510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

K111507

B. Purpose for Submission:

A new device that merges two previously FDA-cleared CDC devices, the CDC Human Influenza Virus Real-time RT-PCR Detection and Characterization Panel (K080570) and the CDC Influenza 2009 A (H1N1)pdm Real-time RT-PCR Panel (K101564).

C. Measurand:

Influenza virus nucleic acids target sequences. Influenza types and subtypes detected: Influenza A, Influenza A/H1, Influenza A/H3, Influenza A/H5 (Asian lineage), Influenza A/H1pdm09, and Influenza B

D. Type of Test:

A panel of oligonucleotide primers and dual-labeled hydrolysis (TaqMan®) probes to be used in rRT-PCR for the in vitro qualitative detection and differentiation of influenza virus type and subtype target sequences in respiratory specimens from human patients with signs or symptoms of respiratory infection and/or from virus culture using nucleic acid isolation, amplification, and detection on the ABI 7500 Fast Dx Real-Time PCR instrument with Sequence Detection Software version 1.4.

E. Applicant:

Centers for Disease Control and Prevention

F. Proprietary and Established Names

CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel

G. Regulatory Information:

1. Regulation section:
   866.3332 - Reagents for detection of specific novel influenza A viruses
2. Classification:
   Class II
3. Product code:
   OQW, NXD, OEP, NSU
4. Panel:
   Microbiology (83)

H. Intended Use:

1. Intended use:

The CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel is intended for use in rRT-PCR assays on an Applied Biosystems (ABI) 7500 Fast Dx Real-Time PCR instrument in conjunction with clinical and epidemiological information:

- For qualitative detection of influenza virus type A or B 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 determination of the subtype of seasonal human influenza A virus as seasonal A/H1, A/H3, and/or A/H1pdm09 from viral RNA in upper respiratory tract clinical specimens (including NPS, NS, TS, NA, NW and NPS/TS) and lower respiratory tract specimens (including BAL, BW, 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 subtype A/H5 (Asian Lineage) from viral RNA in human respiratory specimens and viral culture in conjunction with clinical and epidemiological risk factors.
- To provide epidemiologic information for surveillance of circulating influenza viruses.

Performance characteristics for influenza were established during a season when influenza viruses A/H1 and A/H3 were the predominant influenza A viruses in circulation and during a season when the A/H1pdm09 influenza virus was the predominant influenza A virus in circulation. Performance characteristics may vary with other emerging influenza A viruses.

Testing with the influenza H5a and H5b primer and probe sets should not be performed unless the patient meets the most current U.S. Department of Health and Human Services (DHHS) clinical and epidemiologic criteria for testing suspect A/H5 specimens. The definitive identification of influenza A/H5 (Asian lineage) either directly from patient specimens or from virus cultures requires additional laboratory testing, along with clinical and epidemiological assessment in consultation with national influenza surveillance experts.

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.

2. Indication(s) for use:

   Same as Intended Use

3. Special conditions for use statement(s):

   For prescription use only

4. Special instrument requirements:

   Applied Biosystems ABI 7500 Fast Dx Real-Time PCR Instrument with Sequence Detection Software version 1.4.

I. Device Description:

The CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel is used in rRT-PCR assays on the ABI 7500 Fast Dx Real-Time PCR Instrument. It consists of oligonucleotide primers, dual-labeled hydrolysis (TaqMan®) probes, and positive controls for the in vitro qualitative detection and characterization of the human influenza virus RNA in respiratory tract specimens from human patients presenting with signs and symptoms of respiratory infections. These reagents are used for detection of influenza A and B virus and characterization of influenza A viruses as seasonal A/H1, A/H3, A/H1pdm09, or A/H5 (Asian lineage).
Primers and probes for Influenza A, 2009 Influenza A (swine origin), and B viruses were selected from highly conserved regions of specific gene targets within the matrix protein (M), nucleoprotein (NP), and non-structural protein (NS), respectively. Primers and probes for detection and differentiation of seasonal influenza A/H1, A/H3, A/H1pdm09, and A/H5 viruses are targeted for conserved regions of their respective hemagglutinin (HA) genes. Sizes of targeted regions are between 100–200 nucleotides. Influenza-specific primers were designed to be 19–25 nucleotides long with annealing temperatures of approximately 62–66°C (GC content of 45–60%) and the terminal 3’ nucleotide located at the first or second residue of a predicted amino acid codon.

Device Components (Provided)

The CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel includes the

following:

Lyophilized primer and probe sets

- **InfA** detects all influenza A viruses, but does not detect influenza B viruses
- **InfB** detects all influenza B viruses, but does not detect influenza A viruses
- **H1** specifically detects influenza A, seasonal H1 subtype
- **H3** specifically detects influenza A, seasonal H3 subtype
- **H5a** and **H5b** specifically detects influenza A, H5 Eurasian subtype
- **pdm InfA** detects classical swine influenza viruses, triple reassortant swine influenza viruses, and A/H1pdm09 influenza virus
- **pdm H1** specifically detects the A/H1pdm09 influenza virus
- **RNase P (RP)** detects human RNase P and is used with human clinical specimens to indicate that adequate isolation of nucleic acid resulted from the extraction of the clinical specimen.

Positive Controls

- **Pooled Influenza Positive Control (PIPC)**
  For use as a positive control with the CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel procedure to ensure the detection of seasonal influenza virus A/H1, A/H3, A/H1pdm09 and influenza B. The PIPC 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. PIPC consists of four different beta-propiolactone inactivated influenza viruses (influenza A/H1, A/H3, A/H1pdm09, and influenza B) suspended in cultured human cells (A549). PIPC will yield a positive result with the following primer and probe sets: InfA, InfB, H1, H3, pdm InfA, pdm H1, and RP.
- **Influenza Virus A/H5N1 Positive Control (H5VC)**
  For use as a positive control with the CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel procedure to ensure proper detection of Influenza A/H5 virus (Asian lineage). Noninfectious (beta-propiolactone inactivated) positive control material supplied as a liquid, 500 µl per vial, suspended in 0.01 M PBS at pH 7.2–7.4. The H5VC control consists of a reassortant human vaccine candidate virus (A/Vietnam/1203/04 x PR/8/34) that was generated by reverse genetics and cultured human cells (A549). The H5VC will yield a positive result with the following primer and probe sets: InfA, H5a, H5b, and RP.
- **Human Specimen Control (HSC)**
  For use as a RNA extraction procedural control with the CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel 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 inactivated) cultured human cell material (A549) supplied as a liquid suspended in 0.01 M PBS at pH 7.2–7.4.

Materials Required But Not Provided

rRT-PCR Enzyme Mastermix Options

	Reagent	Quantity	Catalog No.
rRT-PCR Enzyme Mastermix Options	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

RNA Extraction Options

Instrument and Manufacture	Extraction Kit	Catalog No.
Roche MagNA Pure LC 2.0	Total Nucleic Acid Kit	192 extractions: cat # 03 038 505 001
Roche MagNA Pure Compact	Nucleic Acid Isolation Kit I	32 extractions: cat #03 730 964 001
Roche MagNA Pure Compact	RNA Isolation Kit	32 extractions: 04 802 993 001
Qiagen QIAamp	DSP Viral RNA Mini Kit	50 extractions: cat #52904 250 extractions: cat #52906
QIAGEN QIAcube	DSP Viral RNA Mini Kit	50 extractions: cat #52904 250 extractions: cat #52906
bioMérieux NucliSENS® easyMAG® (Automated magnetic extraction reagents sold separately)		EasyMAG® Magnetic Silica (cat # 280133) EasyMAG® Disposables (cat # 280135) EasyMAG® Lysis Buffer (cat # 280134) EasyMAG® Wash Buffers 1,2, and 3 (cat # 280130, 280131, 280132) Biohit Pipette Tips (cat # 280146)

Approved Ancillary Reagents with the CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel

Specific lots for the ancillary reagents that are not manufactured under Quality System Regulations (Roche MagNA Pure and Invitrogen SuperScript), will be qualified for use with the CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel by the CDC Influenza Division. Any lots not specifically qualified by the CDC Influenza Division for use with the CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel are not valid for use with this device, and may affect device performance.

A supplemental cumulative list of the qualified ancillary reagents lots for use with the Flu rRT-PCR Dx Flu Panel is available and can be requested by sending an email to FluSupport@cdc.gov

Any issues related to assay performance or test failure that are suspected to involve ancillary reagents should be reported to the CDC Influenza Division by emailing FluSupport@cdc.gov

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)
- -70º C and -20º C Freezer(s)
- 4º 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)
- Micropipettors (1–10 µL, 10–200 µL and 100–1000 µL)
- Benchtop Microcentrifuge

J. Substantial Equivalence Information:

1. Predicate device name(s):
   CDC Human Influenza Virus Real-time RT-PCR Detection and Characterization Panel
   CDC Influenza 2009 A (H1N1)pdm Real-time RT-PCR Panel

2. Predicate 510(k) number(s):
   K080570
   K101564

3. Comparison with predicate:

Features	CDC Human Influenza Virus Real-time PCR Diagnostic Panel	CDC Human Influenza Virus Real-time RT-PCR Detection and Characterization Panel (K080570)	CDC Influenza 2009 A (H1N1)pdm Real-time RT-PCR Panel (K101564)
Intended Use	The CDC Human Influenza Virus Real-Time PCR Diagnostic Panel is intended for use in Real-time RT-PCR assays on an Applied Biosystems (ABI) 7500 Fast Dx Real-Time PCR Instrument in conjunction with clinical and epidemiological information: 1) for qualitative detection of influenza virus type A or B in symptomatic patients from viral RNA in upper respiratory tract clinical specimens (including nasopharyngeal swabs, nasal swabs, throat swabs nasal aspirates, nasal washes and dual nasopharyngeal/throat swabs), and lower respiratory tract specimens (including bronchoalveolar lavage, bronchial wash, tracheal aspirate, sputum, and lung tissue) from human patients with signs and symptoms of respiratory infection and/or from viral culture, 2) for determination of the subtype of seasonal human influenza A virus as seasonal A/H1, A/H3, and/or A/H1pdm09 from viral RNA in upper respiratory tract clinical specimens (including nasopharyngeal swabs, nasal swabs, throat swabs nasal aspirates, nasal washes and dual nasopharyngeal/throat swabs), and lower respiratory tract specimens (including bronchoalveolar lavage, bronchial aspirate, bronchial	The Human Influenza Virus Real-time RT-PCR Detection and Characterization Panel is intended for use in Real-time RT-PCR assays on an ABI 7500 Fast Dx Real-Time PCR Instrument in conjunction with clinical and epidemiological information: for qualitative detection of influenza virus type A or B in symptomatic patients from viral RNA in nasopharyngeal and/or nasal swab specimens, for determination of the subtype of seasonal human influenza A virus, as seasonal A/HI or A/H3, if present, from viral RNA in nasopharyngeal and/or nasal swab specimens, for presumptive identification of virus in patients who may be infected with influenza A subtype A/H5 (Asian lineage) from viral RNA in human respiratory specimens and viral culture in conjunction with clinical and epidemiological risk factors to provide epidemiologic information for surveillance for influenza viruses.	The CDC rRT-PCR A(H1N1)pdm09 Flu Panel is intended for use in real-time RT-PCR assays on the Applied Biosystems (ABI) 7500 Fast Dx Real-Time PCR Instrument for the *in vitro* qualitative detection of influenza virus type A and 2009 A/H1N1 viral RNA from nasopharyngeal swabs, nasal swabs, throat swabs, nasal aspirates, nasal washes, dual nasopharyngeal / throat swabs and lower respiratory tract specimens from human patients with signs and symptoms of respiratory infection and/or from viral culture, in conjunction with clinical and epidemiological risk factors.

	wash, endotracheal aspirate, endotracheal wash tracheal aspirate, sputum, and lung tissue) from human patients with signs and symptoms of respiratory infection and/or from viral culture, 3) for the presumptive identification of virus in patients who may be infected with influenza A subtype A/H5 (Asian Lineage) from viral RNA in human respiratory specimens and viral culture in conjunction with clinical and epidemiological risk factors, and 4) to provide epidemiologic information for surveillance of the circulating influenza viruses.		
Specimen Types	Nasopharyngeal swabs, nasal swabs, throat swabs, nasal aspirates, nasal washes and dual nasopharyngeal/throat swabs, bronchoalveolar lavage, bronchial wash, tracheal aspirate, sputum, and lung tissue, and virus culture.	Nasopharyngeal swabs, nasal swabs, and/or virus culture	Nasopharyngeal swabs, nasal swabs, throat swabs, nasal aspirates, nasal washes, dual collected nasopharyngeal and throat swabs, bronchoalveolar lavage, bronchial aspirate, bronchial wash, tracheal aspirate, and virus culture.
Technology	Real-time RT-PCR	Real-time RT-PCR	Real-time RT-PCR
Required Instrumentation	Applied Biosystems 7500 Fast Dx Real- Time PCR Instrument	Applied Biosystems 7500 Fast Dx Real- Time PCR Instrument	Applied Biosystems 7500 Fast Dx Real- Time PCR Instrument
Organism Detected	Universal influenza A viruses, Swine-origin influenza A viruses, Influenza B viruses, and Influenza A subtypes: seasonal A/H1, A/H3, A/H1pdm09, and A/H5 (Asian lineage)	Universal influenza A virus, subtypes A/H1 and A/H3; Influenza B virus; Influenza A virus, subtype A/H5 (Asian lineage)	Universal influenza A, Swine-Origin Influenza A, and A/H1pdm09 subtype
Nucleic Acid Extraction	Yes	Yes	Yes

Extraction Method	• QIAamp® Viral RNA Mini Kit, Qiagen Inc. • MagNA Pure Compact - Total Nucleic Acid Kit, Roche Applied Science • MagNA Pure Compact – RNA Isolation Kit, Roche Applied Science • MagNA Pure LC - RNA Isolation Kit II, Roche Applied Science • Qiagen QIAcube with QIAamp® Viral RNA Mini Kit, Qiagen Inc. • NucliSENS® easyMAG®, bioMerieux	• QIAamp® Viral RNA Mini Kit, Qiagen Inc. • RNeasy® Mini Kit, Qiagen, Inc. • MagNA Pure LC RNA Isolation Kit II, Roche Applied Science • MagNA Pure Total Nucleic Acid Kit, Roche Applied Science	• QIAamp® Viral RNA Mini Kit, Qiagen Inc. • MagNA Pure Compact - Total Nucleic Acid Kit, Roche Applied Science • MagNA Pure Compact – RNA Isolation Kit, Roche Applied Science • MagNA Pure LC - RNA Isolation Kit II, Roche Applied Science • Qiagen QIAcube with QIAamp® Viral RNA Mini Kit, Qiagen Inc. • NucliSENS® easyMAG®, bioMerieux
Enzyme Master Mix	Invitrogen SuperScript™ III Platinum® One-Step Quantitative RT-PCR Kits (with or without ROX)	Invitrogen SuperScript™ III Platinum® One-Step Quantitative RT-PCR Kits (with or without ROX)	Invitrogen SuperScript™ III Platinum® One-Step Quantitative RT-PCR Kits (with or without ROX)

K. Standard/Guidance Document referenced (if applicable):

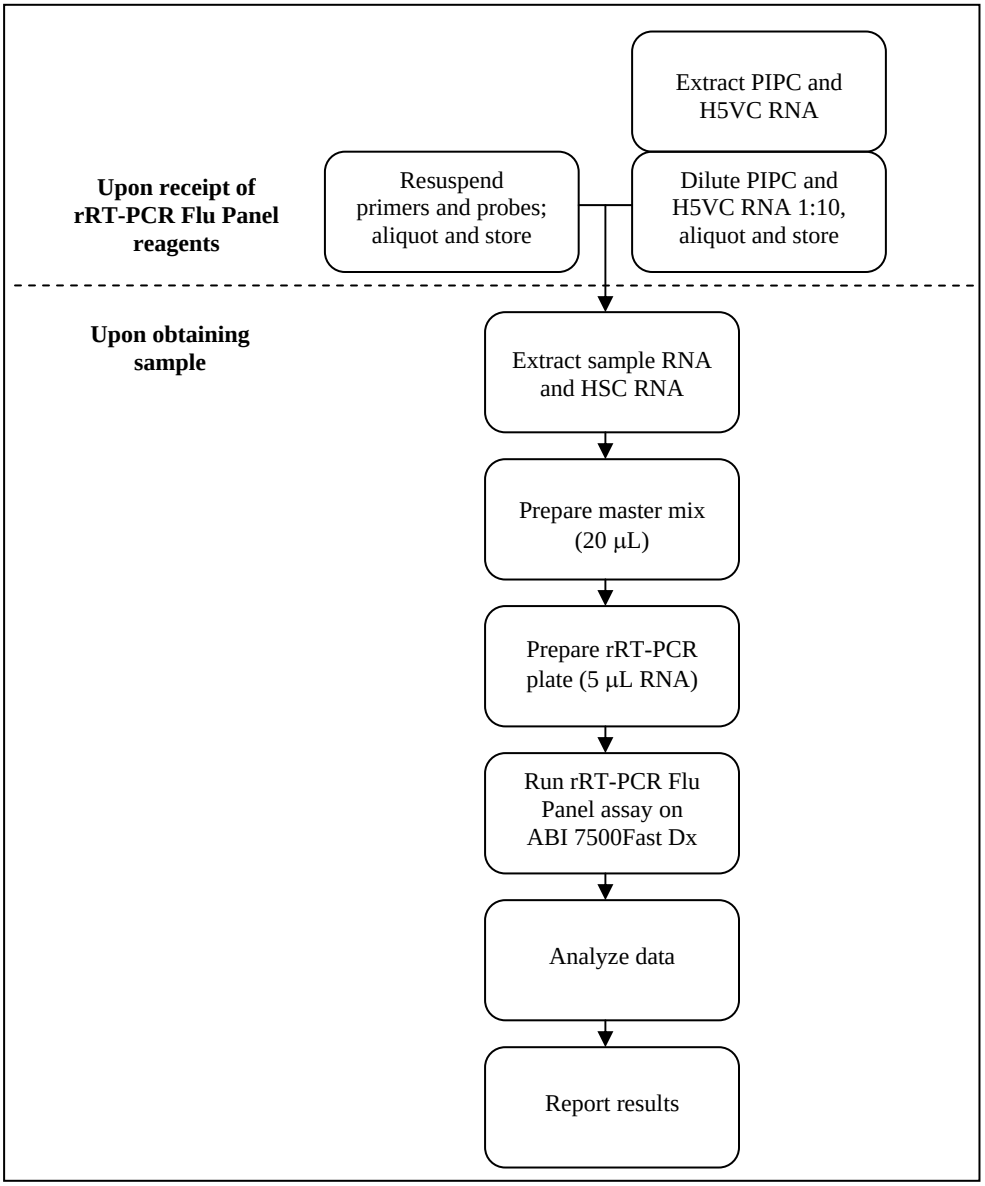
- Guidance for Industry and FDA Staff - Class II Special Controls Guidance Document: Reagents for Detection of Specific Novel Influenza A Viruses, March 22, 2006 *http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm078583.htm*
- Guidance for Industry and FDA Staff, *In Vitro* Diagnostic Devices to Detect Influenza A Viruses: Labeling and Regulatory Path, May 1, 2007 *http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm078538.htm*
- Guidance for Industry and FDA Staff - Class II Special Controls Guidance Document: Testing for Detection and Differentiation of Influenza A Virus Subtypes Using Multiplex Assays October 9, 2009 *http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm180307.htm*
- Guidance for Industry and FDA Staff - Establishing Performance Characteristics of *In Vitro* Diagnostic Devices for Detection or Detection and Differentiation of Influenza Viruses, July 15, 2011 *http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm079171.htm*

L. Test Principle:

The CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel is a panel of oligonucleotide primers and dual-labeled hydrolysis (TaqMan®) probes which may be used in rRT-PCR assays for the *in vitro* qualitative detection and characterization

of the human influenza virus RNA in the respiratory tract. The reagents included in Panel consist of primers, probes, and positive control materials. The primer and probe sets are designed for the detection of influenza type A and B viruses that infect humans and characterization of influenza A viruses as seasonal A/H1, seasonal A/H3, A/H1pdm09, or A/H5. The oligonucleotide primers and probes for detection of Influenza A, 2009 Influenza A (swine origin), and B viruses were selected from highly conserved regions of the matrix (M), nucleoprotein (NP), and non-structural (NS) genes, respectively. Oligonucleotide primers and probes for characterization and differentiation of seasonal influenza A/H1, A/H3, A/H1pdm09, and A/H5 viruses were selected from highly conserved regions of their respective hemagglutinin (HA) genes.

Summary of Influenza testing process:



Interpretation of Results

Extraction and Positive Control 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.

Pooled Influenza Positive Control (PIPC)

The PIPC consists of four different influenza viruses representing influenza A/H1N1, A/H3N2, A/H1pdm09, and influenza B viruses suspended in cultured human cells (A549). Purified RNA from the PIPC will yield a positive result with the following primer and probe sets: InfA, InfB, H1, H3, pdmInfA, pdmH1, and RP.

H5 Virus Control (H5VC)

The H5VC control consists of a reassortant human vaccine candidate virus (A/Vietnam/1203/04 x PR/8/34) that was generated by reverse genetics and cultured human cell (A549) material. Purified RNA from the H5VC will yield a positive result with the following primer and probe sets: InfA, H5a, H5b, and RP.

Human Specimen Control (HSC) (Extraction Control)

The HSC control consists of noninfectious cultured human cell (A549) material. The HSC is used as a 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.

Specimen 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 InfB or InfA along with H1, H3, pdm InfA, or pdm H1 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, InfB, H1, H3, pdmInfA, pdmH1, H5a, and H5b)

➢ When all controls exhibit the expected performance, a specimen is considered negative if all influenza marker (InfA, InfB, H1, H3, pdmInfA, pdmH1, H5a, and H5b) 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 the influenza marker (InfA, InfB, H1, H3, pdmInfA and pdmH1) 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, InfB, H1, H3, pdmInfA, pdmH1, H5a, and H5b) **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 any subtype markers indicating detection (InfA positive without H1, H3, pdmInfA, pdmH1 subtypes detected), the sample has 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: Do not test this sample using the Influenza A/H5 Subtyping Assay unless the patient meets the current WHO epidemiological risk factors.

- When all controls exhibit expected performance and growth curves for InfA, H5a AND H5b markers cross the threshold line within 38.00 cycles (< 38.00 Ct):
    - Report the specimen to be "Presumptive Positive for Influenza A/H5 Virus" and contact the CDC Influenza Division immediately to coordinate transfer of the specimen to CDC for additional testing.
- When all controls exhibit the expected performance and growth curves for InfA and EITHER H5a OR H5b markers cross the threshold line within 38.00 cycles (< 38.00 Ct), extracted RNA from the specimen should be re-tested immediately. If residual RNA is not available, re-extract RNA from residual specimen and re-test. If all controls exhibit the expected performance on the repeated test and either H5a **OR** H5b reactions cross the threshold line within 38.00 cycles (< 38.00 Ct):
    - Report the specimen to be "Inconclusive for Influenza A/H5 virus" and contact the CDC Influenza Division immediately to coordinate transfer of the specimen to CDC for additional testing.

CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel Results Interpretation Guide

The tables below list the expected results for the assays contained within this device. If results are obtained that do not follow these guidelines, re-extract and re-test the sample. If repeat testing yields similar results, contact CDC Technical Support for consultation and possible specimen referral as some results may indicate novel or emerging influenza viruses.

Influenza A/B Typing Assay

InfA	InfB	RP	Result Interpretation[a]	Report for CDC Surveillance	Notes and Special Guidance
+	-	±	Influenza A Detected; Subtyping not performed	Influenza A	
-	+	±	Influenza B Detected	Influenza B	
-	-	+	Influenza NOT Detected	Not Detected	
+	+	±	Report: Influenza A Detected and Influenza B Detected; Refer to CDC for further characterization.	Inconclusive	If original result, re-extract and re-test. If repeat, report as possible co-infection. Specimen should be referred to CDC for further characterization; possible co-infection or LAIV detection
-	-	-	Inconclusive	Inconclusive	If original result, re-extract and re-test. If repeat, report as Inconclusive. Obtain new specimen if possible.

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

While this device can be used as a test for influenza A and B only, CDC strongly recommends that laboratories subtype all the influenza A positives with the Influenza A Subtyping Assay. The CDC recommends maintaining the enhanced surveillance efforts by state and local health departments, hospitals, and clinicians to identify patients that may be transmitting a possible novel or newly emerging influenza virus.

Influenza A Subtyping Assay

InfA	H1	H3	pdm InfA	pdmH1	RP	Result Interpretation[a]	Report for CDC Surveillance	Notes and Special Guidance
+	+	-	-	-	±	Influenza A detected Subtype: H1 detected	Influenza A(H1)	
+	-	+	-	-	±	Influenza A detected Subtype: H3 detected	Influenza A(H3)	
+	-	-	+	+	±	Influenza A and pdm09 A detected Subtype: 2009 H1 detected	Influenza A 2009 H1N1	If one, but not both of the pdm markers is positive, re-extract and test. If same result is obtained, report as Inconclusive and refer to the CDC guidance on referral specimens
-	-	-	-	-	+	Influenza A not detected	Not Detected	
-	-	-	-	-	-	Inconclusive Result	Inconclusive	Re-extract specimen and test. If results are similar, report inconclusive
-	If any of these markers are positive and InfA is negative				±	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.

CDC Guidance for Public Health Follow-Up Actions Regarding Non-Standard Results with Influenza Subtyping

If a laboratory encounters subtyping results that do not coincide with the typical expected results of the Influenza A Subtyping Assay listed in the table above, please refer to the most current guidance from CDC on the criteria for referring specimens and samples to CDC for laboratory confirmation found at the following link: http://www.aphl.org/memberresources/Documents/MR_ID_InfluenzaSpecimenReferralChart.pdf

If the result of the Influenza A Subtyping assay indicates an influenza A unsubtypeable **AND** the patient does not meet the WHO epidemiological risk factors for influenza A/H5, the Laboratory Director should contact CDC (flusupport@cdc.gov) or the CDC Influenza Epidemiology Division **IMMEDIATELY** after generating a repeated result. This result may be indicative of a novel or newly emerging influenza virus and any delay in contacting CDC may result in a critical loss of response time.

Non-Standard Results with the Influenza A Subtyping Assay (After Re-Testing)

InfA	H1	H3	pdm InfA	pdmH1	RP	Result Interpretation	Report for CDC Surveillance	Notes and Special Guidance
+	-	+	+	-	±	Influenza A and pdm09 A detected Subtype: H3 detected	Inconclusive	Possible swine origin triple reassortant; refer specimen to CDC for confirmation
+	+	-	+	-	±	Influenza A and pdm09 A detected Subtype: H1 detected	Inconclusive	Possible swine origin triple reassortant; refer specimen to CDC for confirmation
+	+	+	-	-	±	Influenza A detected Subtype: H1 detected; H3 detected	Inconclusive	Possible co-infection detected; possible LAIV detection; refer specimen to CDC for confirmation
+	-	+	+	+	±	Influenza A detected Subtype: H3 detected; 2009 H1 detected	Inconclusive	Possible co-infection detected; possible LAIV detection; refer specimen to CDC for confirmation
+	-	-	-	-	+	Influenza A detected Subtype undetectable	Inconclusive	Possible 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 A/H5 Subtyping Assay

InfA	H5a	H5b	RP	Result Interpretation	Report for CDC Surveillance	Notes and Special Guidance
+	+	+	±	Influenza A Detected Presumptive positive for Influenza A/H5 virus	Notify CDC	Contact CDC immediately for instructions for coordination of transferring the specimen

+	+	-	±	Influenza A Detected Inconclusive for Influenza A/H5 virus	Re-test; Notify CDC	to CDC for additional testing and further guidance.
+	-	+	±	Influenza A Detected Inconclusive for Influenza A/H5 virus	Re-test; Notify CDC	

Standards-Based Electronic Laboratory Reporting for Influenza

Background

This section contains the recommendations for uniform coding and vocabulary for CDC Human Influenza Virus Real-time RT-PCR Diagnostic Panel. The following information is provided to assist the performing laboratory in complying with new federal guidelines for the meaningful use of electronic health information systems. The implementation of adopted standards should be harmonized across all performing laboratories to ensure semantic interoperability to better support electronic data exchange.

The CDC developer of this assay through collaboration has established Standard English terminology for the test name and test results with the testing community and expert knowledge of the processes involved. It is recognized that this terminology will differ in countries outside the United States. However, through the use of national and international agreements, it is possible to establish a universal set of codes and terms to accurately characterize laboratory observations. Recommendations in this package insert apply to the reporting of results of this assay only within the United States.

Process for achieving uniformity in laboratory test results

The laboratory performing the influenza assay may utilize a Laboratory Information Management Systems (LIMS) with connections to a hospital or medical system Electronic Health Record (EHR). The coding systems include LOINC - Logical Observation Identifiers Names and Codes (LOINC® -- http://www.loinc.org) and SNOMED CT – Systematic Nomenclature of Medicine--Clinical Terms (http://www.ihtsdo.org/). These coding systems have specific capabilities that are essential for achieving uniformity. The test request and results are to be incorporated into a standard Health Level 7 (HL7) electronic format for laboratory test messaging. More information about HL7 can be found at http://www.hl7.org.

LOINC provides for a common understanding of the medical procedure or process related to the specific assay, in this case the process of detecting the presence of influenza virus and the potential sub-typing of the detected influenza virus. The LOINC codes specified here describe the important information about the methodology employed by the assay; recovery and amplification of one or more RNA targets. Multiple LOINC codes are utilized to convey that the assay is composed of multiple components, i.e. it is a panel or a battery of subtests. In the case of the CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel, the LOINC also provides for conveying that an interpretive test summary is appropriate.

SNOMED CT codes provide for unambiguous representation of the test results and allow the application of specific concepts such as “detected” or “positive” or the identification

of detected organism names. Though not further defined in this document, SNOMED CT can also be used to provide for description of the type and source/ location of the specimen being tested or for conveying information about failures of the test procedure or the lack of adequate specimen.

Specific Recommendations for Standards-Based Electronic Data Exchange for Influenza

Laboratories can find more information regarding implementation of HL7 messaging for CDC FLU rRT-PCR Dx Panel, including applicable LOINC test codes and SNOMED result codes at http://www.cdc.gov/flu/professionals/diagnosis/rtpcr-test-kits.htm

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Please refer to previously FDA-cleared, 510(k) Premarket Notifications, K080570 and K101564

b. Linearity/assay reportable range:

Not applicable.

c. Traceability, Stability, Expected values (controls, calibrators, or methods):

There are no changes to the internal positive control, the human RNase P; Human Specimen Control (HSC); Influenza virus A/H5N1 Positive Control (H5VC) and No Template Control (NTC) for the New Assay. Please refer to previously FDA-cleared 510(k) Premarket Notifications, K080570 and K101564 for further information.

A new positive control has been added in the new device, Pooled Influenza Positive Control (PIPC). It 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. PIPC consists of four different beta-propiolactone inactivated influenza viruses (influenza A/H1, A/H3, A/H1pdm09, and influenza B) suspended in cultured human cells (A549). PIPC will yield a positive result with the following primer and probe sets: InfA, InfB, H1, H3, pdm InfA, pdm H1, and RP.

An internal validation study was performed to confirm that it is acceptable to add A/H1N1pdm09 virus to the existing Seasonal Influenza Virus Control (SIVC) to create a new single control material for use with the CDC rRT-PCR Flu Panels. A solution containing a 1:1 mixture of pdmPC (pdm positive control) and SIVC was assembled and tested in triplicate against InfA, InfB, H1, H3, RP, pdm InfA, and pdm H1 markers using procedures described in the CDC rRT-PCR Flu Panel

(IVD) package insert. The test was performed three times. A difference of less than 3 Cts was observed with each of the applicable subtype markers between the pdmPC, SIVC and the pooled mixture.

		pdmPC		SIVC		Pooled pmcPC & SIVC	
		Ave. Ct	SD	Ave. Ct	SD	Ave. Ct	SD
Marker	InfA	28.46	0.34	27.58	0.33	27.77	0.33
	InfB	nd		33.02	0.87	33.92	0.69
	Seasonal H1	nd		34.62	0.52	35.27	0.43
	Seasonal H3	nd		31.48	0.40	33.45	0.71
	pdmInfA	29.03	1.18	nd		29.83	0.33
	pdmH1	31.20	0.25	nd		32.18	0.34
	RP	32.44	0.55	29.21	0.30	30.15	0.38

d. Detection limits:

Please refer to previously FDA-cleared 510(k) Premarket Notifications, K080570 and K101564.

e. Analytical specificity:

Please refer to previously FDA-cleared 510(k) Premarket Notifications, K080570 and K101564.

f. Assay cut-off:

Remains unchanged, please refer to previously FDA-cleared 510(k) Premarket Notifications, K080570 and K101564.

g. Additional specimen types:

Eighteen original lower respiratory specimens were received from U.S. public health laboratories during the 2010-2011 influenza season from hospitalized patients or fatal cases. The CDC rRT-PCR Flu Panel and the CDC rRT-PCR 2009A(H1N1)pdm Flu Panel detected influenza A/H3, A/H1pdm09, and influenza B in bronchoalveolar lavage, bronchial washes, tracheal aspirates, sputum, and lung tissue specimens, verifying detection of influenza viruses in the lower respiratory tract.

In addition, 49 original specimens from U.S. public health laboratories from the 2010-2011 season were tested by CDC rRT-PCR flu Panel. They were 24 Influenza A/H3 (49%) and 25 Influenza B (51%).

2. Comparison studies:

a. Method comparison with gold standard/reference method:

Not Applicable, performance of the assay was evaluated in comparison to the gold standard/reference method, viral culture followed by IFA or DFA and/or sequencing

b. Matrix Comparison:

Not Applicable

3. Clinical studies:

Please refer to previously FDA-cleared, 510(k) Premarket Notifications, K080570 and K101564

In brief, the performance characteristics below are aggregated and combined from the CDC Human Influenza Virus Real-Time RT-PCR Detection (K080570) and Characterization Panel and the CDC Influenza 2009 A (H1N1)pdm Real-Time RT-PCR Panel (K101564). The clinical performance data for 2007-2008 season was a part of CDC EUA submission.

2006 – 2007 Study
Performance characteristics of the CDC Human Influenza Virus Real-Time RT-PCR Detection and Characterization Panel were established during a prospective study at 4 U.S. state public health laboratories during the 2006-2007 respiratory virus season (February-April). Samples used for this study were nasal and nasopharyngeal swabs collected for routine influenza testing at each site.

The reference method was rapid culture (shell vial) followed by direct fluorescent antibody screening and identification. Specimens tested at the public health testing sites followed routine diagnostic influenza rapid culture protocols and the rRT-PCR Flu Panel protocol to demonstrate performance.

A total of 415 specimens were collected and tested at the four public health laboratory testing sites during the 2006-2007 influenza season for this prospective clinical study. A total of 439 specimen results (415 prospective seasonal and 24 retrospective AH5 influenza samples) from nasal, nasopharyngeal swabs, or grown culture in the case of influenza AH5 samples were tested using gold standard virus culture. Influenza AH5 and discrepant prospective seasonal influenza samples were further analyzed by bi-directional sequencing. Please refer to 510(k) Premarket Notification K080570 for additional information.

2007 – 2008 Study
Performance characteristics of the rRT-PCR Flu Panel were demonstrated during a prospective study at four U.S. state public health laboratories during the 2007-

2008 respiratory virus season (February-April). Specimens tested in this study included nasal swabs, nasopharyngeal swabs, dual nasopharyngeal / throat swabs, nasal aspirates and throat swabs collected for routine influenza testing from patients presenting with ILI at each site.

The reference method was rapid culture (shell vial) followed by direct fluorescent antibody screening and identification. Specimens tested at the public health testing sites followed routine diagnostic influenza rapid culture protocols and the rRT-PCR Flu Panel protocol to demonstrate performance and agreement.

A total of 323 dual nasopharyngeal / throat swabs, nasal aspirates, isolates, and throat swabs specimens were collected and tested. A total of 313 results were valid according to the instructions for interpretation within the IVD package insert.

Clinical Sensitivity and Specificity Performance Summary for the CDC rRT-PCR Flu Panel;
2007 – 2008 Season

Analyte Tested	Specimen Type	Sensitivity % (95% CI)	Specificity % (95% CI)
InfA	TS	100.0% (81.6%-100.0%)	98.3% (91.1%-99.7%)
	NA	100.0% (51.0%-100.0%)	100% (72.2%-100.0%)
	NPS/TS	100.0% (56.6%-100.0%)	100.0% (88.6%-100.0%)
	Isolates	100.0% (72.2%-100.0%)	62.1% (54.7%-68.9%)
InfB	TS	90.0% (69.9%-97.2%)	56.1% (43.3%-68.2%)
	NA	100.0% (64.6%-100.0%)	100.0% (64.6%-100.0%)
	NPS/TS	100.0% (56.6%-100.0%)	100.0% (88.6%-100.0%)
	Isolates	95.0% (76.4%-99.1%)	61.6% (54.0%-68.7%)
H1	TS	100.0 (34.2%-100.0%)	97.3% (90.8%-99.3%)
	NA	100% (34.2%-100.0%)	100.0% (75.7%-100.0%)
	NPS/TS	100.0% (64.6%-100.0%)	89.3% (72.8%-96.3%)
	Isolates	100% (70.1%-100.0%)	93.7% (89.1%-96.5%)

H3	TS	93.8% (71.7%-98.9%)	91.8% (82.2%-96.4%)
	NA	100.0% (20.7%-100.0%)	92.3% (66.7%-98.6%)
	NPS/TS	85.7% (48.7%-97.4%)	92.9% (77.4%-98.0%)
	Isolates	100.0% (20.7%-100.0%)	84.2% (78.2%-88.7%)

2009 – 2010 Study

Performance characteristics of the CDC rRT-PCR 2009 A(H1N1)pdm Flu Panel were established during a prospective study at eight U.S. public health laboratories and a Department of Defense (DoD) laboratory during the 2009-2010 respiratory virus season (February-April). A total of 1901 patient specimens collected for routine influenza testing were evaluated at the nine clinical sites: 1191 nasopharyngeal swabs (NPS) and nasal swabs (NS), 50 throat swabs (TS), 519 nasal washes (NW) and nasal aspirates (NA), 99 dual nasopharyngeal swabs / throat swabs (NPS/TS), and 42 lower respiratory specimens. Due to low prevalence of influenza, retrospective specimens were used to supplement prospectively collected specimens.

The reference methods utilized in this study were virus culture followed by Immunofluorescent Antibody (IFA) or Direct Fluorescent Antibody (DFA) for identification of influenza type and bi-directional sequencing for confirmation of 2009 influenza A (H1N1) subtype. InfA analyte results from the CDC rRT-PCR 2009 A(H1N1)pdm Flu Panel were compared to viral culture results in the analysis. Sequencing was performed only with specimens that were first identified as positive for influenza A by virus culture. Pdm InfA and pdm H1 analyte results from the CDC rRT-PCR 2009 A(H1N1)pdm Flu Panel were compared to bi-directional sequencing results in the analysis. Please refer to 510(k) Premarket Notification K101564 for additional information.

4. Clinical cut-off**:** N/A

5. Expected values/Reference range:

   The expected values are derived from the clinical studies performed during the 2006-2007 (K080570), 2009-2010 (K101564).

   From August 30, 2009, through March 27, 2010, World Health Organization (WHO) and National Respiratory and Enteric Virus Surveillance System (NREVSS) collaborating laboratories in the United States tested 422,648 specimens. Of these, 89,585 (21.1%) were positive: 89,298 (99.7%) were positive for influenza A and 287 (0.3%) were positive for influenza B. Among 66,978 influenza A viruses for which subtyping was performed, almost all (66,589 [99.4%]) were 2009 HIN1 viruses.

During October 1, 2006--May 19, 2007, World Health Organization and National Respiratory and Enteric Virus Surveillance System (NREVSS) collaborating laboratories in the United States tested 179,268 respiratory specimens for influenza viruses; 23,753 (13.2%) were positive. Of these, 18,817 (79.2%) were influenza A viruses and 4,936 (20.8%) were influenza B viruses. Among the influenza A viruses, 6,280 (33.4%) were subtyped; 3,912 (62.3%) were influenza A/H1 viruses and 2,368 (37.7%) were influenza A/H3 viruses (http://www.cdc.gov/mmwr/preview/mmwrhtmlml/mm5631a2.htm). In the rRT-PCR Flu Panel multi-center prospective clinical study during the 2006-2007 influenza season, the prevalence as determined by virus culture was as follows: influenza A/H1 (7.0%), influenza A/H3 (23.6%), and influenza B (9.9%).

N. Instrument Name:

Applied Biosystems (ABI) 7500 Fast Dx Real-Time PCR

O. System Descriptions:

1. Modes of Operation:

   The Applied Biosystems 7500 Fast Dx Real-Time PCR instrument integrates a thermal cycler, a fluorimeter and application specific software. The instrument houses the thermal cycler and the fluorimeter, while the application software is run on a PC that is attached to the instrument. Samples are placed in a tube strip or 96-well low-head space plate that is moved to a Peltier-based thermal block and positioned relative to the optics using a tray loading mechanism.

2. Software:

   Sequence Detection Software version 1.4. FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:

   Yes ____X____ or No _____

3. Specimen Identification:

   User manually enters Patient ID/Sample ID.

4. Specimen Sampling and Handling:

   Not applicable

5. Calibration:

   Not applicable

6. Quality Control:

   Quality control is addressed for each specific assay to be run on the instrument (separately cleared).

P. Other Supportive Instrument Performance Characteristics Data Not Covered In the "Performance Characteristics" Section above:

Not applicable

Q. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

R. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.