

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

A. 510(k) Number:

K093415

B. Purpose for Submission:

A new 510(k) application for the Diagnostic Hybrids, Inc. device, D³ FastPoint L-DFA Parainfluenza Virus/Adenovirus Identification Kit, which is intended for the qualitative identification of adenovirus and to screen for the presence of parainfluenza virus types 1, 2, and 3 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 (Adenovirus, Parainfluenza virus types 1, 2 and 3)

D. Type of Test:

Direct Fluorescence Antibody (DFA) test using direct specimens

E. Applicant:

Diagnostic Hybrids Incorporated

F. Proprietary and Established Names:

D³ FastPoint L-DFA Parainfluenza Virus/Adenovirus Identification Kit

G. Regulatory Information:

1. Regulation section:

866.3400

2. Classification:

Class I

3. Product codes:

GQS, GNY

4. Panel:

Microbiology (83)

H. Intended Use:

1. Intended use(s):

The Diagnostic Hybrids, Inc. device, D³ FastPoint L-DFA Parainfluenza Virus/Adenovirus Identification Kit is intended for the qualitative identification of adenovirus and to screen for the presence of parainfluenza virus types 1, 2, and 3 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 parainfluenza virus and adenovirus after examination of the direct specimen result be confirmed by cell culture. Negative results do not preclude parainfluenza virus and adenovirus infection and should not be used as the sole basis for diagnosis, treatment or other management decisions.

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.

I. Device Description:

The D³ FastPoint L-DFA Parainfluenza Virus/Adenovirus 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 (parainfluenza virus) or fluorescein isothiocyanate (FITC) (adenovirus) for the rapid identification of parainfluenza virus and adenovirus in nasal and nasopharyngeal swabs and aspirates/washes from patients with signs and symptoms of respiratory infection.

Kit Components:

1. **D³ FastPoint L-DFA Parainfluenza Virus/Adenovirus Reagent**, 4.0-mL. One dropper bottle containing a mixture of PE-labeled murine monoclonal antibodies directed against parainfluenza virus types 1, 2, or 3 antigens and FITC-labeled murine monoclonal antibodies directed against adenovirus 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. **D³ FastPoint L-DFA Parainfluenza Virus/Adenovirus 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 parainfluenza virus type 3 or adenovirus. The negative wells contain non-infected cells. Each slide is intended to be stained only one time.
5. **D³ FastPoint L-DFA Specimen Slides and Coverslips**, 50-slides with coverslips. Fifty pack of 3-well specimen slides.

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 permeabilized and stained concurrently in a liquid suspension format with the L-DFA Reagent. After incubating at 35°C to 37°C 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 parainfluenza virus types 1, 2 and 3 will exhibit golden yellow fluorescence due to the PE. Cells infected with adenovirus 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. D³ FastPoint L-DFA Parainfluenza Virus/Adenovirus Reagent
2. Re-suspension Buffer
3. D³ FastPoint L-DFA Parainfluenza Virus/Adenovirus Antigen Control Slides
4. 40X PBS Concentrate
5. D³ FastPoint L-DFA Specimen Slides and Coverslips

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. Fine-tip, disposable transfer pipettes.

3. Cover slips (22 x 50mm) for Antigen Control Slides.
4. Adjustable pipettes (20 to 200 and 200 to 1000-μL).
5. Pipette tips (20 to 200 and 200 to 1000-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 (CO₂ or non-CO₂, 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. D³ *Ultra* DFA Respiratory Virus Screening & ID Kit
 Diagnostic Hybrids, Inc. D³ *Duet* DFA RSV/Respiratory Virus Screening Kit

2. Predicate k number(s):

(k061101), (k081928)

3. Comparison with predicates:

The intended use of the D³ FastPoint L-DFA Parainfluenza Virus/Adenovirus Identification Kit is similar to the predicate devices (D³ *Ultra* DFA Respiratory Virus Screening & ID Kit, and D³ *Duet* DFA RSV/Respiratory Virus Screening Kit. Characteristics of the D³ FastPoint L-DFA Parainfluenza Virus/Adenovirus Identification Kit are compared to those of the predicate devices, in the Table below:

Technological Characteristics Comparison of Devices		
D ³ FastPoint L-DFA Parainfluenza Virus/Adenovirus Identification Kit (Subject)	D ³ <i>Ultra</i> DFA Respiratory Virus Screening & ID Kit (Predicate)	D ³ <i>Duet</i> DFA RSV/Respiratory Virus Screening Kit (Predicate)
Target Viruses		
Adenovirus, HPIV-1,2,3	Flu A, Flu B, RSV, Adenovirus, HPIV-1,2,3	Flu A, Flu B, RSV, Adenovirus, HPIV-1,2,3
Monoclonal antibodies (MAbs)		
3 MAbs to Adenovirus and 6 MAbs to HPIV-1,2,3)	15 MAbs to 7 different respiratory viruses	15 MAbs to 7 different respiratory viruses

	(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 HPIV-1,2,3 using fluorescein isothiocyanate (FITC) to label Adenovirus 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		
HPIV-1,2,3	None	RSV
Fluorescein-labeled MAbs		
Adenovirus	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):

- Guidance on Informed Consent for In Vitro Diagnostic Device Studies Leftover Human Specimens that are Not Individually Identifiable (April 2006)
- Statistical Guidance on Reporting Results from Studies Evaluating Diagnostic Tests; Guidance for Industry and FDA Reviewers (March 2007)
- Format for Traditional and Abbreviated 510(k)s - Guidance for Industry and FDA Staff
- 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)

L. Test Principle:

The D³ FastPoint L-DFA Parainfluenza Virus/Adenovirus 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 (parainfluenza virus) or fluorescein (adenovirus) for the rapid identification of parainfluenza virus and adenovirus 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 permeabilized and stained concurrently in a liquid suspension format with the L-DFA Reagent. After incubating at 35°C to 37°C 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 parainfluenza virus types 1, 2 and 3 will exhibit golden yellow fluorescence due to the PE. Cells infected with adenovirus 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. Each of the reproducibility panel consisted of 5 randomized panel members.

The HPIV/Adenovirus panel consisted of the following:

- a. Low level Para 1 (C-35 strain) infected cells.
- b. Low level Adenovirus (ATCC type 1) infected cells.
- c. Low level Para 1 (C-35 strain) infected cells mixed with mid level Adenovirus (ATCC type 1) infected cells.
- d. Low Adenovirus (ATCC type 1) infected cells mixed with mid level Para 1 (C-35 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 2.5×10^5 to 3.5×10^5 total cells.

Each 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 assessed 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 D³ FastPoint L-DFA HPIV/Adenovirus Reagent, the combined data from the four Study Sites demonstrated reproducible detection of HPIV-1 by the R-PE labeled MAbs and reproducible detection of Adenovirus by the FITC-labeled MAbs. The presence of HPIV-1 infected cells was reported in 100% (120/120) of the wells in which the infected cells were expected. The presence of Adenovirus 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 100% (40/40) of the wells in which infected cells were not present. The total percent agreement for the D³ FastPoint L-DFA HPIV/Adenovirus Reagent was 100% (280/280):

HPIV/Adenovirus D³ FastPoint L-DFA Reagent

	Panel Member	Negative	HPIV-1 Low Level	Adenovirus Low Level	Mixed Infection		Mixed Infection		Total % Agreement
					HPIV-1 Mid Level	Adenovirus Low Level	HPIV-1 Low Level	Adenovirus 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	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 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	40/40 (100%)	40/40 (100%)	40/40 (100%)	40/40 (100%)	40/40 (100%)	40/40 (100%)	40/40 (100%)	280/280 (100%)
	95% CI	91.2 – 100%	91.2 – 100%	91.2 – 100%	91.2 – 100%	91.2 – 100%	91.2 – 100%	91.2 – 100%	98.7 – 100%

- 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 D³ FastPoint L-DFA HPIV/Adenovirus Identification Kit have also been used in one the following DHI devices:

1. D³ *Ultra* DFA Respiratory Virus Screening & ID Kit that was cleared for marketing via section 510(k) k061101 on November 20, 2006.
2. D³ *Duet* DFA RSV/Respiratory Virus Screening Kit that was cleared for marketing via section 510(k) k081928 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 parainfluenza virus types 1, 2 and 3 or adenovirus 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 six parainfluenza virus (2 for each parainfluenza virus types 1, 2 and 3) MAbs were labeled with PE. The individual PE-labeled MAbs were used to stain acetone-fixed parainfluenza virus types 1, 2, and 3 model cells (respectively) 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 HPIV/Adenovirus Reagent Kit MAb #	Target Virus	R-PE	FITC
1D8E10	parainfluenza 1	Reactive	Reactive
9F61C9	parainfluenza 1	Reactive	Reactive
2E4D7	parainfluenza 2	Reactive	Reactive
5E4E11	parainfluenza 2	Reactive	Reactive
1F6C8	parainfluenza 3	Reactive	Reactive
4G5(1)E2H9	parainfluenza 3	Reactive	Reactive

Reactivity of PE-labeled MAbs with Permeablized Infected Model Cells

(Model Cells are A549 cell cultures infected with known isolates of parainfluenza virus types 1, 2 and 3 or adenovirus 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 parainfluenza virus types 1, 2, and 3 model cells (respectively) 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 HPIV/Adenovirus Kit MAb #	Target Virus	R-PE	FITC
1D8E10	parainfluenza 1	Reactive	Reactive
9F61C9	parainfluenza 1	Reactive	Reactive
2E4D7	parainfluenza 2	Reactive	Reactive
5E4E11	parainfluenza 2	Reactive	Reactive
1F6C8	parainfluenza 3	Reactive	Reactive
4G5(1)E2H9	parainfluenza 3	Reactive	Reactive

Concentration of PE-labeled MAbs

Final blended solutions of the 6 parainfluenza virus MAbs were formulated to yield optimal fluorescence intensity and lowest background on their respectively 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 parainfluenza virus types 1, 2 and 3 or adenovirus 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 3 adenovirus FITC-labeled MAbs used in the D³ FastPoint L-DFA Parainfluenza Virus/Adenovirus 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 parainfluenza virus types 1, 2 and 3 or adenovirus 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 adenovirus 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 solutions of the 3 adenovirus MAbs were formulated to yield optimal fluorescence intensity and lowest background on their respectively infected cells, permeablized and stained in suspension.

Cell Permeablization and Counterstaining

Selection of Permeablization Reagent

The D³ FastPoint L-DFA Parainfluenza Virus/Adenovirus 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, 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 D³ FastPoint L-DFA Parainfluenza Virus/Adenovirus Reagent.

Determination of Sapogenin Concentration

Experiments were conducted to optimize the concentration of Sapogenin in the D³ FastPoint L-DFA Parainfluenza Virus/Adenovirus 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 D³ FastPoint L-DFA Parainfluenza Virus/Adenovirus Reagent to better ensure adequate Permeablization of clinical nasal pharyngeal cells.

Propidium Iodide Counter-stain

To assist the end user in the use of the D³ FastPoint L-DFA HPIV/Adenovirus kit, improvement to the counter-staining of cells was developed. Propidium Iodide was added to the D³ FastPoint L-DFA HPIV/Adenovirus kit. 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 each D³ FastPoint L-DFA HPIV/Adenovirus 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 HPIV/Adenovirus Reagent:

- Low level (~25 or lower infected cells) parainfluenza virus type model cells were spiked into non-infected cells.
- Low level parainfluenza viruses model cells were spiked into high level (4 +) adenovirus model cells.
- Low level (~25 or lower infected cells) adenovirus model cells were spiked into non-infected cells.
- Low level adenovirus model cells were spiked into high level (4+) parainfluenza virus model cells.

The following table summarizes the study data for the three DFA Reagents:

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 HPIV-1 model cells in non-infected cells	7, 4, 7	6.0
Low HPIV-1 model cells in High Adenovirus model cells	7, 3, 5	5.0
Low HPIV-2 model cells in non-infected cells	3, 3, 7	4.4
Low HPIV-2 model cells in High Adenovirus model cells	8, 4, 4	5.3
Low HPIV-3 model cells in non-infected cells	3, 7, 5	5.0
Low HPIV-3 model cells in High Adenovirus model cells	3, 5, 6	4.6
Low Adenovirus model cells in non-infected cells	13, 15, 9	12.3
Low Adenovirus model cells in High HPIV-1 model cells	7, 9, 8	8.0
Low Adenovirus model cells in High HPIV-2 model cells	17, 13, 12	14.0
Low Adenovirus model cells in High HPIV-3 model cells	9, 18, 23	16.6

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 three D³ FastPoint L-DFA HPIV/Adenovirus Reagent contains 2 parainfluenza virus type 1 MAbs, 2 parainfluenza virus type 2 MAbs, 2 parainfluenza virus type 3 MAbs and 3 adenovirus MAbs. The purpose of combining either two or three 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 adenovirus parainfluenza virus types 1, 2, and 3 were permeabilized. 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 study indicated that there was no evidence of self or cross epitope blocking for all the pairs of MABs.

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 each of the D³ FastPoint L-DFA HPIV/Adenovirus Reagent at 1:16 dilution. This dilution was what was used for the D³ *Ultra* Reagents. 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 September 2009, stability has been demonstrated to 10 months. Additional real-time stability studies are currently being conducted to establish a 20°C to 25°C storage shelf-life claim for all L-DFA Reagents. As of September 2009, stability has also been demonstrated to 10 months for the 20°C to 25°C storage shelf-life claim.

Shelf life for the D³ FastPoint L-DFA Respiratory Virus Identification Kit antigen control slides

The D³ FastPoint L-DFA HPIV/Adenovirus Identification Kit Antigen Control Slides are prepared by combining infected cells (parainfluenza virus and adenovirus in one well). Non-infected cells are spotted onto an additional well for a negative control for each reagent. Stability studies are currently being conducted for the D³ FastPoint L-DFA HPIV/Adenovirus 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 D³ *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 September 2009, stability has been demonstrated to 10 months. Additional real-time stability studies are currently being conducted to establish a 20°C to 25°C storage shelf-life claim for all L-DFA Reagents. As of September 2009, stability has also been demonstrated to 10 months for the 20°C to 25°C storage shelf-life claim.

d. *Analytical Sensitivity (Detection limit):*

Analytical Limit of Detections of the D³ FastPoint L-DFA HPIV/Adenovirus Kit were addressed using dilution series of infected model cells. Model cells for adenovirus (ATCC type 1) and parainfluenza virus types 1, 2, and 3 (ATCC strains C-35, Greer, and C243 respectively) 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 cell 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 this 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 4 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 Parainfluenza Virus/Adenovirus Identification Kit			
Virus Strain	Infected cells/mL	Number of replicates with positive cells	LOD determination
Adenovirus (ATCC type 1)	1000	10/10	100 infected cells/mL
	200	10/10	
	100	9/10	
	50	5/10	
	25	1/10	
	12.5	0/10	
	6	0/10	
	3	0/10	
	1.5	0/10	
	0.8	0/10	
HPIV-1 (ATCC strain C-35)	500	10/10	100 infected cells/mL
	100	10/10	
	50	6/10	
	25	2/10	
	12.5	1/10	
	6	0/10	
	3	0/10	
	1.5	0/10	
	0.8	0/10	
	0.4	0/10	
HPIV-2 (ATCC strain Greer)	500	10/10	25 infected cells/mL
	100	10/10	
	50	10/10	
	25	9/10	
	12.5	6/10	
	6	5/10	
	3	3/10	
	1.5	1/10	
	0.8	0/10	

	0.4	0/10	
HPIV-3 (ATCC strain C243)	1000	10/10	50 infected cells/mL
	200	10/10	
	100	10/10	
	50	9/10	
	25	6/10	
	12.5	2/10	
	6	0/10	
	3	0/10	
	1.5	0/10	
	0.8	0/10	

e. Analytical Reactivity (Inclusivity):

Analytical reactivity (inclusivity) of the D³ FastPoint L-DFA HPIV/Adenovirus Reagent was evaluated using 3 HPIV virus and 10 Adenovirus 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 HPIV/Adenovirus D³ FastPoint L-DFA Reagent. The following table summarizes the data:

Analytical Reactivity (inclusivity) of the D ³ FastPoint L-DFA HPIV/Adenovirus Reagent on various parainfluenza virus and adenovirus strains		
HPIV and Adenovirus Strains	Infected Cell Concentration (as multiples of the respective established LoD concentration)	D ³ FastPoint L-DFA HPIV/Adenovirus Kit Results
Parainfluenza 1 C-35	10x LoD	9 Golden-yellow fluorescent cells
Parainfluenza 2 Greer	10x LoD	11 Golden-yellow fluorescent cells
Parainfluenza 3 C-243	10x LoD	22 Golden-yellow fluorescent cells
Adenovirus 1 VR-1	10x LoD	26 Apple-green fluorescent cells
Adenovirus 3 VR-3	10x LoD	17 Apple-green fluorescent cells
Adenovirus 5 VR-5	10x LoD	15 Apple-green fluorescent cells
Adenovirus 6 VR-6	10x LoD	22 Apple-green fluorescent cells
Adenovirus 7 VR-7	10x LoD	16 Apple-green fluorescent cells
Adenovirus 8 VR-1366	10x LoD	29 Apple-green fluorescent cells
Adenovirus 10 VR-1087	10x LoD	34 Apple-green fluorescent cells
Adenovirus VR-14	10x LoD	37 Apple-green fluorescent cells
Adenovirus Dewitt ATCC Strain	10x LoD	15 Apple-green fluorescent cells
Adenovirus 31 VR-1109	10x LoD	42 Apple-green fluorescent cells

f. Analytical Specificity:

D³ FastPoint L-DFA HPIV/Adenovirus 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 D³ FastPoint L-DFA HPIV/Adenovirus 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, 1.4×10^4 to 1.4×10^5 TCID₅₀ 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 D³ FastPoint L-DFA HPIV/Adenovirus 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 HPIV/Adenovirus Identification Kit			
Organism	Strain or Type	D ³ FastPoint L-DFA HPIV/Adenovirus Reagent Results	Inoculum (TCID ₅₀)
Adenovirus	Type 1	Apple-Green Fluor.	1.4×10^4
	Type 3	Apple-Green Fluor.	1.4×10^4
	Type 5	Apple-Green Fluor.	1.4×10^4
	Type 7	Apple-Green Fluor.	1.4×10^4
	Type 10	Apple-Green Fluor.	1.4×10^4
	Type 16	Apple-Green Fluor.	1.4×10^4
	Type 17	Apple-Green Fluor.	1.4×10^4

Virus Strains Tested for Cross Reactivity with the D ³ FastPoint L-DFA HPIV/Adenovirus Identification Kit			
Organism	Strain or Type	D ³ FastPoint L-DFA HPIV/Adenovirus Reagent Results	Inoculum (TCID ₅₀)
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)	Negative	1.4 x 10 ⁴
	Mal (H1N1)	Negative	1.4 x 10 ⁴
	Hong Kong (H3N2)	Negative	1.4 x 10 ⁴
	Denver (H1N1)	Negative	1.4 x 10 ⁴
	Port Chalmers (H3N2)	Negative	1.4 x 10 ⁴
	Victoria (H3N2)	Negative	1.4 x 10 ⁴
	New Jersey (HSWN1)	Negative	1.4 x 10 ⁴
	WS (H1N1)	Negative	1.4 x 10 ⁴
	PR (H1N1)	Negative	1.4 x 10 ⁴
	Wisconsin (H3N2)	Negative	1.4 x 10 ⁴
	A/NWS/33 (H1N1)	Negative	1.4 x 10 ⁴
	A Mexico/4108/2009 (H1N1)	Negative	1.4 x 10 ⁴
	A California/07/2009 (H1N1)	Negative	1.4 x 10 ⁴
Influenza B	Hong Kong	Negative	1.4 x 10 ⁴
	Maryland	Negative	1.4 x 10 ⁴
	Mass	Negative	1.4 x 10 ⁴
	GL	Negative	1.4 x 10 ⁴
	Taiwan	Negative	1.4 x 10 ⁴
	B/Lee/40	Negative	1.4 x 10 ⁴
	Russia	Negative	1.4 x 10 ⁴
	Long	Negative	1.4 x 10 ⁴
RSV	Wash	Negative	1.4 x 10 ⁴
	9320	Negative	1.4 x 10 ⁴
Parainfluenza 1	C-35	Golden-Yellow Fluor.	1.4 x 10 ⁴
Parainfluenza 2	Greer	Golden-Yellow Fluor.	1.4 x 10 ⁴
Parainfluenza 3	C-243	Golden-Yellow Fluor.	1.4 x 10 ⁴
Parainfluenza 4	M-25	Negative	1.4 x 10 ⁵
Parainfluenza 4b	CH-19503	Negative	1.4 x 10 ⁵
HSV-1	1(f)	Negative	1.4 x 10 ⁵
	MacIntyre	Negative	1.4 x 10 ⁵
HSV-2	Clinical Isolate CWOH-0011	Negative	1.4 x 10 ⁵
	Strain G	Negative	1.4 x 10 ⁵
CMV	Towne	Negative	1.4 x 10 ⁵
	AD169	Negative	1.4 x 10 ⁵
Varicella-zoster	AV92-3	Negative	1.4 x 10 ⁵
Echovirus	4	Negative	1.4 x 10 ⁵
	6	Negative	1.4 x 10 ⁵
	7	Negative	1.4 x 10 ⁵
	22	Negative	1.4 x 10 ⁵
Coxsackievirus	A9	Negative	1.4 x 10 ⁵

Virus Strains Tested for Cross Reactivity with the D ³ FastPoint L-DFA HPIV/Adenovirus Identification Kit			
Organism	Strain or Type	D ³ FastPoint L-DFA HPIV/Adenovirus Reagent Results	Inoculum (TCID ₅₀)
	B1	Negative	1.4 x 10 ⁵
	B3	Negative	1.4 x 10 ⁵
	B4	Negative	1.4 x 10 ⁵
Coronavirus	229E	Negative	1.4 x 10 ⁵
	OC43	Negative	1.4 x 10 ⁵
Rhinovirus	209 Picornavirus	Negative	1.4 x 10 ⁵
Enterovirus 70	VR-836	Negative	1.4 x 10 ⁵
Enterovirus 71	VR-1432	Negative	1.4 x 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 x 10⁹ to 3.5 x 10¹⁰ CFUs depending on the bacterium. These suspensions of Model Cells with bacteria were then processed according to the D³ FastPoint L-DFA HPIV/Adenovirus Identification Kit protocol, using 2X MAb reagents. Except for *Staphylococcus aureus*, which was cross reactive with the D³ FastPoint L-DFA HPIV/Adenovirus 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 HPIV/Adenovirus Identification Kit		
Organism	D ³ FastPoint L-DFA HPIV/Adenovirus Kit Results	CFU tested
Bacteria		
<i>Acholeplasma laidlawii</i>	Negative	Control Slide
<i>Acinetobacter calcoaceticus</i>	Negative	3.6 x 10 ⁹
<i>Bordetella bronchiseptica</i>	Negative	1.1 x 10 ¹⁰
<i>Bordetella pertussis</i>	Negative	4.3 x 10 ⁹
<i>Chlamydia trachomatis</i> (Apache-2)	Negative	LGV-II/Control Slide
<i>Corynebacterium diphtheriae</i>	Negative	5.7 x 10 ⁷
<i>Escherichia coli</i>	Negative	7.5 x 10 ⁸
<i>Gardnerella vaginalis</i>	Negative	Control Slide
<i>Haemophilis influenzae</i> type A	Negative	4.1 x 10 ⁹
<i>Klebsiella pneumoniae</i>	Negative	1.2 x 10 ⁹
<i>Moraxella cartarrhalis</i>	Negative	1.2 x 10 ¹⁰
<i>Mycoplasma hominis</i>	Negative	3.5 x 10 ¹⁰
<i>Mycoplasma orale</i>	Negative	6.6 x 10 ⁹
<i>Mycoplasma pneumoniae</i>	Negative	7.9 x 10 ⁹
<i>Mycoplasma salivarium</i>	Negative	7.7 x 10 ⁸
<i>Proteus mirabilis</i>	Negative	3.6 x 10 ⁹

<i>Pseudomonas aeruginosa</i>	Negative	1.0 x 10 ⁸
<i>Salmonella enteriditis</i>	Negative	8.7 x 10 ⁹
<i>Salmonella typhimurium</i>	Negative	7.5 x 10 ⁹
<i>Staphylococcus aureus</i> *	Positive	6.3 x 10 ⁹
<i>Streptococcus agalactiae</i>	Negative	5.5 x 10 ⁸
<i>Streptococcus pneumoniae</i>	Negative	6.7 x 10 ⁹
<i>Streptococcus pyogenes</i>	Negative	6.9 x 10 ⁹
Yeast		
<i>Candida glabrata</i>	Negative	1.6 x 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 D³ FastPoint L-DFA HPIV/Adenovirus Identification Kit testing direct respiratory specimens were established during prospective studies at 4 geographically diverse U.S. clinical laboratories during the 2009 respiratory virus season (January 2009 – 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 D³ FastPoint L-DFA HPIV/Adenovirus Identification Kit detecting parainfluenza virus and adenovirus from direct specimens was assessed

and compared to DSFA testing using FDA cleared comparator DSFA devices (D³ *Ultra* DFA Respiratory Virus Screening & ID Kit or D³ *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. “True” parainfluenza virus or adenovirus positive was defined as any sample that either tested positive by the comparator DSFA device or viral culture. “True” parainfluenza virus or adenovirus negative was defined as any sample that tested negative by both the comparator DSFA test and viral culture.

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. Seventy-five (75) specimens for adenovirus and 79 specimens for HPIV were excluded from the respective performance analysis due to insufficient sample volume for the comparator methods, resulting in a total of 246 fresh nasal wash/nasopharyngeal aspirate specimens for adenovirus and 242 fresh nasal wash/nasopharyngeal aspirate specimens for HPIV to be included in the respective performance analysis. The tables below summarized the study results of the claimed specimen type at **study site 1**:

Adenovirus			
Fresh nasal/nasopharyngeal wash/aspirate	Comparator DSFA (negatives followed by culture with DFA)		
DHI DSFA	Positive	Negative	Total
Positive	8	0	8
Negative	0	238	238
Total	8	238	246
			95% CI
Sensitivity	8/8	100.0%	63.1-100%
Specificity	238/238	100.0%	98.5-100%

HPIV			
Fresh nasal/nasopharyngeal wash/aspirate	Comparator DSFA (negatives followed by culture with DFA)		
DHI DSFA	Positive	Negative	Total
Positive	6	4	10
Negative	0	232	232
Total	6	236	242
			95% CI
Sensitivity	6/6	100.0%	54.1-100%
Specificity	232/236	98.3%	95.7-99.5%

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 D³ FastPoint L-DFA HPIV/Adenovirus Identification Kit, 19 other types of respiratory specimens were removed from performance analysis. None of the nasal wash/nasopharyngeal aspirate samples for Adenovirus and HPIV were excluded from the respective performance analysis due to insufficient sample volume for the comparator methods, resulting in a total of 86 fresh nasal wash/nasopharyngeal aspirate specimens for Adenovirus and HPIV to be included in the respective performance analysis. The tables below summarized the study results of the claimed specimen type at **study site 2**:

Adenovirus			
Fresh nasal/nasopharyngeal wash/aspirate	Comparator DSFA (negatives followed by culture with DFA)		
DHI DSFA	Positive	Negative	Total
Positive	0	0	0
Negative	0	86	86
Total	0	86	86
			95% CI
Sensitivity	0/0	NA	NA
Specificity	86/86	100.00%	95.8-100%

HPIV			
Fresh nasal/nasopharyngeal wash/aspirate	Comparator DSFA (negatives followed by culture with DFA)		
DHI DSFA	Positive	Negative	Total
Positive	1	0	1
Negative	0	85	85
Total	1	85	86
			95% CI
Sensitivity	1/1	100.0%	NA
Specificity	85/85	100.0%	95.8-100%

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 D³ FastPoint L-DFA HPIV/Adenovirus Identification Kit, 2 other types of respiratory specimens were further removed from performance analysis. None of the remaining nasal wash/nasopharyngeal aspirate samples for Adenovirus and HPIV were excluded from the respective performance analysis due to insufficient sample volume for the comparator methods, resulting in a total of 300 fresh nasal wash/nasopharyngeal aspirate specimens for Adenovirus and HPIV to be included in the respective performance analysis. None of the nasal/nasopharyngeal swab specimens for Adenovirus and HPIV were 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 Adenovirus and HPIV to be included in the respective performance analysis. The tables below summarized the study results of the claimed specimen types at **study site 3**:

Adenovirus			
Fresh nasal/nasopharyngeal wash/aspirate	Predicate DSFA (negatives followed by culture with DFA)		
DHI DSFA	Positive	Negative	Total
Positive	4	0	4
Negative	1	295	296
Total	5	295	300
			95% CI
Sensitivity	4/5	80.0%	28.4-99.5%
Specificity	295/295	100.0%	98.8-100%

HPIV			
Fresh nasal/nasopharyngeal wash/aspirate	Predicate DSFA (negatives followed by culture with DFA)		
DHI DSFA	Positive	Negative	Total
Positive	16	0	16
Negative	2	282	284
Total	18	282	300
			95% CI
Sensitivity	16/18	88.9%	65.3-98.6%
Specificity	282/282	100.0%	98.7-100%

Adenovirus			
Fresh nasal/nasopharyngeal swab	Predicate DSFA (negatives followed by culture with DFA)		
DHI DSFA	Positive	Negative	Total
Positive	0	0	0
Negative	0	140	140
Total	0	140	140
			95% CI
Sensitivity	0/0	NA	NA
Specificity	140/140	100.0%	97.4-100%

HPIV			
Fresh nasal/nasopharyngeal swab	Predicate DSFA (negatives followed by culture with DFA)		
DHI DSFA	Positive	Negative	Total
Positive	7	0	7
Negative	0	133	133
Total	7	133	140
			95% CI
Sensitivity	7/7	100.0%	59.0-100%
Specificity	133/133	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. One hundred and two (102) samples for adenovirus and 101 samples for HPIV were excluded from the respective performance analysis due to insufficient sample volume for the comparator methods, resulting in a total of 541 fresh nasal wash/nasopharyngeal aspirate specimens for adenovirus and 542 fresh nasal wash/nasopharyngeal aspirate specimens for HPIV to be included in the respective performance analysis. The tables below summarized the study results of the claimed specimen type at **study site 4**:

Adenovirus			
Fresh nasal/nasopharyngeal swab	Predicate DSFA (negatives followed by culture with DFA)		
DHI DSFA	Positive	Negative	Total
Positive	1	0	1
Negative	0	540	540
Total	1	540	541
			95% CI
Sensitivity	1/1	100.0%	NA
Specificity	540/540	100.0%	99.3-100%

HPIV			
Fresh nasal/nasopharyngeal swab	Predicate DSFA (negatives followed by culture with DFA)		
DHI DSFA	Positive	Negative	Total
Positive	6	0	6
Negative	1	535	536
Total	7	535	542
			95% CI
Sensitivity	6/7	85.7%	42.1-99.6%
Specificity	535/535	100.0%	99.3-100%

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

Adenovirus			
Fresh nasal/nasopharyngeal wash/aspirate	Predicate DSFA (negatives followed by culture with DFA)		
DHI DSFA	Positive	Negative	Total
Positive	12	0	12
Negative	1	619	620
Total	13	619	632
			95% CI
Sensitivity	12/13	92.3%	64.0-99.8%
Specificity	619/619	100.0%	99.4-100%

HPIV			
Fresh nasal/nasopharyngeal wash/aspirate	Predicate DSFA (negatives followed by culture with DFA)		
DHI DSFA	Positive	Negative	Total
Positive	23	4	27
Negative	2	599	601
Total	25	603	628
			95% CI
Sensitivity	23/25	92.0%	74.0-99.0%
Specificity	599/603	99.3%	98.3-99.8%

Adenovirus			
Fresh nasal/nasopharyngeal swab	Predicate DSFA (negatives followed by culture with DFA)		
DHI DSFA	Positive	Negative	Total
Positive	1	0	1
Negative	0	680	680
Total	1	680	681
			95% CI
Sensitivity	1/1	100.0%	NA
Specificity	680/680	100.0%	99.5-100%

HPIV			
Fresh nasal/nasopharyngeal swab	Predicate DSFA (negatives followed by culture with DFA)		
DHI DSFA	Positive	Negative	Total
Positive	13	0	13
Negative	1	668	669
Total	14	668	682
			95% CI
Sensitivity	13/14	92.9%	66.1-99.8%
Specificity	668/668	100.0%	99.4-100%

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 D³ FastPoint L-DFA HPIV/Adenovirus Identification Kit multicenter prospective clinical study testing direct respiratory specimens, a total of 1519 eligible respiratory specimens were tested using the D³ FastPoint L-DFA HPIV/Adenovirus Identification Kit from four U.S. clinical laboratories across the United States during the 2009 respiratory virus season (January 2009 – March 2009). Prevalence for Adenovirus and HPIV as determined by the D³ FastPoint L-DFA HPIV/Adenovirus Identification Kit direct specimen testing varied from

varied from 0% to 2.5% by site and averaged 0.9% for Adenovirus; and varied from 0.9% to 5.2% by site and averaged 2.6% for HPIV. The number and percentage of positive cases determined by the D³ FastPoint L-DFA HPIV/Adenovirus Identification Kit direct specimen testing, calculated by age group, are presented in the following tables:

All Sites Combined

Age	Total Specimens Evaluated	Adenovirus	HPIV
		# positive (prevalence)	# positive (prevalence)
0 – 1 month	55	0	1 (1.8%)
> 1 month to 2 years	577	11 (1.9%)	29 (5.0%)
> 2 years to 12 years	391	1 (0.3%)	6 (1.5%)
> 12 years to 21 years	173	0	2 (1.2%)
22 years to 30 years	57	0	1 (1.8%)
31 years to 40 years	71	0	0
41 years to 50 years	52	0	0
51 years to 60 years	46	0	0
61 years to 70 years	33	0	0
71 years to 80 years	16	0	0
81 years and above	7	0	1 (14.3%)
Age Not Reported	41	1 (2.4%)	0
Total	1519	13 (0.9%)	40 (2.6%)
*There were 2- adenovirus + RSV, 2- influenza A + hMPV, 1-RSV + adenovirus and 1-RSV + parainfluenza virus co-infections detected			

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.