gt;74.2u/dl</td>
</tr>
<tr>
<td>Manufacturers reference</td>
<td>&gt;61.9u/dl</td>
<td>&gt;65.0u/d</td>
<td>&gt;55.0u/dl</td>
</tr>
</tbody>
</table>

## Sensitivity to genetically confirmed PS deficiency (n=23)

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<th>Factor Va</th>
<th>Factor Xa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Using in-house range</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Using manufacturers range</td>
<td>100%</td>
<td>100%</td>
<td>87%</td>
</tr>
</tbody>
</table>
Protein S activity
Donor homozygous for FV Leiden

- Xa-based
- Xa-based
- Va-based
- TPN-based

Protein S activity levels are shown with different color bars indicating the number of donors (n) at various u/dl concentrations. The chart illustrates the distribution of Protein S activity levels in donors 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