MOLECULAR QC FOR QUALITATIVE SARS-COV-2 TESTING

"YOU CAN OBSERVE A LOT JUST BY WATCHING"

Duane Newton, PhD, D(ABMM), FIDSA
Director, Clinical Microbiology Laboratory
Associate Director, Division of Clinical Pathology
Michigan Medicine, Ann Arbor, MI

DISCLOSURES

- NaviDx – Consultant
- QuantaMatrix – Scientific Advisory Board
- Bio-Rad – Scientific Advisory Board

LEARNING OBJECTIVES

- Describe approaches to quality control for SARS-CoV-2 molecular testing that monitor the entire testing process
- List 2 optimal QC practices

QC GUIDANCE

"If you don't know where you're going, you'll end up someplace else."

- Yogi Berra

QC

- For molecular-based quantitative and qualitative tests, controls are **run at least daily, or more frequently if specified in manufacturer's instructions**, laboratory procedure, or the CAP Checklist, and when changes occur that may impact patient results.

  Note: Controls must be run prior to resuming patient testing when changes occur that may impact patient results, including after a change of analytically critical reagents, major preventive maintenance, change of a critical instrument component, or with software changes, as appropriate.

MIC.65200

QC

Daily quality control must be run as follows:

1. Quantitative tests - three controls at least daily, including a **negative control**, a **low-positive control** and a **high-positive control**, except where a specific exception is given in this checklist
2. Qualitative tests - a **positive and negative control** at least daily

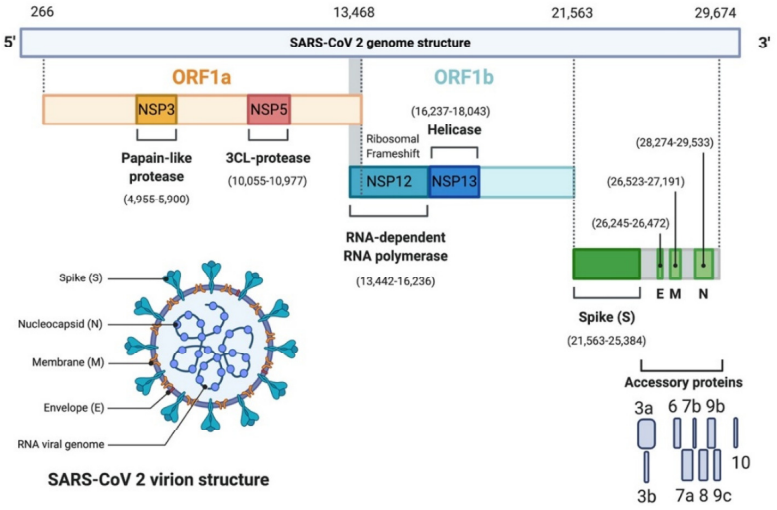
Controls must assess adequacy of extraction and amplification, eg, positive and negative controls that go through the entire testing process.

MIC.65200

KIT AND EXTERNAL CONTROLS

"When you come to a fork in the road, take it."

- Yogi Berra



Pathogens **2020**, *9*(5), 331; https://doi.org/10.3390/pathogens9050331

KIT CONTROLS

Abbott m2000

- *Assay targets*
  - Dual target RdRp and N genes
  - Internal control sequence
    - Proprietary pumpkin seed gene
- *Assay reagents*
  - Positive control
    - Recombinant Sindbis virus containing SARS-CoV-2 sequences
  - Negative control
    - buffer

KIT CONTROLS

Diasorin Simplexa

- *Assay targets*
  - Multiplex target ORF1ab and S gene
  - RNA internal control sequence
    - proprietary
- *Assay reagents*
  - Positive control
    - Synthetic gene fragments of ORF1ab and S gene in UTM
  - No template control
    - Viral transport media

EXTERNAL CONTROLS

Zeptometrix

- Heat-inactivated fluid from a SARS CoV 2 culture (0.5 mL, titer is lot-specific)
  - Isolated from a patient in WA who returned from China in Jan, 2020 (SARS-CoV-2 USA-WA1/2020)
- Matrix
  - Tissue culture media

BEI Resources

- Genomic RNA extracted from a SARS CoV 2 culture (110 uL, titer is lot-specific)
  - Isolated from a patient in WA who returned from China in Jan, 2020 (SARS-CoV-2 USA-WA1/2020)
- Matrix
  - Tris-EDTA (TE) buffer

EXTERNAL CONTROLS

Sera Care AccuPlex

Positive reference material (5000 copies/mL measured using rev trans ddPCR)

- Sindbis virus (replication defective and heat-treated)
  - 2 sections of ORF1a region (417-1899, 3094-3360)
  - 3 sections of RdRp region (13291-13560, 14700-15950, 18577-19051)
  - 1 section of S (Spike) region (21363-26001)
  - 1 section of E (Envelope) region (25801-28200)
  - 1 section of N (Nucleocapsid) region (27952-29673)

Negative reference material

- Sindbis virus
  - Human RNAse P gene (RP)

Matrix

- Viral transport media

EXTERNAL CONTROLS

Exact Diagnostics

- SARS-CoV-2 RNA transcripts (each at 200,000 cp/mL quantified using ddPCR)
  - Envelope (E) gene
  - Nucleocapsid (N) gene
  - ORF1a gene
  - RdRP gene
  - Spike (S) gene
- Human genomic DNA (75,000 cp/mL)
- Matrix
  - Synthetic matrix, including human gDNA

EXTERNAL CONTROLS

Integrated DNA Technologies

Positive control

- Plasmid containing complete nucleocapsid gene from 2019-nCoV, SARS, and MERS virus (200,000 copies/uL)

Negative control

- Plasmid containing portion of RPP30 gene (human RNase P)

Matrix

- Tris-EDTA (TE) buffer

VALUE/USE CASES FOR EXTERNAL CONTROLS

"Ninety percent of this game is half mental."

- Yogi Berra

WHAT CAN WE USE?

	Kit Controls	Other Commercial Controls	Commercial Panels	Patient Samples	Culture Material	Purified DNA/RNA	Cloned Amplicon in Plasmid
Validation Process	X	X	X	X	X	X	X
Daily Run Pos/Neg	X	X		X	X		
Trouble-shooting	X	X		X	X		
New Lot Verification	X	X		X	X		
AMR-Linearity		+/-	X (CAP)	X	X		
Cross-Instrument	X	X	X	X	X	Internal control?	
Std/Cal			X			X	X

Adapted from Linda Cook, CVS 2019

USE CASES/BENEFITS

Roles for external control material:

- Verification and validation
- Lot-to-lot changes
- Independent monitoring across lots, PM, etc
- More stable than pooled patients
- Less work than creating your own
  - Can mimic patient specimens

"IDEAL" CONTROL

- Is real virus or closely mimics real virus
  - Can be used in any manufacturer's assay
- High concentration/titer (precisely quantified)
- Stable to freeze thaw
- gDNA background
  - Kit control vs validation/verification
- Matrix which mimics real specimens
- Controls for total process (extraction to detection)

"The game ain't over 'til it's over."

- Yogi Berra

SARS-CoV-2 Quality Control for High-Throughput, Qualitative Serologic Assays

Elitza Theel, PhD, D(ABMM)
Director, Infectious Diseases Serology Laboratory
Co-Director, Vector-borne Diseases Service Line
Associate Professor, Laboratory Medicine and Pathology
Mayo Clinic
Rochester, MN

Disclosures

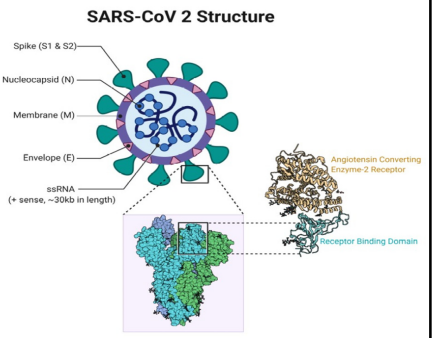
- Advisory Board/Consulting:
  - Roche Diagnostics
- Research funding:
  - Ortho-Clinical Diagnostics

Learning Objectives

- Discuss quality control approaches for high-throughput SARS-CoV-2 antibody tests
- List 2 optimal QC practices

SARS-CoV-2 Serologic Tests with FDA EUA

- 39 serologic assays with FDA EUA (as of 8/18/2020)
- Assays vary!
  - **Format**
    - Lateral Flow Assays
    - Enzyme-Linked Immunosorbent Assays (ELISAs)
    - Chemiluminescent Immunoassays (CLIAs)
  - **Specimen Type**
    - Serum, Plasma
    - Dried Blood Spot (DBS)
  - **Antibody Class Detected**
    - IgM or IgG or Total
  - **SARS-CoV-2 Protein Used**
    - Spike – S1, S2, Receptor Binding Domain (RBD)
    - Nucleocapsid
  - **Reporting**
    - Qualitative vs. Semi-Quantitative



https://www.ncbi.nlm.nih.gov/books/NBK554776/

Commercial, high-throughput serologic assays with EUA

Manufacturer	Specimen Type	Ab Isotype	SARS-CoV-2 Protein	Method	Qual vs. Semi-Quant?	Internal Kit Controls?
Diazyme	Serum (S), Plasma (P)	IgG	Not indicated	CLIA	Qualitative	Yes
Ortho-Clinical	S, P	Total & IgG	S1	CLIA	Qualitative	**No***
Roche	S, P	Total	Nucleocapsid (NC)	CLIA	Qualitative	**No***
Abbott	S, P	IgG	NC	CLIA	Qualitative	**No***
DiaSorin	S, P	IgG	S1/S2	CLIA	Qualitative	**No***
Biocheck	S	IgM/IgG	S1	CLIA	Qualitative	Yes
Siemens	S, P	Total	RBD	CLIA	Qualitative	**No***
Siemens	S, P	IgG	RBD	CLIA	Semi-quantitative	**No***
Beckman Coulter	S, P	IgG	RBD	CLIA	Qualitative	**No***
Babson	S, P	IgG	S1	CLIA	Qualitative	Yes
Luminex	S, P	IgG	NC, RBD, S1	FMIA	Qualitative	**No***
Bio-Rad	S, P	Total	NC	ELISA	Qualitative	Yes
Euroimmun	S, P	IgG	S1	ELISA	Qualitative	Yes
InBios	S	IgM & IgG	Not indicated	ELISA	Qualitative	Yes
Wantai	S, P	Total	RBD	ELISA	Qualitative	**No***
bioMerieux SA	S, P	IgM & IgG	RBD	ELFA	Qualitative	Yes

*QC material provided separately by manufacturer

Commercially Available 3rd Party Quality Control Material for SARS-CoV-2 Serologic Assays

Company	Matrix	Formulation	Shelf-Life	Antibody Isotype(s)	Release Testing Assay?	Positive Target Range?
seracare	Human-sourced material	Liquid, ready to use	Unopened: 2 yrs, Opened: 30 days	IgG, Total Ab	Nucleocapsid based	"Low positive"
TECHNOPATH CLINICAL DIAGNOSTICS	Human-sourced plasma	Liquid, ready to use	Unopened: 1 yr Opened: 10 days	IgG	Nucleocapsid & Spike based	Not defined
BIO-RAD	Human-sourced plasma	Liquid, ready to use	Unopened: 2 yrs, Opened: 30 days	IgG, Total Ab	Nucleocapsid & Spike based	Not defined

General Considerations for Assay Controls

- ✓**Type of control material (should differ from assay calibrator material)**
  - Supplied by assay manufacturer
    - Controls often optimized for the assay
    - May not as readily detect out-of-control events
  - 3rd party controls produced independent of assay
  - Pooled patient samples
- ✓**Control analyte matches assay**
- ✓**Similar to patient sample matrix**
- ✓**Large lot batches**
- ✓**Long shelf life**
- ✓**Cost-effective**

QC Frequency: How often and When?

- **CAP Immunology Checklist**

IMM.34120 **Daily QC - Nonwaived Tests** **Phase II**

Controls are run at least daily, or more frequently if specified in manufacturer's instructions, laboratory procedure, or the CAP Checklist, for quantitative and qualitative tests, and when changes occur that may impact patient results.

- **Method dependent**
  - Batch Testing: Defined start and stop time with all calibrator, control and patient measurements occurring during that time
    - QC run with each batch
  - Continuous Testing: No specific time interval for measurements
    - Manufacturers usually recommend 'at least every 24 hours'
    - "Bracketed QC"
    - Perform QC *before* and *after* major changes that may impact patient results
      - Re-calibration, reagent lot change, major instrument repair, software updates etc.

CLSI C24-ED4:2016 Statistical Quality Control for Quantitative Measurement Procedures

Does the quantitative value of controls matter for a qualitative serologic test?

- **High-throughput qualitative serologic assays use a quantitative value (*i.e.* S/Co) to determine the qualitative result**
- **CLSI recommends 2 levels of control (above and below cut-off threshold)**
- **Positive control level should be *near* the qualitative cut-off threshold**
  - More likely to detect out-of-control analytic performance
  - 'Nearness' to threshold is defined by the laboratory
    - Control at or too close to the threshold may lead to unnecessary run rejection
    - Consider assay precision characteristics
    - Typically 2-3X above threshold
- **CAP Requirement: Verifying the qualitative cut-off threshold**

IMM.33905 **Quantitative Cut-Off Values** **Phase II**

For qualitative tests that use a quantitative cut-off value to distinguish positive from negative results, analytic performance around the cut-off value is verified or established initially, and reverified at least every six months thereafter.

CLSI C24-ED4:2016 Statistical Quality Control for Quantitative Measurement Procedures

Establishing External QC Target Value and Range

***REVISED** 09/17/2019*

IMM.34140 Control Range Verification **Phase II**

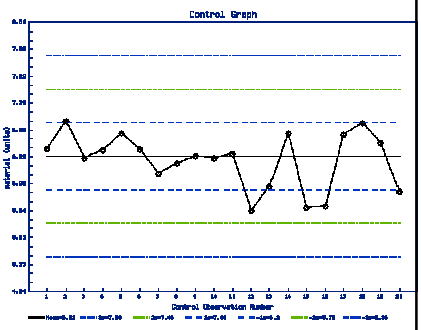
An acceptable control range is established or verified for each lot of control material.

- **Assayed vs. Unassayed QC material values**
  - Vendor assigned QC values should be used as *guide*, not replacement for laboratory established control mean/range
- **Historic QC data available?**
  - Imprecision = assay characteristic; remains the same regardless of QC lot
  - Historic SD may be applied to new QC material lots with a similar target level
  - New lot mean determined using 10 points and historic SD applied
- **No historic QC data available?**
  - Establish new mean/SD
  - 20 data pts on separate (ideally…) days to account for routine assay variability

CLSI C24-ED4:2016 Statistical Quality Control for Quantitative Measurement Procedures

Establishing Control Ranges

- **Levey-Jennings chart to assess initial QC points**
  - Look for drift, shift, outliers before establishing mean/SD range
  - Example: SeraCare Positive Control run on the Ortho-Clinical IgG CIA
- **Re-evaluate initial mean/SD in 1-2 months**
- **Monitor QC values overtime**
  - Westgard Rules
  - Single QC rules – detect large shifts (*e.g.*, 1_{3S})
  - Counting QC rules – detect smaller shifts/trends overtime (*e.g.*, 2_{2S}, 4_{1S})

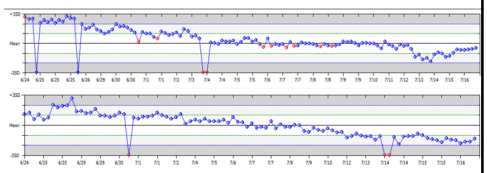


Monitoring QC – A SARS-CoV-2 Case Study

IMM.34362 **Monthly QC Review** **Phase II**

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

- **Implemented 3rd party QC material for Roche Anti-SARS-CoV-2 Total Antibody assay**
  - Downward trending S/Co for the positive control on all cobas e801 instruments:
  - Investigation:
    - Negative control S/Co remained stable
    - Retested previously positive patient samples:
      - S/Co remained stable
    - Tested prior validated pooled patient controls:
      - S/Co remained in expected ranges
  - Conclusion:
    - Degradation of positive control material…
    - Alternative 3rd party control verified and implemented



General Suggestions for Antibody QC

- **3rd party vendor control material preferable to independently monitor assay performance**
- **Qualitative assays based off of a cut-off threshold require 2 levels of control**
  - Above (ideally near cut-off) and below qualitative threshold
  - Establish or verify control range
- **Establish control frequency based on method and laboratory workflow**
  - Often more frequent than once/24 hrs…
- **Remember to monitor QC for trending overtime**

Thank you