

New York State Amended Results and Reporting Criteria for use with the novel influenza A (H1N1) real-time RT-PCR assay using CDC-designed primers, probes and protocol

Amended criteria are required for expedited approval of this molecular assay for the detection of novel influenza A (H1N1) (swine-like) for non-Public Health Laboratories (PHL) holding New York CLEP Virology – General permit category.

Expedited approval and reduced validation requirement necessitate a more conservative Reporting Algorithm than outlined in the hyperlinked protocol:
(<http://www.who.int/csr/resources/publications/swineflu/realtimetypepcr/en/index.html>).

Comment on Specimen types:

1. The FDA's Emergency Use approval for this assay specifies the following specimen types nasopharyngeal swabs (NPS), nasal swabs (NS), throat swabs (TS), nasal aspirates (NA), dual nasopharyngeal/throat swabs (NPS/TS), and culture isolates, only.
2. Full validation is required for approval for testing of bronchoalveolar lavage, tracheal aspirates, sputum, nasopharyngeal washes, and oropharyngeal aspirates or washes.

Interpretation/examination:

1. The Negative Template Control (NTC) reactions for probe/primer sets should not exhibit fluorescence growth curves that cross the threshold line. If a false positive signal occurs with one or more of the primer and probe NTC reactions, sample contamination may have occurred. Invalidate the run and repeat the assay on all specimens.
2. All primary clinical samples should exhibit RP reaction curves that cross the threshold line at or before 37 cycles. This positive result indicates that sufficient RNA from the human RNase P gene (and therefore enough cellular material overall) was gathered, has not deteriorated extensively during transport, and that the specimen is of acceptable quality. In addition to insufficient or low quality sample material, a negative RP result may also indicate:
 - a. Improper or inefficient extraction of nucleic acid from clinical materials resulting in loss of RNA
 - b. Carry-over of RT-PCR inhibitors from clinical specimen resulting in inhibition of PCR reaction
 - c. Improper assay set up and execution
 - d. Reagent or equipment malfunction
3. Primary specimens which exhibit RP CT values greater than 37 or negative, should be reextracted and retested. Extracts should be tested undiluted and at a 1/10 dilution, to evaluate for inhibition. If no inhibition is present, the CT value should be approximately 3 CT higher in the 1/10 dilution compared with the undiluted sample. Note: this is not necessary for specimens that are positive for InfA, swInfA AND swH1 with CT values at or less than 37. For specimens for which the RP CT values are still greater than 37 in the undiluted sample:
 - a. If inhibition is not indicated, report as "Inconclusive due to poor specimen quality."

- b. If inhibition is indicated (CT shift from undiluted to 1/10 is less than 3) report as “Inconclusive: specimen contains PCR inhibitors that may prevent the detection of target virus.”
4. The Human Specimen Control (HSC) should NOT exhibit fluorescence growth curves for primer/probe sets InfA, swInfA, or swH1 that cross the threshold line. If any influenza specific primer/probes exhibit a growth curve that crosses the threshold line, interpret as follows:
 - a. Contamination of RNA extraction reagents may have occurred. Invalidate the run and repeat testing of samples.
 - b. Cross contamination of samples may have occurred during RNA extraction procedures or assay setup. Invalidate the run and repeat the assay
5. Positive Template Control (PTC) reactions should produce a positive result with the InfA, swInfA, and swH1 targets at or before 37 cycles. Positive results may also be produced on the RP target depending on the source of the PTC. If expected positive reactivity is not achieved, invalidate the run and repeat the assay. If unacceptably weak signals continue to occur with the PTC, document, investigate cause, and if necessary, prepare new control material.
6. When all controls meet stated requirements, a specimen is considered positive for influenza A virus if the InfA reaction growth curves cross the threshold line within 37 cycles. If the reaction for influenza A is positive, it may also be positive for swInfA and/or swH1. A specimen is considered positive for novel influenza H1N1 if the InfA and BOTH the swInfA and swH1 reaction growth curves cross the threshold line within 37 cycles.
7. When all controls meet the stated requirements, a specimen is considered negative for influenza virus if growth curves for InfA, swInfA and swH1 do not cross the threshold.
8. When all controls meet the stated requirements, a specimen is considered positive for influenza A virus but negative for novel influenza H1N1, if the growth curve for InfA crosses the threshold line within 37 cycles, but growth curves for NEITHER swInfA nor swH1 cross the threshold.
9. If the reaction growth curves for InfA cross the threshold between 37 and 45, the specimen should be repeated from extraction.
 - a. If the target repeats as positive, crossing the threshold line within 45 cycles, then the sample is considered positive for influenza A.
 - b. If the target repeats as negative (does not cross the threshold) the specimen is negative for InfA unless other targets are positive, in which case the result is inconclusive.
10. When all controls meet the stated requirements and any of the following occur, repeat testing from extraction:
 - a. either swInfA or swH1, but not both, display reaction growth curves that cross the threshold line within 37 cycles
 - b. either swInfA or swH1, or both, cross the threshold line between 37 and 45 cycles (regardless of the result of the other sw target)
11. If the reaction growth curves InfA, swInfA and swH1 cross the threshold line between 37 and 45, the specimen should be repeated from extraction.

- a. If all three targets repeat as positive, crossing the threshold line within 45 cycles, then the sample is considered positive for swine influenza A/H1.
- b. If any of the targets are negative the result is "Equivocal for swine influenza A/H1".

12. See associated algorithm (Novel H1N1 Resulting Algorithm.pdf) for reporting.