K081746

DIAGNOSTIC HYBRIDS
Integrating Science and Humanity™

SECTION 05, 510(K) SUMMARY

Applicant:

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Device Name:

Trade name – D³ *Duet* DFA Influenza A/Respiratory Virus Screening Kit
Common name – Fluorescent antibody test for screening Influenza A
Classification name – Antisera, Cf, Influenza Virus A, B, C
Product Code – GNW
Regulation – 21 CFR 866.3330, Class I, Influenza 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 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.

Device Description:

The Diagnostic Hybrids, Inc. device, D^3 *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.

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 D^3 *Duet* DFA Influenza A/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 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 the individual reagents from the D^3 *Ultra*™ DFA Respiratory Virus Screening & ID Kit (D^3 *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 D^3 *Ultra* on new, separate cell preparations.

Technological Characteristics:

The DHI device, D^3 *Duet*, has been compared directly to the DHI device, D^3 *Ultra*, as the legally marketed device. The technology used in both devices is based on a standard immunofluorescence assay technique utilizing either R-PE or FITC-labeled MAbs. A summary is provided in Table 5.1 below:

TABLE 5.1: Technological Characteristics Comparison

Characteristic	D^3 *Duet* DFA Influenza A/ Respiratory Virus Screening Kit	D^3 *Ultra* DFA Respiratory Virus Screening & ID Kit
Monoclonal antibodies (MAbs)	The Influenza A/Respiratory Virus DFA Screening Reagent contains 12 MAbs to 6 different respiratory viruses (influenza B virus, respiratory syncytial virus, adenovirus, parainfluenza virus type 1, parainfluenza virus type 2, parainfluenza virus type 3), plus 2 MAbs to influenza A virus. One of the 2 MAbs to influenza A virus is different from either of those used in the D^3 *Ultra* Reagent; the second is the same.	The Respiratory Virus DFA Screening Reagent contains 12 MAbs to 6 different respiratory viruses (influenza B virus, respiratory syncytial virus, adenovirus, parainfluenza virus type 1, parainfluenza virus type 2, parainfluenza virus type 3), plus 2 MAbs to influenza A virus.

TABLE 5.1: Technological Characteristics Comparison			
Characteristic		**D³ *Duet* DFA Influenza A/ Respiratory Virus Screening Kit**	**D³ *Ultra* DFA Respiratory Virus Screening & ID Kit**
Labeling method		Direct labeling, - using R-phycoerythrin (R-PE) to label the MAbs to influenza A 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 B virus, respiratory syncytial 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		Influenza A virus (Phycoerythrin-labeled influenza A virus MAbs stain with golden-yellow fluorescence)	None (Fluorescein-labeled influenza A virus MAbs stain with apple-green fluorescence)
Cell Fixative		Cell Fixative is the same for both devices: Acetone	
Performance characteristics			
Staining patterns		**Staining patterns are the same for both devices:** **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. **Respiratory Syncytial Virus:** The fluorescence is cytoplasmic and punctate with small inclusions in the syncytia. **Parainfluenza 1, 2, 3:** The fluorescence is cytoplasmic and punctate with irregular inclusions. Types 2 and 3 cause the formation of syncytia. **Adenovirus:** The fluorescence is cytoplasmic and punctate or bright nuclear or both.	
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.	
		34.3 ± 12.0 culture positives out of 96	34.8 + 9.7 culture positives out of 96
Analytical specificity (for influenza A virus strains; MAbs are reactive with all listed strains)		Mabs to influenza A virus were shown to be reactive with these virus strains:	
		9 Flu A strains (Aichi, VR-547 (H3N2); Mal, VR-98 (H1N1); Hong Kong, VR-544 (H3N2); Denver, VR-546 (H1N1); Port Chalmers, VR-810 (H3N2); Victoria, VR-822 (H3N2); New Jersey, VR-897(H1N1); WS, VR-1520 (H1N1); PR, VR-95 (H1N1))	9 Flu A strains (Aichi, VR-547 (H3N2); Mal, VR-98 (H1N1); Hong Kong, VR-544 (H3N2); Denver, VR-546 (H1N1); Port Chalmers, VR-810 (H3N2); Victoria, VR-822 (H3N2); New Jersey, VR-897(H1N1); WS, VR-1520 (H1N1); PR, VR-95 (H1N1))
Analytical specificity (cross reactivity studies; various strains of microorganisms		Device Screening Reagent is not reactive with these microorganisms:	
	Viruses	32	31
	Bacteria	25	18
	Chlamydia spp.	3	1

TABLE 5.1: Technological Characteristics Comparison			
Characteristic		**D³ *Duet* DFA Influenza A/ Respiratory Virus Screening Kit**	**D³ *Ultra* DFA Respiratory Virus Screening & ID Kit**
and cell lines)	Yeast	1	0
	Protozoan	1	0
	Cell lines	17	17

Non-Clinical Performance:

Staining patterns of the phycoerythrin-labeled influenza A virus MAbs on influenza A 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 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 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 influenza A virus by the R-PE labeled MAbs. 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:

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

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:

Respiratory syncytial 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)	100% (30/30)	100% (30/30)

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:

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

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.

Analytical specificity

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	
		D³ *Duet*	D³ *Ultra*
Influenza A virus (PR, VR-95 H1N1)	$1x10^{-5}$	1, 3, 2, 6	1, 3, 0, 5
	$1x10^{-6}$	1, 0, 1, 1	0, 0, 1, 0
	$1x10^{-7}$	0, 0, 0, 0	0, 0, 0, 0
Influenza B virus (Hong Kong, VR-823)	$1x10^{-4}$	4, 1, 6, 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, 5	1, 3, 2, 4
	$1x10^{-7}$	0, 0, 0, 0	0, 0, 0, 0
RSV (Washington,	$1x10^{-2}$	1, 0, 3, 4	2, 3, 2, 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	
		D³ *Duet*	D³ *Ultra*
VR-1401)	$1x10^{-3}$	0, 1, 1, 0	2, 1, 0, 0
	$1x10^{-4}$	0, 0, 0, 0	0, 0, 0, 0
Parainfluenza 1 (C-35, VR-94)	$1x10^{-4}$	7, 7, 6, 8	9, 8, 4, 6
	$1x10^{-5}$	2, 2, 3, 0	1, 0, 2, 1
	$1x10^{-6}$	0, 0, 0, 0	0, 0, 0, 0
Parainfluenza 2 (Greer, VR-92)	$1x10^{-4}$	4, 0, 3, 1	4, 3, 1, 2
	$1x10^{-5}$	0, 2, 0, 0	0, 1, 1, 1
	$1x10^{-6}$	0, 0, 0, 0	0, 0, 0, 0
Parainfluenza 3 (C 243, VR-93)	$1x10^{-6}$	3, 3, 0, 6	1, 1, 3, 5
	$1x10^{-7}$	1, 0, 1, 1	1, 1, 1, 0
	$1x10^{-8}$	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-$TCID_{50}$ 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* with 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 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	9, 12, 14, 15

Analytical Reactivity (inclusivity) of D³ *Duet* with 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 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. Analytical sensitivity of the phycoerythrin-labeled influenza A MAbs of the D³ *Duet* was determined, and compared to that of the fluorescein-labeled influenza A MAbs of the D³ *Ultra*. Cell monolayers of R-Mix in 96-well plates were inoculated with prepared virus stock of influenza A virus, Victoria strain, VR-822 (H3N2), diluted to give a $TCID_{50}$[a] of 1 per 0.2-mL inoculum. The plates were incubated at 37°C for 24 hours. Monolayers were stained using the procedures in the D³ *Ultra*'s labeling or the D³ *Duet*'s draft labeling. The assay was performed four times. Results indicate that analytical sensitivities of the phycoerythrin-labeled and the fluorescein-labeled influenza A MAbs are not statistically different, by a paired t-test[b].

[a] 50% tissue culture infectivity dose
[b] Microsoft Office Excel, Microsoft Corporation

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 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 D³ *Duet* and the cleared DSFA comparator assay, combined for study sites 1, 2, and 3:

D³ *Duet* R-PE identification of influenza A virus positive specimens			
Direct Specimen (1184 Specimens)		D³ *Ultra* Final Identification (influenza A virus)	
		Pos	Neg
D³ *Duet* 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%

D³ *Duet* FITC detection of influenza B virus, respiratory syncytial virus, adenovirus, and parainfluenza virus types 1, 2, and 3 viruses			
Direct Specimen (1184 Specimens)		D³ *Ultra* Final Identification	
		Pos	Neg
D³ *Duet* 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 D³ *Duet* FITC Positive Specimens for influenza B virus, respiratory syncytial virus, adenovirus, and parainfluenza virus types 1, 2, and 3 viruses, using D³ *Ultra* Identification Reagents						
Virus	**Sensitivity**		**95%CI for Sensitivity**	**Specificity**		**95% CI for Specificity**
	TP / (TP+FN)	**percent**		TN/ (TN+FP)	**percent**	
Influenza B virus	11/11	100%	74.12, 100	1173/1173	100%	99.7, 100
Adenovirus	52/52	100%	93.1, 100	1132/1132	100%	99.7, 100
Parainfluenza type 1	4/4	100%	51.0, 100	1180/1180	100%	99.7, 100
Parainfluenza type 2	1/1	100%	20.1, 100	1183/1183	100%	99.7, 100
Parainfluenza type 3	19/19	100%	83.2, 100	1165/1165	100%	99.7, 100
Respiratory Syncytial Virus	299/299	100%	98.7, 100	885/885	100%	99.6, 100

The D³ *Duet*'s ability to identify influenza A virus using phycoerythrin in direct specimens was compared to the D³ *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 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 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.

Influenza A virus by specimen type						
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

D³ *Duet* FITC detection of influenza B virus, respiratory syncytial virus, adenovirus, and parainfluenza virus types 1, 2, and 3 viruses by specimen type						
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 D³ *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 D³ *Duet*'s phycoerythrin-labeled MAbs identification of influenza A virus positive specimens, and D³ *Duet*'s fluorescein-labeled MAbs detection of influenza B virus, respiratory syncytial virus, adenovirus, and parainfluenza virus types 1, 2, and 3 positive specimens.

Study Site 4 (culture) – D³ *Duet* R-PE identification of influenza A virus positive specimens			
Cell Culture (298 Specimens)		D³ *Ultra* Final Identification (influenza A virus)	
		Pos	Neg
D³ *Duet* 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%

Study Site 4 (culture) – D³ *Duet* FITC detection of influenza B virus, respiratory syncytial virus, adenovirus, and parainfluenza virus types 1, 2, and 3 viruses			
Cell Culture (298 Specimens)		D³ *Ultra* Final Identification	
		Pos	Neg
D³ *Duet* 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 D³ *Duet* FITC Positive Specimens using D³ *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.

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Diagnostic Hybrids, Inc.
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Suite 100
Athens, Ohio 45701

DEC 23 2008

Re: k081746
Trade/Device Name: D^3 *Duet* DFA Influenza A/Respiratory Virus Screening Kit
Regulation Number: 21 CFR 866.3330
Regulation Name: Influenza virus serological reagents
Regulatory Class: Class I
Product Code: GNW
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

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): k081746

Device Name: D^3 *Duet* DFA Influenza A/Respiratory Virus Screening Kit

Indication for Use: The Diagnostic Hybrids, Inc. device, D^3 *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.

Prescription Use		Over-The-Counter Use
X	AND/OR	_____
(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) k081746