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

For Emergency Use Only

Instructions for Use

To Be Used in Conjunction With

CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel Package Insert (LB-029)

Catalog # FluEUA-01 1000 reactions

Centers for Disease Control and Prevention Influenza Division 1600 Clifton Rd NE Atlanta GA 30333



Intended Use

The CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel-Influenza A/H7 (Eurasian Lineage) Assay is intended for use with the 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 3+ 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 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 had 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 is 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 real-time RT-PCR assays (rRT-PCR) on the ABI 7500 Fast Dx Real-Time PCR Instrument. The product 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 Forward Primer	20 nmol	1000
EuH7-R	MR-246	Influenza A Reverse Primer	20 nmol	1000
EuH7-P	MR-247	Influenza A Probe (Fam)	5 nmol	1000

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

Reagent Label	Part #	Description	Quantity / Tube	Notes
EuH7PC	MR-248	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 virus (influenza A/H7 Eurasian Lineage) 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 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 Panel Catalog# FluIVD03

Reagent Label	Part #	Description	Quantity / Tube	Reactions / Tube
InfA-F	SO3304	Influenza A Forward Primer	20 nmol	1000
InfA-R	SO3284	Influenza A Reverse Primer	20 nmol	1000
InfA-P	SO3285	Influenza A Probe (FAM)	5 nmol	1000
RP-F	SO3313	Human RNase P Forward Primer	20 nmol	1000
RP-R	SO3314	Human RNase P Reverse Primer	20 nmol	1000
RP-P	SO3315	Human RNase P Probe (FAM)	5 nmol	1000
Human Specimen Control (HSC)	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		17 tubes x 500 μL / tubes	Five 100 µL extractions per tube

Reagent Label	Part #	Description	Quantity / Tube	Reactions / Tube
		PBS at pH 7.2-7.4.		

rRT-PCR Enzyme Mastermix Options

Reagent	Quantity	Catalog No.
Invitrogen SuperScript™ III Platinum [®] One-Step Quantitative RT-	100 reactions	11732-020
PCR System (without Rox)	500 reactions	
Invitrogen SuperScript™ III Platinum [®] One-Step Quantitative RT-	100 reactions	11745-100
PCR System (With Rox)	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 LC 2.0	Total Nucleic Acid Kit	192 extractions: 03 038 505 001
Roche MagNA Pure Compact	Nucleic Acid Isolation Kit I	32 extractions: 03 730 964 001
Roche MagNA Pure Compact	RNA Isolation Kit	32 extractions: 04 802 993 001
QIAGEN	DSP Viral RNA Mini Kit	50 extractions: 61904
QIAGEN QIAcube	DSP Viral RNA Mini Kit	50 extractions: 61904

Instrument/Manufacturer	Extraction Kit	Catalog No.
bioMérieux NucliSENS [®] easyMAG [®] (Automated magnetic extraction reagents sold separately)		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)

- 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 (Applied Biosystems, Foster City, CA)
- Applied Biosystems 7500 Fast Sequence Detection Consumables (Applied Biosystems, Foster City, CA).
 - 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 μ L, 10-200 μ L and 100-1000 μ L)
- Benchtop microcentrifuge

Procedure

Follow these Instructions for Use in conjunction with the Package Insert (LB-029) for the FDAcleared CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel 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:

- 1. 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. 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 Product Insert LB-029 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	Ν x 5.5 μL
2	Forward Primer	Ν x 0.5 μL
3	Reverse Primer	Ν x 0.5 μL
4	Probe	Ν x 0.5 μL
5	SuperScript™ III RT/Platinum [®] <i>Taq</i> Mix	Ν x 0.5 μL
6	2X PCR Master Mix	Ν x 12.5 μL

То	tal Volume	Ν x 20.0 μL
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OR

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

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

2. An example test set up and sample set up are shown in Figures 2 and 3. Follow procedures, recommendations, and guidelines in Product Insert LB-029 for the addition of master mix, samples and controls.

	1	2	3	4	5	6	7	8	9	10	11	12
Α	InfA											
в	EuH 7											
С	RP											
D												
Е		InfA										
F		EuH 7										
G		RP										
Н												

Figure	2.	Exam	ple	Test	Set	Up
		-Adding				~ r

	1	2	3	4	5	6	7	8	9	10	11	12
Α	NTC	S1	S3	S5	S7	S9	S11	S13	S15	S17	S19	EuH7PC
в	NTC	S1	S3	S5	S7	S9	S11	S13	S15	S17	S19	EuH7PC
С	NTC	S1	S3	S5	S7	S9	S11	S13	S15	S17	S19	EuH7PC
D												
Е		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	
Н												

Figure 3. Example Sample Set Up

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 μ L.
- 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.
- I. 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 μ L.
- 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).</p>
- When all controls exhibit the expected performance, a specimen is considered positive for influenza if the influenza marker (InfA and EuH7) cycle threshold growth curve crosses the threshold line within 38.00 cycles (< 38.00 Ct). 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."</p>
- 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. If no subtype (H1, H3, pdhH1) 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 <u>flusupport@cdc.gov</u> to coordinate the transfer of the specimen to CDC as quickly as possible for confirmatory testing. NOTE: Do not test this sample using the Influenza A/H5 Subtyping Assay unless the patient meets the current WHO epidemiological risk.

InfA	EuH7	RP	Result Interpretation ^a	Report for CDC Surveillance	Notes and Special Guidance
+	+	±	Influenza A Detected; Subtype: Eurasian H7 detected	Influenza A(H7)	
+	-	±	Influenza A Detected; Influenza A/H7 Eurasian lineage not detected	Influenza A	If the specimen has not been tested for A/H1, A/H3, pdmH1 subtypes, proceed with this testing by using the FDA cleared CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel- A/Subtyping kit (Cat# FluIVD03-2). 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	
-	+	±	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.

Specimens Results Interpretation Guide

^aLaboratories should report their diagnostic result as appropriate and in compliance with their specific reporting system.

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 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/H3N2 or A/H1N1pdm09 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

Result Positives ¹	(95% CI) Negatives	¹ (95% Čl)
A/H7 20 100.0	(83.9 – 100.0) 50	100.0 (92.9 -100.0)

¹Proportion 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 (EID₅₀/mL) was extracted using one of the cleared extraction chemistries (the Roche MagNA Pure Compact RNA kit), serially diluted and tested with either Invitrogen SuperScriptTM or Quanta qScriptTM 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 \geq 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	Influenza Virus	LoD (EID ₅₀ /mL)			
Type/Subtype	innuenza virus	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.

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

Analytical Reactivity Study Results (Invitrogen SuperScriptTM)

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

Analytical Reactivity Study Results (Quanta qScript[™])

	Sub-		Conc.	Conc. Quanta qScript [™]	
Strain Designation	type	Lineage	(EID ₅₀ /mL)	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
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¹Eu = Eurasian Lineage; ²N.Am. = North American Lineage.

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	Virus Designation	Conc.	Invitr SuperS	ogen Script [™]	Quanta qScript [™]	
Type/Subtype			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/H1N1pdm09	A/California/07/09	10 ^{8.4}	15.15	Undet	15.13	Undet
A/H1N1pdm09	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.

Ormaniam	Conc. (CFU/mL or	Strain	Invitr SuperS	ogen Script [™]	Quanta qScript [™]	
Organism	Otherwise Specified)	Strain	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 Bacteria and Fungus Tested with the CDC Human Influenza Virus Real-time RT-PCR Diagnostic Panel-Influenza A/H7 (Eurasian Lineage) Assay

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	Conc. (TCID/ml.or		Invitrogen S	uperScript [™]	Quanta o	qScript [™]
Viruses	Otherwise Specified)	Strain	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 and Product Support

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

Send email to: FluSupport@cdc.gov