Verification Examples for Newly Cleared Influenza rRT-PCR Platforms

The Centers for Disease Control and Prevention (CDC) has received Food and Drug Administration (FDA) clearance to add the Rotor-Gene® Q MDx (QIAGEN) & QuantStudio™ Dx (Applied Biosystems) rRT-PCR platforms to be used in conjunction with the CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel (CDC Flu rRT-PCR DX Panel) assays. Adoption of new instrumentation is voluntary, and the Applied Biosystems® 7500 Fast Dx Real-Time PCR instruments remain qualified for use.

Clinical Laboratory Improvement Amendments (CLIA) regulations require the performance (i.e., accuracy and precision) of newly acquired instrumentation to be verified by each laboratory before being used for testing on patient specimens. CDC is not able to provide a verification panel to laboratories transitioning to either the Q MDx or QuantStudio™ Dx platform. To assist public health laboratories with in-house instrument verification, the APHL Influenza and Respiratory Pathogen Subcommittee has developed the following assay verification example as a reference.

Each laboratory should consider their own quality management system (QMS) requirements and relevant regulatory agency/agencies requirements, when planning their instrument verification approach.

Verification Practices

Laboratories are encouraged to use their own specimen repositories as verification specimens. Previously characterized Influenza A and B positive specimens tested as part of national surveillance can be used for this verification. A multiple-system comparison may be necessary for laboratories using multiple thermalcyclers for influenza testing.

Materials Required for the Verification Examples

Verification of Accuracy

- Previously tested influenza-negative specimens: 3-5 specimens
- The following previously tested POSITIVE specimens, at both lower and higher Ct values with a minimum of 60µL extracted RNA/total nucleic acid for each sample:
  - Influenza Type A Subtype H1: 2-4 specimens
  - Influenza Type A Subtype H3: 2-4 specimens
  - Influenza Type B Yamagata lineage: 2-4 specimens
  - Influenza Type B Victoria lineage: 2-4 specimens
- Influenza A, influenza B and RNaseP (RP) primers/probes
- Enzyme Kit

Verification of Precision

- The samples used for the accuracy study also may be used for the precision verification study, but will require multiple nucleic acid extraction aliquots to ensure enough volume for both studies.
- Previously tested influenza-negative specimens: minimum of two specimens
- A minimum of 75µL extracted RNA/total nucleic acid from:
  - One LOW Ct Influenza A positive specimen
  - One LOW Ct Influenza B positive specimen

- One HIGH Ct Influenza A positive specimen
- One HIGH Ct Influenza B positive specimen

- Influenza A, Influenza B and RP primers/probes
- Enzyme Kit

Example Accuracy Verification Practice

- Utilize 11-21 specimens previously tested for influenza. Include 3-5 negative specimens and a mix of Influenza A and B positive specimens. Include specimens with a range of low and high Ct values.
- Starting with the original specimens, perform a fresh nucleic acid extraction and test the extracted specimens on both the new platform and one already in use. This will reduce the likelihood of discordant results from using previous test results. Test all specimens using Influenza A, B and RP primer/probe mixes.
- Compare the results obtained (detected/not detected) between the two instruments. If agreement is not 100% between instruments, consult your laboratory's QMS for acceptance criteria of the accuracy study. Repeat if necessary.

Example Precision Verification Practice

Use extracted RNA from at least four influenza specimens consisting of the following (if specimens with Ct values >32 are not available, dilutions may be made of lower Ct value samples to obtain the higher Ct value):

- Influenza A positive (any subtype), low Ct (e.g. Ct < 28)
- Influenza A positive (any subtype), high Ct (e.g. Ct > 32)
- Influenza B positive (either genotype), low Ct (e.g. Ct < 28)
- Influenza B positive (either genotype), high Ct (e.g. Ct > 32)
- Test five replicates each of the two influenza negative samples using both the influenza A and influenza B primer/probe mixes, five replicates of each of the two influenza A samples using only influenza A primer/probe mix, and five replicates of the two influenza B samples using only influenza B primer/probe mix on one plate. Repeat two more times over two additional days. If possible, have different analysts perform the runs.
- Calculate the mean, standard deviation and coefficient of variation (CV) of the Ct values for each group of within-run and across-run replicates. CV is calculated by dividing the standard deviation by the mean. A target of <10% CV is typically regarded as acceptable, but consult your laboratory's QMS for guidance. For ease of calculation, consider using Microsoft Excel functions for calculating mean, standard deviation and CV.

Questions about the assay? Contact CDC at https://partner.cdc.gov/Sites/NCIRD/FS/default.aspx or at flusupport@cdc.gov.

Questions for APHL and/or other public health laboratories? Contact fluquestions@aphl.org.

Association of Public Health Laboratories

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8515 Georgia Avenue, Suite 700
Silver Spring, MD 20910
Phone: 240.485.2745
Fax: 240.485.2700
www.aphl.org