CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel – Influenza A(H7) [Eurasian Lineage] Assay

For Emergency Use Authorization Only

Instructions for Use
Package Insert

To Be Used in Conjunction With

CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel – Influenza A Subtyping Kit Package Insert (LB-068)

Catalog # FluEUA-01
1000 reactions

Centers for Disease Control and Prevention
Influenza Division
1600 Clifton Rd NE
Atlanta GA 30329-4027

Intended Use

The Centers for Disease Control and Prevention (CDC) Human Influenza Virus Real-Time RT-PCR Diagnostic Panel-Influenza A(H7) [Eurasian Lineage] Assay is intended for use with the U.S Food and Drug Administration (FDA)-cleared CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel in real-time RT-PCR (rRT-PCR) assays on an Applied Biosystems (ABI) 7500 Fast Dx Real-Time PCR Instrument in conjunction with clinical and epidemiological information:

- To identify patients who may be infected with influenza A(H7) [Eurasian Lineage] virus to allow public health authorities to respond to and limit transmission of the virus during a declared public health emergency or threat of emergency.
- For the qualitative detection of influenza A virus in symptomatic patients from viral RNA in upper respiratory tract clinical specimens (including nasopharyngeal swabs [NPS], nasal swabs [NS], throat swabs [TS], nasal aspirates [NA], nasal washes [NW] and dual nasopharyngeal/throat swabs [NPS/TS]) and lower respiratory tract specimens (including bronchoalveolar lavage [BAL], bronchial wash [BW], tracheal aspirate [TA], sputum, and lung tissue) from human patients with signs and symptoms of respiratory infection and/or from viral culture.
- For the presumptive identification of virus in patients who may be infected with influenza A(H7) [Eurasian Lineage] from upper respiratory tract clinical specimens (such as NPS, NS, TS, NA, NW, and NPS/TS) and lower respiratory tract specimens (such as BAL, BW, TA, sputum, and lung tissue) and viral culture in conjunction with clinical and epidemiological risk factors.
- To provide epidemiologic information for surveillance of influenza A(H7) [Eurasian Lineage] viruses.

Testing with the CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel-Influenza A(H7) [Eurasian Lineage] Assay should not be performed unless the patient meets clinical and epidemiologic criteria for testing suspect specimens.

Negative results do not preclude influenza virus infection and should not be used as the sole basis for treatment or other patient management decisions. Conversely, positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease.

If infection with a novel influenza A virus is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions for novel virulent influenza viruses and sent to state or local health department for testing. Viral culture should not be attempted in these cases unless a BSL 3E facility is available to receive and culture specimens.

All users, analysts, and any person reporting results from use of this device should be trained to perform and interpret the results from this procedure by a competent instructor prior to use. CDC Influenza Division will limit the distribution of this device to only those users who have successfully completed a training course provided by CDC instructors or designees.

The CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel-Influenza A(H7) [Eurasian Lineage] Assay is for use under the Food and Drug Administration's Emergency Use Authorization (EUA) only.

Summary and Explanation

On April 1, 2013, the World Health Organization (WHO) first reported 3 human infections with a new influenza A(H7N9) virus in China. Since then, additional cases have been reported. Most reported cases have severe respiratory illness and, in some cases, have died. This is a “novel” (non-human) virus and therefore has the potential to cause a pandemic if it were to change to become easily and sustainably spread from person-to-person. So far, this virus has not been determined to have that capability. However, influenza viruses constantly change and it’s possible that this virus could gain that ability.

The CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel-Influenza A(H7) [Eurasian Lineage] Assay is authorized to be used in rRT-PCR assays on the ABI 7500 Fast Dx Real-Time PCR Instrument. The product when used in conjunction with the FDA-cleared CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel contains oligonucleotide primers and a dual-labeled hydrolysis (TaqMan®) probe used in rRT-PCR for the *in vitro* qualitative detection and characterization of human influenza A(H7) [Eurasian Lineage] viruses from viral RNA in respiratory specimens from patients presenting with influenza-like illness (ILI) and from virus culture. The CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel-Influenza A(H7) [Eurasian Lineage] Assay is to be used in conjunction with the FDA-cleared CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel.

Materials Required (Provided)

Influenza A(H7) [Eurasian Lineage] Assay Kit Contents: Catalog # FluEUA-01

Box #1: Primers and Probes

Reagent Label	Part #	Description	Quantity / Tube	Reactions / Tube
EuH7-F	MR-245	Influenza A(H7) [Eurasian Lineage] Forward Primer	20 nmol	1000
EuH7-R	MR-246	Influenza A(H7) [Eurasian Lineage] Reverse Primer	20 nmol	1000
EuH7-P	MR-247	Influenza A(H7) [Eurasian Lineage] Probe (FAM)	5 nmol	1000

Box #2: Influenza A(H7) [Eurasian Lineage] Positive Control (EuH7PC)

Reagent Label	Part #	Description	Quantity / Tube	Notes
EuH7PC	MR-735	Influenza A(H7) [Eurasian Lineage] Positive Control (EuH7PC). For use as a positive control with the CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel-Influenza A(H7) [Eurasian Lineage] Assay procedure to ensure the detection of influenza A(H7) Eurasian Lineage viruses. The EuH7PC contains noninfectious positive control materials supplied as a liquid, 500 µL per vial, suspended in 0.01 M phosphate buffer saline (PBS) at pH 7.2-7.4. EuH7PC consists of a beta-propiolactone treated influenza A(H7) [Eurasian Lineage] candidate vaccine virus and cultured human cells (A549). EuH7PC will yield a positive result with the following primer and probe sets: InfA, EuH7, and RP.	1 tube x 500 µL / tube	One thousand 5 µL reactions per tube

Materials Required (But Not Provided)

The CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel-Influenza A(H7) [Eurasian Lineage] Assay requires that the InfA and RP assays and Human Specimen Control (HSC) control from the FDA-cleared CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel (Catalog# FluIVD03) are run with the EuH7 assay. These components and their part numbers are listed in the table below:

Required Components from CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Influenza A Subtyping Kit

Reagent Label	Description	Quantity / Tube	Reactions / Tube
InfA-F	Influenza A Forward Primer	20 nmol	1000
InfA-R	Influenza A Reverse Primer	20 nmol	1000
InfA-P	Influenza A Probe (FAM)	5 nmol	1000
RP-F	Human RNase P Forward Primer	20 nmol	1000
RP-R	Human Rnase P Reverse Primer	20 nmol	1000

Reagent Label	Description	Quantity / Tube	Reactions / Tube
RP-P	Human Rnase P Probe (FAM)	5 nmol	1000
Human Specimen Control (HSC) HS0096	Human Specimen Control (HSC): For use as a RNA extraction procedural control to demonstrate successful recovery of RNA as well as extraction reagent integrity. Purified RNA from the HSC material should yield a positive result with the RP primer and probe set and negative results with all influenza specific markers. The HSC consists of noninfectious (beta propiolactone treated) cultured human cell material supplied as a liquid suspended in 0.01 M PBS at pH 7.2-7.4.	17 tubes x 500 µL / tubes	Five 100 µL extractions per tube

rRT-PCR Enzyme Mastermix Options

Reagent	Quantity	Catalog No.
Invitrogen SuperScript™ III Platinum® One-Step Quantitative RT-PCR System (without ROX)	100 reactions	11732-020
	500 reactions	11732-088
Invitrogen SuperScript™ III Platinum® One-Step Quantitative RT-PCR System (with ROX)	100 reactions	11745-100
	500 reactions	11745-500
Quanta BioSciences qScript™ One-Step qRT-PCR kit, Low ROX	50 reactions	95059-050
Quanta BioSciences qScript™ One-Step qRT-PCR kit, Low ROX	200 reactions	95059-200

RNA Extraction Options

Instrument/Manufacturer	Extraction Kit	Catalog No.
Roche MagNA Pure 96	DNA and Viral NA Small Volume Kit	576 extractions 06 543 588 001 External Lysis Buffer 06 374 913 001
QIAGEN	QIAamp DSP Viral RNA Mini Kit	50 extractions: 61904
QIAGEN QIAcube	QIAamp DSP Viral RNA Mini Kit	50 extractions: 61904
QIAGEN EZ1 Advanced XL	EZ1 DSP Virus Kit	48 extractions (62724) Buffer AVL (19073) EZ1 Advanced XL DSP Virus Card (9018703)
	EZ1 RNA Tissue Mini Kit	48 extractions (959034) EZ1 Advanced XL RNA Card (9018705)

Instrument/Manufacturer	Extraction Kit	Catalog No.
bioMérieux NucliSENS® easyMAG® (Automated magnetic extraction reagents sold separately)*	**N/A**	EasyMAG® Magnetic Silica (280133) EasyMAG® Disposables (280135) EasyMAG® Lysis Buffer (280134) EasyMAG® Lysis Buffer, 2 mL (200292) EasyMAG® Wash Buffers 1,2, and 3 (280130, 280131, 280132) Biohit Pipette Tips (280146)

Equipment and Consumables Required (But Not Provided)

- Plasticware and consumables
- Rnase/Dnase-free 1.5 mL polypropylene microcentrifuge tubes
- 100% ethanol (EtOH)
- Disposable gloves
- Molecular grade water (Rnase/Dnase Free)
- -70°C and -20°C freezer(s)
- 4°C refrigerator
- 96-well cold block
- Applied Biosystems 7500 Fast Dx Real-Time PCR Instrument with SDS 1.4 Software (ThermoFisher Scientific)
- Applied Biosystems 7500 Fast Sequence Detection Consumables (ThermoFisher Scientific).
  - ABI MicroAmp™ Fast 8-tube strip 0.1 mL, cat #4358293, or ABI MicroAmp™ Fast Optical 96-Well Reaction Plate with Barcode, 0.1 mL, part #4346906, #4346907, or part #4366932 (alternate to 8-strip tubes)
  - ABI MicroAmp™ Optical 8-cap strip, cat #4323032 (required, do not use film)
- Micropipettors (range between 1-10 µL, 10-200 µL and 100-1000 µL)
- Benchtop microcentrifuge

Warnings and Precautions

- For *In Vitro* Diagnostic Use
- This test has not been FDA cleared or approved;
- This test has been authorized by FDA under an Emergency Use Authorization for use by authorized laboratories;
- This test has been authorized only for the detection of RNA from avian influenza A (H7N9) virus, not for any other viruses or pathogens; and
- This test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of the emergency use of in vitro diagnostic tests for detection of avian influenza A (H7N9) virus under section 564(b)(1) of the Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner
- Follow standard precautions. All patient specimens and positive controls should be considered potentially infectious and handled accordingly.
- Do not eat, drink, smoke, apply cosmetics or handle contact lenses in areas where reagents and human specimens are handled.
- Handle all specimens as if infectious using safe laboratory procedures. Refer to Biosafety in Microbiological and Biomedical Laboratories (BMBL) 6th Edition BMBL (BMBL 6) for standard biological safety guidelines for all procedures.
- Specimen processing should be performed in accordance with national biological safety regulations.
- If infection with a novel influenza A virus is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions for novel virulent influenza viruses and sent to state health departments for testing **IMMEDIATELY**. Virus culture should not be attempted in these cases unless a BSL 3E facility is available to receive and culture specimens. **NOTE: Novel influenza A viruses are new or re-emergent human strains of influenza A that cause cases or clusters of human disease, as opposed to those strains commonly circulating in humans that cause seasonal epidemics and to which human populations have residual or limited immunity (either by vaccination or previous infection)**.
- Perform all manipulations of live virus samples within a Class II (or higher) biological safety cabinet (BSC).
- Use personal protective equipment such as (but not limited to) gloves and lab coats when handling kit reagents while performing this assay and handling materials including samples, reagents, pipettes, and other equipment and reagents.
- Amplification technologies such as PCR are sensitive to accidental introduction of product from previous amplifications reactions. Incorrect results could occur if either the clinical specimen or the real-time reagents used in the amplification step become contaminated by accidental introduction of amplification product (amplicon). Workflow in the laboratory should proceed in a unidirectional manner.
    - Maintain separate areas for assay setup and handling of nucleic acids.
    - Always check the expiration date prior to use. Do not use expired reagent. Do not substitute or mix reagent from different kit lots or from other manufacturers.
    - Change aerosol barrier pipette tips between all manual liquid transfers.
    - During preparation of samples, compliance with good laboratory techniques is essential to minimize the risk of cross-contamination between samples, and the inadvertent introduction of nucleases into samples during and after the extraction procedure. Proper aseptic technique should always be used when working with nucleic acids.
    - Maintain separate, dedicated equipment (e.g., pipettes, microcentrifuges) and supplies (e.g., microcentrifuge tubes, pipette tips) for assay setup and handling of extracted nucleic acids.

- Wear a clean lab coat and powder-free disposable gloves (not previously worn) when setting up assays.
- Change gloves between samples and whenever contamination is suspected.
- Keep reagent and reaction tubes capped or covered as much as possible.
- Primers, probes (including aliquots), and enzyme master mix must be thawed and maintained on cold block at all times during preparation and use.
- Work surfaces, pipettes, and centrifuges should be cleaned and decontaminated with cleaning products such as 5% bleach, "DNAZap™" or "Rnase AWAY®" to minimize risk of nucleic acid contamination. Residual bleach should be removed using 70% ethanol.

- Reagents, master mix, and RNA should be maintained on cold block or on ice during preparation and use to ensure stability.
- Dispose of unused kit reagents and human specimens according to local, state, and federal regulations.

Procedure

Follow these Instructions for Use in conjunction with the Package Insert (LB-068) for the FDA-cleared CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel – Influenza A Subtyping Kit for specimen handling, reagent handling and preparation, nucleic acid extraction, and assay set up.

The following sections have been provided to specifically address the use of CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel-Influenza A(H7) [Eurasian Lineage] Assay.

Tests for each rRT-PCR run:

3. Each sample RNA extract is tested by separate primer/probe sets: InfA, EuH7 and RP. The RP primer and probe set targets the human Rnase P gene and thus serves as an internal positive control for human nucleic acid.

2. No template controls (NTC) and positive template controls for all primer/probe sets should be included in each run.

3. Human Specimen Control (HSC) extraction control provides a secondary negative control that validates the nucleic acid extraction procedure and reagent integrity.

Master Mix Preparation / Plate Setup

1. Follow the procedure as outlined in the CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel – Influenza A Subtyping Kit Instructions for Use (LB-068) for master mix preparation. There are two enzyme system options available for use with the CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel-Influenza A(H7) [Eurasian Lineage] Assay. Figure 1 shows the master mix preparation for each enzyme system.

Figure 1. Steps and Calculations for Master Mix Preparation (Important: Select Appropriate Enzyme System)

Invitrogen SuperScript™ III Platinum® One-Step Quantitative RT-PCR System

Step #	Reagent	Vol. of Reagent Added per Reaction
1	Nuclease-free Water	N x 5.5 µL

2	Combined Primer/Probe Mix	N x 1.5 µL
3	SuperScript™ III RT/Platinum® *Taq* Mix	N x 0.5 µL
4	2X PCR Master Mix	N x 12.5 µL
	Total Volume	**N x 20.0 µL**

OR

Quanta BioSciences qScript™ One-Step qRT-PCR Kit, Low ROX

Step #	Reagent	Vol. of Reagent Added per Reaction
1	Nuclease-free Water	N x 5.5 µL
2	Combined Primer/Probe Mix	N x 1.5 µL
3	Quanta qScript™ One-Step Reverse Transcriptase	N x 0.5 µL
4	One-Step Master Mix (2X)	N x 12.5 µL
	Total Volume	**N x 20.0 µL**

2. An example test set up and sample set up are shown in Figure 2 and Figure 3. Follow procedures, recommendations, and guidelines in Instructions for Use LB-068 for the addition of master mix, samples and controls.

Figure 2. Example Test Set Up

	1	2	3	4	5	6	7	8	9	10	11	12
A	InfA	InfA	InfA	InfA	InfA	InfA	InfA	InfA	InfA	InfA	InfA	InfA
B	EuH7	EuH7	EuH7	EuH7	EuH7	EuH7	EuH7	EuH7	EuH7	EuH7	EuH7	EuH7
C	RP	RP	RP	RP	RP	RP	RP	RP	RP	RP	RP	RP
D												
E		InfA	InfA	InfA	InfA	InfA	InfA	InfA	InfA	InfA	InfA	
F		EuH7	EuH7	EuH7	EuH7	EuH7	EuH7	EuH7	EuH7	EuH7	EuH7	
G		RP	RP	RP	RP	RP	RP	RP	RP	RP	RP	
H												

Figure 3. Example Sample Set Up

	1	2	3	4	5	6	7	8	9	10	11	12
A	NTC	S1	S3	S5	S7	S9	S11	S13	S15	S17	S19	EuH7PC
B	NTC	S1	S3	S5	S7	S9	S11	S13	S15	S17	S19	EuH7PC
C	NTC	S1	S3	S5	S7	S9	S11	S13	S15	S17	S19	EuH7PC
D												
E		S2	S4	S6	S8	S10	S12	S14	S16	S18	HSC	
F		S2	S4	S6	S8	S10	S12	S14	S16	S18	HSC	
G		S2	S4	S6	S8	S10	S12	S14	S16	S18	HSC	
H												

Defining the Instrument Settings

The option of using Invitrogen SuperScript™ III Platinum® One-Step Quantitative RT-PCR System or Quanta BioSciences qScript™ One-Step qRT-PCR kit, Low ROX enzyme kit requires that instrument settings be selected for the appropriate enzyme kit.

1. After detectors have been created and assigned, proceed to instrument set up.
2. Select the **Instrument** tab to define thermal cycling conditions.
3. Modify the thermal cycling conditions as follows:

Invitrogen SuperScript™ III Platinum® One-Step Quantitative RT-PCR System

a. In Stage 1, Set to **30 min** at **50°C**; **1 Rep**.
b. In Stage 2, Set to 2.0 min at 95°C; 1 Rep.
c. In Stage 3, Step 1 set to **15 sec** at **95°C**.
d. In Stage 3, Step 2 set to **30 sec** at **55.0°C**.
e. In Stage 3, Reps should be set to **45**.
f. Under **Settings**, bottom left-hand box, change volume to 25 µL.
g. Under **Settings**, **Run Mode** selection should be **Standard 7500**.
h. Step 2 of Stage 3 should be highlighted in yellow to indicate data collection.

OR

Quanta BioSciences qScript™ One-Step qRT-PCR Kit, Low ROX

i. In Stage 1, Set to **30 min** at **50°C**; **1 Rep**.
j. In Stage 2, Set to 5.0 min at 95°C; 1 Rep.
k. In Stage 3, Step 1 set to **15 sec** at **95°C**.
l. In Stage 3, Step 2 set to **30 sec** at **55.0°C**.
m. In Stage 3, Reps should be set to **45**.
n. Under **Settings**, bottom left-hand box, change volume to 25 µL.
o. Under **Settings**, **Run Mode** selection should be **Standard 7500**.
p. Step 2 of Stage 3 should be highlighted in yellow to indicate data collection.

Interpretation of Results and Reporting

Controls Results and Interpretation

No Template Control (NTC)

- The NTC consists of using nuclease-free water in the rRT-PCR reactions instead of RNA. The NTC reactions for all primer and probe sets should not exhibit fluorescence growth curves that cross the threshold line. If any of the NTC reactions exhibit a growth curve that crosses the cycle threshold, sample contamination may have occurred. Invalidate the run and repeat the assay with strict adherence to the guidelines.

Influenza A(H7) Eurasian Lineage Positive Control (EuH7PC)

- The EuH7PC consists of an influenza virus representing influenza A(H7) Eurasian Lineage viruses suspended in cultured human cells (A549). Purified RNA from the EuH7PC should yield a positive result with the following primer and probe sets: InfA, EuH7, and RP.

Human Specimen Control (HSC) (Extraction Control)

- The HSC control consists of noninfectious cultured human cell (A549) material. The HSC is used as an RNA extraction procedural control to demonstrate successful recovery of RNA as well as extraction reagent integrity. Purified RNA from the HSC should yield a positive result with the RP primer and probe set and negative results with all influenza specific markers.

Control Type	Internal Control Name	Used to Monitor	InfA	EuH7	RP	Expected Ct Values
Positive	EuH7PC	Substantial reagent failure including primer and probe integrity	+	+	+	< 38.00 Ct
Negative	NTC	Reagent and/or environmental contamination	-	-	-	None detected
Extraction	HSC	Failure in lysis and extraction procedure	-	-	+	< 38.00 Ct

If any of the controls do not exhibit the expected performance as described, the assay may have been set up and/or executed improperly, or reagent or equipment malfunction could have occurred. Invalidate the run and re-test.

Specimens Results and Interpretation

RNase P (Extraction Control)

- All clinical samples should exhibit fluorescence growth curves in the RNase P reaction that cross the threshold line within 38.00 cycles (< 38.00 Ct), thus indicating the presence of the human RNase P gene. Failure to detect RNase P in any clinical specimens may indicate:
  - Improper extraction of nucleic acid from clinical materials resulting in loss of RNA and/or RNA degradation.
  - Absence of sufficient human cellular material due to poor collection or loss of specimen integrity.
  - Improper assay set up and execution.
  - Reagent or equipment malfunction.
- If the RP assay does not produce a positive result for human clinical specimens, interpret as follows:
  - If the InfA along with EuH7 are positive even in the absence of a positive RP, the influenza result should be considered valid. It is possible that some samples may fail to exhibit RNase P growth curves due to low cell numbers in the original clinical sample. A negative RP signal does not preclude the presence of influenza virus RNA in a clinical specimen.
  - If all influenza markers **AND** RNase P are negative for the specimen, the result should be considered inconclusive for the specimen. If residual specimen is available, repeat the extraction procedure and repeat the test. If all markers remain negative after re-test, report the results as inconclusive and a new specimen should be collected if possible.
  - The RP assay may be negative when testing virus culture samples.

Influenza Markers (InfA and EuH7)

- When all controls exhibit the expected performance, a specimen is considered negative if influenza markers (InfA, EuH7) cycle threshold growth curves **DO NOT** cross the threshold line within 38.00 cycles (< 38.00 Ct) **AND** the RNase P growth curve **DOES** cross the threshold line within 38.00 cycles (< 38.00 Ct).
- When all controls exhibit the expected performance, a specimen is considered positive for influenza if influenza markers (InfA and EuH7) cycle threshold growth curve crosses the threshold line within 38.00 cycles (< 38.00 Ct).
    - Report the specimen as Presumptive Positive for Influenza A(H7) virus and contact the CDC Influenza Division **IMMEDIATELY** at flusupport@cdc.gov to coordinate the transfer of the specimen to CDC as quickly as possible for confirmatory testing.

  The RNase P may or may not be positive as described above, but the influenza result is still valid. When testing tissue culture derived samples, the RNase P result is likely to yield negative / not detected result due to the absence of the human RNase P target.
- When all controls exhibit the expected performance and the growth curves for the influenza markers (InfA, EuH7) **AND** the RNase P marker **DO NOT** cross the cycle threshold growth curve within 38.00 cycles (< 38.00 Ct), the result is inconclusive. The extracted RNA from the specimen should be re-tested. If residual RNA is not available, re-extract RNA from residual specimen and re-test. If the re-tested sample is negative for all markers and all controls exhibit the expected performance, the result is "Inconclusive."
- When all controls exhibit the expected performance and the cycle threshold growth curve for influenza A (InfA) marker **ONLY** crosses the threshold line within 38.00 cycles (< 38.00 Ct) without the EuH7 indicating detection (InfA positive without A(H7) subtype detected), the sample should be tested for other influenza subtypes using the CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel – Influenza A Subtyping Kit. If no subtype (H3, pdmH1) is detected with the CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel, the sample has the potential for

containing a novel and/or newly emerging influenza A virus. The extracted RNA from the specimen should be re-tested **IMMEDIATELY**. If the fresh unfrozen residual RNA is not available, re-extract RNA from the residual specimen and re-test. If the re-tested sample is again positive for InfA only and all controls exhibit the expected performance, the state public health laboratory director, or designee, should contact the CDC Influenza Division **IMMEDIATELY** at flusupport@cdc.gov to coordinate the transfer of the specimen to CDC as quickly as possible for confirmatory testing. NOTE: Testing this sample using the Influenza A(H5) Subtyping Assay may be considered if the patient meets current WHO epidemiological risk factors.

Specimens Results Interpretation Guide

InfA	EuH7	RP	Result Interpretation[a]	Report for CDC Surveillance	Notes and Special Guidance
+	+	±	Influenza A Detected; Subtype: Eurasian H7 detected Presumptive positive for influenza A(H7)	Influenza A(H7)	Contact CDC immediately (flusupport@cdc.gov) for instructions for coordination of transferring the specimen to CDC for additional testing and further guidance.
+	-	±	Influenza A Detected; Influenza A(H7) Eurasian lineage not detected	Influenza A	If the specimen has not been tested for A(H3) and A(H1)pdm09 subtypes, proceed with this testing by using the FDA cleared CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel - A Subtyping Kit (Cat# FluIVD03-6). If no subtype is detected, the result may indicate a novel or newly emerging influenza. State Lab Director or designee should contact CDC (flusupport@cdc.gov) immediately for instructions for transferring specimen to CDC for further testing and guidance.
-	-	+	Influenza NOT Detected	Not Detected	Not Applicable
-	+	±	Inconclusive Result	Inconclusive	Re-extract specimen and test. If results are similar, report inconclusive.
-	-	-	Inconclusive Result	Inconclusive	Re-extract specimen and test. If results are similar, report inconclusive.

[a]Laboratories should report their diagnostic result as appropriate and in compliance with their specific reporting system.

Limitations

- All users, analysts, and any person reporting diagnostic results should be trained to perform this procedure by a competent instructor. They should demonstrate their ability to perform the test and interpret the results prior to performing the assay independently. CDC Influenza Division will limit the distribution of this device to only those users who have successfully completed training provided by CDC instructors or designees. This device is subject to a special control requiring that distribution be limited to laboratories with (i) experienced personnel who has training in standardized molecular testing procedures and expertise in viral diagnosis, and (ii) appropriate biosafety equipment and containment (21 CFR866.3332(b)(2)).
- Negative results do not preclude influenza virus infection and should not be used as the sole basis for treatment or other patient management decisions.
- A false negative result may occur if a specimen is improperly collected, transported or handled. False negative results may also occur if amplification inhibitors are present in the specimen or if inadequate numbers of organisms are present in the specimen. Children tend to shed virus more abundantly and for longer periods of time than adults. Therefore, testing specimens from adults will have lower sensitivity than testing specimens from children.
- Positive and negative predictive values are highly dependent on prevalence. False negative test results are more likely during peak activity when prevalence of disease is high. False positive test results are more likely during periods of low influenza activity when prevalence is moderate to low.
- The performance of the assay has not been established in individuals who received nasally administered influenza vaccine. Individuals who received nasally administered influenza A vaccine may have positive test results for up to three days after vaccination. http://www.cdc.gov/mmwr/preview/mmwrhtml/rr57e717a1.htm
- Do not use any reagent past the expiration date.
- Optimum specimen types and timing for peak viral levels during infections caused by a novel influenza A virus have not been determined. Collection of multiple specimens from the same patient may be necessary to detect the virus.
- The performance of this test was evaluated with contrived clinical samples due to the limited number of natural clinical specimens available. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of the virus that may mutate in the rRT-PCR target region. A specific novel influenza A virus may not be detected or may be detected less predictably.
- Inhibitors or other types of interference may produce a false negative result. An interference study evaluating the effect of common cold medications was not performed.
- Test performance can be affected because the epidemiology and pathology of disease caused by a specific novel influenza A virus is not fully known. For example, clinicians and laboratories may not know the optimum types of specimens to collect, and when during the course of infection these specimens are most likely to contain levels of virus that can be readily detected.
- Detection of viral RNA may not indicate the presence of infectious virus or that influenza is the causative agent for clinical symptoms.
- The performance of this test has not been established for monitoring treatment of influenza A infection.
- The performance of this test has not been established for screening of blood or blood product for the presence of influenza A.
- This test cannot rule out diseases caused by other bacterial or viral pathogens.

Conditions of Authorization For The Laboratory

- The CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel-Influenza A(H7) [Eurasian Lineage] Assay Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Patients and authorized labeling are available on the FDA website: https://www.fda.gov/medical-devices/emergency-situations-medical-devices/emergency-use-authorizations-medical-devices. Use of the CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel-Influenza A(H7) [Eurasian Lineage] Assay must follow the procedures outlined in these manufacturer's Instructions for Use and the conditions of authorization outlined in the Letter of Authorization. Deviations from the procedures outlined are not permitted under the Emergency Use Authorization. To assist clinical laboratories running the CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel-Influenza A(H7) [Eurasian Lineage] Assay, the relevant Conditions of Authorization are listed verbatim below.
- Authorized laboratories will include with reports of the results of the CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel-Influenza A(H7) [Eurasian Lineage] Assay the authorized Fact Sheet for Healthcare Providers and the authorized Fact Sheet for Patients. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.
- Authorized laboratories will perform the CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel-Influenza A(H7) [Eurasian Lineage] Assay in conjunction with the FDA cleared CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel in rRT-PCR assays.
- Authorized laboratories will perform the CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel-Influenza A(H7) [Eurasian Lineage] Assay as outlined in the manufacturer's Instructions for Use. Deviations from the authorized procedures, including the authorized RT-PCR instruments, authorized extraction methods, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to perform the CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel-Influenza A(H7) [Eurasian Lineage] Assay are not permitted.
- Authorized laboratories will have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.
- Authorized laboratories will collect information on the performance of the test and report to DMD/OIR/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and CDC any suspected occurrence of false positive or false negative results of which they become aware.
- All laboratory personnel using the test should be appropriately trained in RT-PCR techniques and use appropriate laboratory and personal protective equipment when handling this kit, and use the test in accordance with the authorized labeling.
- CDC and authorized laboratories will ensure that any records associated with this EUA are maintained until notified by FDA. Such records will be made available to FDA for inspection upon request.

Performance Characteristics

Clinical Performance

Retrospective Study Results

The CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel-Influenza A(H7) [Eurasian Lineage] Assay clinical performance characteristics were estimated using clinical specimens and contrived specimens. Due to the lack of available clinical specimens containing influenza A(H7), evaluation of the performance of the EuH7 primer and probe set was carried out with an alternative approach. Influenza A(H7) positive samples were prepared according to a method using a characterized

and titered stock of an influenza A(H7N9) virus (Influenza A/Anhui/1/2013) and human A549 cells. The stock virus was added to the A549 cell suspension in high, moderate, and low concentrations with multiple samples at each concentration. The low virus concentration was prepared to approximate the limit of detection (LOD) of the EuH7 assay. The influenza A(H7) negative samples were selected from clinical respiratory specimens from the 2012-2013 influenza season. All specimens were either negative for influenza A or positive for A(H3) or A(H1)pdm09 as determined by the FDA-cleared CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel. Overall, a total of 20 contrived influenza A(H7N9) virus positive specimens and 50 influenza A(H7N9) virus negative clinical specimens were tested in the study. Test results of the study are summarized in the table below:

Performance Summary

Assay Result	# of Positives[1]	% Positive Agreement (95% CI)	# of Negatives[1]	% Negative Agreement (95% CI)
A(H7)	20	100.0 (83.9 – 100.0)	50	100.0 (92.9 -100.0)

[1]Proportion of true positives or true negatives correctly identified.

Analytical Performance

Analytical Sensitivity – Limit of Detection (LOD)

Influenza A/Anhui/1/2013 virus of known 50% infectious dose titer (EID_{50}/mL) was extracted using one of the cleared extraction chemistries (the Roche MagNA Pure Compact RNA kit), serially diluted and tested with either Invitrogen SuperScript™ or Quanta qScript™ in replicates (n= 5) in order to determine an apparent endpoint range. The LOD was confirmed by testing extraction replicates (n=20) of the appropriate virus dilution(s) to determine the lowest concentration where ≥ 95% of the replicates are positive with the assay. Virus dilutions were prepared in VTM containing A549 cells to emulate clinical specimen matrix. The lowest concentration where the endpoints had uniform detection was reported as the LOD.

LOD Summary Comparison Table

Influenza Virus Type/Subtype	Influenza Virus	LOD (EID_{50}/mL)	
		Invitrogen SuperScript™	Quanta qScript™
A(H7N9)	A/Anhui/1/2013	$10^{3.4}$	$10^{3.4}$

Analytical Reactivity (Inclusivity)

The inclusivity of the CDC Human Influenza Virus Real-time RT-PCR Diagnostic Panel-Influenza A(H7) [Eurasian Lineage] Assay was examined by testing replicates (N= 3 to 5) influenza A(H7) viruses at concentrations at or near the established LOD. Virus RNA was isolated using one of the cleared extraction chemistries, the Roche MagNA Pure Compact RNA kit.

Analytical Reactivity Study Results (Invitrogen SuperScript™)

Strain Designation	Sub-type	Lineage	Conc. (EID_{50}/mL)	Invitrogen SuperScript™	
				InfA	EuH7
A/Anhui/1/2013	H7N9	Eu[1]	$10^{4.1}$	5/5	5/5

Strain Designation	Sub-type	Lineage	Conc. (EID_{50}/mL)	Invitrogen SuperScript™	
				InfA	EuH7
A/duck/Vietnam/NCVD-197/2009	H7N3	Eu	$10^{3.1}$	3/3	3/3
A/turkey/Italy/5425/07	H7N3	Eu	$10^{2.9}$	3/3	3/3
A/Shoveler/Egypt/00017-NAMRU3/2007	H7N3	Eu	$10^{3.5}$	3/3	3/3
A/Mexico/7218/2012	H7N3	N.Am.[2]	$10^{3.9}$	3/3	0/3

[1]Eu = Eurasian Lineage; [2]N.Am. = North American Lineage.

Analytical Reactivity Study Results (Quanta qScript™)

Strain Designation	Sub-type	Lineage	Conc. (EID_{50}/mL)	Quanta qScript™	
				InfA	EuH7
A/Anhui/1/2013	H7N9	Eu[1]	$10^{3.4}$	5/5	5/5
A/duck/Vietnam/NCVD-197/2009	H7N3	Eu	$10^{3.1}$	3/3	3/3
A/turkey/Italy/5425/07	H7N3	Eu	$10^{1.9}$	3/3	3/3
A/Shoveler/Egypt/00017-NAMRU3/2007	H7N3	Eu	$10^{2.5}$	3/3	3/3
A/Mexico/7218/2012	H7N3	N.Am.[2]	$10^{2.9}$	3/3	0/3

[1]Eu = Eurasian Lineage; [2]N.Am. = North American Lineage.

In-silico Analysis

We performed an *in-silico* analysis of avian Asian-lineage H7N9 sequences pulled from the GISAID databases either from all time (n=2678 sequences) or from the more recent timeframe of January 2020-July 2025 (n=54 sequences) that had sequence data covering our assay target region. A summary of the mismatch percentages are summarized below:

All time (n=2678):	**0% Mismatches**	**1% Mismatches**	**2% Mismatches**	**3% Mismatches**	**4% Mismatches**	**>5% Mismatches**
Forward Primer	94.8	4.9	0.3	0	0	0
Probe	75.4	24.0	0.6	0	0	0.04
Reverse Primer	65.2	29.4	4.9	0.5	0	0
January 2020 – July 2025 (n=54):	**0% Mismatches**	**1% Mismatches**	**2% Mismatches**	**3% Mismatches**	**4% Mismatches**	**>5% Mismatches**
Forward Primer	87.0	11.1	1.9	0	0	0
Probe	11.1	85.2	3.7	0	0	0
Reverse Primer	20.4	3.7	53.7	22.2	0	0

The *in-silico* analysis of January 2020-July 2025 sequences showed that the sequences with 2 mismatches correspond to locations that are not close to the 3' end of the primer, and hence are not anticipated to affect the sensitivity of the assay. The three mismatches were most commonly found in viruses isolated from chickens in Yunnan, China, in 2021. The CDC influenza division runs monthly analysis to monitor for mutations and potential loss of sensitivity for circulating strains, including for influenza A(H7). While there are a limited number of available sequences for human cases of influenza A(H7) as of July 2025, the performance of the test is not expected to be affected.

Analytical Specificity (Exclusivity)

Reactivity/Specificity of the CDC Human Influenza Virus Real-time RT-PCR Diagnostic Panel -Influenza A(H7) [Eurasian Lineage] Assay (i.e., reactivity for the InfA assay and specificity for the EuH7 assay) was evaluated with additional strains of influenza A (H1N1, H3N2, H1N1pdm09, and H5N1) and influenza B.

Reactivity/Specificity with Other Influenza Viruses

Influenza Type/Subtype	**Virus Designation**	**Conc. (EID_{50}/mL)**	**Invitrogen SuperScript™**		**Quanta qScript™**	
			InfA	**EuH7**	**InfA**	**EuH7**
A(H1N1)	A/Brisbane/59/07	$10^{8.4}$	14.92	Undet	14.78	Undet
A(H1N1)	A/Fujian Gulou/1896/2009	$10^{9.1}$	14.84	Undet	14.52	Undet
A(H3N2)	A/Perth/16/2009	$10^{8.2}$	17.04	Undet	16.63	Undet
A(H3N2)	A/Victoria/361/2011	$10^{9.2}$	14.46	Undet	14.64	Undet
A(H1N1)pdm09	A/California/07/09	$10^{8.4}$	15.15	Undet	15.13	Undet
A(H1N1)pdm09	A/South Carolina/2/2010	$10^{8.2}$	15.45	Undet	15.22	Undet
A(H5N1)	A/Indonesia/NIHRD11771/2011	$10^{9.4}$	15.68	Undet	14.58	Undet
A(H5N1)	A/duck/Vietnam/NCVD-1544/2012	$10^{9.5}$	15.82	Undet	14.46	Undet
B (Victoria lineage)	B/Nevada/03/2011	$10^{8.2}$	Undet	Undet	Undet	Undet
B (Yamagata lineage)	B/Wisconsin/01/2010	$10^{9.2}$	Undet	Undet	Undet	Undet

Non-Influenza Respiratory Viral and Bacterial Pathogens

Cross-reactivity of the CDC Human Influenza Virus Real-time RT-PCR Diagnostic Panel-Influenza A(H7) [Eurasian Lineage] Assay was evaluated by testing additional strains of common respiratory bacteria, fungus, and viruses.

Common Respiratory Bacteria and Fungus Tested with the CDC Human Influenza Virus Real-time RT-PCR Diagnostic Panel-Influenza A(H7) [Eurasian Lineage] Assay

Organism	Conc. (CFU/mL or Otherwise Specified)	Strain	Invitrogen SuperScript™		Quanta qScript™	
			InfA	EuH7	InfA	EuH7
Candida albicans (yeast)	$10^{8.8}$	2001-21-196	Undet	Undet	Undet	Undet
Chlamydia pneumoniae	40 IFU/mL	TW183	Undet	Undet	Undet	Undet
Corynebacterium diphtheriae	10^{10}		Undet	Undet	Undet	Undet
Escherichia coli	$10^{9.6}$	K12	Undet	Undet	Undet	Undet
Streptococcus pyogenes	$10^{7.5}$	7790-06	Undet	Undet	Undet	Undet
Haemophilus influenzae	$10^{6.4}$	M15709	Undet	Undet	Undet	Undet
Lactobacillus plantarum	$10^{8.8}$		Undet	Undet	Undet	Undet
Legionella pneumophila	$10^{10.3}$		Undet	Undet	Undet	Undet
Moraxella catarrhalis	$10^{9.5}$	M15757	Undet	Undet	Undet	Undet
Mycobacterium tuberculosis	95 ng/uL	H37Rv	Undet	Undet	Undet	Undet
Mycoplasma pneumoniae	$10^{7.7}$	M129	Undet	Undet	Undet	Undet
Neisseria elongata	$10^{8.6}$		Undet	Undet	Undet	Undet
Neisseria meningitidis	$10^{7.9}$	M2578	Undet	Undet	Undet	Undet
Pseudomonas aeruginosa	$10^{10.5}$		Undet	Undet	Undet	Undet
Staphylococcus aureus	$10^{10.7}$		Undet	Undet	Undet	Undet
Staphylococcus epidermidis	$10^{10.5}$		Undet	Undet	Undet	Undet
Streptococcus pneumoniae	$10^{6.6}$	249-06 (Blood from Thailand)	Undet	Undet	Undet	Undet
Streptococcus salivarius	$10^{8.4}$	SS1672	Undet	Undet	Undet	Undet

Common Respiratory Viruses Tested with the CDC Human Influenza Virus Real-time RT-PCR Diagnostic Panel-Influenza A(H7) [Eurasian Lineage] Assay

Non-Influenza Respiratory Viruses	Conc. ($TCID_{50}$/ml or Otherwise Specified)	Strain	Invitrogen SuperScript™		Quanta qScript™	
			InfA	EuH7	InfA	EuH7
Human Adenovirus, type 1	$10^{9.2}$	Ad.71	Undet	Undet	Undet	Undet
Human Adenovirus, type 7a	$10^{7.1}$	S-1058	Undet	Undet	Undet	Undet
Human parainfluenza 1	3.0 ng/µL		Undet	Undet	Undet	Undet
Human parainfluenza 2	$10^{3.1}$	Greer	Undet	Undet	Undet	Undet
Human parainfluenza 3	$10^{7.9}$	C-243	Undet	Undet	Undet	Undet
Respiratory syncytial virus	$10^{6.8}$	CH93-18b	Undet	Undet	Undet	Undet
Human Rhinovirus A	$10^{5.8}$	1A	Undet	Undet	Undet	Undet
Enterovirus	$10^{6.9}$	Echo 6	Undet	Undet	Undet	Undet
Human Coronavirus	31.6ng/µL	299E	Undet	Undet	Undet	Undet
Human Coronavirus	50.4ng/µL	OC43	Undet	Undet	Undet	Undet
Herpes Simplex virus	5 X $10^{7.75}$	KOS	Undet	Undet	Undet	Undet
Varicella-zoster virus	5 X $10^{3.75}$	AV92-3:H	Undet	Undet	Undet	Undet
Epstein Barr virus	1.7 ng/µL	B95-8	Undet	Undet	Undet	Undet
Measles	5 X $10^{4.5}$	Edmonston	Undet	Undet	Undet	Undet
Mumps	5 X $10^{6.5}$	Enders	Undet	Undet	Undet	Undet
Cytomegalovirus	5 X $10^{6.25}$	AD-169	Undet	Undet	Undet	Undet

The study demonstrated that the primer and probe sets contained within the CDC Human Influenza Virus Real-time RT-PCR Diagnostic Panel-Influenza A(H7) [Eurasian Lineage] Assay did not cross-react with any of the non-influenza respiratory pathogens or commensal organisms and demonstrated 100% concordance with the expected results.

Contact Information, Ordering, and Product Support

For technical and product support, contact the CDC Influenza Division FluSupport team directly.

For Ordering contact the International Reagent Resource:

Website: InternationalReagentResource.org

Email: contact@internationalreagentresource.org

For technical support contact CDC:

Send email to: FluSupport@cdc.gov