

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE**

A. 510(k) Number: K070206

B. Purpose for Submission: The 510(k) holder would like to introduce D³ DFA Varicella Zoster Virus Identification Kit into interstate commerce.

C. Measurand: Varicella zoster antigen

D. Type of Test: Determination of the presence of varicella zoster in amplified cell culture.

E. Applicant: Diagnostic Hybrids, Inc.

F. Proprietary and Established Names: D³ DFA Varicella Zoster Virus Identification Kit.

G. Regulatory Information:

1. Regulation section: 866.3900
2. Classification: II
3. Product code: GQW
4. Panel: 81

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: 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:

The Diagnostic Hybrids, Inc. D³ DFA Varicella-Zoster Identification Kit includes a blend of two fluorescein-labeled murine monoclonal antibodies directed against VZV antigens. The kit includes the following components which are used for screening and final virus identification in cell cultures inoculated with patient specimens.

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.
- **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 for single use.
- **Wash Solution Concentrate.** One bottle containing a 40X concentrate consisting of 4% sodium azide (after dilution to 1X with water, the concentration of sodium azide in the solution is 0.1%) in Phosphate Buffered Saline (PBS).
- **Mounting Fluid.** An aqueous, buffered, stabilized solution of glycerol and 0.1% sodium azide.

J. Substantial Equivalence Information:

1. Predicate device name(s): 1. Light Diagnostics Varicella-zoster (VZV) Direct Immunofluorescence Assay (DFA).
2. Predicate K number(s): K951799
3. Comparison with predicate:
 - a. Similarities: The similarities to predicate devices are in indicated use, operating principle, materials and formulation.

Similarities		
Item	Device	Predicate
Intended Use	The Diagnostic Hybrids, Inc. D³ DFA Varicella-Zoster Virus Identification Kit is intended for use in the qualitative detection of recombinant glycoprotein E (gE) from the Ellen strain of VZV in cultures by using fluoresceinated monoclonal antibodies (MAb's).	1. The Light Diagnostics Varicella-zoster (VZV) Direct Immunofluorescence Assay (DFA) is intended for the qualitative detection of GPI and immediate early antigen of VZV from vesicular lesions. The kit is intended for use in culture confirmation with standard tube cultures and shell vials.
Basic principle	DFA (Direct Fluorescent Antibody) test -	DFA (Direct Fluorescent Antibody) test -

Similarities		
Item	Device	Predicate
	Immunofluorescence using fluoresceinated monoclonal antibodies in culture to include standard tube, shell vials and multiwell plates.	Immunofluorescence using fluoresceinated monoclonal antibodies in culture to include standard tube and shell vials.
Antibody	Blend of murine monoclonal antibodies (MAbs) directed against two antigenic sites on the VZV recombinant protein, glycoprotein E.	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 correct filter combination for FITC (excitation peak = 490 nm, emission peak = 520nm).	Fluorescence microscope with the correct filter combination for FITC (excitation peak = 490 nm, emission peak = 520nm).
Sample type	Swabs of lesion specimens	Swabs of lesion specimens

b Differences:

Differences		
Item	Device	Predicate
Procedural	Immunofluorescence testing following amplification in cell culture only.	Results considered presumptive for identification of VZV from direct patient specimens using fluoresceinated monoclonal antibodies.

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 which are directed against two antigenic sites on the VZV recombinant protein, glycoprotein E.

The cell cultures are fixed in acetone. The VZV DFA Reagent is added to the monolayers in cell culture to determine the presence of viral antigens. After incubating, the infected cells are stained with a fluorescein conjugated MAb while uninfected cells and are counterstained with an Evan's Blue dye. The stained cells are subsequently rinsed, overlaid with a drop of supplied mounting fluid, and the monolayer is protected with a coverslip. Cells are then examined using a fluorescence microscope.

Interpretation of results: A result is considered positive when a cell cytoplasm and/or nucleus presents with a finely granular bright apple-green fluorescence. Uninfected cells will stain dull red due to the Evan's Blue counter-stain which is included with this device.

M. Performance Characteristics:

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: VZV was diluted to a value of 357-TCID₅₀ and serial 2-fold dilutions were then made to a final value of 1-TCID₅₀. Each dilution of virus was inoculated into 6 shell vials of H&V Mix or MRC-5 cells, centrifuged at 700xg for 60 minutes and incubated at 35-37°C for 48 hours. The Subject Kit or the Predicate Kit was used to stain 3 shell vials of each viral dilution according to the product inserts. The sensitivity of both fluorescent antibody stains is equivalent, with ~ 1.4- to 5.7-TCID₅₀ as the minimum viral dilution detected.*
- e. *Analytical specificity: See results for Cross-reactivity below.*
- 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 VZV culture. Each specimen was evaluated by the D³ DFA VZV Identification Kit and compared to a currently marketed VZV identification kit. A combination of fresh (61) and frozen (193) specimens were tested. The numbers of fresh and frozen specimens tested are summarized below.*

Number of Fresh vs. Frozen Specimens			
Site	Culture		Site Total
	Fresh	Frozen	
1	57	42	99
2	1	31	32
3	0	120	120

- b. *Matrix comparison: Not applicable*

3. Clinical studies: A total of (254) prospectively collected specimens were submitted for VZV culture. Three of the fresh specimens from Site 2 were toxic to cell culture and were not evaluated by either test. One specimen from Site 3 was negative in the multi-well plate culture, but was positive in the tube culture 10-days post inoculation. These evaluations were conducted at two external laboratory sites and one in-house laboratory: (1) A reference laboratory in the Southeastern United States; (2) A reference laboratory in the Southwestern United States; and (3) Diagnostic Hybrids, Inc in-house virology laboratory.

Percent Agreement between the D³ DFA VZV and comparator tests was calculated

and tabulated for all the tested specimens is presented below.

Percent Agreement of All Tests

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

Positive Percent Agreement (PPA)	100%
95% CI- PPA	91.6% to 100%
Negative Percent Agreement (NPA)	99.5%
95% CI – NPA	97.3% to 99.9%

a Clinical Sensitivity:

- i **Study Site 1:** A total of 99 specimens were cultured for VZV using multi-well plates. Briefly, two hundred microliters (200-μL) of each specimen were inoculated using one well per specimen. The inoculated cells were centrifuged at 700xg for 60-minutes, incubated at 35° to 37°C for up to 72-hours then stained in accordance with each respective product insert (DHI and comparison device).

DHI D³ DFA VZV Test and Comparison Device in multi-well plates.

		Comparison Device	
		+	–
D ³ DFA VZV	+	22	0
	–	0	77

	Agreed	95% CI
PPA =	100%	84.6% to 100%
NPA =	100%	95.2% to 100%

- ii **Study Site 2:** A total of 35 specimens were cultured for VZV. Three fresh specimens from this site were toxic to cell culture and were not evaluated. Briefly, 200-μL from the specimens was inoculated into duplicate shell vials. The inoculated cells were incubated at 35° to 37°C for 72-hours then stained in accordance with the respective product insert (DHI and comparison devices). All calculations for confidence intervals were done according to the Exact Method^{Error! Bookmark not defined.}. The results of this testing are summarized below:

DHI D³ DFA VZV Test and Comparison Device in shell vials.

		Comparison Device	
		+	–
D ³ DFA VZV	+	11	1
	–	0	20
Agreed		95% CI	
PPA =	100%	71.5% to 100%	
NPA =	95.1%	76.2% to 99.9%	

- iii **Study Site 3:** A total of 120 specimens were cultured for VZV. Briefly, two hundred microliters (200-μL) of each specimen was inoculated into one well per specimen of a multi-well plate and a single cell culture tube. The inoculated plates were centrifuged at 700xg for 60-minutes, incubated at 35° to 37°C for 72-hours then stained in accordance with each respective product insert (DHI and comparison devices). The results of this testing are summarized in Table 8a. The inoculated tubes were read for CPE daily for 14-days. Tubes exhibiting CPE were scraped and cell spots made on multiwell slides according to the comparison device's product insert procedure (the same procedure was used for both the DHI and the comparison devices). Tubes exhibiting no CPE at 14-days were also scraped and cell spots made to confirm the absence of VZV. The cell spots were fixed with acetone in accordance with each respective product insert (DHI and comparison device). The results of testing at this site, in house, are summarized in the two tables below.

DHI D³ DFA VZV Test and Comparison Device in multi-well plates

		Comparison Device	
		+	–
D ³ DFA VZV	+	8	0
	–	0	112
Agreed		95% CI	
PPA =	100%	63.1% to 100%	
NPA =	100%	96.8% to 100%	

DHI D³ DFA VZV Test and Comparison Device in tube cultures.

		Comparison Device	
		+	–
D ³ DFA VZV	+	9	0
	–	0	111
		Agreed	95% CI
PPA =		100%	66.4% to 100%
NPA =		100%	96.7% to 100%

a. Clinical specificity:

Cross reactivity testing: This device was tested for cross-reactivity against a wide variety of cells and microorganisms compared to the predicate. 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. [See Tables below for cross-reactivity study results.]

Stringent conditions for cross-reactivity testing were achieved by using a high concentration of this device and relatively high titers of microorganisms. The DFA Reagent was prepared at 1.5X the concentration that is provided in the kit.

- Fifty-five (55) virus strains were tested for cross reactivity. Depending on the particular virus, 150 to 2100 TCID₅₀ viruses were inoculated into a shell vial culture and incubated for 24 to 48 hours, to yield a 1+ to 3+ infection, processed and stained with the 1.5X DFAs according to the procedure detailed in the product insert. No cross reactivity was observed for the viruses listed below.

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
Poliovirus	Type 1	Commercially available slides stained ^{Error!} Bookmark not defined.
Poliovirus	Type 2	
Poliovirus	Type 3	
Epstein-Barr	Commercially available slides stained ^{Error!} Bookmark not defined.	
Rubeola		
Mumps		

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. ^d
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 ^{Error!} Bookmark not defined.
Echovirus	6	
Echovirus	9	
Echovirus	11	
Echovirus	30	
Echovirus	34	Commercially available slides stained ^{Error!} Bookmark not defined.
Coxsackievirus	B1	
Coxsackievirus	B2	
Coxsackievirus	B3	
Coxsackievirus	B4	
Coxsackievirus	B5	
Coxsackievirus	B6	

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

- 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 1.5X DFA Reagent according to the procedure as detailed in this product insert and then examined for cross reactivity. No cross reactivity was observed for the following cell lines presented below.

**Cell Lines Tested for Cross Reactivity using
DHI the VZV DFA Reagent**

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

- Twenty-eight microorganisms, including one (1) yeast culture and twenty-seven (27) bacterial cultures, were stained with the 1.5X DFA Reagent according to the procedure as detailed in this product insert, then examined for cross-reactivity. Except for *Staphylococcus aureus*, which was cross reactive with the VZV DFA Reagent (see above), all 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 1.0 and 2.0 (equaling approximately 3.0×10^6 and $6.0 \times 10^6 \times 10^4$ 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 and Yeast Tested for Cross Reactivity with VZV DFA Reagent

BACTERIA	CFU TESTED
<i>Acinetobacter calcoaceticus</i>	9.7×10^5
<i>Bordetella bronchiseptica</i>	1.7×10^5
<i>Bordetella pertussis</i>	4.6×10^6
<i>Corynebacterium diphtheriae</i>	2.5×10^6
<i>Escherichia coli</i>	2.6×10^5
<i>Gardnerella vaginalis</i>	5.0×10^5
<i>Haemophilis influenzae type A</i>	9.3×10^5
<i>Klebsiella pneumoniae</i>	6.4×10^6
<i>Legionella pneumophila</i>	6.5×10^4
<i>Moraxella cartarrhalis</i>	6.4×10^4
<i>Neisseria gonorrhoeae</i>	1.3×10^6
<i>Proteus mirabilis</i>	2.1×10^6
<i>Pseudomonas aeruginosa</i>	1.0×10^7
<i>Salmonella enteriditis</i>	2.5×10^6
<i>Salmonella typhimurium</i>	1.7×10^6
<i>Staphylococcus aureus</i>	1.0×10^7
<i>Streptococcus agalactiae</i>	9.6×10^6
<i>Streptococcus pneumoniae</i>	8.0×10^5
<i>Streptococcus pyogenes</i>	2.9×10^7
<i>Acholeplasma laidlawi</i>	$\sim 6 \times 10^7$
<i>Mycoplasma hominis</i>	$\sim 6 \times 10^4$
<i>Mycoplasma orale</i>	$\sim 6 \times 10^4$
<i>Mycoplasma pneumoniae</i>	$\sim 6 \times 10^4$
<i>Mycoplasma salivarium</i>	$\sim 6 \times 10^7$
<i>Ureaplasma urealyticum</i>	$\sim 6 \times 10^4$
Those listed below were procured as prepared slides:	Proportion of cells reactive
<i>Chlamydophila pneumoniae</i>	10 to 50%
<i>Chlamydia trachomatis</i>	10 to 50%
YEAST	
<i>Candida glabrata</i>	8.7×10^6

- c. Other clinical supportive data (when a. and b. are not applicable): Not applicable.
4. Clinical cut-off: Not applicable.
5. Expected values: The clinical studies used only specimens collected and cultured for the presence of VZV. Most of the specimen types used in the clinical studies was swabs taken from skin lesions (with two taken as respiratory specimens (NP) and one CSF). This sampling is what is normally expected. Specimens were taken from the following body sites (and presented as # positive/# specimens) are described below.

Specimen Sources

Source	Total specimens	Unknown +/Total	Genital +/Total	Penis +/Total	Vaginal +/Total	Cervical +/Total	Rectal +/Total	perineum** +/Total	Eye/Id +/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. Prevalence of VZV within the population tested was quite low (due in part to varicella vaccination programs). The patient demographics are listed 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	Gender not reported	F	M
TOTALS	63	36	10	10	12	80	40
<2y	0/1	0/4	0	0/2	0	0	0/1
2y to 10y	0	0/1	0/1	0/1	0	1/3	0/2
10y to 18y	1/6	1/3	1/1	1/1	0	0/4	0/3
18y to 40y	0/18	1/3	0/1	0/1	0	0/39	0/13
>40y	11/38	7/24	3/6	4/5	0	2/33	5/21
Age not reported	0/0	1/1	0/1	0	1/12	1/1	0

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.