

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY**

A. 510(k) Number:

K080570

B. Purpose for Submission:

New device

C. Measurand:

Influenza virus nucleic acids target sequences. Influenza types and subtypes detected: Influenza A, Influenza A/H1, Influenza A/H3, Influenza A/H5, and Influenza B

D. Type of Test:

A panel of oligonucleotide primers and dual-labeled hydrolysis (TaqMan®) probes for the qualitative detection and differentiation of influenza virus type and subtype target sequences in nasal and nasopharyngeal swabs using nucleic acid isolation, amplification, and detection on the ABI 7500 Fast Dx Real-Time PCR instrument.

E. Applicant:

Centers for Disease Control and Prevention

F. Proprietary and Established Names:

CDC Human Influenza Virus Real-time RT-PCR Detection and Characterization Panel.

Common Name: rRT-PCR Flu Panel

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
NXD, OEP, OCC, NSU	Class II	21 CFR 866.3332 Reagents for detection of specific novel influenza A viruses	Microbiology (83)

H. Intended Use:

1. Intended use(s):

The Human Influenza Virus Real-time RT-PCR Detection and Characterization Panel (rRT-PCR Flu 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/H1 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.

Performance characteristics for influenza A were established when influenza A/H3 and A/H1 were the predominant influenza A viruses in circulation. When other influenza A viruses are emerging, performance characteristics may vary.

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.

All users, analysts, and any person reporting diagnostic results from use of this device should be trained to perform and interpret the results from this procedure by a CDC instructor or designee prior to use. 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.

2. Indication(s) for use:
Same as Intended Use
3. Special conditions for use statement(s):
For prescription use only
4. Special instrument requirements:
ABI 7500 Fast Dx Real-Time PCR instrument

I. Device Description:

The CDC Human Influenza Virus Real-time RT-PCR Detection and Characterization Panel (rRT-PCR Flu Panel) is a panel of oligonucleotide primers and dual-labeled hydrolysis (TaqMan[®]) probes designed to be utilized in real-time RT-PCR assays using the ABI 7500 Fast Dx Real-Time PCR instrument for detection and characterization of human influenza viral RNA. The rRT-PCR Flu Panel influenza A and B primer and probe sets target highly conserved regions of the matrix (M) and non-structural (NS) genes for universal detection of type A and type B influenza viruses. Influenza A subtyping primer and probe sets target highly conserved regions

of subtype-specific hemagglutinin (HA) RNA to detect contemporary A/H1, A/H3, and A/H5 (Asian lineage) influenza viruses in humans.

The rRT-PCR Flu Panel also includes control materials.

- Internal positive control, the human RNase P (RP) primer and probe set detects human RP and is used with each clinical specimen to indicate that adequate isolation of nucleic acid resulted from the extraction of the specimen. A positive RP assay result also ensures that common reagents and equipment are functioning properly, and demonstrates the absence of inhibitory substances.
- A Human Specimen Control (HSC) is a noninfectious cultured human cell material that demonstrates successful recovery of RNA as well as extraction reagent integrity.
- The Seasonal Influenza Virus Control (SIVC) consists of three different influenza viruses representing influenza A/H1, A/H3, and influenza B viruses and cultured human cells. The SIVC demonstrates that the master mix and primer and probe sets for influenza A (InfA), influenza B (InfB), influenza A/H1 (H1), influenza A/H3 (H3), and RP are functioning properly.
- The Influenza Virus A/H5N1 Positive Control (H5VC) consists of a genetically modified reassortant human influenza virus (Influenza A/Vietnam/1203/04 x PR/8/34, BSL2 category) and cultured human cells. The H5VC demonstrates that the master mix and primer and probe sets for InfA, influenza A/H5 (H5a, H5b), and RP are functioning properly.

The use of the CDC rRT-PCR Flu Panel requires **ancillary reagents** for which specific lots have been qualified by the CDC Influenza Division and incorporated in the CDC quality system. Any lots not specifically qualified by CDC for use with the rRT-PCR Flu Panel are not validated for use with this assay and may affect device performance. Lots which are qualified under this process are communicated through a Qualified Reagent Lot List. This list is maintained by CDC and made available to the end users of the CDC rRT-PCR Flu Panel by including it with the Product Insert as well as through the Product Support mechanism identified in the Product Insert.

The following is a list of ancillary reagents that are not supplied with the rRT-PCR Flu Panel and are included in CDC’s reagent qualification program:

	Reagent	Quantity	Catalog No.
rRT-PCR Enzyme Master mix 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
Nucleic Acid	Qiagen QIAamp® Viral RNA Mini Kit *	50 extractions	52904

Purification Kit Options	(Qiagen Inc., Valencia, CA)	250 extractions	52906
	Qiagen RNeasy® Mini Kit * (Qiagen Inc., Valencia, CA)	50 extractions	74104
		250 extractions	74106
	Roche MagNA Pure Total Nucleic Acid Kit * (Roche Applied Science, Indianapolis, IN)	192 extractions	03038505001
	Roche MagNA Pure LC RNA Isolation Kit II * (Roche Applied Science, Indianapolis, IN)	192 extractions	03172627001

J. Substantial Equivalence Information:

- Predicate device name(s):
Influenza A/H5 (Asian lineage) Virus Real-time RT-PCR Primer and Probe Set and the Prodesse Multiplex RT-PCR ProFlu™ Plus Assay
- Predicate K number(s):
K060159
K073029
- Comparison with predicate:

Similarities			
Item	Device	Predicate 1	Predicate 2
	rRT-PCR Flu Panel	Influenza A/H5 (Asian lineage) Virus Real-time RT-PCR Primers and Probe Set (K060159)	Prodesse Multiplex RT-PCR ProFlu+™ Assay (K073029)
Specimen Types	Nasopharyngeal or nasal swab respiratory specimens, or virus culture	Human respiratory specimens or virus cultures	Nasopharyngeal swab specimens
Technology	Real-time RT-PCR	Real-time RT-PCR	Real-time RT-PCR
Extraction Method	<ul style="list-style-type: none"> • QIAamp® Viral RNA Mini Kit, Qiagen Inc. • Qiagen RNeasy® Mini Kit, Qiagen, Inc. • MagNA Pure LC RNA Isolation Kit II, Roche Applied Science • MagNA Pure Total Nucleic Acid Isolation Kit, Roche Applied Science 	<ul style="list-style-type: none"> • QIAamp® Viral RNA Mini Kit, Qiagen, Inc. • RNeasy® Mini Kit, Qiagen, Inc. • MagNA Pure LC Total Nucleic Acid Isolation Kit, Roche Applied Science 	MagNA Pure Total Nucleic Acid Isolation Kit, Roche Applied Science

Differences			
Item	Device	Predicate 1	Predicate 2
	rRT-PCR Flu Panel	Influenza A/H5 (Asian lineage) Virus Real-time RT-PCR Primers and Probe Set (K060159)	Prodesse Multiplex RT-PCR ProFlu+™ Assay (K073029)
Intended Use	Qualitative <i>in-vitro</i> detection of influenza A/H1, A/H3, A/H5 (Asian lineage), and influenza B viruses	Qualitative <i>in vitro</i> detection of influenza A/H5N1 (Asian lineage) virus	Detection/differentiation between influenza A virus, influenza B virus and respiratory syncytial virus (RSV)
Organism Detected	Influenza A/H1, A/H3, A/H5 (Asian lineage), and influenza B viruses	Influenza A virus, subtype H5N1 (Asian lineage)	Influenza type A and type B viruses, RSV
Enzyme Master Mix	Invitrogen SuperScript™ III Platinum® One-Step Quantitative RT-PCR Kits (with or without ROX)	Qiagen QuantiTect™ Probe RT-PCR Kit, Qiagen, Inc.	Supplied in the ProFlu+™ Detection Kit
Required Instrumentation	Applied Biosystems 7500 Fast Dx Real-Time PCR Instrument with SDS software version 1.4	<ul style="list-style-type: none"> •Roche LightCycler® •Cepheid SmartCycler® •Applied Biosystems 7000 Sequence Detection System •Applied Biosystems Prism® 7700 Sequence Detection System 	Cepheid SmartCycler® II instrument

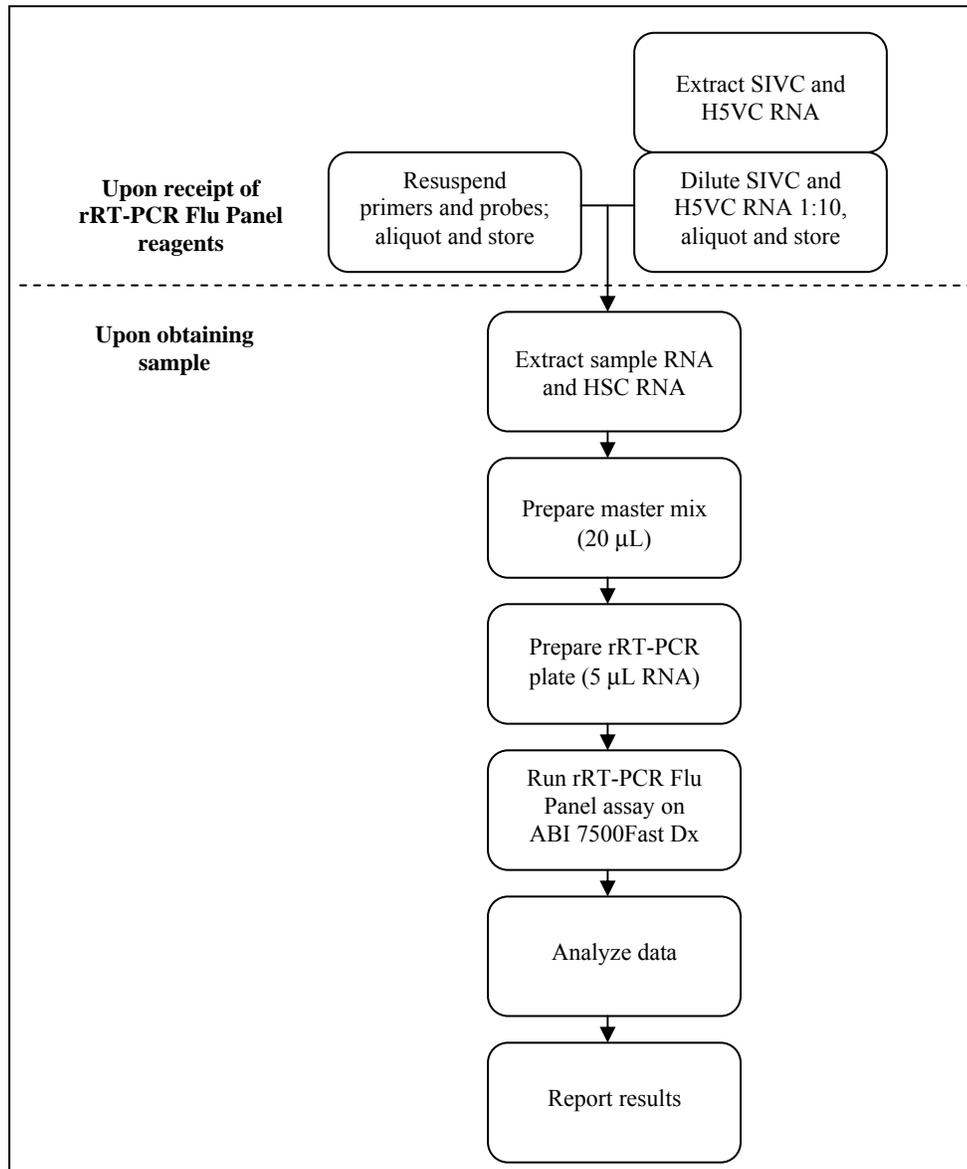
K. Standard/Guidance Documents Referenced (if applicable):

- Class II Special Controls Guidance Document: Reagents for Detection of Specific Novel Influenza A Viruses - <http://www.fda.gov/cdrh/oivd/guidance/1596.pdf>.
- Guidance on In Vitro Diagnostic Devices to Detect Influenza A Viruses: Labeling and Regulatory Path - <http://www.fda.gov/cdrh/oivd/guidance/1594.pdf>.
- Draft Guidance for Industry and FDA Staff: Establishing the Performance Characteristics of In Vitro Diagnostic Devices for the Detection or Detection and Differentiation of Influenza Viruses - <http://www.fda.gov/cdrh/oivd/guidance/1638.pdf>

L. Test Principle:

The rRT-PCR Flu Panel includes a set of oligonucleotide primers and dual-labeled hydrolysis (Taqman®) probes to be used in real-time RT-PCR assays on an ABI 7500 Fast Dx Real-Time PCR instrument. The targeted regions of viral RNA are transcribed into complimentary DNA (cDNA) and amplified by the polymerase chain reaction (PCR). The fluorescently labeled probes anneal to amplified DNA fragments and the fluorescent signal intensity is monitored by the ABI 7500 Fast Dx instrument during each PCR cycle. Amplification of target is recorded as increase of fluorescence over time in comparison to background signal.

Summary of Steps in rRT-PCR Flu Panel Assay:



Interpretation of Results:

Expected Performance of Controls Included in the rRT-PCR Flu Panel:

Control Type	Internal Control Name	Used to Monitor	InfA	InfB	H1	H3	RP	H5a	H5b	Expected Ct Values
Positive	SIVC	Substantial reagent failure including primer and probe integrity	+	+	+	+	+	-	-	≤37 Ct
Positive	H5VC	Substantial reagent failure including primer and probe integrity	+	-	-	-	+	+	+	≤37 Ct
Negative	NTC	Reagent and/or environmental contamination	-	-	-	-	-	-	-	None detected
Extraction	HSC	Failure in lysis and extraction procedure	-	-	-	-	+	-	-	≤37 Ct

If the assay 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.

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 37 cycles, 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, InfB, and/or any subtype reactions are positive even in the presence of a negative 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 assay is “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, H5a, H5b)

When all controls exhibit the expected performance, a specimen is considered negative if influenza marker growth curves DO NOT cross the threshold line within 37 cycles and RNase P growth curve does cross the threshold line within 37 cycles.

When all controls exhibit the expected performance, a specimen is considered a “Presumptive Positive” for influenza if the influenza marker (InfA, InfB, H1, H3, H5a, and/or H5b) growth curve crosses the threshold line within 37 cycles. RP may be positive or negative as described above.

When all controls exhibit the expected performance and the growth curve for influenza A marker only crosses the threshold line within 37 cycles without any subtype detection (InfA - Influenza A positive without a subtype 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. If residual RNA is not available, re-extract RNA from residual specimen and re-test. If the re-tested sample is again positive for InfA only and all controls exhibit the expected performance, contact the CDC Influenza Division to coordinate transfer of the specimen to CDC for confirmatory testing.

When all controls exhibit the expected performance and none of the growth curves for the influenza markers or RP marker cross the threshold line within 37 cycles, 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 expected performance and growth curves for both H5a and H5b markers cross the threshold line within 37 cycles:

- Report the specimen to be “Presumptive Positive for Influenza A/H5 (Asian lineage) 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 either H5a and/or H5b markers cross the threshold line within 37 cycles, 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 37 cycles:

- Report the specimen to be “Inconclusive for Influenza A/H5 (Asian lineage) 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 any of the following occurs, the test is “Invalid”:

- growth curves for influenza A marker and influenza B marker cross the threshold line within 37 cycles, OR
- growth curves for influenza A marker and two or more of the influenza A subtype markers cross the threshold line within 37 cycles, OR
- growth curves for any of the influenza A subtype markers (H1, H3, H5a, H5b) cross the threshold line within 37 cycles but the growth curve for the influenza A type marker (InfA) **does not** cross the threshold line within 37 cycles.

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 repeat testing results remain the same as the original results, report the specimen as “Invalid.”

- If the InfA, InfB, and/or any subtype reactions are positive and RP is negative, the influenza result should be considered valid.
- If a combination of results other than those in the chart is generated, the result is invalid. Repeat extraction and retest the sample. If repeat testing result is unchanged, report as “Invalid” result.

To refer the specimen to the CDC, the following shipping instructions should be followed:

- Ship all specimens and related RNA overnight to CDC.
- Ship frozen specimens on dry ice and non-frozen specimens on cold packs. Ship extracted RNA on dry ice.
- Refer to the International Air Transport Association (IATA - www.iata.org) for requirements for shipment of human or potentially infectious biological specimens.
- Prior to shipping, notify the CDC Influenza Division (see contact information below) that you are sending specimens.
- Send all samples to the following recipient:
Chief, Virus Surveillance and Diagnosis Branch Influenza Division
Centers for Disease Control and Prevention
c/o DASH, MS G-16
Attention: Dr. Stephen Lindstrom
1600 Clifton Rd., Atlanta, GA 30333
Phone: (404) 639-3387 or (404) 639-3591
Fax: (404) 639-2334

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

The rRT-PCR Flu Panel reproducibility and precision studies were performed to 1) evaluate reproducibility of the assay at three laboratory sites using the ABI 7500 Fast Dx Real-Time PCR instruments and SDS software version 1.4 and 2) to compare the assay performance on the original ABI 7500 Fast Real-Time PCR instrument and the ABI 7500 Fast Dx Real-Time PCR instrument (bridging study).

1) The ABI 7500 Fast Dx Real-Time PCR instrument functionality was validated at each site by AB prior to the reproducibility assessment using a panel of nine (9) simulated samples including influenza A/H1N1, A/H3N2, A/H5N1 (reassortant), and Influenza B at two viral RNA concentrations each (a low viral RNA concentration and a 1:10 dilution of the same sample). The low viral RNA concentration generally was one log above the assay cutoff for all analytes, whereas the 1:10 dilution of the same sample approximated a sample at the assay cutoff. Simulated samples in the panel used in the reproducibility evaluation were:

- **Sample #1** Influenza A/H1N1 at a low viral RNA titer range
- **Sample #2** Influenza A/H1N1 at a 1:10 dilution of sample #1
- **Sample #3** Influenza A/H3N2 at a low viral RNA titer range
- **Sample #4** Influenza A/H3N2 at a 1:10 dilution of sample #3
- **Sample #5** Influenza A/H5N1 WT at a low viral RNA titer range
- **Sample #6** Influenza A/H5N1 WT at a 1:10 dilution of sample #5
- **Sample #7** Influenza B Yamagata at a low viral RNA titer range
- **Sample #8** Influenza B Yamagata at a 1:10 dilution of sample #7
- **Sample #9** Influenza Negative (uninfected A549 cells)

The panels and assay controls were tested at each site by two operators on five (5) different days within a 10-day period. Each participating clinical site tested one of the four following RNA purification methods to evaluate reproducibility of the CDC rRT-PCR Flu Panel on the validated ABI 7500 Fast Dx Real-Time PCR instruments: the automated MagNA Pure LC RNA Isolation Kit III (Roche Applied Science), the manual QIAGEN RNeasy[®] RNA extraction (QIAGEN Ltd.), the Roche MagNA Pure LC Automated Isolation System with Total RNA isolation method, and the QIAGEN QIAamp[®] Viral RNA manual extraction method. The manufacturer's instructions for use provided in the package insert were followed. Results generated for each of the extraction methods are summarized in the Table below.

**Reproducibility Study Summary for the CDC rRT-PCR Flu Panel on the ABI 7500
Fast Dx Real-Time PCR Instrument and SDS version 1.4 Software.**

Sample and Analyte Tested	Roche MagNA Pure TNA			Qiagen QIAamp [®] RNA			Qiagen RNeasy [®]			Roche MagNA Pure RNA			Total Agreement w/ Expected Results	95 % CI
	Agreement w/ expected Result	Avg. Ct	% CV	Agreement w/ expected Result	Avg. Ct	% CV	Agreement w/ expected Result	Avg. Ct	% CV	Agreement w/ expected Result	Avg. Ct	% CV		
Sample 1 (low) InfA	10/10	33.73	3.12	10/10	32.24	5.65	10/10	34.34	1.94	10/10	31.55	3.69	40/40	91.2–100.0
Sample 1 (low) H1	10/10	34.48	2.84	10/10	33.78	5.64	10/10	36.67	4.47	10/10	34.08	4.11	40/40	91.2–100.0
Sample 1 (low) RNaseP	10/10	28.62	4.00	10/10	30.08	5.63	10/10	27.67	3.28	10/10	28.22	3.13	40/40	91.2–100.0
Sample 2 (1:10 of Sample 1) InfA	9/10	36.59	3.22	10/10	35.30	5.93	10/10	37.03	4.07	10/10	33.97	3.32	39/40	86.8 - 99.9
Sample 2 (1:10 of Sample 1) H1	6/10	37.92	2.99	9/10	37.76	8.31	7/10	38.28	4.28	9/10	37.21	4.77	31/40	61.6 - 89.2
Sample 2 (1:10 of Sample 1) RNaseP	10/10	29.51	3.80	10/10	31.13	9.58	10/10	28.46	2.47	10/10	28.65	2.12	40/40	91.2–100.0
Sample 3 (low) InfA	10/10	35.39	3.74	10/10	33.32	7.09	10/10	34.01	7.16	10/10	32.29	1.82	40/40	91.2–100.0
Sample 3 (low) H3	9/10	36.25	2.52	10/10	34.98	7.60	10/10	34.07	2.86	10/10	33.28	1.71	39/40	86.8 - 99.9
Sample 3 (low) RNaseP	10/10	29.17	5.32	10/10	30.02	5.33	10/10	28.30	3.05	10/10	28.50	3.34	40/40	91.2–100.0
Sample 4 (1:10 of Sample 3) InfA	7/10	38.44	4.05	10/10	37.03	5.08	5/10	39.05	3.46	10/10	36.18	2.41	32/40	64.4 - 91.0
Sample 4 (1:10 of Sample 3) H3	6/10	38.82	3.34	8/10	39.90	6.14	6/10	38.01	4.81	9/10	36.82	3.49	29/40	56.1 – 85.4
Sample 4 (1:10 of Sample 3) RNaseP	10/10	29.75	4.32	10/10	31.11	7.89	10/10	28.56	4.60	10/10	28.68	3.35	40/40	91.2–100.0
Sample 5 (low) InfA	10/10	33.68	2.70	10/10	31.90	6.66	10/10	33.07	3.14	10/10	30.80	1.74	40/40	91.2–100.0
Sample 5 (low) H5a	10/10	35.69	3.76	10/10	33.65	5.52	10/10	34.58	4.94	10/10	33.03	4.55	40/40	91.2–100.0
Sample 5 (low) H5b	9/10	37.91	3.58	10/10	36.27	9.56	10/10	36.09	4.49	10/10	34.54	3.11	39/40	86.8 - 99.9
Sample 5 (low) RNaseP	10/10	29.44	4.05	10/10	30.16	7.18	10/10	27.97	4.84	10/10	28.46	2.78	40/40	91.2–100.0
Sample 6 (1:10 of Sample 5) InfA	8/10	36.90	6.60	10/10	34.84	4.89	8/10	37.10	5.15	10/10	33.54	2.61	36/40	76.3 – 97.2
Sample 6 (1:10 of Sample 5) H5a	5/10	37.90	3.24	9/10	36.5	4.91	7/10	37.77	7.35	10/10	35.71	2.51	31/40	61.6 – 89.2
Sample 6 (1:10 of Sample 5) H5b	4/10	39.29	ND*	5/10	39.43	3.74	7/10	38.74	3.35	10/10	35.71	5.30	27/40	50.9 – 81.4
Sample 6 (1:10 of Sample 5) RNaseP	10/10	30.05	3.58	10/10	30.12	3.39	10/10	28.59	4.37	10/10	28.66	3.45	40/40	91.2–100.0

Sample 7 (low) Inf B	8/10	34.10	4.25	10/10	33.49	9.12	10/10	34.70	7.70	10/10	32.83	3.61	38/40	83.1 – 99.4				
Sample 7 (low) RNaseP	10/10	29.78	3.55	10/10	30.75	10.16	10/10	28.54	4.42	10/10	28.90	3.13	40/40	91.2–100.0				
Sample 8 (1:10 of Sample 7) Inf B	1/10	36.76	ND*	8/10	37.1	5.77	3/10	38.95	ND*	8/10	37.11	5.81	20/40	33.8 – 66.2				
Sample 8 (1:10 of Sample 7) RNaseP	10/10	29.48	4.46	10/10	30.58	6.30	10/10	28.71	3.35	10/10	28.92	4.07	40/40	91.2–100.0				
Sample 9 Influenza (-) RNaseP	10/10	29.79	3.17	10/10	30.08	5.91	10/10	28.27	4.83	10/10	28.86	3.68	40/40	91.2–100.0				
													213 / 250 85.2 %	319 / 330 96.7 %	303 / 330 91.8 %	326 / 330 98.8 %	1161/1320 88.0 %	86.41–89.6

2) Assay performance, when run on the original ABI 7500 Fast Real-Time PCR instrument and the ABI 7500 Fast Dx Real-Time PCR instrument, was compared utilizing an identical study protocol to that described in paragraph 1) above and using simulated samples for influenza A/H1N1, A/H3N2, A/H5N1 (reassortant), and Influenza B at a low viral RNA concentration, (generally one log above the assay cutoff). Although several panel members displayed, on average, statistically significant higher Ct values in the reproducibility study on the ABI 7500 Fast Dx Real-Time PCR instrument as compared to the original reproducibility study run on the ABI 7500 Fast Real-Time PCR instrument, all values were within clinically acceptable levels. The difference could be attributed to different operators and panel reagent lot-to-lot variability.

b. Linearity/assay reportable range:

Not applicable, qualitative assay

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

Before analyzing the samples prepared using the CDC rRT-PCR Flu Panel on the ABI 7500 Fast Dx Real-Time PCR instrument, the ABI instrument must be prepared following the procedures described in the Package Insert for the CDC Human Influenza Virus Real-time RT-PCR Detection and Characterization Panel and the ABI 7500 Fast Dx Real-Time PCR instrument User Manual.

Assay Controls

Quality Control requirements must be performed in conformance with local, state, and/or federal regulations or accreditation requirements and a laboratory's standard quality control procedures. It is recommended that the user refer to CLSI document C24-A2.

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 stricter adherence to the guidelines.

Seasonal Influenza Virus Control (SIVC)

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

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

A negative amplification/detection control (NTC) and a positive control (SIVC and/or H5VC) should be included in each run. An HSC extraction control must proceed through nucleic acid isolation for each batch of specimens to be tested.

d. Detection limit:

The Limit of Detection (LoD) was determined for each primer and probe set in the rRT-PCR Flu Panel. Ten-fold serial dilutions of two different influenza virus strains of each subtype were tested to identify an end-point for detection. RNA was extracted from each of the characterized viruses with the Qiagen QIAamp[®] Viral RNA Purification kit. The LoD for each primer and probe set (InfA, InfB, H1, H3, H5a, and H5b) was calculated to determine the lowest detectable concentration range of influenza virus (EID₅₀/mL) at which approximately 95% of all replicates tested positive. The lowest concentration of influenza virus detected was determined to be the end-point concentration where the type and subtype primer and probe sets had uniform detection. If the two end-points differed in concentration, the higher (or limiting) point was used.

Limit of Detection Summary

Influenza Virus Tested	Influenza Strain Designation	Limit of Detection (EID ₅₀ /mL)
A/H1N1	A/New Caledonia/20/1999	10 ^{1.2}
	A/Hawaii/15/2001	10 ^{1.5}
A/H3N2	A/New York/55/2004	10 ^{2.2}
	A/Wisconsin/67/2005	10 ^{1.2}
A/H5N1	A/Vietnam/1203/2004×A/Puerto Rico/8/34 reassortant (A/Vietnam/1203/2004 PR8-VNH5N1-PR8/CDC-RG)	10 ^{1.0}
	A/Anhui/01/2005×A/Puerto Rico/8/34 reassortant (A/Anhui/01/2005- PR8-IBCDC-RG5)	10 ^{1.0}
B	B/Florida/07/2004 (B/Victoria/2/87 genetic group)	10 ⁰
	B/Ohio/01/2005 (B/Yamagata/16/88 genetic group)	10 ^{0.5}

e. Analytical specificity:

Analytical specificity of rRT-PCR Flu Panel was evaluated with respect to 1) reactivity (inclusivity) with a number of geographically diverse influenza virus strains and 2) potential cross-reactivity with non-influenza pathogens associated with respiratory tract infections.

1) Reactivity/Inclusivity

Ten (10) influenza virus strains of A/H1N1, A/H3N2, and Influenza B were tested at low virus concentrations at or near the limit of detection (10² TCID₅₀/mL) to demonstrate the flexibility of the primer and probe sets to detect multiple strains of influenza virus. There were 26 influenza A/H5N1 strains tested retrospectively from diverse geographic locations from suspect positive cases received at CDC. The rRT-PCR Flu Panel analytical specificity indicated 100% concordance with expected results for all primer and probe sets included in the device.

Analytical Reactivity Testing Results of the rRT-PCR Flu Panel with Human Seasonal Influenza Viruses A/H1, A/H3, and Influenza B.

	Strain Identification	Stock TCID ₅₀ / mL	rRT-PCR Flu Panel Analyte Tested at 10 ² TCID ₅₀ /mL - (Avg Ct n=3)						Result
			InfA	InfB	H1	H3	H5a	H5b	
Influenza A/H1N1	A/JIANGXI/160/2005	10 ^{5.6}	+	-	+	-	-	-	AH1
	A/Solomon Island/03/2006	10 ^{6.2}	+	-	+	-	-	-	AH1
	A/Taiwan/42/2006	10 ^{4.7}	+	-	+	-	-	-	AH1

	A/Fukushima /141/2006	10 ^{5.7}	+	-	+	-	-	-	AH1
	A/Mexico/1744/2007	10 ^{5.3}	+	-	+	-	-	-	AH1
	A/Mexico/1729/2007	10 ^{4.8}	+	-	+	-	-	-	AH1
	A/Mexico/1677/2007	10 ^{5.7}	+	-	+	-	-	-	AH1
	A/Mexico/949/2007	10 ^{5.1}	+	-	+	-	-	-	AH1
	A/Bangladesh/286/2007	10 ^{6.1}	+	-	+	-	-	-	AH1
	A/Mexico/2010/2007	10 ^{5.1}	+	-	+	-	-	-	AH1
Influenza A/H3N2	A/Hawaii/08/2006	10 ^{7.8}	+	-	-	+	-	-	AH3
	A/Wisconsin/03/2007	10 ^{8.2}	+	-	-	+	-	-	AH3
	A/Henan/Jinshui/147/2007	10 ^{8.1}	+	-	-	+	-	-	AH3
	A/Brisbane/10/2007	10 ^{6.8}	+	-	-	+	-	-	AH3
	A/Mexico/922/2007	10 ^{7.8}	+	-	-	+	-	-	AH3
	A/Afghanistan/2903/2008	10 ^{5.0}	+	-	-	+	-	-	AH3
	A/Mexico/1938/2007	10 ^{5.1}	+	-	-	+	-	-	AH3
	A/Mexico/1995/2007	10 ^{4.3}	+	-	-	+	-	-	AH3
	A/Anhui/1239/2005	10 ^{8.1}	+	-	-	+	-	-	AH3
	A/Mexico/1842/2007	10 ^{6.1}	+	-	-	+	-	-	AH3
	Influenza B	B/Florida/04/2006	10 ^{5.7}	-	+	-	-	-	-
B/Chongqing/Yongchuan/18/2007		10 ^{7.7}	-	+	-	-	-	-	Inf. B
B/Florida/02/2006		10 ^{6.0}	-	+	-	-	-	-	Inf. B
B/Pennsylvania/05/2007		10 ^{6.6}	-	+	-	-	-	-	Inf. B
B/Bangladesh/5972/2007		10 ^{4.7}	-	+	-	-	-	-	Inf. B
B/Bangladesh/3461/2007		10 ^{3.7}	-	+	-	-	-	-	Inf. B
B/Bangladesh/7110/2007		10 ^{4.2}	-	+	-	-	-	-	Inf. B
B/Mexico/1819/2007		10 ^{5.1}	-	+	-	-	-	-	Inf. B
B/Texas/17/2007		10 ^{5.7}	-	+	-	-	-	-	Inf. B
B/Texas/03/2007		10 ^{5.6}	-	+	-	-	-	-	Inf. B

Analytical Reactivity Testing Results of the rRT-PCR Flu Panel with Influenz A/H5N1Virus

Strain Identification	Species	Specimen Type	Country of Origin	Clade	rRT-PCR Flu Panel Analyte Tested						Expected Result
					InfA	InfB	H1	H3	H5a	H5b	
CDC ref #2008706688	Human	egg grown	Egypt	2.2.1	+	-	-	-	+	+	InfA, H5a, H5b
CDC ref #2008706690	Human	egg grown	Egypt	2.2.1	+	-	-	-	+	+	InfA, H5a, H5b
CDC ref #2008706691	Human	egg grown	Egypt	2.2.1	+	-	-	-	+	+	InfA, H5a, H5b
CDC ref #2008706692	Human	egg grown	Egypt	2.2.1	+	-	-	-	+	+	InfA, H5a, H5b
CDC ref #2008706693	Human	egg grown	Egypt	2.2.1	+	-	-	-	+	+	InfA, H5a, H5b
CDC ref #2008706694	Human	egg grown	Egypt	2.2.1	+	-	-	-	+	+	InfA, H5a, H5b
CDC ref #2008706695	Human	egg grown	Egypt	2.2.1	+	-	-	-	+	+	InfA, H5a, H5b
CDC ref #2008706696	Human	egg grown	Egypt	2.2.1	+	-	-	-	+	+	InfA, H5a, H5b
CDC ref #2008706697	Human	egg grown	Egypt	2.2.1	+	-	-	-	+	+	InfA, H5a, H5b
CDC ref #2008706698	Human	egg grown	Egypt	2.2.1	+	-	-	-	+	+	InfA, H5a, H5b
CDC ref #2008706699	Human	egg grown	Egypt	2.2.1	+	-	-	-	+	+	InfA, H5a, H5b
CDC ref #2007726139	Human	throat swab	Egypt	2.2.1	+	-	-	-	+	+	InfA, H5a, H5b
CDC ref #2007726140	Human	throat swab	Egypt	2.2.1	+	-	-	-	+	+	InfA, H5a, H5b
CDC ref #2008706700	Human	egg grown	Pakistan	2.2	+	-	-	-	+	+	InfA, H5a, H5b
CDC ref #200732012	Human	tissue culture	Vietnam	2.3	+	-	-	-	+	+	InfA, H5a, H5b
CDC ref #200732013	Human	tissue culture	Vietnam	2.3	+	-	-	-	+	+	InfA, H5a, H5b
CDC ref #200732014	Human	tissue culture	Vietnam	2.3	+	-	-	-	+	+	InfA, H5a, H5b
CDC ref #2007730165	Human	throat swab	Vietnam	2.3	+	-	-	-	+	+	InfA, H5a, H5b
CDC ref #2007730166	Human	tracheal aspirate	Vietnam	2.3	+	-	-	-	+	+	InfA, H5a, H5b
CDC ref #2007730167	Human	throat swab	Vietnam	2.3	+	-	-	-	+	+	InfA, H5a, H5b
CDC ref #2008740525	Human	tissue culture	Vietnam	2.3	+	-	-	-	+	+	InfA, H5a, H5b
CDC ref #2008740526	Human	tissue culture	Vietnam	2.3	+	-	-	-	+	+	InfA, H5a, H5b
CDC ref #2008740527	Human	tissue culture	Vietnam	2.3	+	-	-	-	+	+	InfA, H5a, H5b
CDC ref #2008740528	Human	tissue culture	Vietnam	2.3	+	-	-	-	+	+	InfA, H5a, H5b

*2 samples were tested with InfA and H5a primer/probe sets only due to low sample volume. Both samples were positive for InfA and H5a, but were not included in the table above (CDC ref#2007700735 – China, and ref#2007700734 – China)

Cross-Reactivity Evaluation

Analytical specificity (cross-reactivity) was evaluated by testing each primer/probe set within the device panel with nucleic acids extracted from 27 organisms (9 viruses, 17 bacteria, and 1 yeast) representing common respiratory pathogens or flora commonly present in specimens collected from the nasopharynx region. All the organisms were propagated, titered, and characterized to confirm identity prior to testing. The identity of the commensal respiratory bacteria was confirmed by 16S ribosomal RNA bi-directional sequencing. The identity of the non-influenza respiratory viruses was confirmed by bi-directional sequencing. Bacteria and yeast were tested at concentrations greater than or equal to 10^6 cfu/mL. Non-influenza respiratory viruses were tested at concentrations greater than 10^6 TCID₅₀/mL with the exception of human parainfluenza type 2 which was tested at $10^{3.1}$ TCID₅₀/mL due to difficulty generating a high titer virus stock in culture and coronaviruses CoV 229E and CoV OC43 (31.6 ng/μl and 50.4 ng/μl, respectively, of total RNA from viral culture).

Each sample (bacteria and virus) was extracted in parallel using both the Qiagen QIAamp[®] Viral RNA Mini Kit and the Qiagen RNeasy[®] Mini Kit. Each RNA sample was tested following the testing procedure previously described for the rRT-PCR Flu Panel and the Applied Biosystems 7500 Fast Real-Time PCR System. The dual extraction method was performed to demonstrate cross method equivalency and ensure no cross reactivity with the influenza primer and probe sets since negative results were expected for all samples.

Analytical Specificity (Cross-reactivity) Test Results with Common Non-influenza Human Respiratory Pathogens

Organism Tested		Referenced Primer & Probe Set Positive (+) or Negative (-) for Reactivity							
Common Respiratory Flora	Strain	cfu / ml	InfA set	Inf B set	H1 set	H3 set	H5a set	H5b set	Result
<i>Bordetella pertussis</i>	A639	$10^{8.3}$	-	-	-	-	-	-	Neg.
<i>Candida albicans</i> (yeast)	2001-21-196	$10^{8.8}$	-	-	-	-	-	-	Neg.
<i>Corynebacterium diphtheriae</i>	NA	10^{10}	-	-	-	-	-	-	Neg.
<i>Escherichia coli</i>	K12	$10^{9.6}$	-	-	-	-	-	-	Neg.
<i>Haemophilus influenzae</i>	M15709	$10^{6.4}$	-	-	-	-	-	-	Neg.
<i>Lactobacillus plantarium</i>	NA	$10^{8.8}$	-	-	-	-	-	-	Neg.
<i>Legionella pneumophila</i>	NA	$10^{10.3}$	-	-	-	-	-	-	Neg.
<i>Moraxella cararrhalis</i>	M15757	$10^{9.5}$	-	-	-	-	-	-	Neg.
<i>Mycobacterium tuberculosis</i> ¹	BCG	0.25 ng /μl	-	-	-	-	-	-	Neg.
<i>Mycoplasma pneumoniae</i>	MI-29	$10^{7.7}$	-	-	-	-	-	-	Neg.
<i>Neisseria elongata</i>	NA	$10^{8.6}$	-	-	-	-	-	-	Neg.
<i>Neisseria meningitidis</i>	M2578	$10^{7.9}$	-	-	-	-	-	-	Neg.
<i>Pseudomonas aeruginosa</i>	NA	$10^{10.5}$	-	-	-	-	-	-	Neg.
<i>Staphylococcus epidermis</i>	NA	$10^{10.5}$	-	-	-	-	-	-	Neg.
<i>Staphylococcus aureus</i>	NA	$10^{10.7}$	-	-	-	-	-	-	Neg.

<i>Streptococcus pneumoniae</i>	249-06 (Thailand)	10 ^{6.6}	-	-	-	-	-	-	-	Neg.
<i>Streptococcus pyrogenes</i>	7790-06	10 ^{7.5}	-	-	-	-	-	-	-	Neg.
<i>Streptococcus salivarius</i>	SS1672	10 ^{8.4}	-	-	-	-	-	-	-	Neg.
Common Non-Influenza Respiratory Viruses	Strain	TCID₅₀ / ml	InfA set	Inf B set	H1 set	H3 set	H5a set	H5b set	Result	
Enterovirus	Echo 6	10 ^{6.9}	-	-	-	-	-	-	Neg.	
Human Adenovirus, type 1	Ad.71	10 ^{9.2}	-	-	-	-	-	-	Neg.	
Human Adenovirus, type 7a	S-1058	10 ^{7.1}	-	-	-	-	-	-	Neg.	
Human Coronavirus virus ¹	OC43	50.4 ng /µl	-	-	-	-	-	-	Neg.	
Human Coronavirus virus ¹	299E	31.6 ng /µl	-	-	-	-	-	-	Neg.	
Human Rhinovirus A	1A	10 ^{5.8}	-	-	-	-	-	-	Neg.	
Human Parainfluenza 2 virus	Greer	10 ^{3.1}	-	-	-	-	-	-	Neg.	
Human Parainfluenza 3 virus	C-243	10 ^{7.9}	-	-	-	-	-	-	Neg.	
Respiratory Syncytial virus	CH93-18b	10 ^{6.8}	-	-	-	-	-	-	Neg.	

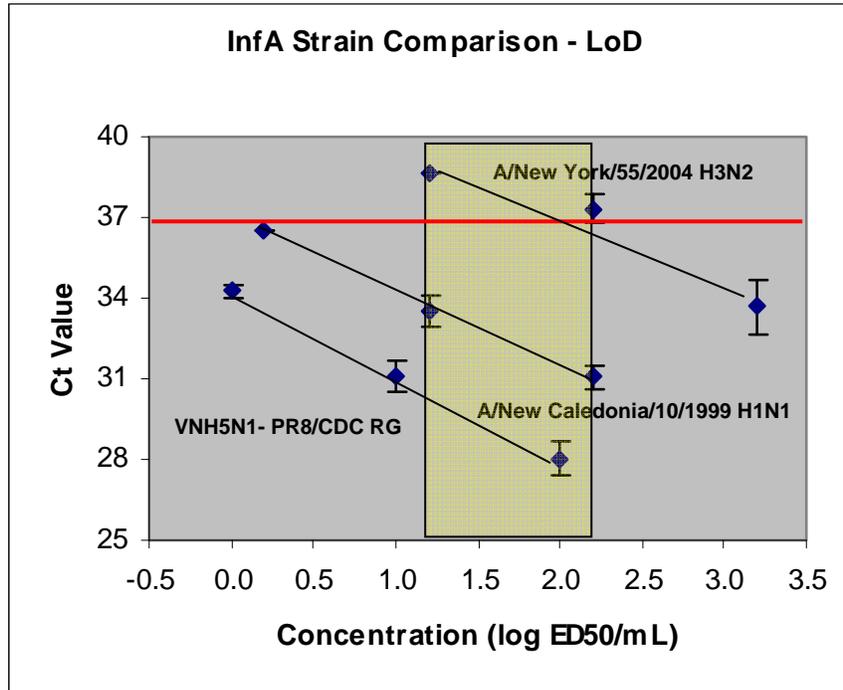
¹Organism quantitated by spectrophotometry (ng/µl)

The study demonstrated that the primer and probe sets contained within the rRT-PCR Flu Panel did not cross-react with any of the non-influenza respiratory pathogens or commensal organisms tested. The data demonstrated 100% concordance with the expected results.

f. Assay cut-off:

To assess the rRT-PCR Flu Panel assay cut-off, the data from the limit of detection study was analyzed. Data were plotted as virus concentration (log TCID₅₀) versus cycle threshold (Ct) value at or below the limits of detection (in yellow) for each marker (e.g. Figure 18-1). The average Ct value at the lower end of the range of the limit of detection plus two standards of deviation afforded the Ct value cut-off for each of the markers in the rRT-PCR Flu Panel assay as shown by the red line (InfA = 37; InfB = 35; H1 = 35; H3 = 36; H5a = 35; H5b = 35). Particularly in the case of the InfA target (Figure 18-1); marked differences in detection between subtypes of influenza A are apparent. As the assay is designed for both seasonal and pandemic use, it is vital to detect any and all strains of influenza as the assay may detect novel influenza A strains. Therefore, the cut-off Ct value for the rRT-PCR assay was set at 37.

Figure 18-1. Assay Cut-Off Plot for the InfA Primer and Probe Set when Compared to Three Influenza A Virus Subtypes.



2. Comparison studies:

a. *Method comparison with predicate device:*

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

b. *Matrix comparison:*

Not applicable

3. Clinical studies:

Performance characteristics of the rRT-PCR Flu Panel were established during a prospective study at 4 U.S. state public health laboratories during the 2006-2007 respiratory virus season (February-April). A total of 439 (415 prospective seasonal specimens collected for routine influenza testing and 24 retrospective AH5 influenza samples) from nasal and nasopharyngeal swabs, or grown in culture in the case of influenza AH5 samples were used for this study.

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 of the assay. Influenza AH5 and discrepant prospective seasonal influenza samples were further analyzed

by bi-directional sequencing.

a and b. Clinical Sensitivity and Specificity:

Influenza A Comparison Results

		Virus Culture “Gold Standard” Results			
		Influenza A Positive	Influenza A Negative	Total	Performance
rRT_PCR Flu Panel	Influenza A Positive	142	21 ^a	163	99.3 % Sensitivity (96.1%-99.9%) 95% CI
	Influenza A Negative	1	251	252	92.3 % Specificity (88.5%-94.9%) 95% CI
	Total	143	272	415	

^a 16 of the 21 samples that were influenza A positive by rRT-PCR and influenza A negative by virus culture were confirmed influenza A positive using bi-directional sequence analysis.

Influenza H1 Comparison Results

		Virus Culture “Gold Standard” Results			
		Influenza A H1 Positive	Influenza A H1 Negative	Total	Performance
rRT_PCR Flu Panel	Influenza A H1 Positive	27	2 ^a	29	93.1 % Sensitivity (78.0%-98.1%) 95% CI
	Influenza A H1 Negative	2	384	386	99.5 % Specificity (98.1%-99.9%) 95% CI
	Total	29	386	415	

^a 1 of the 2 samples that were H1 positive by rRT-PCR and H1 negative by virus culture was confirmed H1 positive using bi-directional sequence analysis.

Influenza H3 Comparison Results

		Virus Culture “Gold Standard” Results			
		Influenza A H3 Positive	Influenza A H3 Negative	Total	Performance
rRT_PCR Flu Panel	Influenza A H3 Positive	98	30 ^a	128	100 % Sensitivity (96.2%-100%) 95% CI
	Influenza A H3 Negative	0	287	287	90.5 % Specificity (86.7%-93.3%) 95% CI
	Total	98	317	415	

^a 28 of the 30 samples that were H3 positive by rRT-PCR and H3 negative by virus culture were confirmed H3 positive using bi-directional sequence analysis.

Influenza B Comparison Results

		Virus Culture “Gold Standard” Results			
		Influenza B Positive	Influenza B Negative	Total	Performance
rRT-PCR Flu Panel	Influenza B Positive	40	7 ^a	47	97.6 % Sensitivity (87.4%-99.6%) 95% CI
	Influenza B Negative	1	367	368	98.1 % Specificity (96.2%-99.1%) 95% CI
	Total	41	374	415	

^a 6 of the 7 samples that were influenza B positive by rRT-PCR and influenza B negative by virus culture were confirmed influenza B positive using bi-directional sequence analysis.

Clinical Sensitivity and Specificity Performance Summary - Prospective Specimen Testing

Analyte Tested	Sensitivity % (95% CI)	Specificity % (95% CI)
InfA	99.3 (96.1 - 99.9)	92.3 (88.5 - 94.9)
InfB	97.6 (87.4 - 99.6)	98.1 (96.2 – 99.1)
H1	93.1 (78.0 - 98.1)	99.5 (98.1 - 99.9)
H3	100.0 (96.2 - 100.0)	90.5 (86.7 - 93.3)

Due to the absence of suspect cases within the United States, a formal prospective clinical evaluation of human and/or avian influenza A/H5N1 virus was not accomplished during the CDC Clinical Evaluation Study. Therefore, the performance specificity and detection capability of the H5a and H5b primer and probe sets in the rRT-PCR Flu Panel could not be evaluated at the U.S. state public health laboratories during the clinical study. Retrospective data from twenty-four (24) suspect A/H5N1 positive specimens from human cases received by the CDC Influenza Division were used to demonstrate clinical performance of the H5a and H5b components of the panel.

**Retrospective Influenza A/H5N1 Data – Culture and Clinical Influenza Samples
Combined H5a and H5b Analytes**

		Reference Method Results			
		Influenza AH5 Positive	Influenza AH5 Negative	Total	Performance
rRT_PCR Flu Panel Results	Influenza H5a & H5b Positive Clinical Specimen	5 ^a	0	5	100 % Percent Positive Agreement (56.6% -100%) 95% CI
	Influenza H5a & H5b Positive Cultured Specimen	19 ^a	0	19	100 % Percent Positive Agreement (83.2% -100%) 95% CI
	Prospective Clinical Specimen	0	415	415	100 % Percent Negative Agreement (99.1%-100%) 95% CI
	Total	24	415	439	

^a Five clinical specimens and 19 specimens tested positive for influenza AH5 directly from the specimen source in the country of origin prior to receipt were included in the study. CDC Influenza Division tested grown virus (culture) from the country of origin and then propagated virus in culture for further confirmatory testing.

Performance Summary - Influenza A/H5N1 Testing with Prospective and Retrospective Specimens

	Analyte Tested	Percent Positive Agreement (%)
Influenza A/H5 Positive Clinical Specimens	H5a / H5b	100 % Percent Positive Agreement (56.6% -100%) 95% CI
Influenza A/H5 Positive Cultured Specimens	H5a / H5b	100 % Percent Positive Agreement (83.2% -100%) 95% CI
Prospective Clinical Specimens	H5a / H5b	100 % Percent Negative Agreement (99.1%-100%) 95% CI

4. Clinical cut-off:

Not applicable

5. Expected values/Reference range:

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%). No dual infections were found in this study. It is recommended that any sample that tests positive for more than one influenza virus be re-tested.

N. Proposed Labeling:

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

O. Conclusion:

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