510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

K093233

B. Purpose for Submission:

This is a new 510(k) application for the Diagnostic Hybrids, Inc. device, D^3 FastPoint L-DFA RSV/MPV Identification Kit, which is intended for the qualitative identification of respiratory syncytial virus and human metapneumovirus 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 (respiratory syncytial virus and human metapneumovirus)

D. Type of Test:

Direct Fluorescence Antibody (DFA) test using direct specimens

E. Applicant:

Diagnostic Hybrids Incorporated

F. Proprietary and Established Names:

D^3 FastPoint L-DFA RSV/MPV Identification Kit

G. Regulatory Information:

1. Regulation section:

   866.3980

2. Classification:

   Class II

3. Product codes:

   OMG, LKT

4. Panel:

   Microbiology (83)

H. Intended Use:

1. Intended use(s):

   The Diagnostic Hybrids, Inc. device, D^3 FastPoint L-DFA RSV/MPV Identification Kit is intended for the qualitative identification of respiratory syncytial virus and human metapneumovirus 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 respiratory syncytial virus after examination of the direct specimen result be confirmed by cell culture. Specimens found to be negative for human metapneumovirus after examination of the direct specimen results should be confirmed by an FDA-cleared human metapneumovirus molecular assay. Negative results do not preclude respiratory syncytial virus and human metapneumovirus 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 D^3 FastPoint L-DFA RSV/MPV 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 (respiratory syncytial virus) or fluorescein (human metapneumovirus) for the rapid identification of respiratory syncytial virus and human metapneumovirus 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 RSV/MPV Reagent**, 4.0-mL. One dropper bottle containing a mixture of PE-labeled murine monoclonal antibodies directed against respiratory syncytial virus antigens and FITC-labeled murine monoclonal antibodies directed against human metapneumovirus 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 RSV/MPV 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 respiratory syncytial virus, or human metapneumovirus. The negative wells contain non-infected cells. Each slide is intended to be stained only one time.
5. **D^3 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 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 1 X 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 respiratory syncytial virus (RSV) will exhibit golden yellow fluorescence due to the PE. Cells infected with human metapneumovirus (hMPV) 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. D^3 FastPoint L-DFA RSV/MPV Reagent
2. Re-suspension Buffer
3. D^3 FastPoint L-DFA RSV/MPV Antigen Control Slides
4. 40X PBS Concentrate
5. D^3 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 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 RSV/Respiratory Virus Screening Kit
   Diagnostic Hybrids, Inc. D^3 DFA MPV Identification Kit

2. Predicate k number(s):

   (k061101), (k081928), (k090073)

3. Comparison with predicates:

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

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

3 MAbs to hMPV	different respiratory viruses (Flu A, Flu B, RSV, Adenovirus, HPIV-1,2,3)	respiratory viruses (Flu A, Flu B, RSV, Adenovirus, HPIV-1,2,3)	hMPV
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			
RSV	None	RSV	None
Fluorescein-labeled MAbs			
hMPV	Flu A, Flu B, RSV, Adenovirus and HPIV 1,2,3	Flu A, Flu B, Adenovirus, and HPIV-1,2,3	hMPV
Cell Fixative			
Sapogenin	Acetone	Acetone	Acetone
Cell Counter-stain			
Propidium Iodide and Evans Blue	Evans Blue	Evans Blue	Evans Blue

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

- Class II Special Controls Guidance Document: Testing for Human Metapneumovirus (hMPV) Using Nucleic Acid Assays (October 2009)
- 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 D^3 FastPoint L-DFA RSV/MPV 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 (respiratory syncytial virus) or fluorescein (human metapneumovirus) for the rapid identification of respiratory syncytial virus and human metapneumovirus 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 1 X 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 respiratory syncytial virus (RSV) will exhibit golden yellow fluorescence due to the PE. Cells infected with human metapneumovirus (hMPV) 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 specimens found to be negative for respiratory syncytial virus after examination of the direct specimen result be confirmed by cell culture. Specimens found to be negative for human metapneumovirus after examination of the direct specimen results should be confirmed by an FDA-cleared human metapneumovirus molecular assay.

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 RSV/hMPV panel consisted of the following:

   a. Low level RSV (Washington strain) infected cells.
   b. Low level hMPV (A1 subtype) infected cells.
   c. Low level RSV (Washington strain) infected cells mixed with mid level hMPV (A1 subtype) infected cells.
   d. Low level hMPV (A1 subtype) infected cells mixed with mid level RSV (Washington 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 RTSV/MPV Reagent, the combined data from the four Study Sites demonstrated reproducible detection of RSV by the R-PE labeled MAbs and reproducible detection of hMPV by the FITC-labeled MAbs. The presence of RSV infected cells was reported in 100% (120/120) of the wells in which the infected cells were expected. The presence of hMPV 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 D^3 FastPoint L-DFA RSV/MPV Reagent was 100% (280/280):

D^3 FastPoint L-DFA RSV/MPV Reagent

	Panel Member	Negative	RSV Low Level	hMPV Low Level	Mixed Infection		Mixed Infection		Total % Agreement
					RSV Mid Level	hMPV Low Level	RSV Low Level	hMPV 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 D^3 FastPoint L-DFA RSV/MPV 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 RSV/Respiratory Virus Screening Kit that was cleared for marketing via section 510(k) k081928 on December 23, 2008.
3. D^3 DFA MPV Identification Kit that was cleared for marketing via section 510(k) k090073 on March 6, 2009.

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 RSV or hMPV, 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 RSV MAbs were labeled with PE. The individual PE-labeled MAbs were used to stain acetone-fixed RSV 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 RSV/MPV Reagent Kit MAb #	**Target Virus**	**R-PE**	**FITC**
3A4D9	RSV	Reactive	Reactive
4F9G3	RSV	Reactive	Reactive

Reactivity of PE-labeled MAbs with Permeablized Infected Model Cells
(Model Cells are A549 cell cultures infected with known isolates of RSV or hMPV, 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 RSV 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**
3A4D9	RSV	Reactive	Reactive
4F9G3	RSV	Reactive	Reactive

Concentration of PE-labeled MAbs

Final blended solution of the 2 RSV 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 RSV or hMPV 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 hMPV FITC-labeled MAbs used in the D^3 FastPoint L-DFA RSV/MPV 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 RSV or hMPV 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 hMPV 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 3 hMPV 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 RSV/MPV 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 RSV/MPV kit.

Determination of Sapogenin Concentration

Experiments were conducted to optimize the concentration of Sapogenin in the D^3 FastPoint L-DFA RSV/MPV 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 RSV/MPV kit 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 RSV/MPV kit, improvement to the counter-staining of cells was developed. Propidium Iodide was added to the D^3 FastPoint L-DFA RSV/MPV 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 RSV/MPV kit 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 RSV/ MPV Reagent:

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

The following table summarizes the study data for the D^3 FastPoint L-DFA RSV/MPV kit:

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 RSV model cells in non-infected cells	28,12,12	17.3
Low RSV in High hMPV model cells	14,12,8	11.3
Low hMPV virus model cells in non-infected cells	5,1,5	3.6
Low hMPV virus in High RSV model cells	4,0,3	2.3

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 D^3 FastPoint L-DFA RSV/MPV kit contains 2 RSV MAbs and 3 hMPV MAbs. The purpose of combining 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 RSV and hMPV 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 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 D^3 FastPoint L-DFA RSV/MPV Kit Reagents 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 RSV/MPV Reagents.

Shelf life for the D^3 FastPoint L-DFA RSV/MPV Kit antigen control slides

The D^3 FastPoint L-DFA RSV/MPV Identification Kit Antigen Control Slides are prepared by combining infected cells (RSV and hMPV 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 RSV/MPV 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. 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 August 2009, stability has also been demonstrated to 9 months for the 20°C to 25°C storage shelf-life claim.

d. Analytical Sensitivity (Detection limit):

Analytical Limit of Detections of the D^3 FastPoint L-DFA RSV/MPV Kit was addressed using dilution series of infected model cells. Model cells for RSV (ATCC Washington strain) and hMPV A1 (clinical 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 D^3 FastPoint L-DFA Influenza A/Influenza B Virus Identification Kit			
Virus Strain	Infected cells/mL	Number of replicates with positive cells	LOD determination
RSV (ATCC Washington strain)	1000	10/10	100 infected cells/mL
	200	10/10	
	100	10/10	
	50	7/10	
	25	7/10	
	12.5	6/10	
	6	1/10	
	3	0/10	
	1.5	0/10	
	0.8	0/10	
hMPV A1 (Clinical strain)	2000	10/10	100 infected cells/mL
	400	10/10	
	200	10/10	
	100	10/10	
	50	6/10	
	25	2/10	
	12.5	0/10	
	6	0/10	
	3	0/10	
	1.5	0/10	

a. Analytical Reactivity (Inclusivity):

Analytical reactivity (inclusivity) of the D^3 FastPoint L-DFA RSV/MPV Kit was evaluated using 3 RSV and 4 hMPV 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 D^3 FastPoint L-DFA RSV/MPV Kit. The following table summarizes the data:

Analytical Reactivity (inclusivity) of the D^3 FastPoint L-DFA RSV/MPV Kit on various RSV and hMPV strains		
RSV and hMPV Strains	**Infected Cell Concentration (as multiples of the respective established LoD concentration**	**D^3 FastPoint L-DFA RSV/MPV Kit Results**
RSV 9320	10x LoD	22 Golden-yellow fluorescent cells
RSV Washington	10x LoD	22 Golden-yellow fluorescent cells
RSV Long	10x LoD	32 Golden-yellow fluorescent cells
hMPV A1	10x LoD	25 Apple-green fluorescent cells
hMPV A2	10x LoD	25 Apple-green fluorescent cells
hMPV B1	10x LoD	25 Apple-green fluorescent cells
hMPV B2	10x LoD	37 Apple-green fluorescent cells

f. Analytical Specificity:

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

Virus Strains Tested for Cross Reactivity with the D^3 FastPoint L-DFA RSV/MPV 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 RSV/MPV Identification Kit protocol, using 2X MAb reagents. Except for *Staphylococcus aureus*, which was cross reactive with the D^3 FastPoint L-DFA RSV/MPV 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 RSV/MPV Identification Kit		
Organism	D^3 FastPoint L-DFA RSV/MPV Kit 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.

i. Comparator Assay Description and Analytical Validation Studies

At the time of the DHI the D^3 FastPoint L-DFA RSV/MPV Identification Kit clinical studies, there were no standard or FDA cleared molecular methods to

detect hMPV in nasopharyngeal aspirates or washes from patients with signs and symptoms of acute respiratory infection. For purposes of the clinical studies, Diagnostic Hybrids, Inc (DHI) established and validated a real-time Reverse Transcriptase (RT-PCR) procedure for use as a comparative test method to detect hMPV RNA in a clinical specimen. This real time RT-PCR assay was developed by DHI using a primer set and TaqMan probe as described in the publication: Maertzdorf, J., CK Wang, JB Brown, JD Quinto, M Chu, M de Graff, BG van den Hoogen, R Spaete, ADME Osterhaus, and RAM Fouchier. "Real-time Reverse Transcriptase PCR Assay for Detection of Human Metapneumoviruses from All Known Lineages" J. Clin. Microbiol. 2004:42:981-986. A nucleic acid (RNA) extraction procedure was also developed and validated, as was the RT-PCR reaction, including primer sets, and gel electrophoresis. Detection of an amplicon in the PCR end-product is presumptive evidence of hMPV RNA presence in a specimen. The amplicons were sequenced by a third party (Agencourt). DHI then compared these sequences against an established/published set of hMPV sequences. Confirmation of the hMPV RNA sequences in the amplicon was interpreted as verification of hMPV RNA in the specimen.

Extraction of RNA from specimens is performed with Qiagen QIAamp Viral RNA Mini Kit according to the manufacturer's suggested protocol (section 12.4). Briefly, the sample is lysed under highly denaturing conditions to inactivate RNase. RNA is then bound to the QIAamp membrane, washed several times to remove contaminants, and eluted in 60 uL RNase-free buffer. The Human Metapneumovirus Real-Time Reverse Transcription PCR Comparator Assay master mix contains oligonucleotide primers and target specific oligonucleotide probes. The primers are complementary to highly conserved sequences within the nucleocapsid gene of hMPV. Reverse transcription of the RNA in the sample into complementary DNA (cDNA) and subsequent amplification of DNA is performed in a Stratagene Mx3000p instrument. In this process, the probe anneals specifically to the template followed by primer extension and amplification. The hMPV real-time RT-PCR Comparator Assay is based on TaqMan chemistry, which utilizes the 5' - 3' exonuclease activity of the Taq polymerase to cleave the probe thus separating the reporter dye from the quencher. This generates an increase in fluorescent signal upon excitation from a light source. With each cycle, additional reporter dye molecules are cleaved from their respective probes, further increasing fluorescent signal. The amount of fluorescence at any given cycle is dependent on the amount of amplification products present at that time. Fluorescent intensity is monitored during each PCR cycle by the Stratagene Mx3000p instrument.

Analytical Sensitivity

The analytical sensitivity of the hMPV real-time RT-PCR/Sequencing Comparator Assay was determined for 4 hMPV strains (subtypes A1, A2, B1 and B2). Viral stocks of 4 hMPV strains (subtypes A1, A2, B1 and B2) were generated in LLC-MK_2 cultured cells. The viral stocks were frozen at -70° C.

The $TCID_{50}$/mL of each stock was determined by cell culture with monoclonal antibody detection. A nasopharyngeal clinical matrix was created from nasopharyngeal swabs placed in transport medium obtained from patients with signs and symptoms of acute respiratory infection. The matrix was tested for the presence of hMPV RNA using the hMPV real-time RT-PCR Comparator Assay. Patient specimens found to be negative were pooled to create sufficient material for all of the dilutions. The quantified viral stocks were diluted with the pooled nasopharyngeal clinical matrix to produce a suspension equivalent to the following $TCID_{50}$/mL levels: 1000, 500, 250, 100, 50, 10, 1, and 0.1. Each $TCID_{50}$/mL level dilution was extracted 10 times (80 per hMPV strain) using the Qiagen Viral RNA Mini Kit. The hMPV real-time RT-PCR/Sequencing Comparator Assay was performed in singlet using 20-µL volumes of each extraction. Analytical detection limits for each of the 4 hMPV genetic sublineages were defined as the lowest dilutions at which at least 9 out of 10 replicates were detected. Detailed analytical sensitivity data for the hMPV real-time RT-PCR/Sequencing Comparator Assay are presented in the following table:

hMPV real-time RT-PCR/Sequencing Comparator Assay Analytical Sensitivity Summary

Viral Strain	Conc $TCID_{50}$/mL	Average C_T	Standard Deviation C_T	Min C_T	Max C_T	Replicates Detected
hMPV A1	1000	33.28	0.26	32.99	33.81	10/10
	500	34.30	0.51	33.46	35.29	10/10
	250	35.25	0.51	34.24	36.18	10/10
	100	37.69	0.87	36.26	39.43	10/10
	50	38.48	0.77	37.6	39.94	9/10
	10	39.5	N/A	39.5	39.5	1/10
	1	N/A	N/A	N/A	N/A	0/10
	0.1	N/A	N/A	N/A	N/A	0/10
hMPV A2	1000	29.67	0.35	32.06	33.17	10/10
	500	30.88	0.57	33.36	35.28	10/10
	250	32.16	0.67	33.98	36.47	10/10
	100	33.30	0.52	35.52	37.38	10/10
	50	34.67	0.59	37.33	39.08	10/10
	10	35.11	0.95	38.51	41.43	7/10
	1	40.96	N/A	40.96	40.96	1/10
	0.1	N/A	N/A	N/A	N/A	0/10
hMPV B1	1000	31.39	0.54	30.90	32.85	10/10
	500	32.22	0.21	31.79	32.53	10/10
	250	32.85	.047	32.07	33.59	10/10
	100	34.37	0.32	33.67	34.77	10/10
	50	35.10	0.53	34.42	35.85	10/10
	10	37.82	1.37	35.51	40.07	9/10
	1	37.25	1.11	36.46	38.03	2/10
	0.1	N/A	N/A	N/A	N/A	0/10

hMPV real-time RT-PCR/Sequencing Comparator Assay Analytical Sensitivity Summary						
Viral Strain	**Conc $TCID_{50}$/mL**	**Average C_T**	**Standard Deviation C_T**	**Min C_T**	**Max C_T**	**Replicates Detected**
hMPV B2	1000	32.96	0.28	32.49	33.34	10/10
	500	35.37	1.08	33.37	37.18	10/10
	250	34.13	0.53	32.8	34.66	10/10
	100	36.8	1.39	36.0	40.63	10/10
	50	36.8	1.16	34.85	38.36	10/10
	10	38.7	1.06	37.2	40.32	6/10
	1	39.46	N/A	39.46	39.46	1/10
	0.1	N/A	N/A	N/A	N/A	0/10

Analytical Specificity

The specificity of the hMPV real-time RT-PCR Comparator Assay was evaluated by analyzing nucleic acid extracts of Diagnostic Hybrids, Inc. Respiratory Viruses Panel (Lot 111406), human gDNA (Biochain, Lot A804328), human total RNA (Lot 11140405, Catalog # 540017-41) and the Negative Clinical Specimen. 140 uL of each sample was extracted with the Qiagen QIAamp Viral RNA Mini Kit, and tested by the DHI hMPV RT-PCR assay.

hMPV real-time RT-PCR Comparator Assay demonstrated 100% specificity and data is presented in the follow table:

hMPV real-time RT-PCR Comparator Assay Analytical Specificity Summary	
Panel Key	**hMPV real-time RT-PCR Comparator Assay Result**
Negative	-
Negative	-
Influenza B B/Taiwan/2/62	-
RSV RSV/B/Wash/18537/62	-
Parainfluenza 2 Greer	-
Negative	-
RSV Long	-
Influenza A A/WS/33	-
Adenovirus Type 1, Adenoid 71	-
Negative	-
RSV 9320	-
Influenza A A/Mal/302/54	-
Influenza A A/Victoria/3/75	-
Negative	-
Parainfluenza 1 C-35	-
Parainfluenza 3 C234	-

hMPV real-time RT-PCR Comparator Assay Analytical Specificity Summary	
Panel Key	**hMPV real-time RT-PCR Comparator Assay Result**
Negative	-
Negative	-
Adenovirus Type 5, Adenoid 75	-
Influenza B B/GL/1734/54	-
Negative	-
Negative	-
Negative	-
Influenza A A/PortChalmers/1/73	-
Human gDNA	-
Human Total RNA	-
Negative Clinical Specimen	-
In-house Positive hMPV Strain	+

Extraction Efficiency

The QIAamp Viral RNA Mini Kit extraction efficiency was determined by extracting serial 10-fold dilutions of cRNA and comparing the actual copies of cRNA in the eluate versus the calculated copies if 100% recovery. The actual copies of cRNA were obtained from the qRT-PCR standard curve constructed by a serial dilution of un-extracted cRNA standards. The extraction efficiency ranges from 25 to 40%. The range was obtained from triplicate extractions repeated over 3 days. Duplicate PCR reactions were performed on each dilution. Detailed results of this study are presented in the following table:

Pre-extraction viral load (copies/mL)	% of Recovery		
	Day 1	Day 2	Day 3
8.58E+08	3.36E+01	2.50E+01	5.72E+01
8.58E+07	3.94E+01	2.35E+01	1.60E+01
8.58E+06	2.74E+01	1.98E+01	4.29E+01
8.58E+05	2.91E+01	2.55E+01	5.17E+01
8.58E+04	2.60E+01	3.36E+01	3.81E+01
8.58E+03	1.69E+01	3.76E+01	2.73E+01
4.19E+03	4.45E+01	1.19E+01	1.80E+01

Sample Freeze and Thaw Study

A study was performed to demonstrate that freezing the specimens would not alter the performance of the hMPV real-time RT-PCR Comparator Assay. Characterized hMPV isolates (subtypes A1, A2, B1, and B2) were spiked separately into fresh nasopharyngeal swab and nasopharyngeal aspirate specimens at known concentrations. The specimens were split into two aliquots:

one was extracted and tested using the hMPV real-time RT-PCR Comparator Assay, the other was frozen at -70°C overnight and then extracted and tested using the hMPV real-time RT-PCR Comparator Assay.

An amplicon from each specimen was analyzed by bi-directional sequencing and confirmed as hMPV (highlighted in the table below).

	A1											
	NPS						NW					
	Fresh			Frozen			Fresh			Frozen		
TCID50 Concentration	Rep. 1	Rep. 2	Rep. 3	Rep. 1	Rep. 2	Rep. 3	Rep. 1	Rep. 2	Rep. 3	Rep. 1	Rep. 2	Rep. 3
1.00E+02	35.46	36.23	35.25	34.85	35.07	35.02	37.76	41.04	38.19	36.27	37.07	37.15
1.00E+01	39.16	40.29	38.66	38.51	36	38.79	40.12	40.38		39.1	42.98	
1.00E+00												
1.00E-01												
	A2											
	NPS						NW					
	Fresh			Frozen			Fresh			Frozen		
TCID50 Concentration	Rep. 1	Rep. 2	Rep. 3	Rep. 1	Rep. 2	Rep. 3	Rep. 1	Rep. 2	Rep. 3	Rep. 1	Rep. 2	Rep. 3
1.00E+02	32.89	32.66	32.77	32.84	33.21	32.98	34.36	34.77	34.62	35.44	34.39	34.53
1.00E+01	36.13	36.55	37.17	36.44	37.11	35.77	38.3	37.89	38.11	37.32	38	38.57
1.00E+00	39.08	39.68	40.37	39.81	40.5	39.84	41.02	41.54		40.72	42.81	
1.00E-01				39.17			41.09					
	B1											
	NPS						NW					
	Fresh			Frozen			Fresh			Frozen		
TCID50 Concentration	Rep. 1	Rep. 2	Rep. 3	Rep. 1	Rep. 2	Rep. 3	Rep. 1	Rep. 2	Rep. 3	Rep. 1	Rep. 2	Rep. 3
1.00E+02	31.23	31.64	32.1	30.51	31.24	31.51	33.96	33.83	34.26	33.16	33.19	33.04
1.00E+01	35.86	35.63	36.04	34.83	34.44	34.55	36.15	36.5	36.52	36.16	35.66	36.12
1.00E+00	38.59	39.38	39.42	36.59	36.67	36.44	40.09	39.62	41.59	41.06	40.51	42.39
1.00E-01	43.18	40.66	41.46		39.59	43.62						
	B2											
	NPS						NW					
	Fresh			Frozen			Fresh			Frozen		
TCID50 Concentration	Rep. 1	Rep. 2	Rep. 3	Rep. 1	Rep. 2	Rep. 3	Rep. 1	Rep. 2	Rep. 3	Rep. 1	Rep. 2	Rep. 3
1.00E+02	32.17	32.82	32.07	34.12	34.5	34.22	35.14	35.02	34.87	35.59	35.6	35.97
1.00E+01	36.51	36.8	37.15	37.52	39.73	39.26	36	36.67	36.81	37.65	37.77	37.77
1.00E+00	37.15	37.57	38.47	41.3						41.72		
1.00E-01	43.6							40.38				

The data indicates that freezing and thawing nasopharyngeal swab and nasopharyngeal aspirate specimens does not alter the performance of the hMPV real-time RT-PCR Comparator Assay significantly.

Based on thorough analytical validations, the hMPV real-time RT-PCR Comparator Assay is an acceptable method to be used to confirm all hMPV negative samples as determined by the FDA cleared comparator DSFA device (D3 Metapneumovirus DFA Reagent) in the DHI D^3 FastPoint L-DFA RSV/MPV Identification Kit Clinical Trial.

j. Bridging Study Comparing the Use of a Disposable Transfer Pipette to an Adjustable Pipette

A limited bridging study of 20-specimens was performed comparing the use of a disposable transfer pipette to an adjustable pipette. The specimens were split and processed concurrently using both a disposable transfer pipette and an adjustable pipette. The number of cells present for interpretation was equivalent for both pipettes. Each pipette yielded sufficient cells to meet the requirement for interpretation (a minimum of 20 columnar epithelia cells).

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 RSV/MPV 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 D^3 FastPoint L-DFA RSV/MPV Kit detecting respiratory syncytial virus 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. "True" RSV positive was defined as any sample that either tested positive by the comparator DSFA device or viral culture. "True" RSV negative was defined as any sample that tested negative by both the comparator DSFA test and viral culture.

Performance of the D^3 FastPoint L-DFA RSV/MPV Kit detecting human metapneumovirus from direct specimens was assessed and compared to DSFA testing using FDA cleared comparator DSFA device (D^3 Metapneumovirus DFA Reagent), followed by confirmation of all frozen negative specimens (as determined by the FDA cleared DSFA comparator device), using a validated hMPV real-time RT-PCR followed by bi-directional sequencing analysis comparator assay. The hMPV real-time RT-PCR comparator assay targets the hMPV Nucleocapsid gene. "True" hMPV positive was defined as any sample that either tested positive by the FDA cleared DSFA comparator device, or had bi-directional sequencing data meeting pre-defined quality acceptance criteria that matched hMPV sequences deposited in the National Center for Biotechnology Information (NCBI) GenBank database (www.ncbi.nlm.nih.gov), with acceptable E-values. "True" hMPV negative was defined as any sample that tested negative by both the FDA cleared DSFA comparator device and the hMPV real-time RT-PCR comparator assay.

The E-values generated from the clinical trials range from a low of 5e-78 to a high of 1e-20. The E-Value from NCBI BLAST Alignment indicates the statistical significance of a given pair-wise alignment and reflects the size of the database and the scoring system used. The lower the E-Value, the more significant the hit. A sequence alignment that has an E-Value of 1e-3 means that this similarity has a 1 in 1000 chance of occurring by chance alone. (http://www.ncbi.nlm.nih.gov/books/bv.fcgi?rid=handbook.section.614). Therefore an E-Value ranging from 1e-20 to 5e-78 has a very low probability of occurring purely by chance.

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%). Thirty-seven (37) specimens for RSV and 3 specimens for hMPV were also excluded from the respective performance analysis due to insufficient sample volume for the comparator culture or real-time RT-PCR method, respectively, resulting in a total of 284 fresh nasal wash/nasopharyngeal aspirate specimens for RSV and 318 fresh nasal wash/nasopharyngeal aspirate specimens for hMPV. The tables below summarized the study results of the claimed specimen type at **study site 1**:

RSV			
Fresh nasal/nasopharyngeal wash/aspirate	**Comparator DSFA (negatives followed by culture with DFA)**		
DHI DSFA	**Positive**	**Negative**	**Total**
Positive	137	1	138
Negative	1	145	146
Total	138	146	284
			95% CI
Sensitivity	137/138	99.3%	96.0-100%
Specificity	145/146	99.3%	96.2-100%

hMPV			
Fresh nasal/nasopharyngeal wash/aspirate	**Comparator DSFA (negatives confirmed by a validated hMPV real-time RT-PCR followed by bi-directional sequencing analysis comparator assay)**		
DHI DSFA	**Positive**	**Negative**	**Total**
Positive	22	0	22
Negative	8	288	296
Total	30	288	318
			95% CI
Sensitivity	22/30	73.3%	54.1-87.7%
Specificity	288/288	100.0%	98.7-100%

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 RSV/MPV Identification Kit, 19 other types of respiratory specimens were removed from performance analysis. None of the nasal wash/nasopharyngeal aspirate samples for RSV 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 RSV to be included in the respective performance analysis. Ten (10) specimens for hMPV were also excluded from the respective performance analysis due to insufficient sample volume for the comparator methods, resulting in a total of 76 fresh nasal wash/nasopharyngeal aspirate specimens for hMPV to be included in the respective performance analysis. The tables below summarized the study results of the claimed specimen type at **study site 2**:

RSV			
Fresh nasal/nasopharyngeal wash/aspirate	**Comparator DSFA (negatives followed by culture with DFA)**		
DHI DSFA	**Positive**	**Negative**	**Total**
Positive	18	0	0
Negative	0	68	86
Total	18	68	86
			95% CI
Sensitivity	18/18	100.0%	81.5-100%
Specificity	68/68	100.0%	94.7-100%

hMPV			
Fresh nasal/nasopharyngeal wash/aspirate	**Comparator DSFA (negatives confirmed by a validated hMPV real-time RT-PCR followed by bi-directional sequencing analysis comparator assay)**		
DHI DSFA	**Positive**	**Negative**	**Total**
Positive	5	0	5
Negative	2	69	71
Total	7	69	76
			95% CI
Sensitivity	5/7	71.4%	29.0-96.3%
Specificity	69/69	100.0%	94.8-100%

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 D^3 FastPoint L-DFA RSV/MPV Identification Kit, 2 other types of respiratory specimens were further removed from performance analysis. None of the remaining nasal wash/nasopharyngeal aspirate samples for RSV and hMPV was 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 RSV and hMPV to be included in the respective performance analysis. None of the nasal/nasopharyngeal swab specimens for RSV 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 RSV to be included in the respective performance analysis. One (1) nasal/nasopharyngeal swab specimen for hMPV was also excluded from the performance analysis due to insufficient sample volume for the comparator methods, resulting in a total of 139 fresh nasal wash/nasopharyngeal aspirate specimens for hMPV to be included in the respective performance analysis. The tables below summarized the study results of the claimed specimen types at **study site 3**:

RSV			
Fresh nasal/nasopharyngeal wash/aspirate	**Predicate DSFA (negatives followed by culture with DFA)**		
DHI DSFA	**Positive**	**Negative**	**Total**
Positive	49	0	49
Negative	2	249	251
Total	51	249	300
			95% CI
Sensitivity	49/51	96.1%	86.5-99.5%
Specificity	249/249	100.0%	98.5-100%

hMPV			
Fresh nasal/nasopharyngeal wash/aspirate	**Comparator DSFA (negatives confirmed by a validated hMPV real-time RT-PCR followed by bi-directional sequencing analysis comparator assay)**		
DHI DSFA	**Positive**	**Negative**	**Total**
Positive	28	0	28
Negative	15	257	272
Total	43	257	300
			95% CI
Sensitivity	28/43	65.1%	49.1-79.0%
Specificity	257/257	100.0%	98.6-100%

RSV			
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%

hMPV			
Fresh nasal/nasopharyngeal swab	**Comparator DSFA (negatives confirmed by a validated hMPV real-time RT-PCR followed by bi-directional sequencing analysis comparator assay)**		
DHI DSFA	**Positive**	**Negative**	**Total**
Positive	9	0	9
Negative	8	122	130
Total	17	122	139
			95% CI
Sensitivity	9/17	52.9%	27.8-77.0%
Specificity	122/122	100.0%	97.0-100%

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. Ninety-six (96) specimens for RSV and 106 specimens for hMPV were also excluded from the respective performance analysis due to insufficient sample volume for the comparator culture or real-time RT-PCR methods, respectively, resulting in a total of 547 fresh nasal wash/nasopharyngeal aspirate specimens for RSV and 537 fresh nasal wash/nasopharyngeal aspirate specimens for hMPV to be included in the respective performance analysis. The tables below summarized the study results of the claimed specimen type at **study site 4**:

RSV			
Fresh nasal/nasopharyngeal swab	**Predicate DSFA (negatives followed by culture with DFA)**		
DHI DSFA	**Positive**	**Negative**	**Total**
Positive	29	0	29
Negative	0	518	518
Total	29	518	547
			95% CI
Sensitivity	29/29	100.0%	88.1-100%
Specificity	518/518	100.0%	99.3-100%

hMPV			
Fresh nasal/nasopharyngeal swab	**Comparator DSFA (negatives confirmed by a validated hMPV real-time RT-PCR followed by bi-directional sequencing analysis comparator assay)**		
DHI DSFA	**Positive**	**Negative**	**Total**
Positive	15	0	15
Negative	12	510	522
Total	27	510	537
			95% CI
Sensitivity	15/27	55.6%	35.3-74.5%
Specificity	510/510	100.0%	99.3-100%

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

RSV			
Fresh nasal/nasopharyngeal wash/aspirate	**Predicate DSFA (negatives followed by culture with DFA)**		
DHI DSFA	**Positive**	**Negative**	**Total**
Positive	204	1	205
Negative	3	462	465
Total	207	463	670
			95% CI
Sensitivity	204/207	98.6%	95.8-99.7%
Specificity	462/463	99.8%	98.8-100%

hMPV			
Fresh nasal/nasopharyngeal wash/aspirate	**Comparator DSFA (negatives confirmed by a validated hMPV real-time RT-PCR followed by bi-directional sequencing analysis comparator assay)**		
DHI DSFA	**Positive**	**Negative**	**Total**
Positive	55	0	55
Negative	25	614	639
Total	80	614	694
			95% CI
Sensitivity	55/80	68.8%	57.4-78.7%
Specificity	614/614	100.0%	99.4-100%

RSV			
Fresh nasal/nasopharyngeal swab	**Predicate DSFA (negatives followed by culture with DFA)**		
DHI DSFA	**Positive**	**Negative**	**Total**
Positive	39	0	39
Negative	1	647	648
Total	40	647	687
			95% CI
Sensitivity	39/40	97.5%	86.8-99.9%
Specificity	647/647	100.0%	99.4-100%

hMPV			
Fresh nasal/nasopharyngeal swab	**Comparator DSFA (negatives confirmed by a validated hMPV real-time RT-PCR followed by bi-directional sequencing analysis comparator assay)**		
DHI DSFA	**Positive**	**Negative**	**Total**
Positive	24	0	24
Negative	20	632	652
Total	44	632	676
			95% CI
Sensitivity	24/44	54.5%	38.8-69.9%
Specificity	632/632	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 D[3] FastPoint L-DFA RSV/MPV 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 RSV/MPV Identification Kit from four U.S. clinical laboratories across the United States during the 2009 respiratory virus seasons (January 2009 – March 2009). Prevalence for each analyte (i.e., RSV and hMPV) as determined by the D[3] FastPoint L-DFA RSV/MPV Identification Kit direct specimen testing varied from 4.5% to 31.0% by site and averaged 13.4% for RSV; varied from 2.2% to 8.4% by site and averaged 5.1% for hMPV. The number and percentage of positive cases determined by the D[3] FastPoint L-DFA RSV/MPV Identification Kit direct specimen testing, calculated by age group, are presented in the following table:

All Sites Combined

Age	**Total Specimens Evaluated**	**RSV**	**hMPV**
		# positive (prevalence)	**# positive (prevalence)**
0 – 1 month	55	15 (27.3%)	2 (3.6%)
> 1 month to 2 years	577	154 (26.7%)	41 (7.1%)
> 2 years to 12 years	391	25 (6.4%)	17 (4.3%)
> 12 years to 21 years	173	4 (2.3%)	3 (1.7%)
22 years to 30 years	57	0	1 (1.8%)
31 years to 40 years	71	1 (1.4%)	3 (4.2%)
41 years to 50 years	52	0	1 (1.9%)
51 years to 60 years	46	1 (2.2%)	3 (6.5%)
61 years to 70 years	33	1 (3.0%)	1 (3.0%)
71 years to 80 years	16	1 (6.3%)	4 (25.0%)
81 years and above	7	1 (14.3%)	0
Age Not Reported	41	0	1 (2.4%)
Total	1519	203 (13.4%)	77 (5.1%)
***** There were 2 - respiratory syncytial virus + metapneumovirus 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.