

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

**A. 510(k) Number:**

k081746

**B. Purpose for Submission:**

New device

**C. Measurand:**

Influenza A, respiratory viral antigens (Influenza B, Respiratory Syncytial Virus (RSV), Adenovirus, Parainfluenza virus types 1, 2 and 3)

**D. Type of Test:**

Direct detection or cell culture method, by immunofluorescence using fluoresceinated monoclonal antibodies (MAbs)

**E. Applicant:**

Diagnostic Hybrids, Inc.

**F. Proprietary and Established Names:**

D<sup>3</sup> *Duet* DFA Influenza A/Respiratory Virus Screening Kit  
Common Name: DFA (Direct Fluorescent Antibody) test kit for the identification of Influenza A, while screening for 6 common respiratory viruses (Influenza B, Respiratory Syncytial Virus (RSV), Adenovirus, Parainfluenza types 1, 2 and 3 virus)

**G. Regulatory Information:**

1. Regulation section:  
866.3330 Influenza virus serological reagents
2. Classification:  
Class I
3. Product code:  
GNW
4. Panel:  
Microbiology (83)

## H. Intended Use:

1. Intended use(s):

The Diagnostic Hybrids, Inc. device, D<sup>3</sup> *Duet* DFA Influenza A/Respiratory Virus Screening Kit, is intended for the qualitative detection and identification of influenza A, while screening for influenza B virus, respiratory syncytial virus, adenovirus, and parainfluenza virus types 1, 2 and 3 viral antigens, in nasal and nasopharyngeal swabs and aspirates or in cell culture. The assay detects viral antigens by immunofluorescence using monoclonal antibodies (MAbs), from patients with signs and symptoms of respiratory infection.

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

Performance characteristics for influenza A virus detection and identification were established when influenza A H3N2 and influenza A H1N1 were the predominant influenza A strains circulating in the United States. When other influenza A viruses are emerging, performance characteristics may vary. If infection with a novel influenza A virus is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions for novel virulent influenza viruses and sent to a state or local health department for testing. Viral culture should not be attempted in these cases unless a BSL 3+ facility is available to receive and culture specimens.

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 correct filter combination for FITC (excitation peak = 490 nm, emission peak = 520nm) and R-phycoerythrin (R-PE), magnification 200 to 400X

## I. Device Description:

The Diagnostic Hybrids, Inc. device, D<sup>3</sup> *Duet* DFA Influenza A/Respiratory Virus Screening Kit, uses a blend of viral antigen-specific murine MAbs. MAbs for influenza A virus are directly labeled with R-phycoerythrin (R-PE) for the rapid detection and identification of influenza A virus. MAbs for influenza B virus, respiratory syncytial virus, adenovirus, and parainfluenza virus types 1, 2, and 3 are directly labeled with fluorescein isothiocyanate (FITC), for rapid detection of these agents.

Kit components:

- D3 Duet DFA Influenza A/Respiratory Virus Screening Reagent - R-phycoerythrin-labeled murine MAbs directed against influenza A virus and a mixture of fluorescein-labeled murine MAbs directed against influenza B, respiratory syncytial virus, adenovirus, and parainfluenza virus types 1, 2, and 3. The buffered, stabilized, aqueous solution also contains Evans Blue as a counter-stain and 0.1% sodium azide as preservative.
- Normal Mouse Gamma Globulin DFA Reagent - a mixture of fluorescein labeled murine gamma globulin that has been shown to be non-reactive with any of the listed respiratory viruses. The buffered, stabilized, aqueous solution contains Evans Blue as a counter-stain and 0.1% sodium azide as preservative.
- Respiratory Virus Antigen Control Slides – five individually packaged control slides containing wells with cell culture-derived positive and negative control cells. Each positive well is identified with the virus infected cells present, i.e., influenza A virus, influenza B virus, respiratory syncytial virus, adenovirus, and parainfluenza virus types 1, 2 and 3. The negative well contains uninfected cultured cells. Each slide is intended to be stained only one time.
- Wash Solution Concentrate - a 40X concentrate consisting of Tween 20 and 4% sodium azide (0.1% sodium azide after dilution in de-mineralized water) in a 40X phosphate buffered saline solution.
- Mounting Fluid - an aqueous, buffered, stabilized solution of glycerol and 0.1% sodium azide.

#### J. Substantial Equivalence Information:

1. Predicate device name(s):

D3 Ultra DFA Respiratory Virus Screening & ID Kit

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

k081746

3. Comparison with predicate:

The similarities to predicate device are in the intended use/detected viruses, operating principle, basic design, materials and formulation.

Difference is that device, unlike predicate, identifies only Influenza A, and does not identify Influenza B, Respiratory Syncytial Virus (RSV), Adenovirus, Parainfluenza 1, Parainfluenza 2 and Parainfluenza 3 virus.

Similarities		
Item	Device	Predicate
Intended Use / Detected viruses	For the qualitative detection and identification of influenza A, while screening for influenza B virus, respiratory syncytial virus, adenovirus, and parainfluenza virus types 1, 2 and 3 viral antigens, in	For the qualitative detection and identification of the respiratory viruses, Influenza A, Influenza B, Respiratory Syncytial Virus (RSV), Adenovirus, Parainfluenza 1, Parainfluenza 2 and

<b>Similarities</b>		
<b>Item</b>	<b>Device</b>	<b>Predicate</b>
	nasal and nasopharyngeal swabs and aspirates or in cell culture	Parainfluenza 3 virus by either direct detection or cell culture method
Basic principle	DFA (Direct Fluorescent Antibody) test - Immunofluorescence using R-phycoerythrin-labeled or fluorescein-labeled monoclonal antibodies (MAbs)	DFA (Direct Fluorescent Antibody) test - Immunofluorescence using fluoresceinated monoclonal antibodies (MAbs)
Antibody	Blend of murine monoclonal antibodies (MAbs) directed against seven respiratory viruses	Blend of murine monoclonal antibodies (MAbs) directed against seven respiratory viruses
Instrumentation (required but not provided)	Fluorescence microscope with the correct filter combination for FITC (excitation peak = 490 nm, emission peak = 520nm) and R-phycoerythrin (R-PE), magnification 200 to 400X	Fluorescence microscope with the correct filter combination for FITC (excitation peak = 490 nm, emission peak = 520nm).
Sample type	Nasal and nasopharyngeal swab and nasal aspirate respiratory specimens	Respiratory specimens

<b>Differences</b>		
<b>Item</b>	<b>Device</b>	<b>Predicate</b>
Virus identification	Identifies Influenza A; detects but does not identify six respiratory viruses	Identifies seven respiratory viruses
Antibody labeling	R-phycoerythrin-labeled murine MAbs and a blend of fluorescein-labeled murine MAbs	Blend of fluorescein-labeled murine MAbs
Assay flow	One-step assay: Influenza A is both detected and identified in a single step. In the same step assay screens for additional six viruses	Two-step assay: first step only screens for seven viruses, while second step identifies each of the seven viruses

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

N/A

#### **L. Test Principle:**

The test kit uses viral antigen-specific murine monoclonal antibodies that are directly labeled with either fluorescein or R-phycoerythrin for rapid detection of respiratory viruses and identification of Influenza A. The cells to be tested, derived from a clinical specimen or cell culture, are placed onto a glass slide, allowed to air dry, and fixed in acetone. After addition of the D<sup>3</sup> Duet DFA Influenza A/Respiratory Virus Screening Reagent and incubation at 35° to 37°C the stained cells are washed with the diluted wash solution, a drop of the supplied Mounting Fluid is added and a coverslip is placed on the prepared cells. The cells are examined using a fluorescence microscope. The influenza A virus infected cells will fluoresce golden-yellow, while cells infected with any of the other six viruses will fluoresce apple-green. Uninfected cells will contain no fluorescence but will be stained red by the Evans Blue counter-stain. If only golden-yellow fluorescent cells are present the specimen can be reported as positive for influenza A antigen. If only apple-green fluorescent cells are present, the particular virus may be identified using an FDA approved assay such as the individual reagents from the D<sup>3</sup> Ultra™ DFA Respiratory Virus Screening & ID Kit (D<sup>3</sup> Ultra) on new, separate cell preparations. If both golden-yellow and apple-green are present, the additional virus may be identified using an FDA approved assay such as the individual reagents from the D<sup>3</sup> Ultra on new, separate cell preparations.

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.

#### **Interpretation of results:**

It is recommended that controls be examined first to ensure proper test performance before examination of the specimens. The entire cell spot or monolayer of cells should be examined before reporting final results. Patient sample results should not be reported unless controls perform as expected.

Uninfected cells will fluoresce dull red due to the Evans Blue counter-stain included in the DFA Reagent.

#### Fluorescent staining pattern of respiratory virus infected cells:

- Influenza A: A positive reaction is one in which golden-yellow fluorescence is observed in the cytoplasm, nucleus or in both. Cytoplasmic staining is often punctate with large inclusions while nuclear staining is uniformly bright.
- Other respiratory viruses: Green fluorescence observed in the cytoplasm and/or nucleus is suggestive of infection with a respiratory virus other than influenza A virus. The staining patterns described below are for reference only; identification of the viral antigens present in the apple-green fluorescent cells must be performed using another FDA cleared device:
  - a) Influenza B virus: The fluorescence is cytoplasmic, nuclear or both. Cytoplasmic staining is often punctate with large inclusions while nuclear staining is uniformly bright.
  - b) Respiratory syncytial virus: The fluorescence is cytoplasmic and punctate with small inclusions in the syncytia.
  - c) Adenovirus: The fluorescence is cytoplasmic and punctate or bright nuclear or both.
  - d) Parainfluenza virus types 1, 2, and 3: The fluorescence is cytoplasmic and

punctate with irregular inclusions. Types 2 and 3 cause the formation of syncytia.

- Co-infection with influenza A virus and other viruses has been reported in a number of studies. The presence of multiple viruses is indicated when a stained slide well exhibits both golden-yellow and apple-green fluorescent cells.

Results from direct specimen testing: The quality of the specimen with respect to the number of epithelial cells in the sample can be assessed by examining the different fields at a magnification of 200X. A satisfactory specimen should have at least 2 columnar epithelial cells per field. A negative result is indicated by the absence of fluorescence in a minimal sampling of 20 columnar epithelial cells. An inadequate sample is indicated by fewer than 20 columnar epithelial cells present in the sample, in which case the test is considered invalid. A new specimen should be obtained and tested or cell culture of the remaining specimen should be initiated.

A satisfactory specimen with no fluorescent cells observed should be reported as “No influenza A, influenza B, respiratory syncytial virus, adenovirus, parainfluenza type 1, parainfluenza type 2, or parainfluenza type 3 viral antigens detected by direct specimen testing”.

If golden-yellow fluorescent cells are found, it should be reported as “influenza A viral antigens detected by direct specimen testing.”

If only apple-green fluorescent cells are found, the identification of the virus(es), other than influenza A may be based on the follow-up assay (e.g. D<sup>3</sup> *Ultra* DFA Kit).

In such cases, identification and reporting of the apple-green fluorescing viral antigen(s) should be performed according the respective device’s instructions.

If co-infection of influenza A virus and one of the other respiratory viruses is detected (exhibited by both golden-yellow and apple-green fluorescent cells present) the identification of the virus(es), other than influenza A, may be based on the follow-up assay, such as D<sup>3</sup> *Ultra* DFA Kit (not provided). In such cases, it should be reported as “Influenza A viral *and* identification and reporting of the apple-green fluorescing viral antigen(s) should be performed according the respective device’s instructions.

Results from Culture Isolation / Confirmation: The entire cell spot or monolayer of cells must be examined for virus-infected, fluorescent cells. If no fluorescent cells are found, the results of testing of the specimen should be reported as, “No Influenza A, Influenza B, Adenovirus, Respiratory Syncytial Virus, Parainfluenza 1, Parainfluenza 2, or Parainfluenza 3 isolated by cell culture.”

If golden-yellow fluorescent cells are found, it should be reported as “influenza A virus isolated in cell culture”.

If only apple-green fluorescing cells are found, the identification of the virus(es), other than influenza A may be based on the follow-up assay (such as D<sup>3</sup> *Ultra* DFA Kit). In such cases, identification and reporting of the apple-green fluorescing viral antigen(s) should be performed according the respective device’s instructions.

4. If co-infection of influenza A virus and one of the other respiratory viruses is detected (exhibited by both golden-yellow and apple-green fluorescent cells present) the identification of the virus(es), other than influenza A, may be based on the follow-up assay, such as D<sup>3</sup> *Ultra* DFA Kit (not provided). In such cases, it should be reported as “Influenza A viral and identification and reporting of the apple-green

fluorescing viral antigen(s) should be performed according the respective device's instructions."

**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 panel of proficiency-level antigen control slides. The panel consisted of slides spotted with cell preparations of the following:

1. Low level influenza A (Victoria strain)
2. Mid level influenza A (Victoria strain)
3. Low level influenza A (Victoria strain) mixed with Mid level RSV (Washington strain)
4. Mid level influenza A (Victoria strain) mixed with Low level RSV (Washington strain)
5. Low level respiratory virus (either influenza virus B {Taiwan strain}, adenovirus type 1, Parainfluenza virus types 1, 2, or 3 (strains C35, Greer, C243 respectively). This panel member was rotated during the 5-days of testing so that each virus is tested twice.
6. Negative – no infected cells present

The low level is estimated to contain between 4 to 10% infected cells per cell spot. The mid level is estimated to contain between 20 to 25% infected cells per cell spot. Both levels were below the level used in quality control slides. Each panel member was re-coded daily to prevent its identification. Each panel was stained twice per day for 5-days by three different laboratories.

The following results were recorded for both the control slide and the panel slide:

1. Presence or absence of Yellow-gold fluorescence.
2. Percent of cells exhibiting Yellow-gold fluorescence
3. Presence or absence of Green fluorescence
4. Percent of cells exhibiting Green fluorescence

The combined data for negative specimens – no infected cells present - from the three sites demonstrates that the R-PE labeled and FITC labeled MAb's reproducibly do not stain uninfected cells. No fluorescent cells were seen in 100% (60/60) of the wells lacking infected cells.

The combined data from the three sites demonstrates reproducible detection of influenza A virus by the R-PE labeled MAb's. The presence of influenza A virus infected cells was reported in 95.3% (143/150) of the wells in which the infected cells were expected:

<b>Influenza A virus detection Summary</b>				
<b>Positive Control Slide</b>	<b>Low Level Slide</b>	<b>Mid-Level Slide</b>	<b>Low Level with Mid-Level RSV</b>	<b>Mid-Level with Low Level RSV</b>
<b>100% (30/30)</b>	<b>100% (30/30)</b>	<b>100% (30/30)</b>	<b>83.3% (25/30)</b>	<b>93.3% (28/30)</b>

The combined data demonstrates the reproducibility of the detection of respiratory syncytial virus by the FITC labeled MAbs. The presence of respiratory syncytial virus infected cells was reported in 100% (90/90) of the wells in which the infected cells were expected:

<b>Respiratory syncytial virus detection Summary</b>		
<b>Positive Control Slide</b>	<b>Low Level Influenza A with Mid-Level RSV</b>	<b>Mid-Level Influenza A with Low Level RSV</b>
<b>100% (30/30)</b>	<b>100% (30/30)</b>	<b>100% (30/30)</b>

The combined data demonstrates that the presence of R-PE fluorescent cells reproducibly does not interfere with the detection of respiratory syncytial virus by the FITC labeled MAbs. The presence of respiratory syncytial virus infected cells was reported in 100% (53/53) of the wells in which the R-PE stained infected cells were present:

<b>Respiratory syncytial virus detection in the presence of R-PE positive cells Summary</b>	
<b>Low Level R-PE stained cells with Mid-Level RSV</b>	<b>Mid-Level R-PE stained cells with Low Level RSV</b>
<b>100% (25/25)</b>	<b>100% (28/28)</b>

The combined data from all three sites demonstrates that the presence of R-PE in the stain reproducibly does not interfere with the FITC staining of other viruses. The presence of influenza B virus infected cells was reported in 100% (36/36) of the wells in which the infected cells were expected. The presence of adenovirus infected cells was reported in 100% (36/36) of the wells in which the infected cells were expected. The presence of parainfluenza virus type 1 virus infected cells was reported in 100% (36/36) of the wells in which the infected cells were expected. The presence of parainfluenza virus type 2 virus infected cells was reported in 100% (36/36) of the wells in which the infected cells were expected. The presence of parainfluenza virus type 3 virus infected cells was reported in 100% (36/36) of the wells in which the infected cells were expected.



<b>Respiratory virus detection in the presence of R-PE Summary</b>					
<b>Adenovirus Control Slide</b>	<b>Low Level Adenovirus</b>	<b>Influenza B Virus Control Slide</b>	<b>Low Level Influenza B Virus</b>	<b>Parainfluenza type 1 Control Slide</b>	<b>Low Level Para-influenza type 1</b>
<b>100% (30/30)</b>	<b>100% (6/6)</b>	<b>100% (30/30)</b>	<b>100% (6/6)</b>	<b>100% (30/30)</b>	<b>100% (6/6)</b>
<b>Parainfluenza type 2 Control Slide</b>	<b>Low Level Parainfluenza type 2</b>	<b>Parainfluenza type 3 Control Slide</b>	<b>Low Level Parainfluenza type 3</b>		
<b>100% (30/30)</b>	<b>100% (6/6)</b>	<b>100% (30/30)</b>	<b>100% (6/6)</b>		

The reproducibility study data demonstrates that the presence of R-PE in the stain reproducibly does not interfere with the detection of the 5 respiratory viruses by their respective FITC labeled MAbs.

*b. Linearity/assay reportable range:*

Not applicable

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

Not applicable

*d. Detection limit:*

Results for analytical detection limit for the seven viruses detected by the D<sup>3</sup> Duet were reported in numbers of fluorescent cells per cell monolayer. Each master stock virus preparation was diluted in a ten-fold manner. Four wells of a 96-well cell culture plate were inoculated with each dilution. The plates were centrifuged at 700 x g for 60 minutes, and then incubated at 35° to 37°C for 24-hours. Four wells from each dilution were stained with the D<sup>3</sup> Duet. Each well was then examined at 200x magnification and the number of fluorescent cells counted. The table below lists the virus identity and strain along with the fluorescent cell count.

<b>Analytical Sensitivity of D<sup>3</sup> Duet compared with that of D<sup>3</sup> Ultra MAbs</b> <b>(values are numbers of fluorescent cells per cell monolayer)</b>			
Virus strain	Virus Dilutions from master stock	Fluorescent staining cells/well	
		D <sup>3</sup> Duet	D <sup>3</sup> Ultra
Influenza A virus (PR, VR-95 H1N1)	1x10 <sup>-5</sup>	1, 3, 2, 6	1, 3, 0, 5
	1x10 <sup>-6</sup>	1, 0, 1, 1	0, 0, 1, 0
	1x10 <sup>-7</sup>	0, 0, 0, 0	0, 0, 0, 0
Influenza B virus (Hong Kong, VR-823)	1x10 <sup>-4</sup>	4, 1, 6, 2	0, 4, 3, 5
	1x10 <sup>-5</sup>	1, 0, 1, 1	0, 0, 2, 2
	1x10 <sup>-6</sup>	0, 0, 0, 0	0, 0, 0, 0
Adenovirus (Type 8, VR-8)	1x10 <sup>-6</sup>	1, 1, 3, 5	1, 3, 2, 4
	1x10 <sup>-7</sup>	0, 0, 0, 0	0, 0, 0, 0
RSV (Washington, VR-1401)	1x10 <sup>-2</sup>	1, 0, 3, 4	2, 3, 2, 0
	1x10 <sup>-3</sup>	0, 1, 1, 0	2, 1, 0, 0
	1x10 <sup>-4</sup>	0, 0, 0, 0	0, 0, 0, 0
Parainfluenza 1 (C-35, VR-94)	1x10 <sup>-4</sup>	7, 7, 6, 8	9, 8, 4, 6
	1x10 <sup>-5</sup>	2, 2, 3, 0	1, 0, 2, 1
	1x10 <sup>-6</sup>	0, 0, 0, 0	0, 0, 0, 0
Parainfluenza 2 (Greer, VR-92)	1x10 <sup>-4</sup>	4, 0, 3, 1	4, 3, 1, 2
	1x10 <sup>-5</sup>	0, 2, 0, 0	0, 1, 1, 1
	1x10 <sup>-6</sup>	0, 0, 0, 0	0, 0, 0, 0
Parainfluenza 3 (C 243, VR-93)	1x10 <sup>-6</sup>	3, 3, 0, 6	1, 1, 3, 5
	1x10 <sup>-7</sup>	1, 0, 1, 1	1, 1, 1, 0
	1x10 <sup>-8</sup>	0, 0, 0, 0	0, 0, 0, 0

Analytical reactivity (inclusivity) of the D<sup>3</sup> Duet was evaluated using 10 influenza A virus and 4 influenza B virus strains. Four wells of a 96-well cell culture plate were inoculated with each viral strain (diluted to less than 20-TCID<sub>50</sub> per 0.2-mL inoculum). The plates were centrifuged at 700xg for 60 minutes, and then incubated at 35° to 37°C for 24-hours. Four wells from each strain were stained with the D<sup>3</sup> Duet, and each well was then examined at 200x magnification and the number of fluorescent cells counted. The table below lists the virus identity and strain along with the fluorescent cell count.

<b>Analytical Reactivity (inclusivity) of D<sup>3</sup> Duet with various influenza A virus and influenza B virus strains (values are numbers of fluorescent cells per cell monolayer)</b>	
Influenza strain	Fluorescent staining cells/cell monolayer
Influenza A Wisconsin/56/2005	3, 2, 1, 0
Influenza A WS, VR-1520 (H1N1)	6, 6, 6, 4
Influenza A Hong Kong, VR-544 (H3N2)	3, 4, 5, 5
Influenza A New Jersey, VR-897 (H1N1)	9, 12, 14, 15
Influenza A Victoria, VR-822 (H3N2)	3, 3, 3, 5
Influenza A PR, VR-95 (H1N1)	3, 9, 9, 6
Influenza A Port Chalmers, VR-810 (H3N2)	6, 6, 9, 10
Influenza A Aichi, VR-547 (H3N2)	3, 7, 9, 11
Influenza A Denver, VR-546 (H1N1)	13, 14, 11, 10
Influenza A Mal, VR-98 (H1N1)	8, 3, 6, 4
Influenza B GL/1739/54, VR-103	7, 6, 7, 7
Influenza B Taiwan/2/62, VR-295	3, 1, 2, 5
Influenza B Hong Kong/5/72, VR-823	3, 2, 0, 1
Influenza B Maryland/1/59, VR-296	5, 6, 6, 8

Based on the data presented above, the assay can reliably detect influenza A virus and influenza B virus strains exhibiting both temporal and geographical diversity at viral levels near the limit of detection in cell culture.

e. *Analytical specificity:*

Cross-Reactivity Testing

The D<sup>3</sup> Duet Influenza A/Respiratory Virus Screening Kit was tested for cross-reactivity against a variety of cells and microorganisms. Stringent conditions for cross-reactivity testing were achieved by using a high concentration of the D<sup>3</sup> Duet DFA Influenza A/Respiratory Virus Screening Reagent and relatively high titers of microorganisms. The D<sup>3</sup> Duet DFA Influenza A/Respiratory Virus Screening Reagent was prepared at 1.5X the concentration that is provided in the kit. No cross-reactivity was observed for 32 virus strains or for 17 host culture cell types. Twenty-five bacterial strains, one yeast, three *Chlamydia sp.* and one protozoan were evaluated for cross-reactivity, including *Staphylococcus aureus*, a protein-A-producing bacterium. Staining of *S. aureus* appeared as small points of fluorescence.

Thirty-two virus strains were tested for cross-reactivity. Depending on the particular virus, 71 to 1,400 TCID<sub>50</sub> were inoculated into shell vial culture and incubated for 24 to 48 hours, to yield a 1+ to 3+ infection, processed and stained with the 1.5X DFA Reagent according to the procedure as detailed in the PI. No cross reactivity was observed for the viruses listed below:

Virus Strains Tested for Cross Reactivity with D <sup>3</sup> Duet DFA Influenza A/Respiratory Virus Screening Reagent					
Organism	Strain or Type	Inoculum (TCID <sub>50</sub> )	Organism	Strain or Type	Inoculum (TCID <sub>50</sub> )
Parainfluenza 4a	M-25, VR-1378	1,400	CMV	Towne, VR-977	430
Parainfluenza 4b	CH19503, VR-377	1,400	CMV	Davis, VR-807	430
Metapneumovirus	Subgroup A1	1,400	CMV	AD169, VR-538	430
Metapneumovirus	Subgroup A2	1,400	Varicella-zoster	Webster, VR-916	430
Metapneumovirus	Subgroup B1	1,400	Varicella-zoster	Ellen, VR-1367	430
Metapneumovirus	Subgroup B2	1,400	Rhinovirus 39	209 Picornavirus, VR-340	1,400
Coronavirus	OC43, VR-1558	1,400	Rubeola		Commercially available slides stained.*
Coronavirus	229E, VR-740	1,400	Mumps		Commercially available slides stained.*
HSV-1	1F, VR-733	71	Echovirus	Types 4, 6, 9, 11, 30, 34	Commercially available slides stained.*
HSV-1	MacIntyre, VR-539	71	Coxsackievirus	Types B1, B2, B3, B4, B5, B6	Commercially available slides stained.*
HSV-2	MS, VR-540	71			
HSV-2	Strain G, VR-734	71			

Seventeen host culture cell types were tested for cross reactivity. Cell cultures were prepared in shell vial format. Confluent monolayers were stained with the 1.5X preparation of the D<sup>3</sup> Duet DFA Influenza A/Respiratory Virus Screening Reagent according to the procedure as detailed in the PI, and then examined for cross reactivity. No cross reactivity was observed for the following:

Cell lines Tested for Cross Reactivity with D <sup>3</sup> Duet DFA Influenza A/Respiratory Virus Screening Reagent			
A549	monolayer	pCMK	cell spot
BGMK	monolayer	pRhMK	cell spot
HEp-2	monolayer	RD	monolayer
LLC-MK2	monolayer	RhMK II	cell spot
MDCK	monolayer	pRK	monolayer
MRC-5	monolayer	R-Mix	monolayer
MRHF	monolayer	Vero	cell spot
Mv1Lu	monolayer	WI-38	cell spot
NCI-H292	monolayer		

Thirty microorganisms, including 25 bacterial and one yeast cultures, three *Chlamydia sp.* and one protozoan commercially available slides, were stained with the 1.5X DFA Reagent according to the procedure as detailed in the product insert, then examined for cross reactivity. Except for *Staphylococcus aureus*, which was cross reactive with the D<sup>3</sup> Duet DFA Influenza A/Respiratory Virus Screening Reagent, all other microorganisms tested

negative. Reactivity with *Staphylococcus aureus* is more than likely due to binding the protein A produced by *Staphylococcus aureus*. Concentrations for each bacterial organism cultured by DHI for cross reactivity testing were determined from suspensions of the bacteria in PBS by spectrophotometer according to McFarland standards of levels 1.0 and 2.0 (equaling approximately  $3.0 \times 10^6$  and  $6.0 \times 10^6$  CFU per mL). Slides were prepared with spots of 0.01-mL of the suspensions to give either  $3.0 \times 10^4$  or  $6.0 \times 10^4$  per spot. At the same time, 1-mL of each suspension was plated on an appropriate agar dish for colony confirmation. According to the confirmation agar cultures, initial concentrations of the bacterial organisms in the study ranged from  $6.4 \times 10^4$  to  $2.9 \times 10^7$  CFU. Microorganisms tested are listed below.

Microorganisms Tested for Cross Reactivity with D <sup>3</sup> Duet DFA Influenza A/Respiratory Virus Screening Reagent	
BACTERIA	CFU TESTED
<i>Acholeplasma laidlawii</i>	$\sim 6 \times 10^7$
<i>Acinetobacter calcoaceticus</i>	$9.7 \times 10^5$
<i>Bordetella bronchiseptica</i>	$1.7 \times 10^5$
<i>Bordetella pertussis</i>	$4.6 \times 10^6$
<i>Corynebacterium diphtheriae</i>	$2.5 \times 10^6$
<i>Escherichia coli</i>	$2.6 \times 10^5$
<i>Gardnerella vaginalis</i>	$5.0 \times 10^5$
<i>Haemophilis influenzae type A</i>	$9.3 \times 10^5$
<i>Klebsiella pneumoniae</i>	$6.4 \times 10^6$
<i>Legionella pneumophila</i>	$6.5 \times 10^4$
<i>Moraxella cartarrhalis</i>	$6.4 \times 10^4$
<i>Mycoplasma hominis</i>	$\sim 6 \times 10^4$
<i>Mycoplasma orale</i>	$\sim 6 \times 10^4$
<i>Mycoplasma pneumoniae</i>	$\sim 6 \times 10^4$
<i>Mycoplasma salivarium</i>	$\sim 6 \times 10^7$
<i>Neisseria gonorrhoeae</i>	$1.3 \times 10^6$
<i>Proteus mirabilis</i>	$2.1 \times 10^6$
<i>Pseudomonas aeruginosa</i>	$1.0 \times 10^7$
<i>Salmonella enteritidis</i>	$2.5 \times 10^6$
<i>Salmonella typhimurium</i>	$1.8 \times 10^6$
<i>Staphylococcus aureus</i> *	$1.0 \times 10^7$
<i>Streptococcus agalactiae</i>	$9.6 \times 10^6$
<i>Streptococcus pneumoniae</i>	$8.0 \times 10^5$
<i>Streptococcus pyogenes</i>	$2.9 \times 10^7$
<i>Ureaplasma urealyticum</i>	$\sim 6 \times 10^4$
<i>Chlamydomphila pneumoniae</i>	Commercially available slides stained.
<i>Chlamydomphila psittaci</i>	Commercially available slides stained.
<i>Chlamydia trachomatis</i>	Commercially available slides stained.
YEAST	
<i>Candida glabrata</i>	$8.7 \times 10^6$
PROTOZOAN	
<i>Trichomonas vaginalis</i>	Commercially available slides stained.

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

f. Assay cut-off:  
Not applicable

2. Comparison studies:

a. *Method comparison with predicate device:*

Direct fresh specimens:

A study was performed prospectively at three sites with 1203 fresh specimens that were received for respiratory virus testing. Each specimen was evaluated by the D<sup>3</sup> *Duet* DFA Influenza A/Respiratory Virus Screening Kit and a cleared DSFA device for the presence of influenza A, influenza B, respiratory syncytial virus, adenovirus, parainfluenza 1, parainfluenza 2 and parainfluenza 3 in cells derived from clinical specimens. A total of nineteen specimens were excluded from analysis due to a site deviations, duplicate specimen, insufficient cell numbers, or high background. These exclusions left 1184 specimen results for analysis.

The following tables detail the summary of the comparison of the D<sup>3</sup> *Duet* and the cleared DSFA comparator assay, combined for study sites 1, 2, and 3:

D <sup>3</sup> <i>Duet</i> R-PE identification of influenza A virus positive specimens			
Direct Specimen (1184 Specimens)		D <sup>3</sup> <i>Ultra</i> Final Identification (influenza A virus)	
		Pos	Neg
D <sup>3</sup> <i>Duet</i> R-PE (influenza A virus)	Pos	99	0
	Neg	1	1084
Positive Percent Agreement (PPA)		99% (99/100)	
95% CI- PPA		94.5, 99.8%	
Negative Percent Agreement (NPA)			100% (1084/1084)
95% CI- NPA			99.7, 100%

D <sup>3</sup> <i>Duet</i> FITC detection of influenza B virus, respiratory syncytial virus, adenovirus, and parainfluenza virus types 1, 2, and 3 viruses			
Direct Specimen (1184 Specimens)		D <sup>3</sup> <i>Ultra</i> Final Identification	
		Pos	Neg
D <sup>3</sup> <i>Duet</i> FITC Screen	Pos	386	0
	Neg	0	798*
Positive Percent Agreement (PPA)		100% (386/386)	
95% CI- PPA		99.0, 100%	
Negative Percent Agreement (NPA)			100% (798/798)
95% CI- NPA			99.5, 100%
* One specimen was screen positive by both devices, but was unable to be identified with the identification reagents.			

Virus Follow-up Identification of 386 D <sup>3</sup> <i>Duet</i> FITC Positive Specimens for influenza B virus, respiratory syncytial virus, adenovirus, and parainfluenza virus types 1, 2, and 3 viruses, using D <sup>3</sup> <i>Ultra</i> Identification Reagents						
Virus	Sensitivity		95%CI for Sensitivity	Specificity		95% CI for Specificity
	TP / (TP+FN)	percent		TN/ (TN+FP)	percent	
<b>Influenza B virus</b>	11/11	100%	74.12, 100	1173/1173	100%	99.7, 100
<b>Adenovirus</b>	52/52	100%	93.1, 100	1132/1132	100%	99.7, 100
<b>Parainfluenza type 1</b>	4/4	100%	51.0, 100	1180/1180	100%	99.7, 100
<b>Parainfluenza type 2</b>	1/1	100%	20.1, 100	1183/1183	100%	99.7, 100
<b>Parainfluenza type 3</b>	19/19	100%	83.2, 100	1165/1165	100%	99.7, 100
<b>Respiratory Syncytial Virus</b>	299/299	100%	98.7, 100	885/885	100%	99.6, 100

The D<sup>3</sup> *Duet*'s ability to identify influenza A virus using phycoerythrin in direct specimens was compared to the D<sup>3</sup> *Ultra*'s ability using fluorescein. The positive percent agreement was 99% (95% CI range of 94.5% to 99.8%). The negative percent agreement was 100% (95% CI range of 99.7% to 100%). When the ability of the D<sup>3</sup> *Duet* to detect the six other respiratory viruses using fluorescein in direct specimens was compared to the D<sup>3</sup> *Ultra*'s ability using fluorescein, the positive percent agreement was 100% (95% CI range of 99.0% to 100%). The negative percent agreement was 100% (95% CI range of 99.5% to 100%).

Specimen type distribution:

Tables below show the study results by the claimed specimen type. Results from sites 1, 2, and 3 have been combined.

<b>Influenza A virus by specimen type</b>						
Specimen type	PPA		95%CI for PPA	NPA		95% CI for NPA
	TP / (TP+FN)	percent		TN/ (TN+FP)	percent	
NPA	61/62	98.4%	91.4, 99.7	525/525	100%	99.3, 100
NPS	38/38	100%	90.8, 100	501/501	100%	99.2, 100
<b>D<sup>3</sup> <i>Duet</i> FITC detection of influenza B virus, respiratory syncytial virus, adenovirus, and parainfluenza virus types 1, 2, and 3 viruses by specimen type</b>						
Specimen type	PPA		95%CI for PPA	NPA		95% CI for NPA
	TP / (TP+FN)	percent		TN/ (TN+FP)	percent	
NPA	196/196	100%	98.1, 100	391/391	100%	99.0, 100
NPS	173/173	100%	97.8, 100	366/366	100%	99.0, 100

#### Cultured specimens:

To evaluate the performance of this device using cultured clinical specimens, a fourth study was performed with 298 frozen specimens to compare performance of the D<sup>3</sup> *Duet* DFA Influenza A/Respiratory Virus Screening Kit with that of the predicate for the presence of Influenza A, Influenza B, Respiratory Syncytial Virus, Adenovirus, Parainfluenza 1, Parainfluenza 2 and Parainfluenza 3 (Para 3) from cultured clinical specimens. At Study Site 4, 298 frozen specimens were processed for cell culture testing in accordance with the procedure in the Comparator product insert (same procedure for both Subject and Comparator devices) using R-Mix Too™ FreshCells™ in 48/24-fill multi-well plates. All specimens at study site 4 were derived from nasopharyngeal specimens. The results of this study are presented below. The table below shows the age distribution for individuals studied at site 4:

Site 4 (culture) – Age Distribution	
0 - 1 month	5
>1 month - 2 years	130
>2 - 12 years	44
>12 - 21 years	28
22 - 30 years	19
31 - 40 years	20
41 - 50 years	10
51 - 60 years	9
61 - 70 years	8
71 - 80 years	6
81 - 90 years	8
>90 years	5
Unknown age	6
Total	298

The following tables detail the results of the cell culture study's comparison of D<sup>3</sup> *Duet*'s phycoerythrin-labeled MABs identification of influenza A virus positive specimens, and D<sup>3</sup> *Duet*'s fluorescein-labeled MABs detection of influenza B virus, respiratory syncytial virus, adenovirus, and parainfluenza virus types 1, 2, and 3 positive specimens.

D <sup>3</sup> <i>Duet</i> R-PE identification of influenza A virus positive specimens			
Cell Culture (298 Specimens)		D <sup>3</sup> <i>Ultra</i> Final Identification (influenza A virus)	
		Pos	Neg
D <sup>3</sup> <i>Duet</i> R-PE (influenza A virus)	Pos	67	0
	Neg	0	231
Positive Percent Agreement (PPA)		100% (67/67)	
95% CI- PPA		94.6, 100%	
Negative Percent Agreement (NPA)			100% (231/231)
95% CI- NPA			98.4, 100%



D <sup>3</sup> <i>Duet</i> FITC detection of influenza B virus, respiratory syncytial virus, adenovirus, and parainfluenza virus types 1, 2, and 3 viruses			
Cell Culture (298 Specimens)		D <sup>3</sup> <i>Ultra</i> Final Identification	
		Pos	Neg
D <sup>3</sup> <i>Duet</i> FITC Screen	Pos	72	0
	Neg	0	226
Positive Percent Agreement (PPA)		100% (72/72)	
95% CI- PPA		95.0,100%	
Negative Percent Agreement (NPA)			100% (226/226)
95% CI- NPA			98.4,100%

A variety of viral respiratory pathogens were isolated. Virus identification of D<sup>3</sup> Duet FITC Positive Specimens using D<sup>3</sup> Ultra Identification Reagents yielded the following isolates: influenza A virus [prevalence 22.5% (67/298)], influenza B virus [prevalence 6.7% (20/298)], respiratory syncytial virus [prevalence 11.1% (33/298)], adenovirus [prevalence 3.4% (10/298)], parainfluenza type 1 virus [prevalence 1.7% (5/298)], parainfluenza type 2 virus [prevalence 1.0% (3/298)], and parainfluenza type 3 virus [prevalence 3.0% (9/298)].

There were sixteen co-infections as follows: three influenza A virus + parainfluenza type 3 virus, one influenza A virus + parainfluenza type 1 virus, one influenza A virus + parainfluenza type 2 virus, two influenza A virus + respiratory syncytial virus, one influenza A virus + adenovirus, one influenza B virus + parainfluenza type 2 virus, one influenza B virus + parainfluenza type 3 virus, one influenza B virus + respiratory syncytial virus, one respiratory syncytial virus + parainfluenza type 1 virus, two respiratory syncytial virus + parainfluenza type 3 virus, one adenovirus + parainfluenza type 1 virus and one adenovirus + parainfluenza type 3 virus.

- b. *Matrix comparison:*  
Not applicable

3. Clinical studies:

- a. *Clinical Sensitivity:*  
Not applicable
- b. *Clinical specificity:*  
Not applicable
- c. Other clinical supportive data (when a. and b. are not applicable):  
Not applicable

4. Clinical cut-off:  
Not applicable

5. Expected values/Reference range:

A study was performed prospectively at three sites with 1184 fresh specimens that were received for respiratory virus testing. Each specimen was evaluated for the presence of influenza A, influenza B, respiratory syncytial virus, adenovirus, parainfluenza 1, parainfluenza 2 and parainfluenza 3 in cells derived from clinical specimens. Prevalence of the respiratory viruses within this population during the 2006/2007 season is noted in the table below:

Respiratory Virus Prevalence 2006/2007 Fresh Specimens (n = 1184)							
h e	Adenovirus	Influenza A	Influenza B	Parainfluenza 1	Parainfluenza 2	Parainfluenza 3	Respiratory Syncytial Virus
No. of positives	52	99	11	4	1	19	300
† Prevalence	4.4%	8.4%	0.9%	0.3%	0.08%	1.6%	25.3%

\*Five co-infections were detected (0.4%): 1-influenza A virus/parainfluenza virus type 3, 2-influenza A virus/ respiratory syncytial virus, 1-adenovirus/respiratory syncytial virus, 1-respiratory syncytial virus/ parainfluenza virus type 3.

The following table summarizes the participant age demographics according to test results for a population of 1184 fresh specimens, prospectively collected and evaluated for performance using the comparator assay:

Participant Age Demographics								
Virus: Age*	Adenovirus	Influenza A	Influenza B	Parainfluenza 1	Parainfluenza 2	Parainfluenza 3	Respiratory Syncytial Virus	Negative
<b>Totals†</b>	<b>52</b>	<b>99</b>	<b>11</b>	<b>4</b>	<b>1</b>	<b>19</b>	<b>300</b>	<b>704</b>
<1m	0	1	0	0	0	1	26	67
1m to 2y	35	46	4	4	1	16	237	358
2y to 12y	16	36	6	0	0	0	31	147
12y to 21y	1	7	0	0	0	1	0	24
21y to 30y	0	1	0	0	0	0	0	20
31 to 40y	0	1	0	0	0	0	1	17
41 to 50y	0	4	1	0	0	0	0	19
51 to 60y	0	2	0	0	0	0	0	12
61 to 70y	0	0	0	0	0	0	0	10
71 to 80y	0	0	0	0	0	0	2	8
81 to 90y	0	1	0	0	0	1	1	17
91 to 100y	0	0	0	0	0	0	0	4
Not reported	0	0	0	0	0	0	2	0

\* Age: m = months, and y = years

† Due to 6 co-infections the total adds up to 1190

**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.