510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

K092882

B. Purpose for Submission:

This is a new 510(k) application for the Diagnostic Hybrids, Inc. device, D^3 FastPoint L-DFA Influenza A/Influenza B Virus Identification Kit, which is intended for the qualitative identification of influenza A virus and influenza B virus in nasal and nasopharyngeal swabs and aspirates/washes specimens from patients with signs and symptoms of respiratory infection by direct detection of immunofluorescence using monoclonal antibodies (MAbs).

C. Measurand:

Respiratory viral antigens (Influenza A and Influenza B)

D. Type of Test:

Direct Fluorescence Antibody (DFA) test using direct specimens

E. Applicant:

Diagnostic Hybrids Inc.

F. Proprietary and Established Names:

D^3 FastPoint L-DFA Influenza A/Influenza B Virus Identification Kit

G. Regulatory Information:

1. Regulation section:

   866.3330

2. Classification:

   Class I

3. Product codes:

   GNX

4. Panel:

   Microbiology (83)

H. Intended Use:

1. Intended use(s):

   The Diagnostic Hybrids, Inc. device, D^3 FastPoint L-DFA Influenza A/Influenza B Virus Identification Kit is intended for the qualitative identification of influenza A virus and influenza B virus in nasal and nasopharyngeal swabs and aspirates/washes specimens from patients with signs and symptoms of respiratory infection by direct detection of immunofluorescence using monoclonal antibodies (MAbs).

   It is recommended that specimens found to be negative for influenza A or influenza B virus after examination of the direct specimen result be confirmed by cell culture. Negative results do not preclude influenza A or influenza B virus infection and should not be used as the sole basis for diagnosis, treatment or other management decisions.

   Performance characteristics for influenza A virus detection and identification were established when influenza A (H3N2) and influenza A (H1N1) were the predominant influenza A strains circulating in the United States. Since influenza strains display antigenic drift and shift from year to year, performance characteristics may vary. If infection with a novel influenza A virus is suspected, based on clinical and epidemiological screening criteria communicated by public health authorities, collect specimens following appropriate infection control precautions and submit to state or local health departments, for testing. Viral culture should not be attempted in these cases unless a BSL 3+ facility[1] is available to receive and culture specimens.[2]

2. Indication(s) for use:

   Same as intended use.

3. Special conditions for use statement(s):

   For prescription use only.

4. Special instrument requirements:

   Fluorescence microscope with the appropriate filter combination for FITC (excitation peak = 490 nm, emission peak = 520 nm) and for R-PE; magnification 200-400X.

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[1] www.cdc.gov

[2] FDA Guidance Document: In Vitro Diagnostic Devices to Detect Influenza A Viruses: Labeling and Regulatory Path; Issued 4/10/2006

I. Device Description:

The D^3 FastPoint L-DFA Influenza A/Influenza B Virus Identification Kit uses a blend (called a "L-DFA Reagent") of viral antigen-specific murine monoclonal antibodies that are directly labeled with either R-PE (influenza A virus) or fluorescein (influenza B virus) for the rapid identification of influenza A virus and influenza B virus in nasal and nasopharyngeal swabs and aspirates/washes from patients with signs and symptoms of respiratory infection.

Kit Components:

1. **D^3 FastPoint L-DFA Influenza A/Influenza B Reagent**, 4.0-mL. One dropper bottle containing a mixture of PE-labeled murine monoclonal antibodies directed against influenza A virus antigens and FITC-labeled murine monoclonal antibodies directed against influenza B virus antigens. The buffered, stabilized, aqueous solution contains Evans Blue and propidium iodide as counter-stains and 0.1% sodium azide as preservative.
2. **40X PBS Concentrate,** 25-mL. One bottle of 40X PBS concentrate containing 4% sodium azide (0.1% sodium azide after dilution to 1X using de-mineralized water).
3. **Re-suspension Buffer,** 6.0-mL. One bottle of a buffered glycerol solution and 0.1% sodium azide.
4. **D^3 FastPoint L-DFA Influenza A/Influenza B Antigen Control Slides**, 5-slides. Five individually packaged control slides containing 2 wells with cell culture-derived positive and negative control cells. Each positive well contains cells infected with either influenza A virus, or influenza B virus. The negative wells contain non-infected cells. Each slide is intended to be stained only one time.

An overview of the procedure is as follows:

The cells to be tested are derived from respiratory specimens from patients with signs and symptoms of respiratory infection. The cells are permeablized and stained concurrently in a liquid suspension format with the L-DFA Reagent. After incubating at 35ºC to 37ºC for 5 minutes, the stained cell suspensions are rinsed with PBS. The rinsed cells are pelleted by centrifugation and then re-suspended with the re-suspension buffer and loaded onto a specimen slide well. The cells are examined using a fluorescence microscope. Cells infected with influenza A virus will exhibit golden yellow fluorescence due to the PE. Cells infected with influenza B virus will exhibit apple-green fluorescence due to the FITC. Non-infected cells will exhibit red fluorescence due to the Evans Blue counter-stain. Nuclei of intact cells will exhibit orange-red fluorescence due to the propidium iodide.

Materials Provided:

1. Influenza A/Influenza B D^3 FastPoint L-DFA Reagent
2. Re-suspension Buffer
3. D^3 FastPoint L-DFA Influenza A/Influenza B Antigen Control Slides
4. 40X PBS Concentrate

Materials Required But Not Provided:

1. Fluorescence microscope with the correct filter combination for FITC (excitation peak = 490 nm, emission peak = 520nm) and for R-PE; magnification 200 to 400X.
2. Fifty pack of 3-well specimen slides.
3. Cover slips (22 x 50mm) for Antigen Control Slides and for specimen slides.
4. Adjustable pipettes (20 to 200 and 200 to1000-µL).
5. Pipette tips (20 to 200 and 200 to1000-µL)
6. 200-mL wash bottle.
7. 1.7-mL centrifuge vials.
8. 15-mL conical centrifuge tube.
9. Sodium hypochlorite solution (1:10 final dilution of household bleach).
10. Humidified chamber (e.g., covered Petri dish with a damp paper towel placed in the bottom) or humidified incubator.
11. Incubator, 35° to 37°C (CO_2 or non-CO_2, depending on the cell culture format used).
12. Centrifuge with free-swinging bucket rotor.
13. De-mineralized water for dilution of 40X PBS Concentrate.
14. Stat-Spin Centrifuge (or bench top centrifuge capable of 2-minutes at 2000xg).

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Diagnostic Hybrids, Inc. D^3 *Ultra* DFA Respiratory Virus Screening & ID Kit
   Diagnostic Hybrids, Inc. D^3 *Duet* DFA Influenza A/Respiratory Virus Screening Kit
   Diagnostic Hybrids, Inc. D^3 *Duet* DFA RSV/Respiratory Virus Screening Kit

2. Predicate k number(s):

   (K061101), (K081928), (K081746)

3. Comparison with predicates:

   The intended use of the D^3 FastPoint L-DFA Influenza A/Influenza B Virus Identification Kit is similar to the predicate devices (D^3 *Ultra* DFA Respiratory Virus Screening & ID Kit and D^3 *Duet* DFA RSV/Respiratory Virus Screening Kit). Characteristics of the D^3 FastPoint L-DFA Influenza A/Influenza B Virus Identification Kit are compared to those of the predicate devices, in the Table below:

Technological Characteristics Comparison of Devices		
D^3 FastPoint L-DFA Influenza A/Influenza B Virus Identification Kit (Subject)	D^3 *Ultra* DFA Respiratory Virus Screening & ID Kit (Predicate)	D^3 *Duet* DFA RSV/Respiratory Virus Screening Kit (Predicate)
Target Viruses		
Flu A, Flu B	Flu A, Flu B, RSV, Adenovirus, HPIV-1,2,3	Flu A, Flu B, RSV, Adenovirus, HPIV-1,2,3
Monoclonal antibodies (MAbs)		
4 MAbs to 2 different respiratory viruses	15 MAbs to7 different respiratory viruses	15 MAbs to7 different respiratory viruses

(Flu A, Flu B)	(Flu A, Flu B, RSV, Adenovirus, HPIV-1,2,3)	(Flu A, Flu B, RSV, Adenovirus, HPIV-1,2,3)
Labeling method		
Direct labeling using R-Phycoerythrin (R-PE) to label the MAbs to FluA using fluorescein isothiocyanate (FITC) to label FluB MAbs with fluorescein	Direct labeling using fluorescein isothiocyanate (FITC) to label Flu A, Flu B, RSV, Adenovirus, HPIV 1,2,3 MAbs with fluorescein	Direct labeling using R-Phycoerythrin (R-PE) to label the MAbs to RSV using fluorescein isothiocyanate (FITC) to label Flu A, Flu B, Adenovirus, and HPIV-1,2,3 MAbs with fluorescein
R-Phycoerythrin-labeled MAbs		
FluA	None	RSV
Fluorescein-labeled MAbs		
FluB	Flu A, Flu B, RSV, Adenovirus and HPIV 1,2,3	Flu A, Flu B, Adenovirus, and HPIV-1,2,3
Cell Fixative		
Sapogenin	Acetone	Acetone
Cell Counter-stain		
Propidium Iodide and Evans Blue	Evans Blue	Evans Blue

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

- Special controls guidance documents will be promulgated
- Guidance on Class II Special Controls Guidance Document: Reagents for Detection of Specific Novel Influenza A Viruses (March 2006) – *http://www.fda.gov/cdrh/oivd/guidance/1596.html*
- Guidance on Informed Consent for In Vitro Diagnostic Device Studies Leftover Human Specimens that are Not Individually Identifiable (April 2006) – *http://www.fda.gov/cdrh/oivd/guidance/1588.html*
- Statistical Guidance on Reporting Results from Studies Evaluating Diagnostic Tests; Guidance for Industry and FDA Reviewers (March 2007) – *http://www.fda.gov/cdrh/osb/guidance/1620.html*
- Format for Traditional and Abbreviated 510(k)s - Guidance for Industry and FDA Staff – *http://www.fda.gov/cdrh/ode/guidance/1567.html*
- 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 (Feb 2008) – *http://www.fda.gov/cdrh/oivd/guidance/1638.pdf*

L. Test Principle:

The D^3 FastPoint L-DFA Influenza A/Influenza B Virus Identification Kit uses a blend (called a "L-DFA Reagent") of viral antigen-specific murine monoclonal antibodies that are directly labeled with either R-PE (influenza A virus) or fluorescein (influenza B virus) for the rapid identification of influenza A virus and influenza B virus in nasal and nasopharyngeal swabs and aspirates/washes from patients with signs and symptoms of respiratory infection.

The cells to be tested are derived from respiratory specimens from patients with signs and symptoms of respiratory infection. The cells are permeablized and stained concurrently in a liquid suspension format with the L-DFA Reagent. After incubating at 35ºC to 37ºC for

5 minutes, the stained cell suspensions are rinsed with PBS. The rinsed cells are pelleted by centrifugation and then re-suspended with the re-suspension buffer and loaded onto a specimen slide well. The cells are examined using a fluorescence microscope. Cells infected with influenza A virus will exhibit golden yellow fluorescence due to the PE. Cells infected with influenza B virus will exhibit apple-green fluorescence due to the FITC. Non-infected cells will exhibit red fluorescence due to the Evans Blue counter-stain. Nuclei of intact cells will exhibit orange-red fluorescence due to the propidium iodide.

It is recommended that results for specimens found to contain no fluorescent cells after examination of the direct specimen result be confirmed by cell culture.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Assay precision, intra-assay variability and inter assay variability were assessed with a reproducibility panel of proficiency-level antigen control slides. The reproducibility panel consisted of 5 randomized panel members.

The Influenza A/B panel consisted of the following:

a. Low level influenza A (Victoria strain) infected cells.
b. Low level influenza B (Taiwan strain) infected cells.
c. Low level influenza A (Victoria strain) infected cells mixed with mid level influenza B (Taiwan strain) infected cells.
d. Low level influenza B (Victoria strain) infected cells mixed with mid level influenza A (Victoria strain) infected cells.
e. Mid level non-infected (negative) cells.

The low level is estimated to contain between 4 to 10% infected cells in the sample. The mid level is estimated to contain between 20 to 25% infected cells in the sample. Each sample contains 2.5 $x10^5$ to 3.5 $x10^5$ total cells.

The panel was tested daily in two separate runs for 5-days by four different laboratories (40 total runs). The following results were recorded:

a. Presence or absence of golden-yellow fluorescence.
b. Percent of cells exhibiting golden-yellow fluorescence.
c. Presence or absence of apple-green fluorescence.
d. Percent of cells exhibiting apple-green fluorescence.

Note: "Processing of specimen", although a source of variability, was done according to each laboratory's established practices. The product insert for this device instructs the laboratory to process a specimen according to Clinical Microbiology Handbook (H.D. Isenberg, 2004, publ. by ASM; sections 10.7.1-10.7.10). As such, testing reproducibility of "processing of specimen" is beyond

the scope of this reproducibility study. This study accessed reproducibility of the test alone, i.e., "chemistry of assay" (DFA staining) and "interpretation of result". "Interpretation of result" is considered to be the largest source for variability for this test. Interpretation of test is subjective, according to potential variability in an individual technician's competence, experience, and/or diligence in microscopic evaluations of stained cells.

A total of 280 data points were included in the reproducibility study data analysis (1 panel X 7 members/run X 2 runs/day X 5 days X 4 sites = 280).

For the D^3 FastPoint L-DFA Influenza A/Influenza B Reagent, the combined data from the four Study Sites demonstrated reproducible detection of influenza A virus by the R-PE labeled MAbs and reproducible detection of influenza B virus by the FITC-labeled MAbs. The presence of influenza A virus infected cells was reported in 100% (120/120) of the wells in which the infected cells were expected. The presence of influenza B virus infected cells was reported in 100% (120/120) of the wells in which the infected cells were expected. The absence of infected cells was reported in 95% (38/40) of the wells in which infected cells were not present. The total percent agreement for the D^3 FastPoint L-DFA Influenza A/Influenza B Reagent was 99.3% (278/280):

D^3 FastPoint L-DFA Influenza A/Influenza B Reagent

	Panel Member	Negative	Flu A Low Level	Flu B Low Level	Mixed Infection		Mixed Infection		Total % Agreement
					Flu A Mid Level	Flu B Low Level	Flu A Low Level	Flu B Mid Level	
	Concentration	No infected cells	4 to 10% infected cells	4 to 10% infected cells	20 to 30% infected cells	4 to 10% infected cells	4 to 10% infected cells	20 to 30% infected cells	
Site 1	Agreement with Expected result	8/10 (80%)	10/10 (100%)	10/10 (100%)	10/10 (100%)	10/10 (100%)	10/10 (100%)	10/10 (100%)	**68/70 (97.1%)**
Site 2	Agreement with Expected result	10/10 (100%)	10/10 (100%)	10/10 (100%)	10/10 (100%)	10/10 (100%)	10/10 (100%)	10/10 (100%)	**70/70 (100%)**
Site 3	Agreement with Expected result	10/10 (100%)	10/10 (100%)	10/10 (100%)	10/10 (100%)	10/10 (100%)	10/10 (100%)	10/10 (100%)	**70/70 (100%)**
Site 4	Agreement with Expected result	10/10 (100%)	10/10 (100%)	10/10 (100%)	10/10 (100%)	10/10 (100%)	10/10 (100%)	10/10 (100%)	**70/70 (100%)**
	Total Agreement with Expected result	38/40 (95%)	40/40 (100%)	40/40 (100%)	40/40 (100%)	40/40 (100%)	40/40 (100%)	40/40 (100%)	**278/280 (99.3%)**
	95% CI	83.1 – 99.4%	91.2 – 100%	91.2 – 100%	91.2 – 100%	91.2 – 100%	91.2 – 100%	91.2 – 100%	**97.4 – 99.9%**

b. Linearity/assay reportable range:

Not applicable.

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

Development and Characterization of Reagents

Development and Characterization of MAbs

Development and characterization of each MAb includes immunogen preparation, immunization, hybridoma preparation, clone selection, MAb purification, determination of relative binding affinities, Western blot testing and isotype identification. All of the monoclonal antibodies (MAbs) included in the D^3 FastPoint L-DFA Influenza A/Influenza B Virus Identification Kit have also been used in one the following DHI devices:

1. D^3 *Ultra* DFA Respiratory Virus Screening & ID Kit that was cleared for marketing via section 510(k) k061101 on November 20, 2006.
2. D^3 *Duet* DFA Influenza A/Respiratory Virus Screening Kit that was cleared for marketing via section 510(k) k081746 on December 23, 2008.

Performance Evaluation of PE-labeled MAbs

Reactivity of PE-labeled MAbs with Acetone-Fixed Infected Model Cells
(Model Cells are A549 cell cultures infected with known isolates of influenza A virus or influenza B virus, at a high (0.1) MOI. These cultures are incubated for 20 to 22 hours at 35°C to 37°C, and then processed by scraping the monolayers and resuspending the cells in a viral transport medium.)

An antibody may exhibit high affinity for its target antigen until labeled with a reporter moiety such as PE due to blocking or modification of the antigen-binding site. Each of the two influenza A virus MAbs were labeled with PE. The individual PE-labeled MAbs were used to stain acetone-fixed influenza A virus in order to verify that each of the MAbs remains reactive with its target after labeling with PE. The same model cells were stained concurrently with FITC-labeled MAbs for comparison. This comparison of the reactivity is summarized in the following table:

PE-labeled MAb versus FITC-labeled MAb Reactivity Comparison in Acetone-fixed Cells			
D^3 FastPoint L-DFA Reagent Kit MAb #	**Target Virus**	**R-PE**	**FITC**
A(6)B11	influenza A	Reactive	Reactive
2H3C5	influenza A	Reactive	Reactive

Reactivity of PE-labeled MAbs with Permeablized Infected Model Cells
(Model Cells are A549 cell cultures infected with known isolates of influenza A virus or influenza B virus, at a high (0.1) MOI. These cultures are incubated for 20 to 22 hours at 35°C to 37°C, and then processed by scraping the monolayers and resuspending the cells in a viral transport medium.)

Studies were conducted to demonstrate that the PE-labeled MAbs would stain infected cells in liquid suspension that have been permeablized. The individual PE-labeled MAbs were used to stain influenza A virus model cells that had been permeablized in order to verify that each of the PE-labeled MAbs remains reactive with its target. The same cells were stained with FITC-labeled MAbs. This comparison of the reactivity is summarized in the following table:

PE-labeled MAb versus FITC-labeled MAb Reactivity Comparison in Permeablized Cells			
D^3 FastPoint L-DFA Reagent Kit MAb #	**Target Virus**	**R-PE**	**FITC**
A(6)B11	influenza A	Reactive	Reactive
2H3C5	influenza A	Reactive	Reactive

Concentration of PE-labeled MAbs

Final blended solution of the 2 influenza A virus MAbs was formulated to yield optimal fluorescence intensity and lowest background on the infected cells, permeablized and stained in suspension.

Performance Evaluation of FITC-labeled MAbs

Reactivity of FITC-labeled MAbs with Acetone-Fixed Infected Model Cells
(Model Cells are A549 cell cultures infected with known isolates of influenza A virus or influenza B virus at a high (0.1) MOI. These cultures are incubated for 20 to 22 hours at 35°C to 37°C, and then processed by scraping the monolayers and resuspending the cells in a viral transport medium.)

The 2 influenza B virus FITC-labeled MAbs used in the D^3 FastPoint L-DFA Influenza A/Influenza B Virus Identification Kit have all previously been FDA cleared for use with acetone-fixed cells. No additional testing was performed.

Reactivity of FITC-labeled MAbs with Permeablized Infected Model Cells
(Model Cells are A549 cell cultures infected with known isolates of influenza A virus or influenza B virus at a high (0.1) MOI. These cultures are incubated for 20 to 22 hours at 35°C to 37°C, and then processed by scraping the monolayers and resuspending the cells in a viral transport medium.)

Studies were conducted to demonstrate that the FITC-labeled MAbs would stain infected cells in solution that have been permeablized. The individual FITC-labeled MAbs were used to stain influenza B virus model cells that had been permeablized in order to verify that each of the FITC-labeled MAbs remains

reactive with its target. All FITC-labeled MAbs reacted with the appropriate permeablized model cells as expected, and similar to acetone-fixed cells.

Concentration of FITC-labeled MAbs

Final blended solution of the 2 influenza B virus MAbs was formulated to yield optimal fluorescence intensity and lowest background on the infected cells, permeablized and stained in suspension.

Cell Permeablization and Counterstaining

Selection of Permeablization Reagent

The D^3 FastPoint L-DFA Influenza A/Influenza B Virus Identification Kit uses Sapogenin to permeablize the cell membrane instead of acetone to allow the MAbs to react with their respective antigens. Permeablization involves treatment of cells with a mild surfactant. This treatment will dissolve portions of the cell membranes and allow larger dye molecules and antibodies access to the cell's interior. This allows the cells to maintain their three dimensional structure while being stained with labeled antibodies and counter-stain. By doing this, cells can remain in liquid suspension. Studies were conducted to compare performance using acetone with that using another permeablizing reagent, Sapogenin. Sapogenins are the aglycones, or non-saccharide portions of the family of natural products known as saponins. The amphipathic nature of saponins gives them activity as surfactants that can be used to enhance penetration of macromolecules such as proteins through cell membranes. Using influenza A virus and influenza B virus and respiratory syncytial virus model cells, acetone and Sapogenin were tested at various concentrations. Acetone was tested at concentrations from 20% to 100%. Sapogenin was used at 0.1% based on what has been published in the literature. Data generated from the study indicated that at all acetone concentrations Sapogenin had greater numbers of infected cells in the liquid format. Based on these studies, Sapogenin was chosen as the Permeablization reagent in the D^3 FastPoint L-DFA Reagents.

Determination of Sapogenin Concentration

Experiments were conducted to optimize the concentration of Sapogenin in the D^3 FastPoint L-DFA Influenza A/Influenza B Virus Identification Kit. Non-infected model cells were treated with different concentrations of Sapogenin and counter-stain for 5-minutes. The cells were then counted, and the values compared. Data generated from the study indicated that there was no difference in numbers of cells when Sapogenin concentrations at 0.1% to 0.025% were used. When 0.2% Sapogenin was used, reduced numbers of cells were noted, which was an indication that Sapogenin at that concentration may cause cell disruption. The 0.1% level was chosen to be used in the D^3 FastPoint L-DFA Reagents to better ensure adequate Permeablization of clinical nasal pharyngeal cells.

Propidium Iodide Counter-stain

To assist the end user in the use of the D^3 FastPoint L-DFA Reagent Kits, improvement to the counter-staining of cells was developed. Propidium Iodide was added to the D^3 FastPoint L-DFA Flu A/B Reagent. The stained cell's nuclei fluoresce red. This improves the ability to assess specimen quality compared to standard acetone fixation, Evans Blue stained DSFA specimens. Subjective studies were conducted to determine the optimal concentration of Propidium Iodide. Higher concentrations of Propidium Iodide (16-µg/mL or higher) began interfering with the ability to see low level fluorescence generated by either the PE- or FITC-stained cells. Lower levels of Propidium Iodide (4-µg/mL or lower) made it difficult to see the stained nuclei. 8-µg/mL was the optimal concentration to allow easy identification of cells with no quenching of PE or FITC fluorescence. A low level of Evans Blue (25-µg/mL compared to 250-µg/mL in each of the predicate respiratory devices) is also included to help reduce background of non-specific antibody staining sometimes seen in clinical specimens.

Reagent Interference Studies of MAbs

Studies were conducted to demonstrate that the final blend of PE- and FITC-labeled MAbs in the D^3 FastPoint L-DFA Influenza A/Influenza B Reagent did not affect the ability to detect low level positive infected cells that are stained by one fluor when they are in the same sample, with a high level of positive infected cells which are stained by the other fluor in the same well. The following cell preparations were permeablized and stained with the appropriate reagent.

For the Influenza A/ Influenza B Reagent:

a. Low level (~25 or lower infected cells) infected influenza A virus model cells were spiked into non-infected cells.
b. Low level influenza A virus model cells were spiked into high level (4 +) influenza B virus model cells.
c. Low level (~25 or lower infected cells) infected influenza B virus model cells were spiked into non-infected cells.
d. Low level influenza B virus model cells were spiked into high level (4+) of influenza A virus model cells.

The following table summarizes the study data for the DFA Reagent:

Staining Interference of High Level Infected Model Cells		
Test Condition	**Infected Cell Counts of Low Level Model Cells**	**Average Infected Cell Counts of Low Level Model Cells**
Low Flu A virus model cells in non-infected cells	8, 6, 13	9.0
Low Flu A virus in High Flu B virus model cells	9, 9, 10	9.3
Low Flu B virus model cells in non-infected cells	17, 9, 16	14.0
Low Flu B virus in High Flu A virus model cells	8, 9, 12	9.6

For each combination of low level infected cells spiked into high level of infected cells, there was not a significant difference in detection compared to the low level positive cells spiked into non-infected cells (control).

Binding Competition Studies of MAbs

The Influenza A/Influenza B L-DFA Reagent contains 2 influenza A virus MAbs and 2 influenza B virus MAbs. The purpose of combining two MAbs specific per virus is to ensure that all strains will be detected. Studies were conducted to determine if the individual MAbs compete with one another for the same binding sites since originally, the clones were selected for their individual and highest level of staining intensity of the respective virus antigens.

Model cells of influenza A virus and influenza B virus were permeablized. Cells were stained with each unlabeled clone of the appropriate virus. The cells were then stained with the PE- or FITC-labeled MAbs for each pair. Labeled MAbs were used individually, each at their standard concentrations used in the assay.

Results of the initial study indicated that there was no evidence of self or cross epitope blocking for all the pairs of MAbs, except for the two influenza A MAbs. The two influenza A MAbs were labeled with FITC and tested using the same protocol. The MAbs again blocked each other. This suggested that the blocking is unrelated to the labeling chemistry. The testing was repeated using acetone fixation of the model cells in place of the permeablization. The MAbs again blocked each other. This suggested that the blocking is unrelated to the fixation chemistry.

For the influenza A virus MAbs, blocking of the epitopes is strain dependant. Even though these clones appear to bind to the same epitope for influenza A virus using the H3N2 virus strain, they are still used because of an "additive" effect on the brightness of fluorescent cells, i.e. doubling the concentration of one clone and using it alone is not as bright as using the two clones with the same final concentration.

Stability Studies

Shelf life for the complete kit

Kits were tested at time intervals during storage according to the study plan. Characteristics monitored were performance, as well as pH, color and clarity. Among the acceptance criteria was fluorescence (as opposed to no fluorescence) observed in processed, infected model cells at a high level of infection (2+ to 4+) for the D^3 FastPoint L-DFA Influenza A/Influenza B Reagent at 1:16 dilution. Stability studies have been conducted in two phases: (1) using kits produced during the development phase according to draft written procedures, and (2) using kits produced according to established procedures by manufacturing staff (both phases are on-going). To establish the final shelf life of the device, real-time

testing (under labeled storage conditions of 2°C to 8°C) is also being conducted. As of August 2009, stability has been demonstrated to 9 months. Additional stability studies are currently being conducted to establish a 20°C to 25°C storage shelf-life claim for the D^3 FastPoint L-DFA Influenza A/Influenza B Reagent.

Shelf life for the D^3 FastPoint L-DFA Influenza A/Influenza B Kit antigen control slides

The D^3 FastPoint L-DFA Influenza A/Influenza B Virus Identification Kit Antigen Control Slides are prepared by combining infected cells (influenza A virus and influenza B virus in one well). Non-infected cells are spotted onto an additional well for a negative control for the reagent. Stability studies are currently being conducted for the D^3 FastPoint L-DFA Flu A/B Identification Kit Antigen Control Slides; however, since they are prepared using the same procedure and same infected cell cultures as the control slides in the D^3 *Ultra* Kit, a shelf life of 18 months is anticipated. Stability studies have been conducted in two phases: (1) using slides produced during the development phase according to draft written procedures, and (2) using slides produced according to established procedures by manufacturing staff (both phases are on-going). To establish shelf life of the device, real-time testing (under labeled storage conditions of 2°C to 8°C) is also being conducted. As of August 2009, stability has been demonstrated to 9 months.

d. Analytical Sensitivity (Detection limit):

Analytical Limit of Detections of the D^3 FastPoint L-DFA Flu A/B Reagent Kit was addressed using dilution series of infected model cells. Model cells for influenza A virus (ATCC Victoria strain) and influenza B virus (ATCC Taiwan strain) were diluted with non-infected cells to produce a suspension equivalent to 1,000 infected cells per milliliter. This level theoretically yields approximately 25 infected cells per 25-µL of suspension. This suspension was then serially diluted to a theoretical level of less than 1 cell per milliliter. (NOTE: This level was the target to begin with a low positive level. Actual starting levels vary, however, and are within 1 dilution of the 25 infected cells target level). 25-µL aliquots from each dilution level were spotted onto 10 replicate microscope slides, and then stained according to the instructions for use described in the product insert. Each cell spot was examined at 200x magnification. Results were reported as numbers of positive replicates for each set of 10. Analytical detection limits for each of the 2 analytes were defined as the lowest dilutions at which at least 9 out of 10 replicates were detected. Results are summarized in the table below:

Limit of Detections of the D³ FastPoint L-DFA Influenza A/Influenza B Virus Identification Kit			
Virus Strain	Infected cells/mL	Number of replicates with positive cells	LOD determination
Flu A (ATCC Victoria strain)	500	10/10	50 infected cells/mL
	100	10/10	
	50	10/10	
	25	5/10	
	12.5	3/10	
	6	2/10	
	3	0/10	
	1.5	2/10	
	0.8	0/10	
	0.4	0/10	
Flu B (ATCC Taiwan strain)	2000	10/10	50 infected cells/mL
	400	10/10	
	200	10/10	
	100	10/10	
	50	10/10	
	25	7/10	
	12.5	4/10	
	6	2/10	
	3	0/10	
	1.5	0/10	

a. Analytical Reactivity (Inclusivity):

Analytical reactivity (inclusivity) of the D³ FastPoint L-DFA Influenza A/Influenza B Reagent was evaluated using 13 influenza A virus and 7 influenza B virus strains. Low concentration infected cell suspensions (approximately 4% cells infected, 25-50 infected cells) were prepared for each viral strain. The suspensions were stained with the D³ FastPoint L-DFA Influenza A/Influenza B Reagent. The following table summarizes the data:

Analytical Reactivity (inclusivity) of the D³ FastPoint L-DFA Influenza A/Influenza B Reagent on various influenza A virus and influenza B virus strains		
Influenza Strains	**Infected Cell Concentration (as multiples of the respective established LoD concentration**	**D³ FastPoint L-DFA Influenza A/ Influenza BReagent Results**
Influenza A Mexico/4108/2009 (H1N1) from CDC*	20x LoD	19 Golden-yellow fluorescent cells
Influenza A California/07/2009 (H1N1) from CDC*	20x LoD	26 Golden-yellow fluorescent cells
Influenza A Wisconsin/56/2005 (H3N2)	20x LoD	39 Golden-yellow fluorescent cells
Influenza A WS, VR-1520 (H1N1)	20x LoD	67 Golden-yellow fluorescent cells
Influenza A Hong Kong, VR-544 (H3N2)	20x LoD	13 Golden-yellow fluorescent cells
Influenza A New Jersey, VR-897 (H1N1)	20x LoD	15 Golden-yellow fluorescent cells
Influenza A A/NWS/33 (H1N1)	20x LoD	10 Golden-yellow fluorescent cells
Influenza A Victoria, VR-822 (H3N2)	20x LoD	10 Golden-yellow fluorescent cells
Influenza A PR, VR-95 (H1N1)	20x LoD	20 Golden-yellow fluorescent cells
Influenza A Port Chalmers, VR-810 (H3N2)	20x LoD	8 Golden-yellow fluorescent cells
Influenza A Aichi, VR-547 (H3N2)	20x LoD	28 Golden-yellow fluorescent cells
Influenza A Denver, VR-546 (H1N1)	20x LoD	30 Golden-yellow fluorescent cells
Influenza A Mal, VR-98 (H1N1)	20x LoD	21 Golden-yellow fluorescent cells

Influenza B GL/1739/54, VR-103	20x LoD	13 Apple-green fluorescent cells
Influenza B Taiwan/2/62, VR-295	20x LoD	44 Apple-green fluorescent cells
Influenza B Hong Kong/5/72, VR-823	20x LoD	21 Apple-green fluorescent cells
Influenza B Maryland/1/59, VR-296	20x LoD	22 Apple-green fluorescent cells
Influenza B Russia, VR-790	20x LoD	36 Apple-green fluorescent cells
Influenza B B/Lee/40	20x LoD	41 Apple-green fluorescent cells
Influenza B Massachusetts, VR-523	20x LoD	67 Apple-green fluorescent cells

*Although the D^3 FastPoint L-DFA Influenza A/Influenza B Reagent has been shown to detect the 2009 H1N1 virus in two culture isolates provided by the CDC, and well-characterized by the CDC rRT-PCR Swine Flu Detection Panel (under an EUA), the performance characteristics of this device with clinical specimens that are positive for the 2009 H1N1 influenza virus have not been established. The D^3 FastPoint L-DFA Influenza A/Influenza B Reagent can distinguish between influenza A and B viruses, but it can not differentiate influenza subtypes.

f. Analytical Specificity:

D^3 FastPoint L-DFA Influenza A/Influenza B Virus Identification Kit was tested for cross-reactivity against a variety of microorganisms. Stringent conditions for cross-reactivity testing were achieved by using both the 1.5 X concentration of MAbs and relatively high titers of microorganisms. No cross-reactivity was observed for 59 virus strains. Twenty-two (22) bacterial strains, one yeast, and one *Chlamydia sp.* were also evaluated for cross-reactivity, including *Staphylococcus aureus,* a protein-A-producing bacterium. Except for *Staphylococcus aureus*, which was cross reactive with the D^3 FastPoint L-DFA Influenza A/Influenza B Virus Identification Kit, all other microorganisms tested negative.

Staining of *S. aureus* appeared as small points of fluorescence. The Protein A produced by the bacterium, *Staphylococcus aureus*, may bind the Fc portion of some fluorescein-labeled monoclonal antibodies. Such binding can be distinguished from viral antigen binding on the basis of morphology, i.e., *S. aureus*-bound fluorescence appears as small (~1-micron diameter), bright dots. Results from testing direct respiratory specimens with bacterial contamination must be interpreted with caution. The following language was added to the "Limitations of Procedure" section of the product insert to address this issue: **"Light background staining may occur with specimens contaminated with *Staphylococcus aureus* strains containing large amounts of protein A. Protein A will bind to the Fc portions of conjugated antibodies. Such binding can be distinguished from viral antigen binding on the basis of morphology, for example, *S. aureus*-bound fluorescence appears as small (~1 micron diameter), bright dots. Therefore, results from testing direct respiratory specimens with bacterial contamination must be interpreted with caution."**

- Fifty-nine (59) virus strains were tested for cross reactivity. Depending on the particular virus, 1.4 x 10^4 to 1.4 x 10^5 $TCID_{50}$ viruses were inoculated into multi-well plate cultures and incubated for 24 to 72 hours to yield a 1+ to 4+ cytopathic effect. For each virus, a confirmation stain was done with the appropriate MAb to ensure the desired titer was achieved. These cells were then prepared as Model Cells (scraped and resuspended in UTM). Each cell suspension of infected Model Cells was processed according to the D^3 FastPoint L-DFA Influenza A/Influenza B Virus Identification Kit protocol, using 2X MAb and was examined at 200X magnification. No cross reactivity was observed for the viruses listed below:

Virus Strains Tested for Cross Reactivity with the D^3 FastPoint L-DFA Influenza A/Influenza B Virus Identification Kit			
Organism	Strain or Type	D^3 FastPoint L-DFA Influenza A/B Reagent Results	Inoculum ($TCID_{50}$)
Adenovirus	Type 1	Negative	1.4 x 10^4
	Type 3	Negative	1.4 x 10^4
	Type 5	Negative	1.4 x 10^4
	Type 7	Negative	1.4 x 10^4
	Type 10	Negative	1.4 x 10^4
	Type 16	Negative	1.4 x 10^4
	Type 17	Negative	1.4 x 10^4
Metapneumovirus (hMPV)	Subtype A1	Negative	1.4 x 10^4
	Subtype A2	Negative	1.4 x 10^4
	Subtype B1	Negative	1.4 x 10^4
	Subtype B2	Negative	1.4 x 10^4
Influenza A	Aichi (H3N2)	Golden-Yellow Fluor.	1.4 x 10^4
	Mal (H1N1)	Golden-Yellow Fluor.	1.4 x 10^4
	Hong Kong (H3N2)	Golden-Yellow Fluor.	1.4 x 10^4
	Denver (H1N1)	Golden-Yellow Fluor.	1.4 x 10^4
	Port Chalmers (H3N2)	Golden-Yellow Fluor.	1.4 x 10^4
	Victoria (H3N2)	Golden-Yellow Fluor.	1.4 x 10^4
	New Jersey (HSWN1)	Golden-Yellow Fluor.	1.4 x 10^4
	WS (H1N1)	Golden-Yellow Fluor.	1.4 x 10^4
	PR (H1N1)	Golden-Yellow Fluor.	1.4 x 10^4
	Wisconsin (H3N2)	Golden-Yellow Fluor.	1.4 x 10^4
	A/NWS/33 (H1N1)	Golden-Yellow Fluor.	1.4 x 10^4
	A Mexico/4108/2009 (H1N1)	Golden-Yellow Fluor.	1.4 x 10^4
	A California/07/2009 (H1N1)	Golden-Yellow Fluor.	1.4 x 10^4
Influenza B	Hong Kong	Apple-Green Fluor.	1.4 x 10^4
	Maryland	Apple-Green Fluor.	1.4 x 10^4
	Mass	Apple-Green Fluor.	1.4 x 10^4
	GL	Apple-Green Fluor.	1.4 x 10^4
	Taiwan	Apple-Green Fluor.	1.4 x 10^4
	B/Lee/40	Apple-Green Fluor.	1.4 x 10^4
	Russia	Apple-Green Fluor.	1.4 x 10^4
RSV	Long	Negative	1.4 x 10^4
	Wash	Negative	1.4 x 10^4
	9320	Negative	1.4 x 10^4

Virus Strains Tested for Cross Reactivity with the D^3 FastPoint L-DFA Influenza A/Influenza B Virus Identification Kit			
Organism	Strain or Type	D^3 FastPoint L-DFA Influenza A/B Reagent Results	Inoculum ($TCID_{50}$)
Parainfluenza 1	C-35	Negative	1.4×10^4
Parainfluenza 2	Greer	Negative	1.4×10^4
Parainfluenza 3	C-243	Negative	1.4×10^4
Parainfluenza 4	M-25	Negative	1.4×10^5
Parainfluenza 4b	CH-19503	Negative	1.4×10^5
HSV-1	1(f)	Negative	1.4×10^5
	MacIntyre	Negative	1.4×10^5
HSV-2	Clinical Isolate CWOH-0011	Negative	1.4×10^5
	Strain G	Negative	1.4×10^5
CMV	Towne	Negative	1.4×10^5
	AD169	Negative	1.4×10^5
Varicella-zoster	AV92-3	Negative	1.4×10^5
Echovirus	4	Negative	1.4×10^5
	6	Negative	1.4×10^5
	7	Negative	1.4×10^5
	22	Negative	1.4×10^5
Coxsackievirus	A9	Negative	1.4×10^5
	B1	Negative	1.4×10^5
	B3	Negative	1.4×10^5
	B4	Negative	1.4×10^5
Coronavirus	229E	Negative	1.4×10^5
	OC43	Negative	1.4×10^5
Rhinovirus	209 Picornavirus	Negative	1.4×10^5
Enterovirus 70	VR-836	Negative	1.4×10^5
Enterovirus 71	VR-1432	Negative	1.4×10^5

- Twenty four (24) microorganisms, including 22 bacterial, 1 yeast, and 1 *Chlamydia sp.* were tested for cross-reactivity. Bacteria were cultured, processed as suspensions, then spiked into non-infected Model Cells suspensions at levels (as CFUs, colony-forming units) ranging from 1.6×10^9 to 3.5×10^{10} CFUs depending on the bacterium. These suspensions of Model Cells with bacteria were then processed according to the D^3 FastPoint L-DFA Influenza A/Influenza B Virus Identification Kit protocol, using 2X MAb reagents. Except for *Staphylococcus aureus*, which was cross reactive with the D^3 FastPoint L-DFA Influenza A/Influenza B Virus Identification Kit, all other microorganisms tested negative. Reactivity with *Staphylococcus aureus* is more than likely due to binding the protein A produced by *Staphylococcus aureus*. Microorganisms tested are listed in the table below:

Microorganisms Tested for Cross Reactivity with D^3 FastPoint L-DFA Influenza A/Influenza B Virus Identification Kit		
Organism	D^3 FastPoint L-DFA Influenza A/B Reagent Results	CFU tested
Bacteria		
Acholeplasma laidlawii	Negative	Control Slide
Acinetobacter calcoaceticus	Negative	3.6×10^9
Bordetella bronchiseptica	Negative	1.1×10^{10}
Bordetella pertussis	Negative	4.3×10^9
Chlamydia trachomatis (Apache-2)	Negative	LGV-II/Control Slide
Corynebacterium diphtheriae	Negative	5.7×10^7
Escherichia coli	Negative	7.5×10^8
Gardnerella vaginalis	Negative	Control Slide
Haemophilis influenzae type A	Negative	4.1×10^9
Klebsiella pneumoniae	Negative	1.2×10^9
Moraxella cartarrhalis	Negative	1.2×10^{10}
Mycoplasma hominis	Negative	3.5×10^{10}
Mycoplasma orale	Negative	6.6×10^9
Mycoplasma pneumoniae	Negative	7.9×10^9
Mycoplasma salivarium	Negative	7.7×10^8
Proteus mirabilis	Negative	3.6×10^9
Pseudomonas aeruginosa	Negative	1.0×10^8
Salmonella enteriditis	Negative	8.7×10^9
Salmonella typhimurium	Negative	7.5×10^9
*Staphylococcus aureus**	Positive	6.3×10^9
Streptococcus agalactiae	Negative	5.5×10^8
Streptococcus pneumoniae	Negative	6.7×10^9
Streptococcus pyogenes	Negative	6.9×10^9
Yeast		
Candida glabrata	Negative	1.6×10^6

***** Reactivity with *Staphylococcus aureus* is more than likely due to binding the protein A produced by *Staphylococcus aureus*.

g. Assay cut-off:

Not applicable.

h. Interfering Substances:

Not applicable.

2. Comparison studies:

a. Method comparison with predicate device:

Not applicable.

b. Matrix Description and Comparison:

Not applicable.

3. Clinical studies:

a. Prospective Clinical Studies Testing Direct Respiratory Specimens

Performance of the D^3 FastPoint L-DFA Influenza A/Influenza B Virus Identification Kit testing direct respiratory specimens were established during prospective studies at 4 geographically diverse U.S. clinical laboratories during the 2008/2009 respiratory virus seasons (January 2008 – March 2009). All specimens used in the studies meeting the inclusion and exclusion criteria represented excess, remnants of respiratory specimens that were prospectively collected from symptomatic individuals suspected of respiratory infection, and were submitted for routine care or analysis by each site, and that otherwise would have been discarded. Individual specimens were delinked from all patient identifiers and given a study sample code. All clinical sites were granted waivers of informed consent by their IRBs for this study.

Performance of the D^3 FastPoint L-DFA Influenza A/Influenza B Virus Identification Kit detecting influenza A and influenza B from direct specimens was assessed and compared to DSFA testing using FDA cleared comparator DSFA devices (D^3 *Ultra* DFA Respiratory Virus Screening & ID Kit or D^3 *Duet* DFA RSV/Respiratory Virus Screening Kit), followed by viral culture confirmation of all negative specimens (as determined by the FDA cleared DSFA comparator devices), using FDA cleared DFA reagents.

Study Site 1 evaluated a total of 323 fresh respiratory specimens submitted, January 2009 through March 2009, to the laboratory for respiratory virus testing. Slides were prepared from Phosphate Buffered Saline (PBS)-washed cells from the fresh specimens and processed according to the prescribed protocol. The slides were stained in accordance with the procedure in the product insert.

The following table shows the age and gender distribution for individuals studied at site 1:

Site 1 – Age and Gender Distribution		
Sex	F	M
Total	150	173
Age		
0 – 1 month	13	7
> 1 month to 2 years	100	131
> 2 years to 12 years	35	35
> 12 years to 21 years	2	0
22 years to 30 years	0	0
31 years to 40 years	0	0
41 years to 50 years	0	0
51 years to 60 years	0	0
61 years to 70 years	0	0
71 years to 80 years	0	0
81 years and above	0	0
Age Not Reported	0	0
Total	150	173

Of the 323 fresh respiratory specimens tested, all were nasal wash/nasopharyngeal aspirate specimens. Of the 323 fresh nasal wash/nasopharyngeal aspirate specimens tested, 2 nasal wash/nasopharyngeal aspirate specimens were excluded from the performance analysis due to insufficient sample volume for the investigational device testing (0.62%). 70 specimens for Flu A and 79 specimens for Flu B were excluded from the respective performance analysis due to insufficient sample volume for the comparator culture method, resulting in a total of 251 fresh nasal wash/nasopharyngeal aspirate specimens for Flu A and 242 fresh nasal wash/nasopharyngeal aspirate specimens for Flu B. The tables below summarized the study results of the claimed specimen type at **study site 1**:

Flu A			
Fresh nasal/nasopharyngeal wash/aspirate	**Comparator DSFA (negatives followed by culture with DFA)**		
DHI DSFA	**Positive**	**Negative**	**Total**
Positive	21	1	22
Negative	4	225	229
Total	25	226	251
			95% CI
Sensitivity	21/25	84.0%	63.9-95.5%
Specificity	225/226	99.6%	97.6-100%

Flu B			
Fresh nasal/nasopharyngeal wash/aspirate	**Comparator DSFA (negatives followed by culture with DFA)**		
DHI DSFA	**Positive**	**Negative**	**Total**
Positive	2	0	2
Negative	0	240	240
Total	2	240	242
			95% CI
Sensitivity	2/2	100.0%	15.8-100%
Specificity	240/240	100.0%	98.5-100%

Study Site 2 evaluated a total of 105 fresh respiratory specimens submitted, February 2009 through March 2009, to the laboratory for respiratory virus testing. Slides were prepared from Phosphate Buffered Saline (PBS)-washed cells from the fresh specimens and processed according to the prescribed protocol. The slides were stained in accordance with the procedure in the product insert.

The following table shows the age and gender distribution for individuals studied at site 2:

Site 2 – Age and Gender Distribution		
Sex	F	M
Total	48	57
Age: 0 – 1 month	2	4
> 1 month to 2 years	15	17

> 2 years to 12 years	6	5
> 12 years to 21 years	4	6
22 years to 30 years	2	2
31 years to 40 years	4	6
41 years to 50 years	1	4
51 years to 60 years	6	5
61 years to 70 years	3	6
71 years to 80 years	3	2
81 years and above	2	0
Age Not Reported	0	0
Total	48	57

Of the 105 fresh respiratory specimens tested, 86 were nasal wash/nasopharyngeal aspirate specimens. Due to insufficient sample numbers to establish performance of the D^3 FastPoint L-DFA Influenza A/Influenza B Virus Identification Kit, 19 other types of respiratory specimens were removed from performance analysis. None of the nasal wash/nasopharyngeal aspirate samples for Flu A and Flu B were excluded from the respective performance analysis due to insufficient sample volume for the comparator culture method, resulting in a total of 86 fresh nasal wash/nasopharyngeal aspirate specimens for Flu A and Flu B to be included in the respective performance analysis. The tables below summarized the study results of the claimed specimen type at **study site 2**:

Flu A			
Fresh nasal/nasopharyngeal wash/aspirate	**Comparator DSFA (negatives followed by culture with DFA)**		
DHI DSFA	**Positive**	**Negative**	**Total**
Positive	6	2	8
Negative	0	78	78
Total	6	80	86
			95% CI
Sensitivity	6/6	100.0%	54.1-100%
Specificity	78/80	97.5%	91.3-99.7%

Flu B			
Fresh nasal/nasopharyngeal wash/aspirate	**Comparator DSFA (negatives followed by culture with DFA)**		
DHI DSFA	**Positive**	**Negative**	**Total**
Positive	4	0	4
Negative	1	81	82
Total	5	81	86
			95% CI
Sensitivity	4/5	80.0%	28.4-99.5%
Specificity	81/81	100.0%	95.5-100%

Study Site 3 evaluated a total of 443 fresh respiratory specimens submitted, February 2009 through March 2009, to the laboratory for respiratory virus testing. Slides were prepared from Phosphate Buffered Saline (PBS)-washed cells from the fresh specimens and processed according to the prescribed protocol. The slides

were stained in accordance with the procedure in the product insert.

The following table shows the age and gender distribution for individuals studied at site 3:

Site 3 – Age and Gender Distribution			
Sex	F	M	Sex Not Reported
Total	231	209	3
Age			
0 – 1 month	17	10	1
> 1 month to 2 years	116	132	2
> 2 years to 12 years	48	39	0
> 12 years to 21 years	8	15	0
22 years to 30 years	5	2	0
31 years to 40 years	9	4	0
41 years to 50 years	8	4	0
51 years to 60 years	5	1	0
61 years to 70 years	6	1	0
71 years to 80 years	6	0	0
81 years and above	2	0	0
Age Not Reported	1	1	0
Total	231	209	3

Of the 443 fresh respiratory specimens tested, 301 were nasal wash/nasopharyngeal aspirate specimens, and 140 were nasal/nasopharyngeal swab specimens. One (1) nasal wash/nasopharyngeal aspirate specimen was excluded from the performance analysis due to the fact that the sample was tested by the investigational device greater than 48 hours post sample collection. Due to insufficient sample numbers to establish performance of the D^3 FastPoint L-DFA Influenza A/Influenza B Virus Identification Kit, 2 other types of respiratory specimens were further removed from performance analysis. None of the remaining nasal wash/nasopharyngeal aspirate samples for Flu A and Flu B were excluded from the respective performance analysis due to insufficient sample volume for the comparator culture method, resulting in a total of 300 fresh nasal wash/nasopharyngeal aspirate specimens for Flu A and Flu B to be included in the respective performance analysis. None of the nasal/nasopharyngeal swab specimens for Flu A and Flu B was excluded from the respective performance analysis due to insufficient sample volume for the comparator methods, resulting in a total of 140 nasal/nasopharyngeal swab specimens for Flu A and Flu B to be included in the respective performance analysis. The tables below summarized the study results of the claimed specimen types at **study site 3**:

Flu A			
Fresh nasal/nasopharyngeal wash/aspirate	**Predicate DSFA (negatives followed by culture with DFA)**		
DHI DSFA	**Positive**	**Negative**	**Total**
Positive	29	0	29
Negative	6	265	271
Total	35	265	300
			95% CI
Sensitivity	29/35	82.9%	66.4-93.4%
Specificity	265/265	100.0%	98.6-100%

Flu B			
Fresh nasal/nasopharyngeal wash/aspirate	**Predicate DSFA (negatives followed by culture with DFA)**		
DHI DSFA	**Positive**	**Negative**	**Total**
Positive	3	0	3
Negative	1	296	297
Total	4	296	300
			95% CI
Sensitivity	3/4	75.0%	19.4-99.4%
Specificity	296/296	100.0%	98.8-100%

Flu A			
Fresh nasal/nasopharyngeal swab	**Predicate DSFA (negatives followed by culture with DFA)**		
DHI DSFA	**Positive**	**Negative**	**Total**
Positive	10	0	10
Negative	1	129	130
Total	11	129	140
			95% CI
Sensitivity	10/11	90.9%	58.7-99.8%
Specificity	129/129	100.0%	97.2-100%

Flu B			
Fresh nasal/nasopharyngeal swab	**Predicate DSFA (negatives followed by culture with DFA)**		
DHI DSFA	**Positive**	**Negative**	**Total**
Positive	2	0	2
Negative	1	137	138
Total	3	137	140
			95% CI
Sensitivity	2/3	66.7%	9.4-99.2%
Specificity	137/137	100.0%	97.3-100%

Study Site 4 evaluated a total of 648 fresh respiratory specimens submitted, February 2009 through March 2009, to the laboratory for respiratory virus testing. Slides were prepared from Phosphate Buffered Saline (PBS)-washed cells from the fresh specimens and processed according to the prescribed protocol. The slides were stained in accordance with the procedure in the product insert.

The following table shows the age and gender distribution for individuals studied at site 4:

Site 4 – Age and Gender Distribution			
Sex	F	M	Sex Not Reported
Total	331	279	38
Age			
0 – 1 month	0	1	0
> 1 month to 2 years	29	35	0
> 2 years to 12 years	109	114	0
> 12 years to 21 years	77	61	0
22 years to 30 years	31	15	0
31 years to 40 years	27	21	0
41 years to 50 years	21	14	0
51 years to 60 years	21	8	0
61 years to 70 years	9	8	0
71 years to 80 years	4	1	0
81 years and above	2	1	0
Age Not Reported	1	0	38
Total	331	279	38

Of the 648 fresh respiratory specimens tested, all were nasal/nasopharyngeal swab specimens. Three (3) nasal/nasopharyngeal swab specimens were excluded from the performance analysis due to insufficient sample volume for both the investigational device and the comparator DSFA device testing (0.46%). One (1) additional nasal/nasopharyngeal swab specimen was excluded from the performance analysis due to insufficient sample volume for the investigational device testing (0.15%). One (1) nasal/nasopharyngeal swab specimen was also excluded from the performance analysis due to un-interpretable result generated by the investigational device because of high background. 93 samples for Flu A and 72 samples for Flu B were excluded from the respective performance analysis due to insufficient sample volume for the comparator culture method, resulting in a total of 549 fresh nasal wash/nasopharyngeal aspirate specimens for Flu A, 570 fresh nasal wash/nasopharyngeal aspirate specimens for Flu B to be included in the respective performance analysis. The tables below summarized the study results of the claimed specimen type at **study site 4**:

Flu A			
Fresh nasal/nasopharyngeal swab	**Predicate DSFA (negatives followed by culture with DFA)**		
DHI DSFA	**Positive**	**Negative**	**Total**
Positive	47	1	48
Negative	7	495	502
Total	54	496	550
			95% CI
Sensitivity	47/54	87.0%	75.1-94.6%
Specificity	495/496	99.8%	98.9-100%

Flu B			
Fresh nasal/nasopharyngeal swab	**Predicate DSFA (negatives followed by culture with DFA)**		
DHI DSFA	**Positive**	**Negative**	**Total**
Positive	201	1	202
Negative	27	342	369
Total	228	343	571
			95% CI
Sensitivity	201/228	88.2%	84.0-92.4%
Specificity	342/343	99.7%	98.4-100%

The following tables summarized study results from **all clinical sites combined**, stratified by the claimed specimen types:

Flu A			
Fresh nasal/nasopharyngeal wash/aspirate	**Predicate DSFA (negatives followed by culture with DFA)**		
DHI DSFA	**Positive**	**Negative**	**Total**
Positive	56	3	59
Negative	10	568	578
Total	66	571	637
			95% CI
Sensitivity	56/66	84.8%	73.9-92.5%
Specificity	568/571	99.5%	98.5-99.9%

Flu B			
Fresh nasal/nasopharyngeal wash/aspirate	**Predicate DSFA (negatives followed by culture with DFA)**		
DHI DSFA	**Positive**	**Negative**	**Total**
Positive	9	0	9
Negative	2	617	619
Total	11	617	628
			95% CI
Sensitivity	9/11	81.8%	48.2-97.7%
Specificity	617/617	100.0%	99.4-100%

Flu A			
Fresh nasal/nasopharyngeal swab	**Predicate DSFA (negatives followed by culture with DFA)**		
DHI DSFA	**Positive**	**Negative**	**Total**
Positive	57	1	58
Negative	8	624	632
Total	65	625	690
			95% CI
Sensitivity	57/65	87.7%	77.2-94.5%
Specificity	624/625	99.8%	99.1-100%

Flu B			
Fresh nasal/nasopharyngeal swab	**Predicate DSFA (negatives followed by culture with DFA)**		
DHI DSFA	**Positive**	**Negative**	**Total**
Positive	203	1	204
Negative	28	479	507
Total	231	480	711
			95% CI
Sensitivity	203/231	87.9%	83.7-92.1%
Specificity	479/480	99.8%	98.8-100%

c. Retrospective Clinical studies

Not applicable.

d. Other clinical supportive data (when a. and b. are not applicable):

Not applicable.

4. Clinical cut-off**:**

Not applicable.

5. Expected values/Reference range:

In the D^3 FastPoint L-DFA Influenza A/Influenza B Virus Identification Kit multicenter prospective clinical study testing direct respiratory specimens, a total of 1519 eligible respiratory specimens were tested using the D^3 FastPoint L-DFA Influenza A/Influenza B Virus Identification Kit from four U.S. clinical laboratories across the United States during the 2008/2009 respiratory virus seasons (January 2008 – March 2009). Prevalence for each analyte (i.e., Flu A and Flu B) as determined by the D^3 FastPoint L-DFA Influenza A/Influenza B Virus Identification Kit direct specimen testing varied from 7.1% to 8.8% by site and averaged 7.6% for Flu A; varied from 0.6% to 31.2% by site and averaged 14.0% for Flu B. The number and percentage of positive cases determined by the D^3 FastPoint L-DFA Influenza A/Influenza B Virus Identification Kit direct specimen testing, calculated by age group, are presented in the following tables:

Site 1

Age	**Total Specimens Evaluated**	**Flu A**	**Flu B**
		# positive (prevalence)	**# positive (prevalence)**
0 – 1 month	20	0	0
> 1 month to 2 years	231	13 (5.6%)	2 (0.9%)
> 2 years to 12 years	70	10 (14.3%)	0

> 12 years to 21 years	2	0	0
22 years to 30 years	0	0	0
31 years to 40 years	0	0	0
41 years to 50 years	0	0	0
51 years to 60 years	0	0	0
61 years to 70 years	0	0	0
71 years to 80 years	0	0	0
81 years and above	0	0	0
Age Not Reported	0	0	0
Total	323	23 (7.1%)	2 (0.6%)

Site 2

Age	**Total Specimens Evaluated**	**Flu A**	**Flu B**
		# positive (prevalence)	**# positive (prevalence)**
0 – 1 month	6	0	0
> 1 month to 2 years	32	1 (3.1%)	1 (3.1%)
> 2 years to 12 years	11	0	0
> 12 years to 21 years	10	1 (10.0%)	1 (10.0%)
22 years to 30 years	4	1 (25.0%)	1 (25.0%)
31 years to 40 years	10	3 (30%)	1 (10%)
41 years to 50 years	5	0	0
51 years to 60 years	11	1 (9.1%)	0
61 years to 70 years	9	0	0
71 years to 80 years	5	1 (20%)	0
81 years and above	2	0	0
Age Not Reported	0	0	0
Total	105	8 (7.6%)	4 (3.8%)

Site 3

Age	**Total Specimens Evaluated**	**Flu A**	**Flu B**
		# positive (prevalence)	**# positive (prevalence)**
0 – 1 month	28	0	0
> 1 month to 2 years	250	11 (4.4%)	2 (0.8%)
> 2 years to 12 years	87	17 (14.8%)	1 (1.1%)
> 12 years to 21 years	23	6 (26.1%)	1 (4.3%)
22 years to 30 years	7	1 (14.3%)	1 (14.3%)
31 years to 40 years	13	3 (23.1%)	0
41 years to 50 years	12	1 (8.3%)	0
51 years to 60 years	6	0	0
61 years to 70 years	7	0	0
71 years to 80 years	6	0	0
81 years and above	2	0	0
Age Not Reported	2	0	0
Total	443	39 (8.8%)	5 (1.1%)

Site 4

Age	**Total Specimens Evaluated**	**Flu A**	**Flu B**
		# positive (prevalence)	**# positive (prevalence)**
0 – 1 month	1	0	0
> 1 month to 2 years	64	2 (3.1%)	15 (23.4%)
> 2 years to 12 years	223	16 (7.2%)	103 (46.8%)
> 12 years to 21 years	138	12 (8.7%)	39 (28.3%)
22 years to 30 years	46	1 (2.2%)	12 (26.1%)
31 years to 40 years	48	6 (12.5%)	8 (16.7%)
41 years to 50 years	35	4 (11.4%)	5 (14.3%)

51 years to 60 years	29	2 (6.9%)	3 (10.32%)
61 years to 70 years	17	2 (11.8%)	2 (11.8%)
71 years to 80 years	5	1 (20%)	1 (20%)
81 years and above	3	0	0
Age Not Reported	39	2 (5.1%)	14 (35.9%)
Total	648	48 (7.4%)	202 (31.2%)

All Sites Combined

Age	**Total Specimens Evaluated**	**Flu A**	**Flu B**
		# positive (prevalence)	**# positive (prevalence)**
0 – 1 month	55	0	0
> 1 month to 2 years	577	27 (4.7%)	20 (3.5%)
> 2 years to 12 years	391	43 (11.0%)	104 (26.6%)
> 12 years to 21 years	173	19 (11.0%)	41 (23.7%)
22 years to 30 years	57	3 (5.3%)	14 (24.6%)
31 years to 40 years	71	9 (12.7%)	9 (12.7%)
41 years to 50 years	52	5 (9.6%)	5 (9.6%)
51 years to 60 years	46	3 (6.5%)	3 (6.5%)
61 years to 70 years	33	2 (6.1%)	2 (6.1%)
71 years to 80 years	16	2 (12.5%)	1 (6.3%)
81 years and above	7	0	0
Age Not Reported	41	2 (4.9%)	14 (34.1%)
Total	1519	115 (7.6%)	213 (14.0%)

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.