

D³ DUET DFA RSV/RESPIRATORY
VIRUS SCREENING KIT



SECTION 05, 510(K) SUMMARY

Applicant:

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Device Name:

Trade name – D³ Duet DFA RSV/Respiratory Virus Screening Kit
Common name – Fluorescent antibody test for detecting respiratory syncytial virus,
and screening for other respiratory viruses
Classification name – Respiratory Syncytial Virus, Antigen, Antibody, Ifa
Product Code – GNW
Regulation – 21 CFR 866.3480, Class I, respiratory syncytial virus serological
reagents; Panel Microbiology (83)

Legally marketed device to which equivalence is claimed:

K061101, D³ Ultra DFA Respiratory Virus Screening & ID Kit

Intended Use: The Diagnostic Hybrids, Inc. device, D³ Duet DFA RSV/Respiratory Virus Screening Kit, is intended for the qualitative detection and identification of respiratory syncytial virus, while screening for influenza A virus, influenza B 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

Device Description:

The MAbs furnished with the D³ *Duet* DFA RSV/Respiratory Virus Screening Kit (D³ *Duet*) are the same MAbs that are contained in the Diagnostic Hybrids, Inc. (DHI) device D³ *Ultra* DFA Respiratory Virus Screening & ID Kit (D³ *Ultra*) [510(k) number K061101, November 20, 2006].

The Diagnostic Hybrids, Inc. device, D³ *Duet* DFA RSV/Respiratory Virus Screening Kit, uses a blend of viral antigen-specific murine MAbs. MAbs for RSV are directly labeled with R-phycoerythrin (R-PE) for the rapid detection and identification of RSV. MAbs for influenza A virus, influenza B 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 RSV/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 A, influenza B, 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.

The cells to be tested, derived from a clinical specimen or cell culture, are placed onto a glass slide and allowed to air dry. The cells are fixed in acetone. The D³ *Duet* DFA RSV/Respiratory Virus Screening Reagent is added to the cells which are then incubated for 15 to 30 minutes at 35° to 37°C in a humidified chamber or humidified incubator. The stained cells are then 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 respiratory syncytial 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 respiratory syncytial virus antigen. If only apple-green fluorescent cells are present, the particular virus is identified using the individual reagents from the D³ *Ultra*TM DFA Respiratory Virus Screening & ID Kit (D³ *Ultra*) on new, separate cell preparations. If both golden-yellow and apple-green are present, the additional virus may be identified using the individual reagents from the D³ *Ultra* on new, separate cell preparations.

Technological Characteristics:

The DHI device, D³ *Duet*, has been compared directly to the DHI device, D³ *Ultra*, as the legally marketed device. The technology used in both devices is based on a standard immunofluorescence assay technique utilizing either phycoerythrin- or fluorescein-labeled MAbs. A summary is provided in Table 5.1 below:

Characteristic	D ³ <i>Duet</i> DFA RSV/ Respiratory Virus Screening Kit	D ³ <i>Ultra</i> DFA Respiratory Virus Screening & ID Kit
Monoclonal antibodies (MAbs)	The RSV/Respiratory Virus DFA Screening Reagent contains 12 MAbs to 6 different respiratory viruses (influenza A virus, influenza B virus, adenovirus, parainfluenza virus type 1, parainfluenza virus type 2, parainfluenza virus type 3), plus 2 MAbs to respiratory syncytial virus.	The Respiratory Virus DFA Screening Reagent contains 12 MAbs to 6 different respiratory viruses (influenza A virus, influenza B virus, adenovirus, parainfluenza virus type 1, parainfluenza virus type 2, parainfluenza virus type 3), plus 2 MAbs to respiratory syncytial virus.

Characteristic	D ³ Duet DFA RSV/ Respiratory Virus Screening Kit	D ³ Ultra DFA Respiratory Virus Screening & ID Kit	
Labeling method	Direct labeling, - using R-phycoerythrin (R-PE) to label the MAbs to respiratory syncytial virus antigens - using fluorescein isothiocyanate (FITC) to label all other MAbs with fluorescein moiety	Direct labeling, - using fluorescein isothiocyanate (FITC) to label all MAbs with fluorescein moiety	
Fluorescein-labeled MAbs	Influenza A virus, influenza B virus, adenovirus, parainfluenza virus type 1, parainfluenza virus type 2, parainfluenza virus type 3	Influenza A virus, influenza B virus, respiratory syncytial virus, adenovirus, parainfluenza virus type 1, parainfluenza virus type 2, parainfluenza virus type 3	
Phycoerythrin-labeled MAbs	Respiratory syncytial virus (Phycoerythrin-labeled influenza A virus MAbs stain with golden-yellow fluorescence)	None (Fluorescein-labeled respiratory syncytial virus MAbs stain with apple-green fluorescence)	
Cell Fixative	Cell Fixative is the same for both devices: Acetone		
Performance characteristics			
Staining patterns	<p>Staining patterns are the same for both devices:</p> <p>Influenza A and B: The fluorescence is cytoplasmic, nuclear or both. Cytoplasmic staining is often punctate with large inclusions while nuclear staining is uniformly bright.</p> <p>Respiratory Syncytial Virus: The fluorescence is cytoplasmic and punctate with small inclusions in the syncytia.</p> <p>Parainfluenza 1, 2, 3: The fluorescence is cytoplasmic and punctate with irregular inclusions. Types 2 and 3 cause the formation of syncytia.</p> <p>Adenovirus: The fluorescence is cytoplasmic and punctate or bright nuclear or both.</p>		
Analytical sensitivity, according to 96-well cell culture plates infected with Flu A diluted to give a TCID ₅₀ of 1 per 0.2-mL inoculum (reported as average of 4 runs)	There is no significant difference between the two devices for analytical sensitivity.		
	27.7 ± 1.7 culture positives out of 96	28.7 ± 1.3 culture positives out of 96	
Analytical specificity (for influenza A virus strains; MAbs are reactive with all listed strains)	MAbs to respiratory syncytial virus were shown to be reactive with these virus strains:		
	3 Respiratory syncytial virus strains: Long, VR-26 Group A, Wash, VR-1401 Group B, 9320, VR-955 Group B	3 Respiratory syncytial virus strains: Long, VR-26 Group A, Wash, VR-1401 Group B, 9320, VR-955 Group B	
Analytical specificity (cross reactivity studies; various strains of microorganisms and cell lines)	Device Screening Reagent is not reactive with these numbers of microorganisms.*		
	Viruses	32	31
	Bacteria	25	18
	Chlamydia spp.	3	1
	Yeast	1	0
	Protozoan	1	0
Cell lines	17	17	
* Lists of these microorganisms are provided in Section 18 of this 510(k) submission.			

Non-Clinical Performance:

Staining patterns of the phycoerythrin-labeled respiratory syncytial virus MAbs on respiratory syncytial virus infected cells were similar to those of the Predicate device.

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 RSV (Washington strain)
2. Mid level RSV (Washington 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 MAbs 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 respiratory syncytial virus by the R-PE labeled MAbs. The presence of respiratory syncytial virus infected cells was reported in 98% (147/150) of the wells in which the infected cells were expected:

Respiratory syncytial virus detection Summary				
Positive Control Slide	Low Level Slide	Mid-Level Slide	Low Level with Mid-Level Influenza A	Mid-Level with Low Level Influenza A
100% (30/30)	100% (30/30)	100% (30/30)	90% (27/30)	100% (30/30)

The combined data demonstrates the reproducibility of the detection of Influenza A virus by the FITC labeled MAbs. The presence of Influenza A virus infected cells was reported in 96.7% (87/90) of the wells in which the infected cells were expected:

Influenza A virus detection Summary		
Positive Control Slide	Low Level Influenza A with Mid-Level RSV	Mid-Level Influenza A with Low Level RSV
100% (30/30)	90% (27/30)	100% (30/30)

The combined data demonstrates that the presence of R-PE fluorescent cells does not interfere with the detection of influenza A virus by the FITC labeled MAbs in a reproducible manner. The presence of influenza A virus infected cells was reported in 94.7% (54/57) of the wells in which the R-PE stained infected cells were present:

Influenza A virus detection in the presence of R-PE positive cells summary	
Low Level R-PE stained cells with Mid-Level influenza A virus	Mid-Level R-PE stained cells with Low Level influenza A virus
100% (27/27)	90% (27/30)

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.

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

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.

Detection limit:

Results for analytical detection limit for the seven viruses detected by the D³ 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 xg for 60 minutes, and then incubated at 35° to 37°C for 24-hours. Four wells from each dilution were stained with the D³ 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.

Analytical Sensitivity of D³ Duet compared with that of D³ Ultra MABs			
(values are numbers of fluorescent cells per cell monolayer)			
Virus strain	Virus Dilutions from master stock	Fluorescent staining cells/well	
		D ³ Duet	D ³ Ultra
Influenza A virus (PR, VR-95 H1N1)	1x10 ⁻⁵	4, 1, 5, 4	1, 3, 0, 5
	1x10 ⁻⁶	1, 2, 0, 3	0, 0, 1, 0
	1x10 ⁻⁷	0, 0, 0, 0	0, 0, 0, 0
Influenza B virus (Hong Kong, VR-823)	1x10 ⁻⁴	3, 3, 4, 2	0, 4, 3, 5
	1x10 ⁻⁵	1, 0, 1, 1	0, 0, 2, 2
	1x10 ⁻⁶	0, 0, 0, 0	0, 0, 0, 0
Adenovirus (Type 8, VR-8)	1x10 ⁻⁶	1, 1, 3, 3	1, 3, 2, 4
	1x10 ⁻⁷	0, 0, 0, 0	0, 0, 0, 0
RSV (Washington, VR-1401)	1x10 ⁻²	1, 1, 3, 3	2, 3, 2, 0
	1x10 ⁻³	2, 0, 0, 1	2, 1, 0, 0
	1x10 ⁻⁴	0, 0, 0, 0	0, 0, 0, 0
Parainfluenza 1 (C-35, VR-94)	1x10 ⁻⁴	6, 5, 8, 6	9, 8, 4, 6
	1x10 ⁻⁵	0, 2, 4, 2	1, 0, 2, 1
	1x10 ⁻⁶	0, 0, 0, 0	0, 0, 0, 0
Parainfluenza 2 (Greer, VR-92)	1x10 ⁻⁶	5, 4, 2, 1	4, 3, 1, 2
	1x10 ⁻⁷	0, 0, 1, 0	0, 1, 1, 1
	1x10 ⁻⁸	0, 0, 0, 0	0, 0, 0, 0
Parainfluenza 3 (C-243, VR-93)	1x10 ⁻⁶	1, 2, 0, 3	1, 1, 3, 5
	1x10 ⁻⁷	1, 0, 1, 0	1, 1, 1, 0

Analytical Sensitivity of D³ Duet compared with that of D³ Ultra MAbs (values are numbers of fluorescent cells per cell monolayer)			
Virus strain	Virus Dilutions from master stock	Fluorescent staining cells/well	
		D ³ Duet	D ³ Ultra
	1x10 ⁻⁸	0, 0, 0, 0	0, 0, 0, 0

Analytical reactivity (inclusivity): of the D³ 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-TCID₅₀ 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 D³ 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.

Analytical Reactivity (inclusivity) of D³ Duet on various influenza A virus and influenza B virus strains (values are numbers of fluorescent cells per cell monolayer)	
Influenza strain	Fluorescent staining cells/cell monolayer
Influenza A WS, VR-1520 (H1N1)	10, 8, 7, 7
Influenza A Hong Kong, VR-544 (H3N2)	12, 11, 11, 12
Influenza A New Jersey, VR-897 (H1N1)	8, 11, 10, 14
Influenza A Victoria, VR-822 (H3N2)	7, 9, 10, 11
Influenza A PR, VR-95 (H1N1)	6, 9, 8, 11
Influenza A Port Chalmers, VR-810 (H3N2)	8, 11, 15, 9
Influenza A Aichi, VR-547 (H3N2)	16, 15, 14, 13
Influenza A Denver, VR-546 (H1N1)	6, 9, 9, 8
Influenza A Mal, VR-98 (H1N1)	16, 13, 11, 15
Influenza A A/NWS/33, VR-219	12, 17, 15, 10

Analytical Reactivity (inclusivity) of D³ Duet on various influenza A virus and influenza B virus strains (values are numbers of fluorescent cells per cell monolayer)	
Influenza strain	Fluorescent staining cells/cell monolayer
(H1N1)	
Influenza B Russia/69, VR-790	13, 14, 12, 15
Influenza B Mass/3/66, VR-523	12, 19, 14, 13
Influenza B Hong Kong/5/72, VR-791	8, 8, 9, 11
Influenza B Maryland/1/59, VR-296	16, 12, 13, 12

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.

Analytical specificity:

Cross-Reactivity Testing

The D³ Duet RSV/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 D³ Duet DFA Influenza RSV/Respiratory Virus Screening Reagent and relatively high titers of microorganisms. The D³ Duet DFA RSV/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 TCID₅₀ 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 this product insert. No cross reactivity was observed for the viruses listed below:

Virus Strains Tested for Cross Reactivity with D ³ Duet DFA RSV/Respiratory Virus Screening Reagent					
Organism	Strain or Type	Inoculum (TCID ₅₀)	Organism	Strain or Type	Inoculum (TCID ₅₀)
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

Virus Strains Tested for Cross Reactivity with D ³ Duet DFA RSV/Respiratory Virus Screening Reagent					
Organism	Strain or Type	Inoculum (TCID ₅₀)	Organism	Strain or Type	Inoculum (TCID ₅₀)
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	IF, 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 D³ Duet DFA RSV/Respiratory Virus Screening Reagent according to the procedure as detailed in the product insert, and then examined for cross-reactivity. No cross-reactivity was observed for the following:

Cell lines Tested for Cross Reactivity with D ³ 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 D³ 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 x 10⁶ and 6.0 x 10⁶ CFU per mL). Slides

were prepared with spots of 0.01-mL of the suspensions to give either 3.0×10^4 or 6.0×10^4 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 6.4×10^4 to 2.9×10^7 CFU. Microorganisms tested are listed below.

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

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

Clinical Performance:

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 D³ Duet DFA Influenza A/Respiratory Virus Screening Kit and a cleared DSFA device for the presence of respiratory syncytial virus, influenza A, influenza B, adenovirus, parainfluenza 1, parainfluenza 2 and parainfluenza 3 in cells derived from clinical specimens. Seventeen specimens were excluded from analysis due to a variety of reasons (site deviations, duplicate specimen, insufficient cell

numbers, or high background). These exclusions left 1187 specimen results for analysis.

The following tables detail the summary of the comparison of the D³ Duet and the cleared DSFA comparator assay, combined for study sites 1, 2, and 3:

D ³ Duet R-PE identification of respiratory syncytial virus positive specimens			
Direct Specimen (1187 Specimens)		D ³ Ultra Final Identification (respiratory syncytial virus)	
		Pos	Neg
D ³ Duet R-PE (respiratory syncytial virus)	Pos	300	0
	Neg	0	887
Positive Percent Agreement (PPA)		100% (300/300)	
95% CI- PPA		97.8, 100%	
Negative Percent Agreement (NPA)		100% (887/887)	
95% CI- NPA		99.6, 100%	

D ³ Duet FITC detection of influenza A virus, influenza B virus, adenovirus, and parainfluenza virus types 1, 2, and 3			
Direct Specimen (1187 Specimens)		D ³ Ultra Final Identification	
		Pos	Neg
D ³ Duet FITC Screen	Pos	187	0
	Neg	0	1000*
Positive Percent Agreement (PPA)		100% (186/186)	
95% CI- PPA		98.0, 100%	
Negative Percent Agreement (NPA)		100% (1001/1001)	
95% CI- NPA		99.6, 100%	

* One specimen was screen positive by both devices, but was unable to be identified with the identification reagents.

Virus Follow-up Identification of 187 D ³ Duet FITC Positive Specimens for influenza A virus, influenza B virus, adenovirus, and parainfluenza virus types 1, 2, and 3 viruses, using D ³ Ultra Identification Reagents						
Virus	Sensitivity		95%CI for Sensitivity	Specificity		95% CI for Specificity
	TP / (TP+FN)	percent		TN/ (TN+FP)	percent	
Influenza A virus	100/100	100%	96.3, 100	1087/1087	100%	99.7, 100
Influenza B virus	11/11	100%	74.1, 100	1176/1176	100%	99.7, 100

Adenovirus	52/52	100%	93.1, 100	1135/1135	100%	99.7, 100
Parainfluenza type 1	4/4	100%	51.0, 100	1183/1183	100%	99.7, 100
Parainfluenza type 2	1/1	100%	20.7, 100	1186/1186	100%	99.7, 100
Parainfluenza type 3	19/19	100%	83.2, 100	1168/1168	100%	99.7, 100

The D³ Duet's ability to identify respiratory syncytial virus using phycoerythrin in direct specimens was compared to the D³ Ultra's ability using fluorescein. The positive percent agreement was 100% (95% CI range of 98.7% to 100%). The negative percent agreement was 100% (95% CI range of 99.6% to 100%). When the ability of the D³ Duet to detect the six other respiratory viruses using fluorescein in direct specimens was compared to the D³ Ultra's ability using fluorescein, the positive percent agreement was 100% (95% CI range of 97.8% to 100%). The negative percent agreement was 100% (95% CI range of 99.6% 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.

Respiratory syncytial virus by specimen type Study Sites 1, 2, and 3 Combined						
Specimen type	PPA		95% CI for PPA	NPA		95% CI for NPA
	TP / (TP+FN)	percent		TN/ (TN+FP)	percent	
NPA	155/155	100%	97.6, 100	435/435	100%	99.1, 100
NPS	132/132	100%	97.2, 100	410/410	100%	99.1, 100

D ³ Duet FITC detection of influenza A virus, influenza B virus, adenovirus, and parainfluenza virus types 1, 2, and 3 viruses by specimen type Study Sites 1, 2, and 3 Combined						
Specimen type	PPA		95% CI for PPA	NPA		95% CI for NPA
	TP / (TP+FN)	percent		TN/ (TN+FP)	percent	
NPA	103/103	100%	96.4, 100	484/484	100%	99.2, 100
NPS	79/79	100%	95.4, 100	460/460	100%	99.2, 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 D³ Duet DFA RSV/Respiratory Virus Screening Kit with that of the predicate for the presence of respiratory syncytial virus, influenza A virus, influenza B virus, adenovirus, parainfluenza virus types 1, 2 and 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 D³ Duet's phycoerythrin-labeled MAbs identification of respiratory syncytial virus specimens positive specimens, and D³ Duet's fluorescein-labeled MAbs detection of influenza A virus, influenza B virus, adenovirus, and parainfluenza virus types 1, 2, and 3 positive specimens.

Study Site 4 (culture) – D ³ Duet R-PE identification of respiratory syncytial virus positive specimens			
Cell Culture (298 Specimens)		D ³ Ultra Final Identification (respiratory syncytial virus)	
		Pos	Neg
D ³ Duet R-PE (respiratory syncytial virus)	Pos	33	0
	Neg	0	265
Positive Percent Agreement (PPA)		100% (33/33)	
95% CI- PPA		89.5, 100%	
Negative Percent Agreement (NPA)		100% (265/265)	
95% CI- NPA		98.6, 100%	

Study Site 4(culture) – D ³ Duet FITC detection of influenza A virus, influenza B virus, adenovirus, and parainfluenza virus types 1, 2, and 3			
Cell Culture (298 Specimens)		D ³ Ultra Final Identification	
		Pos	Neg

D ³ Duet FITC Screen	Pos	104	0
	Neg	0	194
Positive Percent Agreement (PPA)		100% (104/104)	
95% CI- PPA		96.4,100%	
Negative Percent Agreement (NPA)			100% (194/194)
95% CI- NPA			98.1,100%

A variety of viral respiratory pathogens were isolated. Virus identification of D³ Duet FITC Positive Specimens using D³ Ultra Identification Reagents yielded the following isolates: respiratory syncytial virus [prevalence 11.1% (33/298)], influenza A virus [prevalence 22.5% (67/298)], influenza B virus [prevalence 6.7% (20/298)], 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.



Food and Drug Administration
2098 Gaither Road
Rockville MD 20850

Gail R. Goodrum
Vice President of Regulatory Affairs
Diagnostic Hybrids, Inc.
1055 East State Street
Suite 100
Athens, Ohio 45701

DEC 23 2008

Re: k081928

Trade/Device Name: D³ *Duet* DFA RSV/Respiratory Virus Screening Kit
Regulation Number: 21 CFR 866.3480
Regulation Name: Respiratory syncytial virus serological reagents
Regulatory Class: Class I
Product Code: LKT
Dated: November 26, 2008
Received: November 28, 2008

Dear Ms. Goodrum:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to such additional controls. Existing major regulations affecting your device can be found in Title 21, Code of Federal Regulations (CFR), Parts 800 to 895. In addition, FDA may publish further announcements concerning your device in the Federal Register.

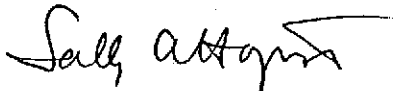
Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Parts 801 and 809); and good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820). This letter

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This letter will allow you to begin marketing your device as described in your Section 510(k) premarket notification. The FDA finding of substantial equivalence of your device to a legally marketed predicate device results in a classification for your device and thus, permits your device to proceed to the market.

If you desire specific advice for your device on our labeling regulation (21 CFR Part 801), please contact the Office of In Vitro Diagnostic Device Evaluation and Safety at 240-276-0450. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21CFR Part 807.97). For questions regarding postmarket surveillance, please contact CDRH's Office of Surveillance and Biometric's (OSB's) Division of Postmarket Surveillance at 240-276-3474. For questions regarding the reporting of device adverse events (Medical Device Reporting (MDR)), please contact the Division of Surveillance Systems at 240-276-3464. You may obtain other general information on your responsibilities under the Act from the Division of Small Manufacturers, International and Consumer Assistance at its toll-free number (800) 638-2041 or (240) 276-3150 or at its Internet address <http://www.fda.gov/cdrh/industry/support/index.html>.

Sincerely yours,



Sally A. Hojvat, M.Sc., Ph.D.
Director
Division of Microbiology Devices
Office of *In Vitro* Diagnostic Device
Evaluation and Safety
Center for Devices and
Radiological Health

Enclosure

SECTION 04, INDICATIONS FOR USE

510(k) Number (if known): k081928

Device Name: D³ Duet DFA RSV/Respiratory Virus Screening Kit

Indication for Use: The Diagnostic Hybrids, Inc. device, D3 Duet DFA RSV/Respiratory Virus Screening Kit, is intended for the qualitative detection and identification of respiratory syncytial virus, while screening for influenza A virus, influenza B 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.

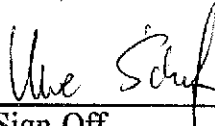
It is recommended that specimens found to be negative after examination of the direct specimen result be confirmed by cell culture. Negative results do not preclude influenza virus infection and should not be used as the sole basis for diagnosis, treatment or other management decisions.

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. 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.

Prescription Use X AND/OR Over-The-Counter Use _____
 (Part 21 CFR 801 Subpart D) (21 CFR 801 Subpart C)

(PLEASE DO NOT WRITE BELOW THIS LINE-CONTINUE ON ANOTHER PAGE IF NEEDED)

Concurrence of CDRH, Office of Device Evaluation (ODE)



 Division Sign-Off

**Office of In Vitro Diagnostic Device
 Evaluation and Safety**

510(k) k081928