QC in Virology Labs Today

- “In kit” Quality Controls are provided with reagent/test kits
  - May be labelled as “Positive”, “Negative”, “Cut Off”
  - Run each time the assay is performed
- Reagent/test kits may also include a calibrator (std or cut off)
  - Run each time the assay is performed
- Laboratories may subscribe to an External Assessment Scheme (EQAS)
  - Run periodically (monthly/quarterly)
- Additional controls may be included
  - Often run periodically on an arbitrary basis
- Seroconversion Panels
  - To assess assay sensitivity
What is the Difference Between These Materials?

• Some definitions from CLIA for Qualitative Tests…
  • Controls
    – Any reagent of a known reactive level that is **not** used to calculate the assay cutoff
  • Calibrators
    – Any reagent supplied by the manufacturer that is used to calculate the assay cutoff
  • Qualitative tests must have a **calibrator AND two controls**
    – At least one Calibrator
    – At least one Positive control
    – At least one Negative control
  • Control
    – Can be an in-house prepared control or a commercially purchased external control
What Are The Uses Of These Materials?

- **In-Kit internal controls**
  - Used during the initial **calibration phase** (determining the cutoff)
  - Checking the instrument satisfies the specs defined by the manufacturer

- **Independent internal controls**
  - Used as unknown samples after the calibration phase in the **sample run**
  - Can be assayed or unassayed
  - Ideally should be weakly reactive
  - Results should be monitored on Control Charts.
  - They are not an alternative to in-kit controls but supplement them

- **Calibrators or cutoff reagents**
  - To be used before every run for instrument set up
  - They can never be used as controls !!
Internal v External Control

• Internal QC
  – Material run every day or each time assay performed
  – Provides continuous monitoring
  – Assesses assay reproducibility (precision)

• External QC
  – Material run on a periodic basis (weekly/monthly)
  – Provides a snapshot of performance
  – Assesses accuracy

• Monthly Interlaboratory Program
  – Uses internal qc data
  – Assesses trueness
Precision or Random error

Trueness

“True” Value

Accuracy (one measure)

Value

Precision or Random error
Internal Quality Control

• **Purpose**
  – To monitor precision (reproducibility)

• **How**
  – Define a range of acceptability for results
  – Monitor continuously to assess precision
  – Implement corrective actions where appropriate

• Stable results = reproducible assay
External Quality Control

• **Purpose**
  – To assess accuracy
  – Ensure patient results are comparable in different labs

• **How**
  – Unknown sample tested periodically
  – Assay performance across laboratories compared

• Good accuracy = correct results reported
Virology Testing

• Tests are normally considered in terms of
  – Sensitivity
    • Probability of the assay giving a positive result in samples from subjects known to have the disease (TP/TP+FN)
  – Specificity
    • Probability of the assay giving a negative result in samples from subjects known to be free of the disease (TN/TN+FP)

• “Accuracy” and “precision” are rarely considered (unless in terms of providing a clinically correct result)

• But how can we be sure the assay is reproducible throughout the life of the reagent / kit?
  – Requires independent internal qc monitoring
Suggestions for Performing IQC In Virology

1. Use the same lot no. of control material over as long time period as possible
   – Allows changes in reagent sensitivity/specificity to be identified

2. Quantify the qc results rather than record a simple positive or negative
   – Use the signal/cut off ratio not the absorbance value (O.D.)
Suggestions for Performing IQC In Virology

3. Plot results on a Levey Jennings Chart
   – Look for any trends in the data

4. Assess the stability of the system and take corrective actions where necessary
Westgard Rules?

• A set of Statistical Rules
  – Make judgements on reproducibility
  – However there is no information to suggest what level of precision is required for virology tests

• Simple observation of trends within 3sd is probably sufficient (at least for now)
Troubleshooting QC

• When 3s are exceeded ➔ triggers an alarm
  - Check if lot reagent or procedure changed (majority of situations)
  - Wrong storage, wrong use of control material (fewer situations)

• Don’t be as critical as biochemists are! No official indication exists regarding the necessary precision for a virology assay
• Precision with virology tests can show CV >>20-30 %
• It’s much more important to know and quantify your performances
Impact of Changes in Assay Performance

• Altered assay sensitivity or specificity can change the interpretation of a patient result near the assay “cut off”

• The number of patients in this area are very few but the impact of producing a wrong result on just one patient is very big
Changes in Assay Performance

- **Cut off**
  - Abs: 200

- **In Kit Control**
  - Abs: 600

- **B-R Control**
  - Abs: 800

- **Ratio S/Co**
  - $R_K = 3$
  - $R_B = 4$

- **Abs**
  - 180
  - 540
  - 780

- **Ratio S/Co**
  - $R_K = 3$
  - $R_B = 4.3$
Changes in Assay Performance

specificity  
Cut-off  
sensitivity

normal
positive

False Neg  False Pos

imprecision 5%

imprecision 10%
What is the risk for the lab?

- The problem is not in providing a wrong result with a high positive or a negative patient.
- The issue will always be with those patient sample results close to the assay cut off
  - Probability of finding an unknown weak seroconverting patient is probably only 1 in 100,000’s per year so the risk is small
  - **BUT** if a wrong result is provided on a borderline patient the implications would be very serious

- Using an Independent control can help eliminate this risk
  - Low positive result simulates a weak patient sample
  - Allows monitoring of assay from reagent lot to lot
Study Conducted By Italian Blood Transfusion Service

CONTINUOUS QUALITY MONITORING IN INFECTIOUS DISEASE TESTING CONDUCTED WITH AN INTEGRATED SYSTEM INTRA AND INTER LABORATORIES

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PREMISE
Monitoring analytical quality in infectious disease testing has rarely been conducted in an efficient, complete and continuous way because of the lack of standardized quality control materials with long shelf life and because of the lack of specific software applications able to monitor the stability of the analytical system in terms of measure reproducibility. Recently, Bio-Rad Laboratories introduced an integrated solution with a software application (Only Real Time®) able to manage quality control in infectious disease obtained with multiparametric quality control materials (Bio-Rad VIROTROL® I, II, III, SYBV, TUB, TOL, CON).

BIO-RAD VIROTROL® I CONTROL SAMPLE
Human liquid multiparametric serum for the following tests: HBsAg, anti-HCV, anti-HIV-1, anti-HIV-2, anti-HIV-3, anti-HIV-4, CMV

INTERNAL QC MATERIALS
1. The sample used for Internal Quality Control in infectious disease testing must be provided by a third party manufacturer and not by the manufacturer of the analytical system in use.
2. The sample used for Internal QC should be "weakly positive", meaning that it should contain analytes in a concentration close to the cut-off or the limit of the clinical interpretation.
3. The Control material is the most sensitive tool for monitoring changes in methods/feasts and their implementation and accuracy.

STATISTICAL EVALUATION
- Quality Control results are evaluated in real time with Lerner-Jennings charts (warning rule 1.5; rejection rule 1.3). The Quality Control dynamic range is 1-3 times the ratio signal/cut-off (S/CO). Quality Control results are submitted monthly to Bio-Rad Laboratories and single analysis specific reports are available monthly on their website (www.qcnet.com).

“The use of 3rd party qc materials with a dynamic range close to the cut off, long shelf life and an appropriate intra and inter laboratory integrated statistical analysis comparing data to peer groups and method consensus is a very powerful tool for laboratories that need to manage and control the quality of infectious disease assays”
Are You Satisfied With Current QC Practices in Your Virology Lab?

- Do you use in-kit controls alone (which only validate the calibration) or do you also run additional QC (which assures the quality of the patient results)?
- Do you monitor potential changes that may be introduced when the reagent / kit lot changes?
  - How confident do you feel you will be in detecting these changes?
- If a requesting physician questions a result, how do you trace back and verify the assay quality on a specific day?
- Do you record a quantifiable result for QC data (ie a number)?
  - If so what do you use? (OD, ratio?)
- At audit, how can you provide a record of all QC activities?
Summary

• Do not use only in-kit controls and rely on them alone
• QC results should be traceable (ie monitored long term)
• Independent controls ideally should be weakly reactive to simulate a low positive patient sample
• Independent controls cannot be used to compare sensitivity between methods (seroconversion panels needed for this)
• Avoid recording a positive qc test result as “positive”
  – It’s important to know the strength of the reaction and compare that to other runs
• Differing reagent lots can make a difference to patient interpretation:
  - Strong positive or negative patient results are never affected
  - But a weak positive sample could become negative (or the reverse) when changing reagent lots
Available Brochures

- Infectious Disease Controls
  Blood Virus, Molecular and Serological Quality Controls

- Using Quality Controls for Infectious Disease Testing
  Compelling Reasons to Monitor Your Test Procedures

- QC Data Management Solutions
  Optimize Your Laboratory Performance With Powerful QC Data Management Solutions
Hepatitis & Retrovirus Controls

**Virotrol I**: anti-HIV-1, anti–HTLV-I, anti-HCV, anti-CMV, anti HBc, HBsAg

**Virotrol II**: anti-HBs, anti-HAV

**Virotrol III**: anti-HAV, anti-HAV IgM, anti-HBc IgM, HBeAg

**Virotrol IV**: anti-HBe

**Virotrol HIV-2**: anti-HIV-2

**Viroclear**: Negative Control
Congenital Diseases Controls

**ToRCH** : anti-Toxo IgG, anti-Rubella IgG, anti-CMV IgG, anti-HSV-1 IgG, anti-HSV-2 IgG

**ToRCH-M** : anti-Toxo IgG & IgM, anti-Rubella IgG & IgM, anti-CMV IgG & IgM, anti-HSV-1 IgG, anti-HSV-2 IgG

**Viroclear ToRCH** : Negative Control

**Virotrol MuMZ** : Mumps-IgG, Measles-IgG, VZV-IgG
Other Disease Controls

**Virotrol EBV** : anti-VCA IgG, anti-VCA IgM, anti EBNA IgG  
**Viroclear EBV** : Negative Control  
**Pyloritrol** : anti-Helicobacter Pylori IgG  
**Syphilis Total** : anti-Syphilis IgG, anti-Syphilis IgM, RPR  
**Virotrol Chagas** : Chagas Fever  
**Virotrol Lyme** : Borrelia B.  
**Amplitrol III** : HBV, HIV HCV in NAT  
**Ampliclear CT/GC** : negative control