K070206

AUG 30 2007

DIAGNOSTIC HYBRIDS™

510 (k) Summary

August 29, 2007

A. 510(k) Number:
K070206

B. Purpose for Submission:
New device

C. Measurand:
Varicella Zoster Virus (VZV)

D. Type of Test:
Cell culture method, by immunofluorescence using fluoresceinated monoclonal antibodies (MAbs)

E. Applicant:
Diagnostic Hybrids, Inc.
350 West State Street
Athens, OHIO 45701
Tel. 740-593-1784
Fax. 740-597-1546
Contact person: Gail R. Goodrum

F. Proprietary and Established Names:
D^3 DFA Varicella-Zoster Virus Identification Kit
Common Name: DFA (Direct Fluorescent Antibody) test kit for the identification of VZV in cell cultures inoculated with patient specimens

G. Regulatory Information:

1. Regulation section:
   866.3900 antiserum, cf, varicella-zoster
2. Classification:
   Class II
3. Product code:
   GQX
4. Panel:
   Microbiology (83)

H. Intended Use:

1. Intended use(s):
   The Diagnostic Hybrids, Inc D3 DFA Varicella-zoster Virus Identification Kit is intended for use in the qualitative detection of varicella-zoster virus (VZV) in cell cultures by immunofluorescence using fluoresceinated monoclonal antibodies (MAbs). Negative results do not preclude an infection and should not be used as the sole basis for diagnosis, treatment or other management decision. Performance testing has not been done on direct patient specimen testing.
2. Indication(s) for use:
   The Diagnostic Hybrids, Inc D3 DFA Varicella-zoster Virus Identification Kit is intended for use in the qualitative detection of varicella-zoster virus (VZV) in cell cultures by immunofluorescence using fluoresceinated monoclonal antibodies

(MAbs). Negative results do not preclude an infection and should not be used as the sole basis for diagnosis, treatment or other management decision. Performance testing has not been done on direct patient specimen testing.

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:

The Diagnostic Hybrids, Inc. D3 DFA VARICELLA-ZOSTER IDENTIFICATION KIT includes a DFA Reagent that contains a blend of two fluorescein-labeled murine monoclonal antibodies directed against VZV antigens. The kit includes the following components:

Kit Components:

- VZV DFA Reagent. A blend of two fluorescein labeled murine monoclonal antibodies directed against a recombinant glycoprotein E (gE) from the Ellen strain of VZV. The buffered, stabilized, aqueous solution contains Evan's Blue as a counter-stain and 0.1% sodium azide as preservative.
- Mounting Fluid. An aqueous, buffered, stabilized solution of glycerol and 0.1% sodium azide.
- VZV Antigen Control Slides. Individually packaged control slides containing wells with cell culture derived positive and negative control cells. Each VZV Positive well is identified. The Negative wells contain uninfected cells. Each slide is intended to be stained only one time.
- PBS Concentrate. One bottle containing a 40X concentrate consisting of 4% sodium azide in Phosphate Buffered Saline (after dilution to 1X with water, the concentration of sodium azide in the solution is 0.1%).

J. Substantial Equivalence Information:

1. Predicate device name(s):
   1. Light Diagnotics Varicella-zoster (VZV) Direct Immunofluorescence Assay (DFA)
   2. Light Diagnostics Simulfluor HSV/VZV Immunofluorescence Assay

   Predicate 510(k) number(s):
   1. K951799
   2. K990141
3. Comparison with predicate:
   The similarities to predicate devices are in indicated use, operating principle, basic design, materials and formulation.

Similarities		
Item	Device	Predicate
Intended Use	For the qualitative detection of Varicella-Zoster Virus (VZV) in cultures by immunofluorescence using	1. The Light Diagnotics Varicella-zoster (VZV) Direct Immunofluorescence Assay (DFA) is intended for the qualitative detection and identification of GPI and

Similarities		
Item	Device	Predicate
	fluoresceinated monoclonal antibodies (MAb's). Negative results do not preclude an infection and should not be used as the sole basis for diagnosis, treatment or other management decision. Performance testing has not been done on direct patient specimen testing.	immediate early antigen of VZV from vesicular lesions. The kit is intended for use in culture confirmation with standard tube cultures and shell vials and is presumptive in the detection and identification of VZV from direct specimens. 2. The Light Diagnostics Simulfluor HSV/VZV Immunofluorescence Assay is intended for the simultaneous detection and identification of herpes simplex viruses (HSV) 1 and 2 and varicella-zoster virus (VZV) from patients with vesicular, oral, genital, or skin lesions, using direct specimens and culture confirmation. Specimens found to be negative on direct specimen examination must be confirmed with culture.
Basic principle	DFA (Direct Fluorescent Antibody) test - Immunofluorescence using fluoresceinated monoclonal antibodies (MAbs)	1. DFA (Direct Fluorescent Antibody) test-Immunofluorescence using fluoresceinated monoclonal antibodies. 2. DFA (Direct Fluorescent Antibody) test-Immunofluorescence using fluoresceinated monoclonal antibodies.
Antibody	Blend of murine monoclonal antibodies (MAbs) directed against two antigenic sites on the VZV recombinant protein, glycoprotein E.	Predicates 1 and 2: Blend of murine monoclonal antibodies (MAbs) directed against two antigens, glycoprotein I and the immediate early antigen of VZV.
Instrumentation (required but not provided)	Fluorescence microscope with the filter combination for FITC (excitation peak = 490 nm, emission peak = 520nm).	Fluorescence microscope with filter combination for FITC (excitation peak = 490 nm, emission peak = 520nm).
Sample type	Swabs of lesion specimens	Swabs of lesion specimens

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

N/A

L. Test Principle:

The test kit uses viral antigen-specific murine monoclonal antibodies that are directly labeled with fluorescein for rapid detection and identification of VZV.

The cells to be tested, derived from cell culture, are fixed in acetone. The VZV DFA Reagent is added to the cells to determine the presence of viral antigens. After

incubating at 35°C to 37°C, the stained cells are rinsed with the diluted PBS Concentrate, 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. VZV-infected cells will be stained with viral specific apple-green fluorescence when stained with the VZV DFA Reagent while uninfected cells will contain no fluorescence but will be stained dull red by the Evan's Blue counter-stain.

Interpretation of results:

It is recommended that controls be examined first to ensure proper test performance before examination of the specimens. A positive reaction is one in which bright apple-green fluorescence is observed in the infected cells. Uninfected cells will stain dull red due to the Evan's Blue counter-stain included in the VZV DFA Reagent.
If no fluorescent cells are found, report result as, "No varicella-zoster virus detected.
If fluorescent cells are found, report result as, "varicella-zoster virus isolated by cell culture".
Technologists should not confuse the dried out edge of monolayer or cell clumps which may brightly fluoresce due to entrapment of antibody with virus-specific staining. Occasionally, dead, rounded cells due to specimen toxicity or improper cell storage may nonspecifically stain a dull olive green due to trapped antibody. Adequate humidity while staining and adequate washing between steps will help to eliminate this type of nonspecific staining.

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. *Detection limit:*
      The Predicate and Subject MAbs were compared by inoculating 96-well cell culture plates with the appropriate virus stock at a level of 1-$TCID_{50}$ per well. The plates were incubated at 37°C for 48 hours and then stained with either the Subject Kit or the Predicate Kit. All plates were stained according to the product inserts. This assay was performed 4 times with an average of 21.8 and 22.3 positive wells for the Subject and Predicate kits, respectively. The results indicate no statistical difference between the Subject and Predicate kits by a paired t-test.
      Analytical specificity:
      The VZV DFA Reagent was tested for cross-reactivity against a wide variety of cells and microorganisms. No cross-reactivity was observed for 55 virus strains (cultured and processed for staining) or for 20 host culture cell types. Twenty-seven (27) bacterial cultures and one (1) yeast culture 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 while all other bacterial cultures were negative. [Protein A will specifically bind to the Fc portions of conjugated antibodies. 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. No cross-reactivity was observed for the other 26 bacterial cultures or for the one yeast culture.

Stringent conditions for cross-reactivity testing were achieved by using a high concentration of the VZV DFA Reagent and relatively high titers of microorganisms. The DFA Reagent was prepared at 1.5X the concentration that is provided in the kit. Viruses were prepared as infected cell monolayers (150 to 2100 $TCID_{50}$ viruses, depending on the particular virus, were inoculated into a shell vial culture and incubated for 24 to 48 hours, to yield a 3+ to 4+ infection), and processed and stained with the 1.5X DFAs according to the procedure detailed in the product inserts. Some viruses were tested as commercially prepared slides. Bacterial strains were cultured, processed as suspensions, then spotted on microscope slides (at CFU's ranging from $6.4x10^4$ to $6x10^7$/well in a 10 µL dot, depending on the bacterium), then stained with the 1.5X DFAs according to the procedure in the product insert. Cell cultures were stained as confluent monolayers.

Virus Strains Tested for Cross Reactivity with VZV DFA Reagent

Organism	Strain or Type	Inoculum Concentration ($TCID_{50}$)
Adenovirus	Type 1	350
Adenovirus	Type 5	350
Adenovirus	Type 6	350
Adenovirus	Type 7	350
Adenovirus	Type 8	350
Adenovirus	Type 10	350
Adenovirus	Type 14	350
Adenovirus	Type 18	350
Adenovirus	Type 31	350
Influenza A	Aichi	2,100
Influenza A	Mal	2,100
Influenza A	Hong Kong	2,100
Influenza A	Denver	2,100
Influenza A	Port Chalmers	2,100
Influenza A	Victoria	2,100
Influenza A	PR	2,100
Influenza B	Hong Kong	350
Influenza B	Maryland	350
Influenza B	Mass	350
Influenza B	Taiwan	350
Influenza B	GL	350
Influenza B	Russia	350

Organism	Strain or Type	Inoculum Concentration ($TCID_{50}$)
RSV	Long	350
RSV	Wash	350
RSV	9320	350
Parainfluenza 1	C-35	Commercially available slides stained.[1]
Parainfluenza 2	Greer	
Parainfluenza 3	C 243	
HSV-1	1F	150
HSV-1	CWOH 0026	150
HSV-1	CWOH 0015	150
HSV-1	MacIntyre	150
HSV-2	MS	150
HSV-2	Strain G	150
CMV	Towne	700
CMV	Davis	700
CMV	AD169	700
Echovirus	4	Commercially available slides stained.[1]
Echovirus	6	
Echovirus	9	
Echovirus	11	
Echovirus	30	
Echovirus	34	
Coxsackievirus	B1	Commercially

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

Poliovirus	Type 1	Commercially available slides stained.[1]
Poliovirus	Type 2	
Poliovirus	Type 3	
Epstein-Barr	Commercially available slides stained.[1]	
Rubeola		
Mumps		

Coxsackievirus	B2	available slides stained.[1]
Coxsackievirus	B3	
Coxsackievirus	B4	
Coxsackievirus	B5	
Coxsackievirus	B6	

Cell Lines Tested for Cross Reactivity with VZV DFA Reagent

A549	Mv1Lu	RD
BGMK	HFF	RhMK II
HEp-2	McCoy	R-Mix
LLC-MK2	NCI-H292	Vero
MDCK	pCMK	WI-38
MRC-5	pRhMK	Vero 76
MRHF	pRK	

Bacteria and Yeast Tested for Cross Reactivity with VZV DFA Reagent

BACTERIA	**CFU TESTED**
Acinetobacter calcoaceticus	9.7×10^5
Bordetella bronchiseptica	1.7×10^5
Bordetella pertussis	4.6×10^6
Corynebacterium diphtheriae	2.5×10^6
Escherichia coli	2.6×10^5
Gardnerella vaginalis	5.0×10^5
Haemophilis influenzae type A	9.3×10^5
Klebsiella pneumoniae	6.4×10^6
Legionella pneumophila	6.5×10^4
Moraxella cartarrhalis	6.4×10^4
Neisseria gonorrhoeae	1.3×10^6
Proteus mirabilis	2.1×10^6
Pseudomonas aeruginosa	1.0×10^7
Salmonella enteriditis	2.5×10^6
Salmonella typhimurium	1.7×10^6
Staphylococcus aureus	1.0×10^7
Streptococcus agalactiae	9.6×10^6
Streptococcus pneumoniae	8.0×10^5
Streptococcus pyogenes	2.9×10^7
Acholeplasma laidlawi	$\sim 6 \times 10^7$
Mycoplasma hominis	$\sim 6 \times 10^4$
Mycoplasma orale	$\sim 6 \times 10^4$
Mycoplasma pneumoniae	$\sim 6 \times 10^4$
Mycoplasma salivarium	$\sim 6 \times 10^7$
Ureaplasma uralyticum	$\sim 6 \times 10^4$
These were procured as prepared slides:	Proportion of cells reactive

Chlamydophila pneumoniae	10 to 50%
Chlamydia trachomatis	10 to 50%
YEAST	
Candida glabrata	8.7×10^6

f. Assay cut-off:
Not applicable

2. Comparison studies:

a. Method comparison with predicate device:
This study included two hundred and fifty-four (254) prospectively collected specimens submitted for Varicella-zoster virus culture. Each specimen was evaluated by D^3 DFA VZV Identification Kit and a currently marketed Varicella-zoster virus identification kit (comparison device). Tube cultures were tested as evidence of infection (e.g. CPE) was observed; if no evidence of infection was observed after 14-days, the tubes were tested at that time. Shell vial and multi-well plate cultures were tested at a minimum of 72-hours. All 254 specimens were cultured; however, 3 of the specimens were not evaluated because they produced toxic cell culture monolayers, leaving a total of 251 specimens to be included in the Performance Characteristics. The evaluations were conducted at three laboratory sites. Percent Agreement between the D^3 DFA VZV and comparison tests was calculated and tabulated for all tested specimens. These results are summarized in the table below:

Percent Agreement of All Tests

		Comparison Device +	Comparison Device –
D^3 DFA VZV	+	42	1
	–	0	208

Positive Percent Agreement [2] (PPA)	100%
95% CI [3]- PPA	91.6% to 100%
Negative Percent Agreement [4] (NPA)	99.5%
95% CI – NPA	97.3% to 99.9%

b. Matrix comparison:
n/a

3. Clinical studies:

[2] "Positive Percent Agreement", or "PPA", values were calculated according to {[Total Number of Positive Results in Agreement by both DHI and Comparison Tests) divided by [(Total Number of Positive Results in Agreement by both DHI and Comparison Tests) plus (Number of Results Positive by the Comparison Test but Negative by the DHI test)]} multiplied by 100%.

[3] "95% CI" refers to 95% Confidence Intervals, which were calculated according to Exact method (Clopper, C. and S. Pearson, Biometrika 26:404-413, 1934).

[4] "Negative Percent Agreement", or "NPA", values were calculated according to {[Total Number of Negative Results in Agreement by both DHI and Comparison Tests) divided by [(Total Number of Negative Results in Agreement by both DHI and Comparison Tests) plus (Number of Results Negative by the Comparison Test but Positive by the DHI test)]} multiplied by 100%.

a. Clinical Sensitivity:
Not applicable.

b. Clinical specificity:
Not applicable.

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

4. Clinical cut-off:
Not applicable

5. Expected values/Reference range:

The clinical studies used only specimens collected and cultured for the presence of VZV. Most of the specimen types used in the clinical studies were swabs taken from skin lesions (with two taken as respiratory specimens (NP) and one CSF). Specimens were taken from the following body sites (and presented as # positive/# specimens).

Specimen sources

Source	Total specimens	Unknown +/Total	Genital +/Total	Penis +/Total	Vaginal +/Total	Cervical +/Total	Rectal +/Total	Perineum** +/Total	Eyelid +/Total	Face +/Total	Mouth* +/Total	Skin‡ +/Total	NP+/Total	CSF/Brain +/Total
Site 1	99	0/8	0/1	0/0	0/0	0/0	0/1	0/11	0/1	4/14	0/2	17/61	0/0	0/0
Site 2	35	0/0	0/0	1/2	0/0	0/0	0/0	1/3	0/0	0/2	0/0	9/27	0/1	0/0
Site 3	120	2/51	0/6	0/1	0/9	0/1	0/0	0/3	0/0	1/9	0/5	4/33	1/1	0/1

*mouth: mouth, lip, tongue, gum, throat
**perineum: groin, buttock, gluteal, coccyx, sacral, pubic, perianal
‡skin: skin lesion, skin, finger, wrist, chest, axilla, abdomen, thigh, blister

Demographics by age and gender for the specimens that were tested at the 3 study sites are tabulated below. Of the specimens evaluated in these studies (which had been submitted to the laboratories as swabs taken from lesions for both HSV and VZV testing), a large proportion were from patients between the ages of 18 and 40. The specimen demographics are listed in the Table below.

Demographics by Age and Gender

	Site 1 Values are # pos / total		Site 2 Values are # pos / total		Site 3 Values are # pos / total	
Age	F	M	F	M	F	M
TOTALS	63	36	10	10	80	40
<2y	0/1	0/4	0	0/2	0	0/1
2y to 10y	0	0/1	0/1	0/1	1/3	0/2
10y to18y	1/6	1/3	1/1	1/1	0/4	0/3
18y to 40y	0/18	1/3	0/1	0/1	0/39	0/13
>40y	11/38	7/24	3/6	4/5	2/33	5/21
Age not reported	0/0	1/1	0/1	0	1/1	0
Age/gender not reported	0		1/12		0	

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
2098 Gaither Road
Rockville MD 20850

AUG 30 2007

Gail R. Goodrum
Vice President, Regulatory and Quality Affairs
DIAGNOTIC HYBRIDS, INC.
350 West State Street
Athens, OH 45701

Re: k070206
Trade/Device Name: D^3 DFA Varicella-zoster Virus Identification Kit
Regulation Number: 21 CFR 866.3900
Regulation Name: Varicella-zoster virus Serological Reagents
Regulatory Class: Class II
Product Code: GQW
Dated: July 27, 2007
Received: July 31, 2007

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.

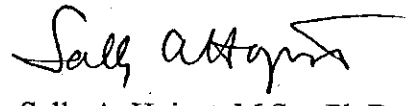
If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to such additional controls. Existing major regulations affecting your device can be found in Title 21, Code of Federal Regulations (CFR), Parts 800 to 895. In addition, FDA may publish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

Indications for Use

510(k) Number (if known): _K070206_

Device Name: Diagnostic Hybrids D^3 DFA Varicella-zoster Virus Identification Kit

Indications for Use: The Diagnostic Hybrids, Inc D3 DFA Varicella-zoster Virus Identification Kit is intended for use in the qualitative detection of varicella-zoster virus (VZV) in cell cultures by immunofluorescence using fluoresceinated monoclonal antibodies (MAbs). Negative results do not preclude an infection and should not be used as the sole basis for diagnosis, treatment or other management decision.
Performance testing has not been done on direct patient specimen testing.

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 OF NEEDED)

Concurrence of CDRH, Office of Device Evaluation (ODE)

Division Sign-Off

Office of In Vitro Diagnostic Device Evaluation and Safety

510(k) K070206