Meaningful Internal and External Quality Control Measures to Secure Safety of Blood Donations

Abbott Symposium
Kiev, Ukraine
19th April 2019
Overview

- Introduction to NRL
- Laboratory Quality Assurance
- External Quality Assessment Schemes
- Laboratory Quality Control
- Quality Control Samples
- Monitoring QC Results
- Traditional Approach to Setting Control Limits
- QConnect Limits
NRL

Not-for-profit organisation that exists to promote the quality of tests and testing for infectious diseases, globally.

Major Stakeholders:
- Australian Government (DoH, DFAT, TGA)
- WHO, US CDC, Global Fund, UNDP, MSF
- Australian Red Cross Blood Service
- Test kit manufacturers
- Laboratories (blood screening and clinical)
NRL

Credentials:

- WHO Collaborating Centre
- Accreditation as a Medical Testing Laboratory; Compliant with ISO/IEC 15189: 2007
- Accredited as EQAS Provider to ISO 17043:2010
Laboratory Quality Assurance

Specimen Collection
- Test selection
- Patient preparation
- Specimen collection
- Specimen preservation

Laboratory
- Specimen reception
- Unique lab identifier
- Analysis
- Analytical validation
- Clinical validation

Reporting and Interpretation
- Results dispatched
- Results interpreted
- Clinical management

QC
- Quality Assurance
- EQAS
- Assurance/Management
External Quality Assessment Schemes

- EQAS is a requirement of most laboratory standards
- Assesses a laboratory’s testing process in its entirety
- Monitor consistency and accuracy of test results
- Provide objective evidence of quality
- Review performance of different assays
- Compare performance of laboratories
- Identify errors – random vs systemic
- Identify training needs
- Information exchange in the region
  - Provide advice on assays used
  - Provide advice on testing strategies used
- Assist in identification and resolution of testing problems
External Quality Assessment Schemes - Serology

- Blinded panels of samples provided periodically
- Samples should be similar to true patient samples
  - Serology samples should not be diluted
  - Pooled in equal volume and like reactivity
- Do not use “tricky” samples
- Know the true results
  - Use a validated testing strategy
  - Include confirmatory testing
  - Do not use “Consensus” results as reference
- Select some samples representing different disease states
  - Early infection
  - Different serotypes
External Quality Assessment Schemes - NAT

- NAT samples can be diluted to selected viral load
- Where possible, calibrate to WHO international standard
- Constructed to ask specific questions
  - Detection of genotypes
  - Reproducibility
  - Repeatability

- All EQAS samples should have
  - Homogeneity and stability confirmed
  - Sufficient numbers of samples to detect problems
  - Sufficient participants to provide statistically significance
External Quality Assessment Schemes

EQAS for infectious disease testing should:

- Be provided by an accredited organization (where possible)
- Tested within a defined period of time
- Results submitted and analyzed
- Report written within weeks of closing date
- Report reviewed by participants
- Initiate investigation of unacceptable results
- Results used for educational purposes
External Quality Assessment Schemes

- EQAS is a requirement of most standards
- Assesses a laboratory’s testing process in its entirety
- Monitor consistency and accuracy of test results
- Provide objective evidence of quality
- Review performance of different assays
- Compare performance of laboratories
- Identify errors – random vs systemic
- Identify training needs
- Information exchange in the region
  - Provide advice on assays used
  - Provide advice on testing strategies used
- Assist in identification and resolution of testing problems
External Quality Assessment Schemes - NRL

Serology
- HIV, Hepatitis (A, B and C) & Syphilis - laboratory and PoCT
- ToRCH – toxoplasma, rubella, CMV IgG/IgM
- HTLV

Nucleic Acid Testing
- Viral load for HIV, HBV, HCV, CMV
- Qualitative for HAV, Parvo B19, HSV, Lepto, CTNG, HPV
- PoCT for STI, TB, HIV, HCV, HBV (Dry Tube Sample)
- Genotyping for HIV, HCV, HIV Tropism
External Quality Assessment Schemes - NRL

More than 2,000 participants in more than 70 countries
Laboratory Quality Control
Sources of Variation

- Reagent lots
- Instrument and equipment
- Calibrations and maintenance
- Operators
- Storage and transport conditions
- Environmental conditions
Laboratory Quality Control

- Quality control (run control) sample
- Procedure for testing and collecting results
- Process for monitoring results
- Criteria for accepting or rejection results
- Action plan if the results are rejected

- Always test the manufacturer’s kit controls as these are used to validate the assay
Quality Control Sample

- Sufficient volume for extended use
- Stable over a long period
- Composition similar to patient sample
- Results within the clinically significant range
- Must not “saturate” the assay
- Must be on the linear part of the curve (dynamic range)
Ideal QC/Assay Combination

Increasing analyte conc.

Increasing signal
Ideal QC/Assay Combination

Increasing analyte conc.

Increasing signal

mean
What We Know About QC
Monitoring Results

- QC samples are a tool, not the end point
- Results collected after each test run
- Displayed graphically
- Have acceptance rules
- React immediately if unexpected results are detected
Monitoring Results
Control Limits

- Very unexpected (0.3%)
- kinda expected (5%)
- Mean
- 3 SD
- 2 SD
- 1 SD
- 0 SD
- 1 SD
- 2 SD
- 3 SD

Date/Run number/Time
Traditional methods for setting QC limits rely on mean ± xSD

...of what data set?
Control Limits

- RiliBÄK standard (2015)
  15
  20
  20
  20 (recalculate periodically)
Traditional Approaches to QC

- Assumes normal distribution of QC results
- Data set used to establish limits are representative of future results
- Patient and QC sample results change proportionally

NOT TRUE
Traditional Approaches to QC
Traditional Approaches to QC
Traditional Approaches to QC
Incorrect Assumption 1

15-20 results are not predictive of future results for infectious disease serology QC

- Too much reagent lot variation
- Does not include sufficient “normal” variation
- Causes confusion
Traditional Approaches to QC

Assuming QC commutability, percentage misinterpretation of chemistry results can be determined
Traditional Approaches to QC

Two populations in infectious disease serology

Delta value usually > 10 SD

Likelihood of a true low positive sample is low
True low positive samples occur during seroconversion
- Are low level for hours-days only
- Infection can be detected by NAT
- Extremely rare event (but it happens)
<table>
<thead>
<tr>
<th>Sample</th>
<th>93093LI00</th>
<th>95367LI00</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative kit control</td>
<td>0.06</td>
<td>0.07</td>
</tr>
<tr>
<td>Positive kit control</td>
<td>4.11</td>
<td>3.09</td>
</tr>
<tr>
<td>QConnect BLUE (DM18210)</td>
<td>3.15</td>
<td>1.98</td>
</tr>
<tr>
<td>Z332203</td>
<td>4.65</td>
<td>3.79</td>
</tr>
<tr>
<td>Z328261</td>
<td>4.75</td>
<td>2.82</td>
</tr>
<tr>
<td>Z332233</td>
<td>5.14</td>
<td>4.75</td>
</tr>
<tr>
<td>Z332216</td>
<td>7.05</td>
<td>5.33</td>
</tr>
<tr>
<td>Z332217</td>
<td>7.74</td>
<td>7.07</td>
</tr>
<tr>
<td>Z332241</td>
<td>10.28</td>
<td>9.98</td>
</tr>
<tr>
<td>Z332205</td>
<td>10.79</td>
<td>10.75</td>
</tr>
<tr>
<td>Z332204</td>
<td>11.24</td>
<td>9.85</td>
</tr>
<tr>
<td>Z332242</td>
<td>11.60</td>
<td>11.43</td>
</tr>
<tr>
<td>Z332243</td>
<td>11.88</td>
<td>11.65</td>
</tr>
<tr>
<td>Z332225</td>
<td>16.05</td>
<td>18.90</td>
</tr>
<tr>
<td>Lab no</td>
<td>HCV</td>
<td>S/CO</td>
</tr>
<tr>
<td>----------------</td>
<td>-----</td>
<td>------</td>
</tr>
<tr>
<td>94123-2572</td>
<td>RE</td>
<td>1.4</td>
</tr>
<tr>
<td>75264-8867</td>
<td>RE</td>
<td>1.84</td>
</tr>
<tr>
<td>97337-0579</td>
<td>RE</td>
<td>1.18</td>
</tr>
<tr>
<td>93818-8480</td>
<td>RE</td>
<td>2.83</td>
</tr>
<tr>
<td>98386-6942</td>
<td>RE</td>
<td>1.48</td>
</tr>
</tbody>
</table>
Incorrect Assumption 2

Decrease is QC reactivity does not result in a change in sensitivity and specificity of the assay

- Positive and negative populations are well removed from cut-off
- Only a minimal drop in reactivity is seen
- Only seroconversion samples have true border-line results
- Very occasional change in test result may occur
- Infection would be detected by NAT
Traditional Approaches to QC

- Low QC samples are made from diluted plasma from chronic infections.
- Antibody profiles are different to those in early infection (seroconversion).
- Therefore concept of using a low positive QC to ensure the detection of an early infection “window” is also flawed.
HIV Seroconversion

- gp160
- gp120
- p66
- p51
- gp41
- p32
- p24
- p17
HIV western blot

- Different antibody responses in different individuals
- Assay response depends on conjugates, substrates and antigens used
Incorrect Assumption 3

Diluted samples ≠ early infection

- Different antibody profiles in seroconversion
New look QC samples
Easier to distinguish. Simply locate your assay and we’ve pre-matched the right QC sample.

Online ordering.
Order what you want when you want it. Online ordering with cheaper and prompt shipping.

EDCNet reborn.
Streamlined data collection. Customisable graphs and reports. Full flexibility puts you in control.

Completely redesigned software. Even more graphing features. Everything about EDCNet is now better and easier.

Uncertainty of Measurement
Fulfil regulatory requirements easily with tailored MU calculations and reports.

QC Limits demystified
Customised QC limits based on your needs. Control limits specific for common assays. Harness the power of peer data sharing and robustness of pre-determined control limits.

Reference Materials
Need to know something about QC? Access a wealth of information, including references, regulations and case studies.

Have a QC problem?
You are not alone. Pose a question. Search for common problems. Learn from the experience of others.

Connect with others
Meet members and form networks. Access advice from peers and experts in the field. Share data, ideas and advice.

QConnect
Follow the trend
Your single portal to access everything you need.
<table>
<thead>
<tr>
<th>Assay</th>
<th>QC Lot Number</th>
<th>Number</th>
<th>Max</th>
<th>Min</th>
<th>SD</th>
<th>Mean</th>
<th>Total</th>
<th>No QC batches</th>
<th>QConnect Limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abbott ARCHITECT HIV Ag/Ab Combo CMIA</td>
<td>1</td>
<td>5653</td>
<td>6.51</td>
<td>0.11</td>
<td>0.68</td>
<td>3.49</td>
<td>115,828</td>
<td>12</td>
<td>2.0 - 5.1</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>6897</td>
<td>5.64</td>
<td>0.07</td>
<td>0.64</td>
<td>3.18</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>3695</td>
<td>5.60</td>
<td>0.14</td>
<td>0.58</td>
<td>3.46</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>243</td>
<td>5.18</td>
<td>2.05</td>
<td>0.42</td>
<td>3.42</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>7774</td>
<td>7.53</td>
<td>1.31</td>
<td>0.36</td>
<td>2.95</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>20095</td>
<td>6.32</td>
<td>0.06</td>
<td>0.43</td>
<td>2.95</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>4574</td>
<td>4.86</td>
<td>1.77</td>
<td>0.45</td>
<td>3.09</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>24955</td>
<td>6.42</td>
<td>0.11</td>
<td>0.60</td>
<td>3.52</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>2941</td>
<td>6.21</td>
<td>1.39</td>
<td>0.49</td>
<td>3.49</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>24848</td>
<td>6.49</td>
<td>0.14</td>
<td>0.62</td>
<td>3.90</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>3115</td>
<td>6.24</td>
<td>1.98</td>
<td>0.51</td>
<td>4.74</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>4141</td>
<td>6.91</td>
<td>2.52</td>
<td>0.59</td>
<td>4.86</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
QConnect Limits

- Published methodology*
- Minimal QC lot-to-lot variation
- Limits based on more than 10 year’s of data
- Data outside limits are flagged and quarantined
- NRL reviews all unusual results periodically
- Uses 1000s of data points rather than 10s
- More predictive of “unexpected” variation than traditional QC methods

Data Set for Westgard Comparison
# Application of Westgard Rules

<table>
<thead>
<tr>
<th>Number of datasets investigated (greater than 100 results)</th>
<th>Percentage outside range</th>
<th>Percentage of Results Failing Westgard Rules</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>First 10 results</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12S</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12S</td>
</tr>
<tr>
<td>103 (79)</td>
<td>Less than 10%</td>
<td>48.5%</td>
</tr>
<tr>
<td></td>
<td>10% to 20%</td>
<td>25.2%</td>
</tr>
<tr>
<td></td>
<td>Greater than 20%</td>
<td>26.2%</td>
</tr>
</tbody>
</table>

Greater than 20% failure rate across data set

- 103 datasets consisting of 21,500 QC results
- Test runs passed manufacturer’s validation
- 14 different assays, 6 infectious disease analytes
- Laboratories from 5 countries

Incorrect Assumption 4

Westgard Rules Inappropriate for Infectious Disease Serology QC

- Detects “normal” variation as “errors”
- Causes numerous “false rejections”
- Results in unnecessary investigations by labs and manufacturers
- Wastes resources, reduces confidence
Conclusions

- 15-20 QC results are not predictive
- A change in QC does not mean change in sensitivity/specificity
- Low level diluted QC does not mimic seroconverter
- Westgard rules are inappropriate for infectious disease serology
Conclusions

- All assays have “normal” or expected variation
- Infectious disease assays’ lot to lot variation is normal
- QC ranges must account for this variation or unnecessary waste of resources
- Traditional QC methods do not account for these normal variation
Conclusions

- QC results tell a story
- Challenge is to understand what it means
- QConnect provides
  - QC sample
  - EDCNet
  - QConnect limits
  - Scientific and technical support
  - Troubleshooting
Case Studies
Scenario 1
Scenario 1
What is the cause of the results in the red box?

1. No problem
2. Reagent variation
3. QC failure
4. Instrument failure
5. Unknown failure
Scenario 1

Answer:

Classic reagent lot variation
Scenario 2
What is the cause of the changes?

1. No problem
2. Reagent variation
3. QC failure
4. Instrument failure
5. Unknown failure
Chart by Instrument

Date


Subchannel A  Subchannel B

Trend: Instrument Test Process: Detection

- PRISM/Channel 2 (1048)/Subchannel A
- PRISM/Channel 2 (1048)/Subchannel B
- PRISM/Channel 2 (1092)/Subchannel A
- PRISM/Channel 2 (1092)/Subchannel B
- PRISM/Channel 2 (1113)/Subchannel A
- PRISM/Channel 2 (1113)/Subchannel B

NRL Range: 2.12 to 4.64
Chart by Reagent Lot Number
Adding mean and SD

- Mean
- Mean ± 2SD
- Mean ± 3SD
- NRL Limits
- Mean ± 3SD
- Mean ± 2SD
- Mean ± 1SD
Scenario 2

- **Normal QC performance**
  - $n = 593$
  - Data within NRL Limits = 99.3% ($n=589$)
  - Number of points within $±2SD$ ($n=569$) and $±3SD$ ($n=591$)

- Compare this with statistical likelihood
  - $±2SD = 95\%$ (actual 95.95%) 
  - $±3SD = 99.7\%$ (actual 99.66%)
Scenario 3
Scenario 3

- Traditional statistical analysis - all is OK
- Laboratory - feeling something was not OK
- Results regularly outside QConnect Limits
- Manufacturer’s maintenance – ‘all clear’
  - But… results continued to be outside Qconnect Limits
What is the cause of the changes?

1. No problem
2. Reagent variation
3. QC failure
4. Instrument failure
5. Unknown failure
Plot by Instrument  (n= 5 instruments)
Scenario 3

Answer:

- Instruments contributing to variation

- Look for all sources of variation
  - Equipment problem - needed a part replacement/repaired
  - EDCNet allows multiple ways to look at data

- Once repaired, instrument operated as expected
Scenario 4
Anti-HBs testing on Abbott Architect
What is the cause of the changes?

1. No problem
2. Reagent variation
3. QC failure
4. Instrument failure
5. Unknown failure
Comparison with Peers
Trending by Reagent Lot
Trending by Operator
Trending by Instrument
Resolution of Issue
Scenario 4

Answer:

Instrument Issue
Conclusions

Collect metadata with QC results
- Date
- Instrument(s) identification
- Reagent lot number(s)
- Operators
- QC lot number
- Calibration and maintenance data
Conclusions

Monitoring Quality Control in a systematic manner can identify unexpected variation that may, in time, contribute to incorrect patient results.

QC is done in addition to kit controls and EQA and QMS.

Do not blindly follow without evidence – you are a scientist.
Thank-you!