DIAGNOSTIC HYBRIDS
Integrating Science and Humanity

510(K) SUMMARY – K081164

D^3 DFA CYTOMEGALOVIRUS IMMEDIATE EARLY ANTIGEN IDENTIFICATION KIT

Applicant:
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June 06, 2008

Device Name:
Trade name – D^3 DFA CYTOMEGALOVIRUS IMMEDIATE EARLY ANTIGEN IDENTIFICATION KIT
Common name – Fluorescent antibody test for identification of Cytomegalovirus (CMV)
Classification name – Antisera, Conjugated Fluorescent, Cytomegalovirus
Product Code – LIN
Regulation – 21 CFR 866.3175, Cytomegalovirus serological reagents

Legally marketed devices to which equivalence is claimed:

1. K951821, Light Diagnostics CMV Direct Immunofluorescence Assay (Millipore)
   Intended Use: Light Diagnostics Cytomegalovirus Direct Immunofluorescence Assay is intended for pre-cytopathic effect (CPE) detection and identification of immediate early antigen of human CMV in cell culture. This product is not FDA approved for use in testing blood or plasma donors and is not intended for use in direct detection of CMV in clinical specimens.

2. K904036, Bartels Cytomegalovirus Immediate Early Antigen Indirect Fluorescent Antibody (Trinity Biotech PLC)
   Intended Use: Bartels Cytomegalovirus Immediate Early Antigen Indirect Fluorescent Antibody is intended for qualitative detection of CMV pre-cytopathic effect (CPE) immediate early antigen in centrifugation-enhanced inoculated cell cultures. This product is not FDA cleared (approved) for use in testing (i.e., screening) blood or plasma donors.

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.

Patient samples are inoculated onto susceptible cell monolayers and cultured. After a defined incubation period, the cells to be tested for the presence of CMV-IEA are fixed in acetone. The CMV-IEA DFA Reagent is added to the cells which are then incubated for 15 to 30 minutes at 35° to 37°C, the stained cells are washed with the supplied PBS Solution (diluted), and a drop of the supplied Mounting Fluid is placed on the prepared cells. The cells are examined using a fluorescence microscope. The cells infected with CMV and expressing the CMV-IEA will have apple-green fluorescent nuclei while uninfected cells will contain no fluorescence but will be stained red by the Evans Blue counter-stain.

If no fluorescent cells are found, report result as "No cytomegalovirus detected".
If fluorescent cells are found in the CMV-IEA DFA Reagent stained monolayer, report result as "Cytomegalovirus isolated by cell culture".

Intended Use:

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.

Technological Characteristics:

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 5.1 below:

TABLE 5.1: 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

Non-Clinical Performance:

Staining patterns of the fluorescein-labeled MAbs on CMV infected cells were similar to those of the predicate devices.

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-$TCID_{50}$ 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[a].

Detection limit for the subject device CMV-IEA ID Kit was addressed under the conditions: CMV, at a starting concentration 350-$TCID_{50}$ per mL, was serially diluted to a final value of 0.7-$TCID_{50}$ per mL using 2-fold dilutions. Each dilution was inoculated into confluent monolayers of Hs27 cells contained in multi-well plates, centrifuged at 700 xg 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

[a] Microsoft Office Excel, Microsoft Corporation

comparable, with 0.7-$TCID_{50}$ 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[a].

Analytical specificity was evaluated according to cross reactivity studies against a number of strains of viruses, strains of bacteria, and different cell lines. No cross-reactivity or non-specific staining with any of the other agents was observed, except for *Staphylococcus aureus* (Protein A producing bacteria will bind the Fc portion of some of the fluorescein-labeled MAb), which was cross-reactive with the CMV-IEA DFA Reagent. Microorganisms and cell lines which were evaluated against the CMV-IEA DFA Reagent are listed in Table 5.2, below:

TABLE 5.2: Microorganisms tested for Cross-Reactivity with D³ CMV-IEA DFA Reagent

Virus (no reactivity with CMV-IEA DFA Reagent)	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.[b]	
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 stained.
Influenza B	Hong Kong	1,400			
Influenza B	Maryland	1,400	Coxsackievirus	Types B1, B2, B3, B4, B5, B6	
Influenza B	Mass	1,400			
Influenza B	Taiwan	1,400	Poliovirus	Types 1, 2, 3	
Influenza B	GL	1,400			
Influenza B	Russia	1,400			

BACTERIA	Result	CFU TESTED	BACTERIA	Result	CFU TESTED
Acholeplasma laidlawii	Negative	~6×10^7	Mycoplasma salivarium	Negative	~6×10^7
Acinetobacter calcoaceticus	Negative	9.7×10^5	Neisseria gonorrhoeae	Negative	1.3×10^6
Bordetella bronchiseptica	Negative	1.7×10^5	Proteus mirabilis	Negative	2.1×10^6
Bordetella pertussis	Negative	4.6×10^6	Pseudomonas aeruginosa	Negative	1.0×10^7
Corynebacterium diphtheriae	Negative	2.5×10^6	Salmonella enteriditis	Negative	2.5×10^6
Escherichia coli	Negative	2.6×10^5	Salmonella typhimurium	Negative	1.8×10^6

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

Gardnerella vaginalis	Negative	5.0×10^5	Staphylococcus aureus	POSITIVE[c]	1.0×10^7
Haemophilis influenzae type A	Negative	9.3×10^5	Streptococcus agalactiae	Negative	9.6×10^6
Klebsiella pneumoniae	Negative	6.4×10^6	Streptococcus pneumoniae	Negative	8.0×10^5
Legionella pneumophila	Negative	6.5×10^4	Streptococcus pyogenes	Negative	2.9×10^7
Moraxella cartarrhalis	Negative	6.4×10^4	Ureaplasma uralyticum	Negative	$\sim 6 \times 10^4$
Mycoplasma hominis	Negative	$\sim 6 \times 10^4$	Chlamydiophila pneumoniae	Negative	Commercially available slides stained.
Mycoplasma orale	Negative	$\sim 6 \times 10^4$	Chlamydiophila psittaci	Negative	Commercially available slides stained.
Mycoplasma pneumoniae	Negative	$\sim 6 \times 10^4$	Chlamydia trachomatis	Negative	Commercially available slides stained.
YEAST			**PROTOZOAN**		
Candida glabrata	Negative	8.7×10^6	Trichomonas vaginalis	Negative	Commercially available slides stained.
CELL LINES (no reactivity with CMV-IEA DFA Reagent)					
A549	Monolayer	MRC-5	Monolayer	RhMK II	Cell Spot
BGMK	Monolayer	MRHF	Monolayer	RK (passage 1)	Monolayer
HEp-2	Monolayer	Mv1Lu	Monolayer	R-Mix	Monolayer
HFF / Hs27	Monolayer	NCI-H292	Monolayer	Vero	Cell spot
LLC-MK2	Monolayer	pCMK	Cell spot	Vero 76	Cell spot
McCoy	Monolayer	pRhMK	Cell spot	WI-38	Cell spot
MDCK	Monolayer	RD	Monolayer		

Clinical Performance:

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).

Study site 1 collected and cultured a total of 314 fresh specimens during August, 2006. There were no specimens excluded from final analysis. A wide variety of specimen sources were cultured from a diverse age population. The D³ DFA Cytomegalovirus Immediate Early Antigen Identification Kit positive percent agreement compared with Bartels Cytomegalovirus Immediate Early Antigen kit for cultured specimens had a positive percent agreement of 94.1% (exact 95% CI range of 73.0, 98.9) and a negative percent agreement of 99.7% (exact 95% CI range of 98.1, 99.9).

Study site 2 cultured a total of 300 specimens (72 fresh and 228 archival) specimens from August 31 through November 8, 2006. The archival specimens were collected from June, 2005 through September, 2006. They were stored at -70° C until re-

[c] Staining of *S. aureus* appeared as small points of fluorescence while all other cultures were negative. This will be noted in labeling in the section "Limitations of the Assay": The Protein A produced by the bacterium, *Staphylococcus aureus*, will bind the Fc portion of some of the fluorescein-labeled monoclonal antibodies used in this kit. Such binding can be distinguished from viral antigen binding on the basis of morphology, i.e., *S. aureus*-bound fluorescence appears as small (~1 micron diameter), bright dots. Results from cell cultures with bacterial contamination must, therefore, be interpreted with caution."

cultured for this study. The specimens were not selected for this study based on previous culture results. Of the 228 archival specimens, seven were excluded from the final analysis due to toxicity in cell culture. The D^3 DFA Cytomegalovirus Immediate Early Antigen Identification Kit combined archival and fresh positive percent agreement, compared with Light Diagnostics CMV Direct Immunofluorescence Assay, for cultured specimens had positive percent agreement of 100% (exact 95% CI range of 94.7, 100) and a negative percent agreement of 98.7% (exact 95% CI range of 96.1, 99.5).

Study site 3 tested specimens from February 2007 through May 2007. A total of 146 fresh specimens were cultured. Of these 146 specimens, 18 were excluded from the final analysis due to toxicity, contamination, and specimen acceptability. The D^3 DFA Cytomegalovirus Immediate Early Antigen Identification Kit positive percent agreement, compared with Light Diagnostics Cytomegalovirus Direct Immunofluorescence Assay kit for culture confirmation specimens, had a positive percent agreement of 83.3% (exact 95% CI range of 43.6, 97.0) and a negative percent agreement of 100% (exact 95% CI range of 96.9, 100).

Study site 4 cultured 300 specimens that were collected at a clinical reference laboratory located in the Eastern U.S. in February, 2007. These frozen prospectively collected specimens were stored at -70° C until cultured for this study. The specimens were not selected for this study based on previous culture results. Of these 300 archival specimens, 9 were excluded from the final analysis due to toxicity and non-specific fluorescence. The D^3 DFA Cytomegalovirus Immediate Early Antigen Identification Kit positive percent agreement, compared with Light Diagnostics Cytomegalovirus Direct Immunofluorescence Assay kit for culture confirmation specimens, had a positive percent agreement of 83.3% (exact 95% CI range of 55.2, 95.3) and a negative percent agreement range of 100% (exact 95% CI 98.6, 100).

The overall study results indicate that the D^3 DFA Cytomegalovirus Immediate Early Antigen Identification Kit performs comparably to the legally marketed CMV immunofluorescence assay kits for the qualitative detection and identification of CMV IEA in cell cultures.

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

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JUN 13 2008

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Re: k081164

Trade/Device Name: D^3 DFA Cytomegalovirus Immediate Early Antigen Identification Kit
Regulation Number: 21 CFR 866.3175
Regulation Name: Cytomegalovirus Serological Reagents
Regulatory Class: Class II
Product Code: LIN
Dated: April 21, 2008
Received: April 24, 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

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

Indications for Use

510(k) Number (if known): K081164

Device Name: D^3 DFA Cytomegalovirus Immediate Early Antigen Identification Kit

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

Prescription Use ___X___ (Part 21 CFR 801 Subpart D) AND/OR Over-The-Counter Use _____ (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) K081164