K090073

D^3 DFA METAPNEUMOVIRUS IDENTIFICATION KIT

SECTION 05, 510(K) SUMMARY

DIAGNOSTIC HYBRIDS
Integrating Science and Humanity™

Applicant:

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MAR 6 2009

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Date of preparation of 510(k) summary:

December 22, 2008

Device Name:

Trade name – D^3 DFA Metapneumovirus Identification Kit
Common name – Fluorescent antibody test for detecting human metapneumovirus
Classification name – (blank)
Product Code – OMG
Regulation – 21 CFR 866.3980, Class II, Respiratory viral panel multiplex nucleic acid assay reagents; Panel Microbiology (83)

Legally marketed device to which equivalence is claimed:

K082688, Pro hMPV+ Assay

Intended Use: The Pro hMPV+ Assay is a Real Time RT-PCR *in vitro* diagnostic test for the qualitative detection of human Metapneumovirus (hMPV) nucleic acid isolated and purified from nasopharyngeal swab (NP) specimens obtained from individuals exhibiting signs and symptoms of acute respiratory infection. This assay targets a highly conserved region of the Nucleocapsid gene of hMPV. The detection of hMPV nucleic acid from symptomatic patients aids in the diagnosis of human respiratory hMPV infection if used in conjunction with other clinical and laboratory findings. This test is not intended to differentiate the four genetic sub-lineages of hMPV.
Negative results do not preclude hMPV infection and should not be used as the sole basis for diagnosis, treatment or other management decisions.

Device Description:

The D³ DFA Metapneumovirus Identification Kit uses a blend of three hMPV antigen-specific murine MAbs that are directly labeled with fluorescein for detection of hMPV. The reagent detects but does not differentiate between the four recognized subtypes of hMPV (subtypes A1, A2, B1, and B2).

Kit Components:

1. Metapneumovirus DFA Reagent, 5-mL. One dropper bottle containing a blend (see below for MAb discussion) of fluorescein-labeled murine monoclonal antibodies directed against MPV. The buffered, stabilized, aqueous solution contains Evans Blue as a counter-stain and 0.1% sodium azide as a preservative.
2. hMPV Antigen Control Slides, 5 slides. Five individually packaged control slides, each with a well containing cell culture-derived MPV positive cells and a well containing cell culture-derived negative cells. Each slide is intended to be stained only one time. Control material has been treated to be non-infectious; however normal laboratory precautions are required when the material is handled.
3. 40X PBS Concentrate, 25-mL. One bottle containing a 40X concentrate consisting of 4% sodium azide (0.1% sodium azide after dilution to 1X using de-mineralized water) in PBS.
4. Mounting Fluid, 7-mL. One dropper bottle containing an aqueous, buffer-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, allowed to air dry and are fixed in acetone. The Metapneumovirus DFA Reagent is added to the cells which are then incubated for 15 to 30 minutes at 35°C to 37°C in a humidified chamber or humidified incubator. The stained cells are then washed with the diluted phosphate buffered saline (PBS), 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 hMPV infected cells will fluoresce apple-green. Uninfected cells will contain no fluorescence but will be stained red by the Evans Blue counter-stain.

It is recommended that specimens found to contain no fluorescent cells after examination of the direct specimen be confirmed by an FDA cleared hMPV molecular assay.

Intended Use:

The Diagnostic Hybrids, Inc. device, D³ DFA Metapneumovirus Identification Kit, is intended for the qualitative detection and identification of human metapneumovirus (hMPV) in nasal and nasopharyngeal swabs and aspirates/washes or cell culture. The assay detects hMPV antigens by immunofluorescence using a

blend of three monoclonal antibodies (MAbs), from patients with signs and symptoms of acute respiratory infection. This assay detects but is not intended to differentiate the four recognized genetic sub-lineages of hMPV.

Negative results do not preclude hMPV infection and should not be used as the sole basis for diagnosis, treatment or other management decisions. It is recommended that specimens found to be negative after examination of the direct specimen results be confirmed by an FDA cleared hMPV molecular assay.

Technological Characteristics, Compared to Predicate Device:

TABLE 1: Technological Characteristics Comparison of Devices

D³ DFA Metapneumovirus Identification Kit (Subject)	Pro hMPV+ Assay (Predicate)	DHI Human Metapneumovirus Real-Time, Reverse Transcription PCR Assay (Reference)
Target:		
Searches of the National Center for Biotechnology Information (NCBI) databases[a] yielded presumptive evidence that the target for each of the 3 MAb clones is the MPV nucleoprotein. Nine proteins are known to be encoded in the hMPV genome.[b] Of the nine proteins, only the nucleoprotein is of a size equivalent to the 46 kDa size noted on the Western of the 3 MAb clones.	The Pro hMPV+ Supermix contains oligonucleotide primers and target-specific oligonucleotide probes. The primers are complementary to highly conserved regions of genetic sequences of the nucleocapsid of hMPV.	The DHI Human Metapneumovirus Real-Time, Reverse Transcription PCR Assay master mix contains oligonucleotide primers and target-specific oligonucleotide probes. The primers are complementary to highly conserved sequences within the nucleocapsid gene of hMPV.
Specimen:		
Nasal and nasopharyngeal swabs and aspirates or cell culture.	Nasopharyngeal swab specimens using a polyester, rayon or nylon tipped swab and placed into viral transport medium	Nasal and nasopharyngeal swabs and aspirates or cell culture.
Detection Methods:		
The assay detects specific hMPV viral antigens by immunofluorescence using monoclonal antibodies (MAbs). 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 hMPV DFA 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 phosphate buffered saline (PBS), 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 hMPV infected cells will fluoresce apple-green. Uninfected cells will	Reverse transcription of the RNA in the sample into complementary DNA (cDNA) and subsequent amplification of DNA is performed in a Cepheid SmartCycler®II instrument. In this process, the probe anneals specifically to the template followed by primer extension and amplification. The Pro hMPV+ Assay is based on TaqMan chemistry, which utilizes the 5' - 3' exonuclease activity of the Taq polymerase to cleave the probe thus separating the reporter dye from the quencher. This generates an increase in fluorescent signal upon excitation from a light source. With each cycle,	Reverse transcription of the RNA in the sample into complementary DNA (cDNA) and subsequent amplification of DNA is performed in a Stratagene Mx3000p instrument. In this process, the probe anneals specifically to the template followed by primer extension and amplification. The DHI Human Metapneumovirus Real-Time, Reverse Transcription Assay is based on TaqMan chemistry, which utilizes the 5' - 3' exonuclease activity of the Taq polymerase to cleave the probe thus separating the reporter dye from the quencher. This generates an increase in fluorescent signal upon excitation from a light source. With each cycle,

[a] NCBI (National Center for Biotechnology Information) internet web site http://www.ncbi.nlm.nih.gov/

[b] Proteins encoded in hMPV genome are nucleoprotein, phosphoprotein, matrix protein, fusion glycoprotein precursor, matrix protein M2-1, matrix protein M2-2, small hydrophobic protein, attachment glycoprotein G, RNA dependent RNA polymerase.

TABLE 1: Technological Characteristics Comparison of Devices		
D³ DFA Metapneumovirus Identification Kit (Subject)	**Pro hMPV+ Assay (Predicate)**	**DHI Human Metapneumovirus Real-Time, Reverse Transcription PCR Assay (Reference)**
contain no fluorescence but will be stained red by the Evans Blue counter-stain.	additional reporter dye molecules are cleaved from their respective probes, further increasing fluorescent signal. The amount of fluorescence at any given cycle is dependent on the amount of amplification products present at that time. Fluorescent intensity is monitored during each PCR cycle by the SmartCycler II instrument.	additional reporter dye molecules are cleaved from their respective probes, further increasing fluorescent signal. The amount of fluorescence at any given cycle is dependent on the amount of amplification products present at that time. Fluorescent intensity is monitored during each PCR cycle by the Stratagene Mx3000p instrument.
Analytical sensitivity:		
Analytical detection limits on direct specimens for the D³ DFA Metapneumovirus Identification Kit were addressed using quantified cultures of characterized isolates of each of the 4 recognized genetic sublineages of hMPV (A1, A2, B1, and B2). The infected culture cells from a 1,000 infected cells/mL culture were serially diluted with a suspension of uninfected LLC-MK_2 cells. 25-µL aliquots from each dilution level were spotted onto 10 replicate microscope slides, then fixed and stained according to the instructions for use described in this product insert. Each cell spot was examined at 200x magnification. Results were reported as numbers of positive replicates for each set of 10. Analytical detection limits for each of the 4 hMPV genetic sublineages were defined as the lowest dilutions at which at least 9 out of 10 replicates were detected. Based on this testing the LoD for each subtype was A1= 25 infected cells/mL, A2 = 200 infected cells/mL, B1 = 50 infected cells/mL, B2 = 100 infected cells/mL. Detection limit on cell culture amplified specimens of the D³ DFA Metapneumovirus Identification Kit was addressed using a cell culture system. Analytical detection limits on cell culture amplified specimens for hMPV subtypes A1, A2, B1, and B2 were established with results reported in numbers of fluorescent cells per cell monolayer. Each master stock virus preparation was diluted in a ten-fold manner. Eight wells of a 48-well R-Mix cell culture plate were inoculated with	The analytical sensitivity (limit of detection or LoD) of the Pro hMPV+ Assay was determined using quantified ($TCID_{50}$/mL) cultures of 2 hMPV (subtype A2 and subtype B2) strains serially diluted in nasopharyngeal clinical matrix. Each viral strain was extracted using the Roche MagNA Pure LC and tested in replicates of 20 per concentration of virus. Analytical sensitivity (LoD) as defined as the lowest concentration at which ³ 95% of all replicates tested positive, ranged from 102 to 101 $TCID_{50}$/mL.**D Concentration** hMPV subtype A2 102 TCID50/mL hMPV subtype B2 101 TCID50/mL	Analytical validation of the real-time hMPV RT-PCR followed by bi-directional sequencing analysis comparator assay included analytical sensitivity and reactivity study, analytical specificity study, and extraction efficiency study. The analytical sensitivity (limit of detection or LoD) of the real-time hMPV RT-PCR followed by bi-directional sequencing analysis comparator assay was determined using quantified ($TCID_{50}$/mL) stocks of the 4 hMPV (subtypes A1, A2, B1 and B2) strains diluted in hMPV negative nasopharyngeal clinical matrix, and ranged from 10 – 50 $TCID_{50}$/mL.

TABLE 1: Technological Characteristics Comparison of Devices

D³ DFA Metapneumovirus Identification Kit (Subject)	Pro hMPV+ Assay (Predicate)	DHI Human Metapneumovirus Real-Time, Reverse Transcription PCR Assay (Reference)

0.2-mL volumes of each dilution. The plates were centrifuged at 700 xg for 60 minutes, and then incubated at 35°C to 37°C for 48-hours. Each well was stained with the D³ DFA Metapneumovirus Identification Kit then examined at 200x magnification and the number of fluorescent cells counted. In this study, the detection limit for the test on cell culture amplified specimens is defined as the lowest inoculum level at which positive wells (i.e. containing fluorescent cells) are observed, in terms of $TCID_{50}$. Table 1.1 below summarizes the results:

TABLE 1.1: Limit of Detection of the D³ DFA Metapneumovirus Identification Kit for Cell Culture Amplified Specimens (values are numbers of fluorescent staining cells per cell monolayer)

Virus Strain	Conc. of Inoculum	Fluorescent staining cells/well
hMPV A1	50-$TCID_{50}$	47,39,41,31,26,30, 21,29
	5-$TCID_{50}$	0,0,0,3,1,0,2,0
	0.5-$TCID_{50}$	0,0,0,0,0,0,0,0
hMPV A2	50-$TCID_{50}$	10,13,23,13,23,15, 17,12
	5-$TCID_{50}$	3,1,1,4,2,2,0,0
	0.5-$TCID_{50}$	0,0,0,0,0,0,0,0
hMPV B1	50-$TCID_{50}$	36,56,23,41,28,29, 34,28
	5-$TCID_{50}$	4,7,0,3,1,0,4,4
	0.5-$TCID_{50}$	0,0,0,0,0,0,0,0
hMPV B2	50-$TCID_{50}$	25,49,36,41,53,68, 43,27
	5-$TCID_{50}$	0,3,1,1,5,6,3,5
	0.5-$TCID_{50}$	0,0,0,0,0,0,0,0

Analytical specificity (cross reactivity studies; various strains of microorganisms and cell lines):

Viruses	59	26	14
Bacteria	25	21	0
Chlamydia spp.	3	2	0
Yeast	1	1	0
Protozoan	1	0	0

TABLE 1: Technological Characteristics Comparison of Devices			
D³ DFA Metapneumovirus Identification Kit (Subject)		Pro hMPV+ Assay (Predicate)	DHI Human Metapneumovirus Real-Time, Reverse Transcription PCR Assay (Reference)
Cell lines	17	0	0
Human genomic DNA			1
Human total RNA			1

Analytical Performance:

The MAbs all recognize a hMPV protein, approximately 46 kiloDaltons in size, which corresponds with the size of hMPV nucleoprotein, but do not compete with one another for binding sites (as demonstrated by SDS-PAGE analyses, against lysates of cell culture infected with hMPV subtypes A1, A2, B1, and B2).

Analytical specificity of the MPV DFA Reagent was evaluated against other respiratory viruses (multiple strains of adenovirus, influenza A, influenza B, respiratory syncytial virus, and parainfluenza types 1, 2, and 3), as well as strains of 8 other viruses, and 30 other microorganisms that could be encountered in a respiratory specimen, cell culture contamination, or laboratory processing.

Reproducibility

Assay reproducibility was assessed at 3 laboratory sites with a panel of proficiency-level antigen control slides. The reproducibility panel consisted of 5 panel members. Each panel member was a 2-well slide spotted with the same cell preparation in each well. The cell preparations used to construct the slides are the following:

1. Non-infected LLC-MK_2 cells.
2. Low level hMPV (A1 strain) grown in LLC-MK_2 cells (manufactured to contain between 4 to 10% infected cells).
3. Mid level hMPV (A1 strain) grown in LLC-MK_2 cells (manufactured to contain between 20 to 30% infected cells).
4. High level hMPV (A1 strain) grown in LLC-MK_2 cells (manufactured to contain between 50 to 75% infected cells).

Each panel was tested daily in two separate runs for 5-days by three different laboratories (30 total runs). The panel members were randomized with different slide identification numbers to act as a "blinded" panel. An hMPV Antigen Control Slide (two-well slide, one well contains cell culture-derived hMPV positive cells and one well contains cell culture-derived negative cells) was stained according to the D³ DFA Metapneumovirus Identification Kit instructions for use with each run. The following results were recorded for both the control slides and the panel slides:

1. Presence or absence of green fluorescence.
2. Percent of cells exhibiting green fluorescence.

A single lot of D³ MPV Kit was used. A total of 210 data points were included in the reproducibility study data analysis (7 samples and controls/run X 2 runs/day X 5 days X 3 sites = 210). The combined data from the three sites demonstrated that the detection of hMPV occurs in a reproducible manner. The presence of hMPV infected cells was reported in 100% (120/120) of the wells in which infected cells were present. The combined data from the three sites also demonstrated that no hMPV was detected in non-infected cells. The absence of hMPV was reported in 100% (90/90) of the wells in which infected cells were not present. The total percent agreement for the D³ DFA Metapneumovirus Identification Kit was 100% (210/210). The Table below summarizes the reproducibility study results:

Reproducibility Study Results

	Panel Member	hMPV A1 Low Level	hMPV A1 Mid Level	hMPV A1 High Level	hMPV A1 Negative	Positive Control	Negative Control	Total % Agreement
	Concentration	4 to 10% infected cells	20 to 30% infected cells	50 to 75% infected cells	Non-infected cells	50 to 75% infected cells	Non-infected cells	
Site 1	Agreement with Expected result	10/10 (100%)	10/10 (100%)	10/10 (100%)	20/20 (100%)	10/10 (100%)	10/10 (100%)	**70/70 (100%)**
Site 2	Agreement with Expected result	10/10 (100%)	10/10 (100%)	10/10 (100%)	20/20 (100%)	10/10 (100%)	10/10 (100%)	**70/70 (100%)**
Site 3	Agreement with Expected result	10/10 (100%)	10/10 (100%)	10/10 (100%)	20/20 (100%)	10/10 (100%)	10/10 (100%)	**70/70 (100%)**
	Total Agreement with Expected result	30/30 (100%)	30/30 (100%)	30/30 (100%)	60/60 (100%)	30/30 (100%)	30/30 (100%)	**210/210 (100%)**
	95% CI	88.4%-100%	88.4%-100%	88.4%-100%	94.0%-100%	88.4%-100%	88.4%-100%	**98.3%-100%**

Clinical Performance:

Performance characteristics of the DHI D³ DFA Metapneumovirus Identification Kit testing direct respiratory specimens were established during prospective studies at 3 geographically diverse U.S. clinical laboratories during the 2005 and 2006 respiratory virus seasons (December 2005 – April 2006 and December 2006 – March 2007). All specimens used in the studies meeting the inclusion and exclusion criteria represented excess, remnants of respiratory specimens that were prospectively collected from symptomatic individuals suspected of respiratory infection, and were submitted for routine care or analysis by each site, and that otherwise would have been discarded. Individual specimens were delinked from all patient identifiers and given a study sample code. All clinical sites were granted waivers of informed consent by their IRBs for this study.

Performance of the D³ DFA Metapneumovirus Identification Kit was assessed and compared to a predetermined algorithm that used composite comparator methods at clinical study site 1 and 2. The composite comparator methods consisted of viral culture and a validated real-time RT-PCR comparator assay targeting the hMPV nucleocapsid gene followed by bi-directional sequencing analysis[c]. "True" hMPV positive was defined as any sample that either tested positive by viral culture, or had bi-directional sequencing data meeting pre-defined quality acceptance criteria that matched hMPV sequences deposited in the National Center for Biotechnology Information (NCBI) GenBank database (www.ncbi.nlm.nih.gov), with acceptable E-values[d]. "True" hMPV negative was defined as any sample that tested negative by both viral culture and the hMPV real-time RT-PCR comparator assay..

Performance of the D³ DFA Metapneumovirus Identification Kit at clinical study site 3 was evaluated and compared only to the validated hMPV real-time RT-PCR followed by bi-directional sequencing analysis comparator assay described above. Any sample that had bi-directional sequencing data meeting pre-defined quality acceptance criteria that matched hMPV sequences deposited in the National Center for Biotechnology Information (NCBI) GenBank database (www.ncbi.nlm.nih.gov), with acceptable E-values, was considered as hMPV positive, and the real-time hMPV RT-PCR comparator assay negatives were considered as hMPV negatives at this site.

Study Site 1

Study Site 1 evaluated a total of 1564 fresh respiratory specimens submitted, December 2006 through March 2007, to the laboratory for respiratory virus testing. Slides were prepared from Phosphate Buffered Saline (PBS)-washed cells from the fresh specimens and fixed according to the prescribed protocol. The slides were stained in accordance with the procedure in the product insert.

[c] Analytical validation of the real-time hMPV RT-PCR followed by bi-directional sequencing analysis comparator assay included analytical sensitivity and reactivity study, analytical specificity study, and extraction efficiency study. The analytical sensitivity (limit of detection or LoD) of the real-time hMPV RT-PCR followed by bi-directional sequencing analysis comparator assay was determined using quantified ($TCID_{50}$/mL) stocks of the 4 hMPV (subtypes A1, A2, B1 and B2) strains diluted in hMPV negative nasopharyngeal clinical matrix, and ranged from 10 – 50 $TCID_{50}$/mL.

[d] The E-values generated from the clinical trials range from a low of 2e-77 to a high of 2e-67. The E-Value from NCBI BLAST Alignment indicates the statistical significance of a given pair-wise alignment and reflects the size of the database and the scoring system used. The lower the E-Value, the more significant the hit. A sequence alignment that has an E-Value of 1e-3 means that this similarity has a 1 in 1000 chance of occurring by chance alone. (http://www.ncbi.nlm.nih.gov/books/bv.fcgi?rid=handbook.section.614).
Therefore an E-Value ranging from 2e-67 to 2e-77 has a very low probability of occurring purely by chance.

Table 2 shows the age and gender distribution for individuals studied at study site 1:

TABLE 2: Site 1 – Age and Gender Distribution		
Sex	F	M
Total	687	877
Age: <1m	42	50
≥ 1m to < 2y	444	617
≥2y to <12y	164	185
≥ 12y to < 18y	30	20
≥ 18y to < 21y	4	3
≥ 21y	3	2
Age Not Reported	0	0

Of the 1564 fresh respiratory specimens tested, 1509 were nasal wash/nasopharyngeal aspirate specimens. Due to insufficient sample numbers to establish performance of the D³ DFA Metapneumovirus Identification Kit, 55 other types of respiratory specimens were removed from performance analysis. Of the 1509 fresh nasal wash/nasopharyngeal aspirate specimens tested, 27 were further excluded from the performance analysis due to insufficient volume for the comparator methods, resulting in a total of 1482 fresh nasal wash/nasopharyngeal aspirate specimens for analysis. Table 3 below shows the study results of the claimed specimen type at study site 1:

TABLE 3: Study Site 1- Comparison of Results using D³ DFA MPV Kit, with Results using the Composite Comparator Methods			
Fresh Nasal Wash/Nasopharyngeal Aspirate	Composite Comparator Methods		
DHI DSFA	Positive	Negative	Total
Positive	122	3	125
Negative	108	1249	1357
Total	230	1252	1482
			95% CI
Sensitivity	122/230	53.0%	46.6%-59.5%
Specificity	1249/1252	99.8%	99.3%-99.9%

Study Site 2

Study Site 2 evaluated a total of 371 fresh respiratory specimens submitted, December 2005 through January 2006, to the laboratory for respiratory virus testing. Slides were prepared from Phosphate Buffered Saline (PBS)-washed cells from the fresh specimens and fixed according to the prescribed protocol. The slides were stained in accordance with the procedure in the product insert.

Table 4 below shows the age and gender distribution for individuals studied at study site 2:

TABLE 4: Site 2 – Age and Gender Distribution		
Sex	F	M
Total	155	216
Age: <1m	2	5
≥ 1m to < 2y	50	83
≥2y to <12y	26	37
≥ 12y to < 18y	2	5
≥ 18y to < 21y	1	0
≥ 21y	74	86
Age Not Reported	0	0

Of the 371 fresh respiratory specimens tested, all were nasal/nasopharyngeal swab specimens. 3 were excluded from the performance analysis due to insufficient volume for the comparator methods, resulting in a total of 368 fresh nasal/nasopharyngeal swab specimens for analysis. Table 5 below shows the study results of the claimed specimen type at study site 2:

TABLE 5: Study Site 2- Comparison of Results using D³ DFA MPV Kit, with Results using the Composite Comparator Methods			
Fresh Nasal/Nasopharyngeal Swab	Composite Comparator Methods		
DHI DSFA	Positive	Negative	Total
Positive	41	1	42
Negative	17	309	326
Total	58	310	368
			95% CI
Sensitivity	41/58	70.7%	57.3%-81.9%
Specificity	309/310	99.7%	98.2%-100%

Study Site 3

Study Site 3 evaluated a total of 174 fresh respiratory specimens submitted, March 2006 through April 2006, to the laboratory for respiratory virus testing. Slides were prepared from Phosphate Buffered Saline (PBS)-washed cells from the fresh specimens and fixed according to the prescribed protocol. The slides were stained in accordance with the procedure in the product insert.

Table 6 below shows the age and gender distribution for individuals studied at study site 3:

TABLE 6: Site 3 – Age and Gender Distribution			
Sex	F	M	Sex Not Reported
Total	78	95	1
Age: <1m	1	1	0
≥ 1m to < 2y	19	37	0
≥2y to <12y	16	17	0
≥ 12y to < 18y	3	6	0
≥ 18y to < 21y	2	0	0
≥ 21y	26	22	0
Age Not Reported	11	12	1

Of the 174 fresh respiratory specimens tested, 62 were nasal wash/nasopharyngeal aspirate specimens, and 110 were nasal/nasopharyngeal swab specimens. Of the 62 nasal wash/nasopharyngeal aspirate specimens, 30 were excluded from the performance analysis due to insufficient volume for the comparator method, resulting in a total of 32 fresh nasal wash/nasopharyngeal aspirate specimens for analysis. Of the 110 nasal/nasopharyngeal swab specimens, 44 were excluded from the performance analysis due to insufficient volume for the comparator method, resulting in a total of 66 fresh nasal/nasopharyngeal swab specimens for analysis. Table 7 and 8 below show the study results of the claimed specimen types at **study site 3**:

TABLE 7: Study Site 3- Comparison of Results using D[3] DFA MPV Kit, with Results using the hMPV real-time RT-PCR followed by bi-directional sequencing analysis comparator assay			
Fresh Nasal Wash/Nasopharyngeal Aspirate	Comparator Assay		
DHI DSFA	Positive	Negative	Total
Positive	9	0	9
Negative	0	23	23
Total	9	23	32
			95% CI
Positive Percent Agreement*	9/9	100.0%	66.4%-100%
Negative Percent Agreement*	23/23	100.0%	85.2%-100%

TABLE 8: Study Site 3- Comparison of Results using D[3] DFA MPV Kit, with Results using the hMPV real-time RT-PCR followed by bi-directional sequencing analysis comparator assay			
Fresh Nasal/Nasopharyngeal Swab	Comparator Assay		
DHI DSFA	Positive	Negative	Total
Positive	3	0	3
Negative	1	62	63
Total	4	62	66
			95% CI

Positive Percent Agreement*	3/4	75.0%	19.4%-99.4%
Negative Percent Agreement*	62/62	100.0%	94.2%-100%

*Since the performance of the D³ DFA Metapneumovirus Identification Kit at clinical study site 3 was not assessed against the predetermined composite comparator methods, positive and negative percent agreements, instead of sensitivity and specificity, are used in the performance presentation.

Cultured Cells Testing

Performance characteristics of the DHI D³ DFA Metapneumovirus Identification Kit testing cultured cell specimens were established during a prospective study at DHI during the 2007 respiratory virus seasons (January – April 2008). All specimens used in the studies meeting the inclusion and exclusion criteria represented excess, remnants of respiratory specimens that were prospectively collected from symptomatic individuals suspected of respiratory infection, and were submitted for routine care or analysis by each collection site, and that otherwise would have been discarded. Individual specimens were delinked from all patient identifiers and given a study sample code.

Performance of the D³ DFA Metapneumovirus Identification Kit testing cultured cell specimens was evaluated and compared to the same validated hMPV real-time RT-PCR followed by bi-directional sequencing analysis comparator assay as described earlier, at clinical study site 4. Any cultured cell specimen that had bi-directional sequencing data meeting pre-defined quality acceptance criteria that matched hMPV sequences deposited in the National Center for Biotechnology Information (NCBI) GenBank database (www.ncbi.nlm.nih.gov), with acceptable E-values, was considered as hMPV positive, and the real-time hMPV RT-PCR comparator assay negative cultured cell specimens were considered as hMPV negatives.

Study Site 4

Study Site 4 evaluated a total of 74 freeze-thawed nasopharyngeal swab specimens that were cultured and stained in accordance with the D[3] DFA Metapneumovirus Identification Kit procedure. Table 9 below shows the study results testing cultured cell specimens at study site 4:

TABLE 9: Study Site 4- Comparison of Results using D[3] DFA MPV Kit, with Results using the hMPV real-time RT-PCR followed by bi-directional sequencing analysis comparator assay

Freeze-thawed Nasopharyngeal Swab Amplified in Cell Culture	DHI hMPV RT-PCR Followed by Sequencing Comparator Assay		
DHI DFA	Positive	Negative	Total
Positive	5	0	5
Negative	1	68	69
Total	6	68	74
			95% CI
Positive Percent Agreement	5/6	83.3%	35.9%-99.6%
Negative Percent Agreement	68/68	100.0%	99.7%-100%

DEPARTMENT OF HEALTH & HUMAN SERVICES

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Re: K090073
Trade/Device Name: D3 DFA Metapneumovirus Identification Kit
Regulation Number: 21 CFR 866.3980
Regulation Name: Respiratory viral panel multiplex nucleic acid assay
Regulatory Class: Class II
Product Code: OMG
Dated: December 22, 2008
Received: January 12, 2009

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 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

Indication for Use

510(k) Number (if known): k090073

Device Name: D^3 DFA Metapneumovirus Identification Kit

Indication For Use:

The Diagnostic Hybrids, Inc. device, D^3 DFA Metapneumovirus Identification Kit, is intended for the qualitative detection and identification of human metapneumovirus (hMPV) in nasal and nasopharyngeal swabs and aspirates/washes or cell culture. The assay detects hMPV antigens by immunofluorescence using a blend of three monoclonal antibodies (MAbs), from patients with signs and symptoms of acute respiratory infection. This assay detects but is not intended to differentiate the four recognized genetic sub-lineages of hMPV.

Negative results do not preclude hMPV infection and should not be used as the sole basis for diagnosis, treatment or other management decisions. It is recommended that specimens found to be negative after examination of the direct specimen results be confirmed by an FDA cleared hMPV molecular assay.

Prescription Use X And/Or Over the Counter Use ____.
(21 CFR Part 801 Subpart D) (21 CFR Part 801 Subpart C)

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

Concurrence of CDRH, Office of In Vitro Diagnostic Device Evaluation and Safety (OIVD)

Division Sign-Off
Office of In Vitro Diagnostic Device
Evaluation and Safety

510(k) k090073