



Quality Control of Diagnostic Molecular Assays: The U.S. Perspective

Joseph D. C. Yao, M.D.

**Division of Clinical Microbiology,
Department of Laboratory Medicine and Pathology**

National Molecular Microbiology Diagnostic Users Group
Annual Meeting, Toronto, Canada
October 23, 2012

Outline

1. Importance of good QC
2. QC of qualitative assays
3. QC of quantitative assays

Classification of Assays used in U.S. Clinical Laboratories

Assay classification

FDA approval & manufacturing

In vitro diagnostic
(IVD)

FDA-cleared or FDA-approved
+ cGMP manufacture

Research use-only
(RUO)

± cGMP manufacture

Laboratory-developed
(LDT)

None

cGMP = Current good manufacturing practice (1996)

Quality Control

A process by which the quality of all factors involved in production is reviewed, with emphasis on:

- Elements such as **controls**, job management, defined and well-managed processes, **performance** and integrity criteria, and identification of records;
- Competence, such as **knowledge**, **skills**, **experience**, and **qualifications**;
- Soft elements, such as **personnel integrity**, confidence, **organizational culture**, **motivation**, **team spirit**, and quality relationships.

Quality Control vs. Quality Assurance

Quality control emphasizes testing of products to uncover defects and reporting to management who decides whether to allow or deny product release.

Quality assurance attempts to improve and stabilize production and its associated processes to avoid, or at least minimize, issues which led to the defect(s) in the first place.

Importance of Quality Control

- Ensure accuracy and reproducibility of lab test results used in patient care;
- Ensure integrity and confidence in test results;
- Provide safety in management of patients;
- Comply with local, regional, and national regulations and lab accreditation requirements;
- Maintain staff morale and reputation of laboratory

College of American Pathologists Lab Accreditation - Microbiology Checklist (1)

MIC.11017 QC Verification

Phase II

Control results are reviewed for acceptability before reporting patient results.

Evidence of Compliance:

- Written policy / procedure stating that controls are reviewed and acceptable prior to reporting patient results, **AND**
- Evidence of corrective action taken when QC results are not acceptable

CAP Microbiology Checklist (6)

MIC.63275 QC Acceptability Limits

Phase II

Acceptability limits are defined for all control procedures, control materials, and standards.

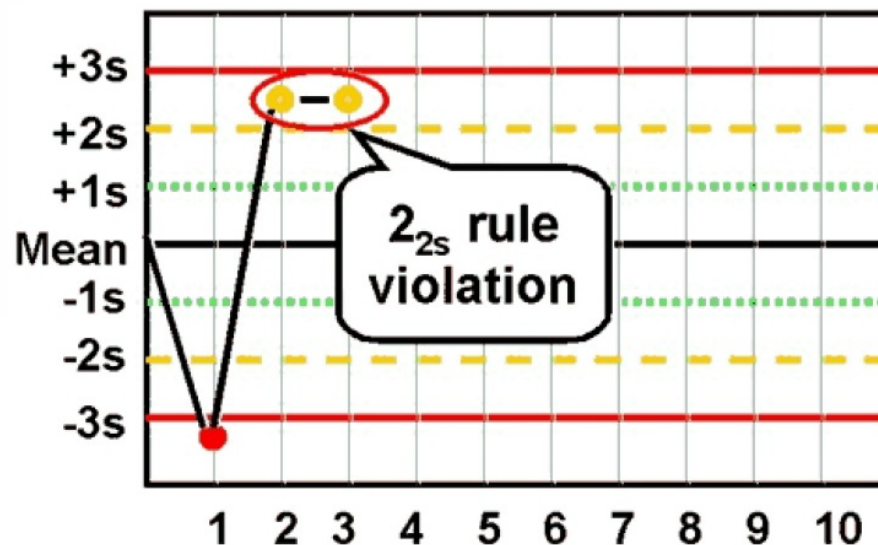
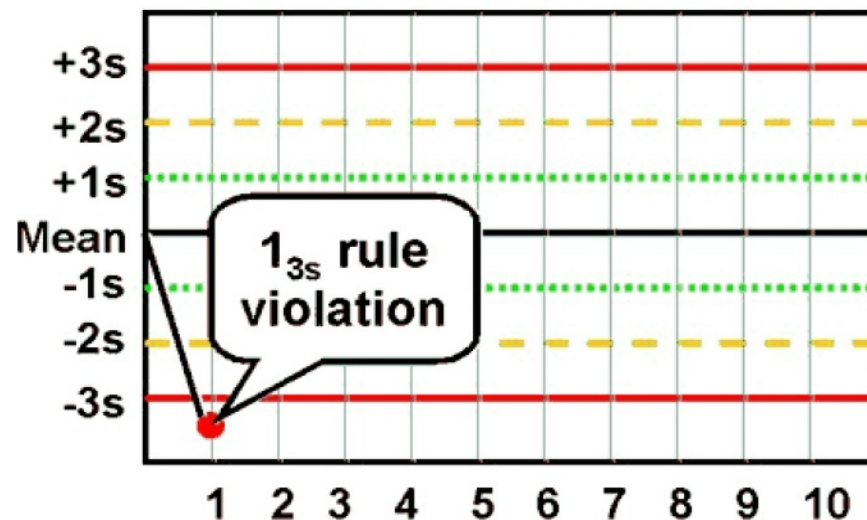
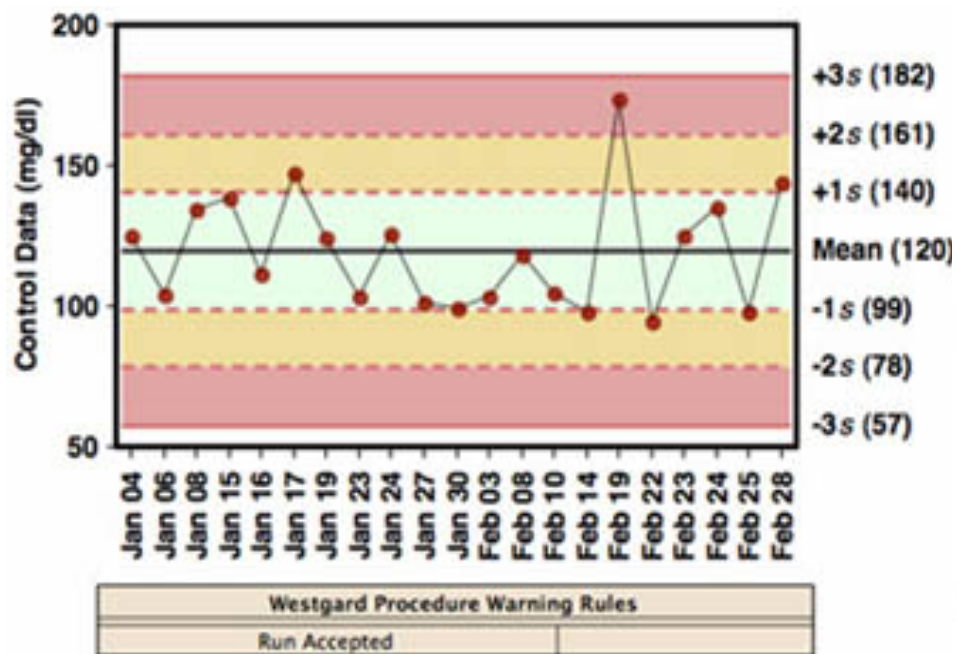
Acceptability limits must be defined for all control procedures, control materials, and standards. These controls must be appropriate for the range of sensitivities tested and should, ideally, focus on result ranges that are near clinical decision points.

Quantitative assays: Westgard rules (eg. 1_{3s} , 2_{2s}) for acceptance

Evidence of Compliance:

- Written QC procedure(s) defining acceptability limits

Westgard Rules



CAP Microbiology Checklist (2)

MIC.11018

QC Corrective Action

Phase II

There is documentation of corrective action when control results exceed defined acceptability limits.

- *Re-evaluate test results obtained in an analytically unacceptable test run or since the last acceptable test run to determine if there is a significant clinical difference in patient / client results;*
- *Re-evaluation may or may not include re-testing patient samples, depending on the circumstances;*
- *If patient samples are no longer available, test results can be re-evaluated to search for evidence of an out-of-control condition that might have affected patient results.*

CAP Microbiology Checklist (3)

MIC.11020 Monthly QC Review

Phase II

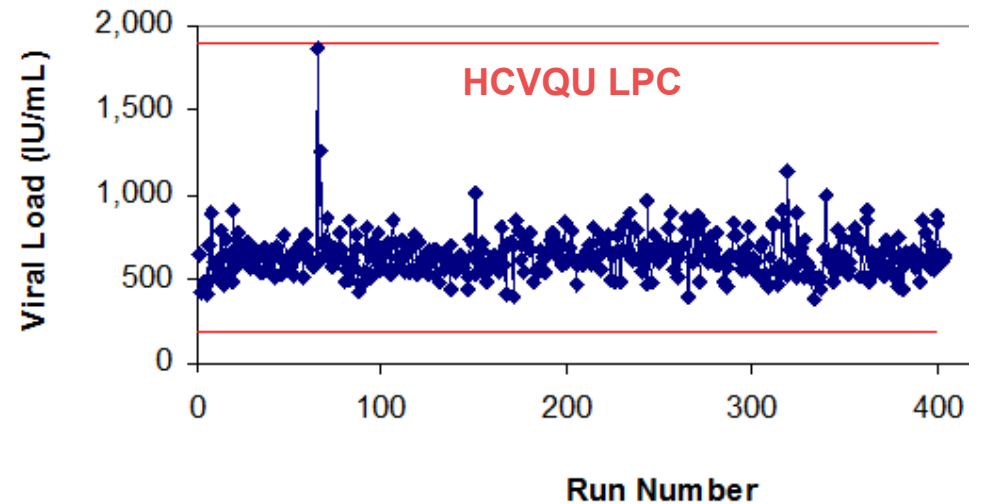
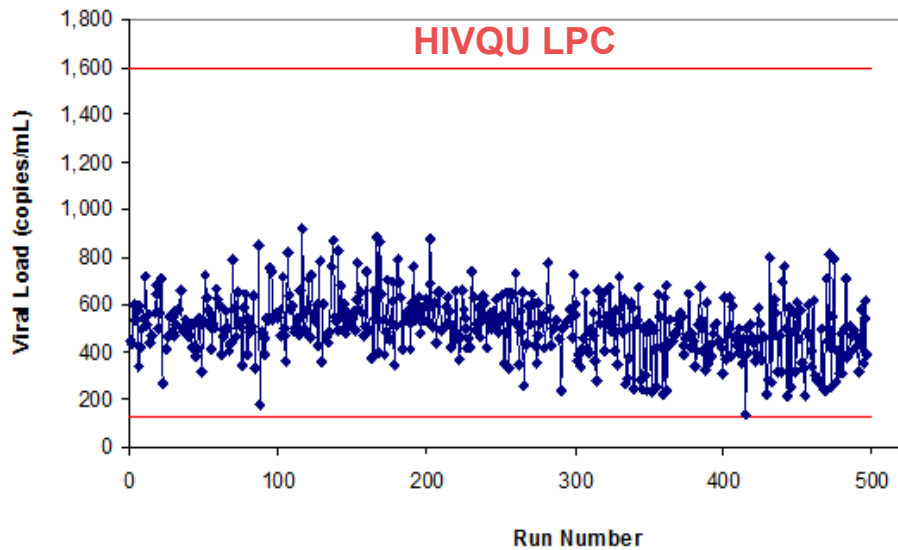
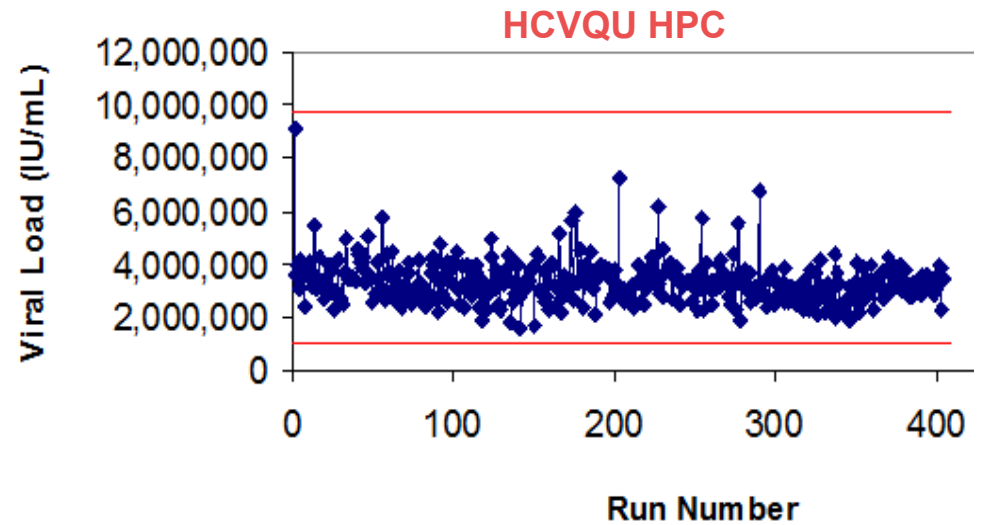
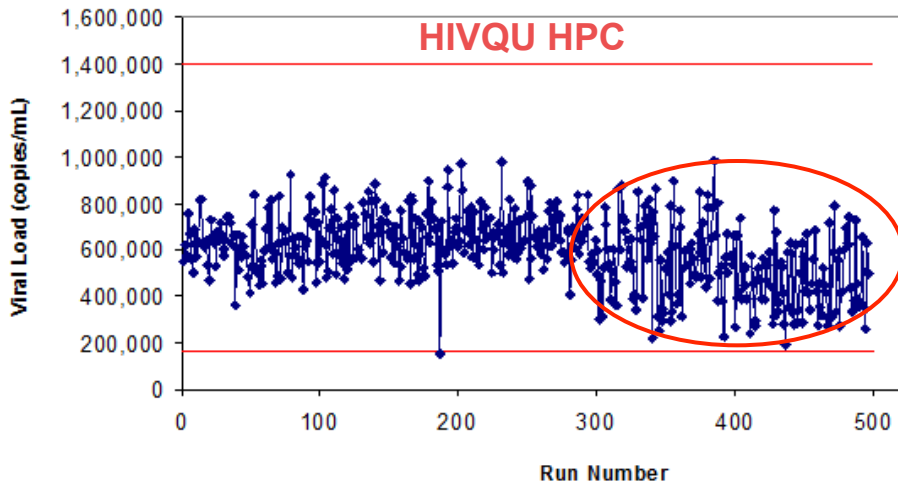
Quality control data are reviewed and assessed at least monthly by the laboratory director or designee.

QC data for tests performed less frequently than monthly should be reviewed when the tests are performed.

Evidence of Compliance:

- Records of QC review with documented follow-up for outliers, trends, or omissions

QC Trend Monitoring of VL Assays



CAP Microbiology Checklist (4)

Controls are samples that act as surrogates for patient specimens. They are processed just like patient specimens to monitor the ongoing performance of the entire analytic process in every run.

- **Qualitative molecular** tests typically include **(+) and (-) controls** and, in some instances, **a sensitivity control** to show that low level target is detectable.
- **Quantitative molecular tests** typically include a **(-) control** and **≥ 2 levels of control at relevant decision points** to verify that calibration status is maintained within acceptable limits.
- *Controls must assess adequacy of extraction and amplification, e.g. positive and negative controls that go through the entire testing process.*

Commercial Sources of NAT Control Materials

AcroMetrix Corp., Benicia, CA (Life Technologies Inc.)

<http://www.invitrogen.com/site/us/en/home/brands/Product-Brand/acrometrix.html?CID=AcrometrixLife>

SeraCare Life Sciences, Milford, MA

Somagen Diagnostics, Inc., Edmonton, AB

www.somagen.com

ZeptoMetrix Corp., Buffalo, NY

www.zeptometrix.com

CAP Microbiology Checklist (5)

MIC.63264 Multiplex QC

Phase II

For multiplex tests, controls for each analyte are either included in each run or rotated so that all analytes are tested periodically.

Evidence of Compliance:

- Written procedure defining multiplex test QC AND
- Records of multiplex test QC

CAP Microbiology Checklist (7)

MIC.63277 QC Statistics Phase II

For quantitative assays, quality control statistics are performed monthly to define analytic imprecision and to monitor trends over time.

The laboratory must use statistical methods such as calculating SD and CV monthly to evaluate variance in numeric QC data.

Evidence of Compliance:

- Written procedure for monitoring of analytic imprecision including statistical analysis of data

Qualitative Molecular Assays

MIC.64915 Qualitative Cut-Off

Phase I

For qualitative tests that use a cut-off value to distinguish positive from negative, the cut-off value is established initially, and verified with every change in lot or at least every 6 months.

*The **limit of detection (LoD)** that distinguishes a positive from a negative result should be established or verified when the test is initially placed in service, and **verified with every change in lot** (e.g. new master mix), **instrument maintenance, or at least every six months thereafter**. Note that a low-positive control that is close to the limit of detection can satisfy this checklist requirement, but must be external to the kit (e.g. weak-positive patient sample or reference material prepared in appropriate matrix).*

Comparability of Test Results Using Same Assay and Reagents

Rationale:

- To compare results within peer group of assay users;
- To detect reagent or instrument problems not readily discovered otherwise.

Requirements:

- Peer group uses exactly same assay method, reagents and external controls of same lot no.
- Web-based data entry and comparison of data via commercial vendors (eg, AcroMetrix, Bio-Rad, SeraCare)

Costs (eg, HIV-1 VL reagent + control only)

- Testing daily: $\$45 \times 7 \text{ days} \times 52 \text{ wks} = \$15,652 / \text{yr}$
- Testing weekly: $\$45 \times 52 \text{ wks} = \$2,236 / \text{yr}$

Take-Home Points

- Proper QC is necessary to ensure accuracy and reproducibility of lab test results for patient care;
- Daily review of QC failures;
- Weekly and/or monthly review of QC outliers and trends;
- Optimize reagent and control lot sizes for lab-developed assays to avoid frequent lot QC;
- “Over QC” is wasteful and costly.

Resources

CLSI documents:

C24-A3 Statistical quality control for quantitative measurement procedures: principles and definitions; approved guideline – third ed. 2006.

GP35-A Development and use of quality indicators for process improvement and monitoring of laboratory quality; approval guideline. 2010.

MM6-A2 Quantitative molecular methods for infectious diseases; approved guideline – second ed. 2010.

College of American Pathologists Laboratory Accreditation Program. Microbiology checklist, 07.31.2012. http://www.cap.org/apps/cap.portal?_nfpb=true&_pageLabel=accreditation