

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

A. 510(k) Number: K081164

B. Purpose for Submission: The 510(k) holder would like to introduce D³ DFA Cytomegalovirus Immediate Early Antigen Identification Kit into interstate commerce.

C. Measurand: Human cytomegalovirus (CMV) immediate early antigen (IEA)

D. Type of Test: DFA to detect the presence of CMV immediate early antigen amplified in cell culture using fluoresceinated monoclonal antibodies.

E. Applicant: Diagnostic Hybrids, Inc.

F. Proprietary and Established Names: Diagnostic Hybrids' D³ DFA Cytomegalovirus Immediate Early Antigen Identification Kit

G. Regulatory Information:

1. Regulation section:
21CFR 866.3175 Cytomegalovirus serological reagents
2. Classification:
Class II
3. Product code:
LIN – Antiserum, Conjugated Fluorescent, Cytomegalovirus
4. Panel:
Microbiology (83)

Product Code	Classification	Regulation Section	Panel
LIN	Class II	21CFR 866.3175	Microbiology (83)

H. Intended Use:

Intended use(s): The Diagnostic Hybrids, Inc. device, D³ DFA Cytomegalovirus Immediate Early Antigen Identification Kit, is intended for use in the qualitative detection and identification of human cytomegalovirus (CMV) immediate early antigen (IEA) in cell cultures by immunofluorescence using fluoresceinated monoclonal antibodies (MAbs).

This product is not intended for use in testing blood or plasma donors and is not intended for use in direct detection of cytomegalovirus in clinical specimens.

2. Indication(s) for use: Same as Intended Use.
3. Special conditions for use statement(s): For prescription use only

4. Special instrument requirements: Fluorescence microscope with the correct filter combination for FITC (excitation peak = 490 nm, emission peak = 520nm).

I. Device Description:

Two murine derived monoclonal antibodies (MAbs) are used in the Diagnostic Hybrids, Inc. (DHI) device, D³ DFA Cytomegalovirus Immediate Early Antigen Identification Kit (CMV-IEA ID Kit), and are directed against CMV immediate early antigen (pp 72). The MAbs used in the Kit have been shown to be highly specific, with no cross-reactivity to other cultured viruses. The MAbs have been labeled by DHI using Fluorescein Isothiocyanate (FITC).

Kit Components

1. CMV-IEA DFA Reagent, 10-mL. One dropper bottle containing a mixture of two murine MAbs directed against CMV immediate early antigen (pp 72). The MAbs are both IgG1 (k) isotype. The buffered, stabilized, aqueous solution contains Evans Blue as a counter-stain and 0.1% sodium azide as preservative.
2. CMV Antigen Control Slides, 5-slides. Individually packaged control slides containing wells with cell culture derived positive and negative control cells. Each slide contains one Negative well of uninfected cells and one Positive well of CMV infected cells. Each slide is intended to be stained only one time.
3. Mounting Fluid, 7-mL. One dropper bottle of an aqueous, buffered, stabilized solution of glycerol (ph 8.2 ± 0.2) and 0.1% sodium azide.
4. 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 a phosphate buffered saline (PBS) solution.

J. Substantial Equivalence Information:

1. Predicate device names:

- a. K951821, Light Diagnostics CMV Direct Immunofluorescence Assay (Millipore)
- b. K904036, Bartels Cytomegalovirus Immediate Early Antigen Indirect Fluorescent Antibody (Trinity Biotech PLC)

2. Predicate K numbers:

- a. K951821
- b. K904036

1. Comparison with predicate:

The Diagnostic Hybrids, Inc. device, D³ DFA Cytomegalovirus Immediate Early Antigen Identification Kit, has been compared directly to Bartels Cytomegalovirus Immediate Early Antigen Kit and the Light Diagnostics CMV Direct Immunofluorescence Assay as the legally marketed devices. The technology used in all devices is based on a standard immunofluorescence assay technique utilizing fluorescein-labeled MAbs and viral isolation in cell culture. A summary is provided in Table 1 below:

TABLE 1: Technological Characteristics Compared Among DHI Device and Predicate Devices			
Characteristic	Subject Device DHI	Predicate Bartels (K904036)	Predicate Light Diagnostics (K951821)
Immediate Early Antigen	X	X	X
MAB Directly Labeled with Fluorescein	X	--	X
MAB Indirect labeled with Fluorescein (requires labeled secondary anti-mouse antibody)	--	X	--
Culture Confirmation	X	X	X

Similarities		
Item	Device	Comparison Device
tended Use	For the qualitative detection of CMV in inoculated cell cultures by immunofluorescence using fluoresceinated monoclonal antibodies (MAbs). Performance has not been established with direct patient specimens.	a. Light Diagnostics b. Bartels
asic principle	Direct Fluorescent Antibody (DFA) test - Immunofluorescence using fluoresceinated MAbs	a. Light Diagnostics
ntibody ntigen	Murine monoclonal antibodies against epitopes of CMV	a. Light Diagnostics b. Bartels
Instrumentation (required but not provided)	Fluorescence microscope with filter combination for fluorescein (excitation peak = 490 nm, emission peak = 520nm).	a. Light Diagnostics b. Bartels

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

L. Test Principle:

The D³ DFA Cytomegalovirus Immediate Early Antigen Identification Kit uses a blend of CMV-IEA antigen-specific murine MAbs that are directly labeled with fluorescein for the rapid detection of CMV-IEA in cell culture. Clinical specimens are inoculated into permissible cultured cell monolayers. These cells are fixed in acetone 1- to 4-days post-inoculation. The CMV-IEA DFA Reagent is added to the cells to detect the presence of

any CMV-IEA antigens present. After incubating for 15 to 30 minutes at 35° to 37°C, the stained cells are washed with the supplied Phosphate Buffered Saline (PBS) and, using the supplied Mounting Fluid, processed further for examination using a fluorescence microscope equipped with the correct filter combination for Fluorescein Isothiocyanate (FITC) at a magnification of 200-400X. Virus infected cells will contain bright apple-green fluorescent nuclei while uninfected cells will contain no apple-green fluorescence but will fluoresce red from the Evan's Blue.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility: Not applicable*

b. *Linearity/assay reportable range: Not applicable*

c. *Traceability, Stability, Expected values (controls, calibrators, or methods): Not applicable*

d. *Analytical Sensitivity:*

Analytical sensitivity was studied for purposes of demonstrating the effectiveness of the D³ CMV-IEA DFA Reagent with that of a comparator device. This was done by first inoculating two 96-well cell culture plates (Hs27) with CMV diluted to a value of 1-TCID₅₀ and incubating at 37°C for 48 hours; then, one plate was stained with the subject D³ CMV-IEA DFA Reagent and the other plate was stained using the CMV-IEA DFA Reagent from the comparator device. This assay was performed three times, with an average of 35.3 ±2.3 positive wells out of a total 96 wells detected with the subject, and an average of 38.3 ±2.1 positive wells out of a total 96 wells with the predicate. These results were not statistically different by a paired t-test.

Detection limit for the subject device CMV-IEA ID Kit was addressed under the conditions: CMV, at a starting concentration 350-TCID₅₀ per mL, was serially diluted to a final value of 0.7-TCID₅₀ per mL using 2-fold dilutions. Each dilution was inoculated into confluent monolayers of Hs27 cells contained in multi-well plates, centrifuged at 700 x g for 60 minutes and incubated at 35° to 37°C for 48 hours. The subject CMV-IEA ID Kit or the comparator device was used to stain 3 monolayers of each viral dilution according to the respective device's product insert. The number of positive cells per well was counted. The experiment was performed three times. The results suggest that the detection limit of both fluorescent antibody stains are comparable, with 0.7-TCID₅₀ as the minimum viral dilution detected, as indicated by at least one well having no detectable infection. These results were not statistically different by a paired T-test.

Analytical Specificity (Cross-reactivity Testing):

The D³ DFA Cytomegalovirus Immediate Early Antigen Identification Kit DFA Reagent was tested for cross-reactivity against a wide variety of cells and microorganisms. Stringent conditions for cross-reactivity testing were achieved by using a high concentration of the CMV-IEA DFA Reagent and relatively high titers of microorganisms. The DFA Reagent was prepared at 2X the concentration that is provided in the kit. No cross-reactivity was observed for 58 virus strains or for 20 host culture cell types. Twenty-five (25) bacterial cultures, one yeast culture and three bacterial (*Chlamydia* sp.) and one protozoan commercially available slides, were stained and examined for cross-reactivity, including *Staphylococcus aureus*, a protein-A-producing bacterium. Staining of *S. aureus* appeared as small points of fluorescence (see Limitations of Procedure). Fifty-eight (58) virus strains were tested for cross-reactivity. Depending on the particular virus, 140 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 2X DFA Reagent according to the procedure as detailed in this product insert. No cross-reactivity was observed for the viruses listed below

Organism	Strain or Type	Inoculum (TCID ₅₀)	Organism	Strain or Type	Inoculum (TCID ₅₀)
Adenovirus	Type 1	1,400	RSV	Long	1,400
Adenovirus	Type 3	1,400	RSV	Wash	1,400
Adenovirus	Type 5	1,400	RSV	9320	1,400
Adenovirus	Type 6	1,400	Parainfluenza 1	C-35	1,400
Adenovirus	Type 7	1,400	Parainfluenza 2	Greer	1,400
Adenovirus	Type 8	1,400	Parainfluenza 3	C 243	1,400
Adenovirus	Type 10	1,400	Parainfluenza 4a	M-25	1,400
Adenovirus	Type 13	1,400	Parainfluenza 4b	CH19503	1,400
Adenovirus	Type 14	1,400	HSV-1	IF	140
Adenovirus	Type 31	1,400	HSV-1	MacIntyre	140
Influenza A	Aichi	1,400	HSV-2	MS	140
Influenza A	Malaya	1,400	HSV-2	Strain G	140
Influenza A	Hong Kong	1,400	VZV	Webster	140
Influenza A	Denver	1,400	VZV	Ellen	140
Influenza A	Port Chalmers	1,400	VZV	AV92-3	140
Influenza A	Victoria	1,400	Epstein-Barr	Commercially available slides stained.*	
Influenza A	New Jersey	1,400	Rubeola		
Influenza A	PR	1,400	Mumps		
Influenza A	WS	1,400	Echovirus	Types 4, 6, 9, 11, 30, 34	Commercially available slides
Influenza B	Hong Kong	1,400			

* Test material is from commercially available prepared slides. Each positive well contains 10 to 50% reactive cells.

Organism	Strain or Type	Inoculum (TCID ₅₀)	Organism	Strain or Type	Inoculum (TCID ₅₀)
Influenza B	Maryland	1,400	Coxsackievirus	Types B1, B2, B3, B4, B5, B6	stained.*
Influenza B	Mass	1,400			
Influenza B	Taiwan	1,400			
Influenza B	GL	1,400	Poliovirus	Types 1, 2, 3	
Influenza B	Russia	1,400			

Influenza A	PR	1,400	Mumps		
Influenza A	WS	1,400	Echovirus	Types 4, 6, 9, 11, 30, 34	Commercially available slides stained.*
Influenza B	Hong Kong	1,400	Coxsackievirus	Types B1, B2, B3, B4, B5, B6	
Influenza B	Maryland	1,400	Poliovirus	Types 1, 2, 3	
Influenza B	Mass	1,400			
Influenza B	Taiwan	1,400			
Influenza B	GL	1,400			
Influenza B	Russia	1,400			

Twenty (20) host culture cell types were tested for cross-reactivity. Cell cultures were prepared in shell vial format. Confluent monolayers were stained with the 2X DFA Reagent according to the procedure as detailed in this product insert, then examined for cross-reactivity. No cross-reactivity was observed for the following cell lines

Cell Lines and Viruses Cross-reactivity Testing

Organism	Strain or Type	Lot Number	Chlamydiae DFA Reagent	TCID ₅₀ or CFU
Cell Line	A-549	C560316	-	n/a
Cell Line	BGMK	C530316	-	n/a
Cell Line	HEp-2	C570316	-	n/a
Cell Line	HFF (Hs27)	C870315	-	n/a
Cell Line	LLC-MK2	C860316S	-	n/a
Cell Line	McCoy	C540316	-	n/a
Cell Line	MDCK	C830316S	-	n/a
Cell Line	MRC-5	C510315A	-	n/a
Cell Line	MRHF	C440314	-	n/a
Cell Line	Mv1Lu	C580317S	-	n/a
Cell Line	NCI-H292	C590313S	-	n/a
Cell Line	pCMK	470309	-	n/a
Cell Line	pRhMK	A490310	-	n/a
Cell Line	RD	C760317S	-	n/a
Cell Line	RhMK II	490311YS	-	n/a
Cell Line	RK (passage 1)	C480314PS	-	n/a

Cell Line	R-Mix	C960314S	-	n/a
Cell Line	Vero	C840331S	-	n/a
Cell Line	Vero 76	C670329S	-	n/a
Cell Line	WI-38	850318W	-	n/a

Thirty (30) microorganisms, including 25 bacterial cultures, one yeast and three bacterial (*Chlamydia sp.*) and one protozoan commercially available slides, were stained with the 2X DFA Reagent according to the procedure as detailed in this product insert, then examined for cross-reactivity. Except for *Staphylococcus aureus*, this was cross-reactive with the CMV-IEA DFA Reagent (see above), all other microorganisms tested negative. 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 3.0×10^6 and 6.0×10^6 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. Results of testing are listed below.

Bacteria, Yeast, and Protozoa Cross Reactivity Testing

Organism	Strain or Type	Lot Number	Chlamydiae DFA Reagent	TCID₅₀ or CFU
Bacteria	<i>Acholeplasma laidlawi</i>	031404	-	~1.0e7
Bacteria	<i>Acinetobacter calcoaceticus</i>	934332	-	9.7e5
Bacteria	<i>Bordetella bronchiseptica</i>	031404	-	1.8e5
Bacteria	<i>Bordetella pertussis</i>	031404	-	4.7e6
Bacteria	<i>Chlamydiophila pneumoniae</i>	CP-0176	+	Control Slide*
Bacteria	<i>Chlamydiophila psittaci</i>	FP-12-050218	+	Control Slide*
Bacteria	<i>Chlamydia trachomatis</i>	052705	+	Control Slide*
Bacteria	<i>Corynebacterium diphtheriae</i>	031404	-	2.5e6
Bacteria	<i>Escherichia coli</i>	335472	-	2.6e5
Bacteria	<i>Gardnerella vaginalis</i>	3457511	-	5.0e5
Bacteria	<i>Haemophilis influenzae type A</i>	031404	-	9.3e5
Bacteria	<i>Klebsiella pneumoniae</i>	031404	-	6.4e6
Bacteria	<i>Legionella pneumophila</i>	031404	-	6.5e4
Bacteria	<i>Moraxella cartarrhalis</i>	031404	-	6.4e4
Bacteria	<i>Mycoplasma hominis</i>	031404	-	~1.0e4
Bacteria	<i>Mycoplasma orale</i>	031404	-	~1.0e4

Bacteria	<i>Mycoplasma pneumoniae</i>	031404	-	~1.0e4
Bacteria	<i>Mycoplasma salivarium</i>	031404	-	~1.0e7
Bacteria	<i>Neisseria gonorrhoeae</i>	060805	-	1.3e6
Bacteria	<i>Proteus mirabilis</i>	440498	-	2.1e6
Bacteria	<i>Pseudomonas aeruginosa</i>	031404	-	1.0e7
Bacteria	<i>Salmonella enteritidis</i>	3457511	-	2.5e6
Bacteria	<i>Salmonella typhimurium</i>	363162	-	1.8e6
Bacteria	<i>Staphylococcus aureus</i>	081100	+	1.0e7
Bacteria	<i>Streptococcus agalactiae</i>	370784	-	9.6e6
Bacteria	<i>Streptococcus pneumoniae</i>	031404	-	8.0e5
Bacteria	<i>Streptococcus pyogenes</i>	031404	-	2.9e7
Bacteria	<i>Ureaplasma urealyticum</i>	031404	-	~1.0e4
Yeast	<i>Candida glabrata</i>	992206	-	8.7e6
Protozoa	<i>Trichomonas vaginalis</i>	410721	-	

f. Assay cut-off: Not applicable

2. Comparison studies:

a. *Method comparison with predicate device:* A total of 1060 specimens were cultured and stained with one of two comparative devices and the D³ DFA Cytomegalovirus Immediate Early Antigen Identification Kit at three external clinical laboratory sites and at the DHI internal laboratory. A total of 34 specimens were excluded from final analysis, resulting in a total of 1026 results reported. Reasons for exclusion were specimen toxicity to cell culture (29), bacterial contamination of cell culture (1), non-specific fluorescence seen prohibiting interpretation (2), and unacceptable specimens (2).

b. *Matrix comparison: Not applicable*

3. Clinical studies:

Percent Agreement between the D³ DFA Cytomegalovirus Immediate Early Antigen Identification Kit (D³ CMV-IEA ID Kit) and Comparator Devices was calculated for all the above analyzed specimens. [See Table below.]

Overall Comparison of D³ CMV-IEA ID Kit with Comparator Kits

		Comparator Device	
		+	-
D ³ CMV-IEA ID Kit	+	100	4
	-	4	918
Total:		1026	

Positive percent agreement (PPA) = 96.1% (100/104), 95% CI 90.5-98.5

Negative percent agreement (NPA) = 99.6% (918/922), 95% CI 98.9-99.8

a. *Clinical Sensitivity: Not applicable*

b. *Clinical specificity: Not applicable*

c. *Other clinical supportive data (when a. and b. are not applicable):*

5. Expected values/Reference range:

The clinical studies described in Section X (‘Specific Performance Characteristics’) used only specimens that were collected and cultured for the presence of CMV.

The specimen sources and positivity with the comparison devices are described in the Tables below.

Summary of Overall Specimen Source by Site												
Site	Total	Blood	Body Fluid	Genital	G.I.- upper	G.I.- lower	Resp. - upper	Resp. - lower	Skin/Lesions	Tissue	Urine	Unknown
1	314	45	16	1	4	12	10	131	1	21	73	0
2	293	0	0	0	0	0	271	15	0	6	1	0
3	128	11	8	0	4	4	13	62	2	0	23	1
4	291	72	16	0	7	0	30	95	1	12	58	0
Total:	1026	128	40	1	15	16	324	303	4	39	155	1
Blood: blood, buffy coat Body Fluids: fluid, amniotic fluid, central spinal fluid (CSF/CF), pericardial fluid, pleural Genital: Genital G.I. – upper: gastrointestinal (upper), mouth, esophageal, ranula basil G.I. – lower: gastrointestinal (lower), stomach, colon, sigmoid, bowel, stool, pleura Resp. – upper: respiratory (upper), nasal (NS), nasopharyngeal (NP) aspirates, throat, nares Resp. – lower: respiratory (lower), lung, lobes, bronchial, bronchial alveolar lavage (BAL), bronchial washing (BW), brushings, tracheal aspirates (TA), sputum, Skin/Lesions: abdomen, conjunctiva Tissue: biopsy (BX), liver, heart, lymph nodes, brain, bone marrow, Urine: urine Unknown: investigator unable to specify source												

The specimen Age/Sex demographics for the specimens cultured are tabulated in the Table below.

Age and Gender Distribution				
Age Range	Values are # Positive / Total			
	Male	Female	Gender Unknown	Total
0 - 1 month	0 / 37	3 / 17	0 / 0	3 / 54
>1 month - 2 years	31 / 176	33 / 151	0 / 0	64 / 327
>2 - 12 years	6 / 34	2 / 31	0 / 0	8 / 65
>12 - 21 years	0 / 12	0 / 9	0 / 0	0 / 21
22 - 30 years	0 / 14	0 / 21	0 / 0	0 / 35
31 - 40 years	3 / 48	1 / 49	0 / 0	4 / 97
41 - 50 years	5 / 43	4 / 38	0 / 0	9 / 81
51 - 60 years	5 / 63	1 / 31	0 / 0	6 / 94
>60 years	6 / 110	4 / 105	0 / 0	10 / 215
Unknown age	0 / 14	0 / 4	0 / 19	0 / 37

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.