

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

A. 510(k) Number: K063798

B. Purpose for Submission: Premarket notification

C. Measurand: Herpes simplex virus (HSV)

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

E. Applicant: Diagnostic Hybrids, Inc.

F. Proprietary and Established Names:

Proprietary Name: D³ DFA Herpes Simplex Virus Identification Kit

Common Name: DFA (Direct Fluorescent Antibody) test kit for the detection of HSV in cell cultures inoculated with patient specimens.

G. Regulatory Information:

1. Regulation section: 866.3305 Herpes simplex virus serological reagents
2. Classification: Class II
3. Product code: GQN [Antigens, CF (including CF control), herpesvirus hominis, 1, 2]
4. Panel: Microbiology (83)

H. Intended Use:

1. Intended use(s):

Diagnostic Hybrids, Inc D³ DFA Herpes Simplex Virus Identification Kit is intended for use in the qualitative detection of human herpes simplex virus (HSV) 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 decisions.

Performance using direct specimen testing has not been evaluated.

2. Indication(s) for use: Same as Intended Use.
3. Special conditions for use statement(s): For prescription use only.
4. Special instrument requirements: This device requires the use of a fluorescence microscope to read device stained cultures. One should use with the correct filter combination for FITC (excitation peak = 490 nm, emission peak = 520nm).

I. Device Description:

Diagnostic Hybrids D³ DFA Herpes Simplex Virus Identification Kit includes a DFA Reagent that contains a blend of four fluorescein-labeled murine monoclonal antibodies directed against a blend of 4 murine MABs directed against two antigens of HSV-1 and two antigens of HSV-2. The kit includes the following components:

1. HSV DFA Reagent – A blend of fluorescein labeled murine monoclonal antibodies directed against 4 murine MABs directed against two antigens of HSV-1 and two antigens of HSV-2 produced from HSV-infected cell culture. The buffered, stabilized, aqueous solution contains Evan's Blue as a counter-stain and 0.1% sodium azide as preservative.
2. HSV Antigen Control Slides - Individually packaged control slides containing wells with cell culture derived positive and negative control cells. Each HSV positive well is identified. The negative wells contain uninfected cells. Each slide is intended to be stained only one time.
3. PBS Concentrate - A 40X concentrate consisting of 4% sodium azide in phosphate buffered saline (after dilution to 1X in water, the concentration of sodium azide in the solution is 0.1%).
4. Mounting Fluid - an aqueous, buffered, stabilized solution of glycerol and 0.1% sodium azide.

J. Substantial Equivalence Information:

1. Predicate device name(s): Bartels[®] Herpes Simplex Virus Fluorescent Monoclonal Antibody Test.
2. Predicate K number(s): K902662
3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Intended Use	For the qualitative detection of herpes simplex virus in cell cultures by immunofluorescence using fluoresceinated monoclonal antibodies. Negative results do not preclude an infection and should not be used as the sole basis for diagnosis, treatment or other management decisions. Performance using direct specimen testing has not been evaluated.	Bartels [®] Herpes Simplex Virus Fluorescent Monoclonal Antibody Test is intended for use in the qualitative identification of HSV culture isolates.
Basic principle	Direct Fluorescent Antibody (DFA) test - Immunofluorescence using fluoresceinated MABs.	DFA (Direct Fluorescent Antibody) test - Immunofluorescence using

Similarities		
Item	Device	Predicate
		fluoresceinated MAbs.
Antibody	Blend of 4 murine MAbs directed against two antigens of HSV-1 and two antigens of HSV-2.	Mouse MAbs specific to HSV-1 and HSV-2 antigens.
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	Specimens of vesicular fluid; swabbed areas or lesions contained in transport medium	Specimens of vesicular fluid; swabbed areas or lesions contained in transport medium.

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

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

L. Test Principle:

The test kit uses a blend of four viral antigen-specific murine MAbs which are directly labeled with fluorescein for rapid identification of HSV in cell culture.

The cells to be tested, derived from cell culture, are fixed in acetone. The HSV 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. HSV-infected cells will be stained with viral specific apple-green fluorescence when stained with the HSV DFA Reagent while uninfected cells will contain no fluorescence but will be stained dull red by the Evan's Blue counter-stain.

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*: This testing is not considered necessary during premarket notification process because performance testing has been done using a comparative device in amplified cell culture (cell culture is not part of the review process for this device).

e. *Analytical specificity*:

- i The HSV D³ DFA Reagent was tested for cross-reactivity against a wide variety of cells and microorganisms. No cross-reactivity was observed for 59 viral strains (cultured and processed for staining) or for 17 host culture cell types.
 1. Depending on the particular virus used, a concentration of 150 to 715 TCID₅₀ of selected virus was inoculated into shell vial or multiwell plate cultures and incubated for 24 to 48 hours to yield what was considered a 1+ to 3+ cytopathic effect. Only confluent monolayer cell cultures were stained. The culture was processed and stained with the DFA reagent at a 1.5X working dilution concentration and processed according to the procedure detailed in each respective product insert. Stained cells were examined at 200x magnification.
- ii Twenty-seven (27) bacterial cultures, one yeast and one protozoan 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 cultures were negative.**
 1. Bacteria and yeast were cultured, processed as suspensions, and then spotted onto microscopic slides (having CFUs ranging from 6.4×10^4 to 2.93×10^7 /well, depending on the bacterium), and stained with the DFA reagent at a 1.5X working dilution concentration and processed according to each respective product insert. Stained slides were examined at 400X magnification. Some of the microorganisms listed below were procured from an external source as prepared microscope slides (marketed for quality control use).
 2. 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 punctuate dots.
- iii Various conditions for cross-reactivity testing were achieved by using a relatively high concentration of the HSV D³ DFA Reagent (1.5X) and testing relatively high titers of microorganisms commensurate with infectious states in human. The HSV D³ DFA Reagent was prepared at 1.5X the concentration that is provided in the kit.

No cross reactivity was observed for the viruses, cell lines, bacteria listed in the next 3 Tables:

Virus Strains Tested for Cross Reactivity with D ³ HSV DFA Reagent					
Organism	Strain or Type	Inoculum (TCID ₅₀)	Organism	Strain or Type	Inoculum (TCID ₅₀)
Adenovirus	Type 1	715	Influenza B	Hong Kong	715
Adenovirus	Type 3	715	Influenza B	Maryland	715
Adenovirus	Type 5	715	Influenza B	Mass	715
Adenovirus	Type 6	715	Influenza B	Taiwan	715
Adenovirus	Type 7	715	Influenza B	GL	715
Adenovirus	Type 8	715	Influenza B	JH-001 isolate	715
Adenovirus	Type 10	715	Influenza B	Russia	715
Adenovirus	Type 13	715	RSV	Long	715
Adenovirus	Type 14	715	RSV	Wash	715
Adenovirus	Type 18	715	RSV	9320	715
Adenovirus	Type 31	715	Parainfluenza 1	C-35	715
Adenovirus	Type 40	715	Parainfluenza 2	Greer	715
Adenovirus	Type 41	715	Parainfluenza 3	C 243	715
Influenza A	Aichi	715	Parainfluenza 4a	M-25	715
Influenza A	Mal	715	Parainfluenza 4b	CH19503	715
Influenza A	Hong Kong	715	CMV	Towne	700
Influenza A	Denver	715	CMV	Davis	700
Influenza A	Port Chalmers	715	CMV	AD169	700
Influenza A	Victoria	715	VZV	Webster	500
Influenza A	New Jersey	715	VZV	Allen	500
Influenza A	PR	715	Epstein-Barr	Commercially available slides stained. ¹	
Influenza A	WS	715	Rubeola		
			Mumps		
Echovirus	Types 4, 6, 9, 11, 30, 34	Commercially available slides stained. ¹	HPV	Types 6, 11	Commercially available slides stained. ¹
Coxsackievirus	Types B1, B2, B3, B4, B5, B6	Commercially available slides stained. ⁱ			
Cell Lines Tested for Cross Reactivity with D ³ HSV DFA Reagent					
A-549		NCI-H292			
BGMK		pCMK			
HEp-2		pRhMK			
LLC-MK2		RhMK II			
MDCK		pRK			
MRC-5		RD			
MRHF		R-Mix			
Mv1Lu		Vero & WI-38			

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

Microorganisms Tested for Cross Reactivity with D ³ HSV DFA Reagent	
BACTERIA	CFU TESTED
<i>Acinetobacter calcoaceticus</i>	9.7 x 10 ⁵
<i>Bordetella bronchiseptica</i>	1.7 x 10 ⁵
<i>Bordetella pertussis</i>	4.6 x 10 ⁶
<i>Corynebacterium diphtheriae</i>	2.5 x 10 ⁶
<i>Escherichia coli</i>	2.6 x 10 ⁵
<i>Gardnerella vaginalis</i>	5.0 x 10 ⁵
<i>Haemophilis influenzae type A</i>	9.3 x 10 ⁵
<i>Klebsiella pneumoniae</i>	6.4 x 10 ⁶
<i>Legionella pneumophila</i>	6.5 x 10 ⁴
<i>Moraxella cartarrhalis</i>	6.4 x 10 ⁴
<i>Neisseria gonorrhoeae</i>	1.3 x 10 ⁶
<i>Proteus mirabilis</i>	2.1 x 10 ⁶
<i>Pseudomonas aeruginosa</i>	1.0 x 10 ⁷
<i>Salmonella enteriditis</i>	2.5 x 10 ⁶
<i>Salmonella typhimurium</i>	1.7 x 10 ⁶
<i>Staphylococcus aureus</i>	1.0 x 10 ⁷
<i>Streptococcus agalactiae</i>	9.6 x 10 ⁶
<i>Streptococcus pneumoniae</i>	8.0 x 10 ⁵
<i>Streptococcus pyogenes</i>	2.9 x 10 ⁷
<i>Acholeplasma laidlawi</i>	~6 x 10 ⁷
<i>Mycoplasma hominis</i>	~6 x 10 ⁴
<i>Mycoplasma orale</i>	~6 x 10 ⁴
<i>Mycoplasma pneumoniae</i>	~6 x 10 ⁴
<i>Mycoplasma salivarium</i>	~6 x 10 ⁷
<i>Ureaplasma urealyticum</i>	~6 x 10 ⁴
<i>Chlamydophila pneumoniae</i>	Commercially available slides stained. ¹
<i>Chlamydia trachomatis</i>	Commercially available slides stained. ¹
YEAST	
<i>Candida glabrata</i>	8.7 x 10 ⁶
PROTOZOAN	
<i>Trichomonas vaginalis</i>	[Commercially available slides stained.]

f. Assay cut-off: Not applicable

2. Comparison studies:

a. Method comparison with predicate device:

Comparative studies included five hundred and thirty (530) prospectively collected specimens submitted for HSV culture. Each specimen was evaluated by the D³ DFA HSV Identification Kit and compared to a currently marketed HSV identification kit. A combination of fresh (250) and frozen (280) specimens were tested. Although fresh and frozen specimen results are normally presented separately, combining results for performance characteristics of this device is

considered acceptable because direct patient specimen testing was not performed at all sites. Only cell culture confirmation with this device was performed. Evaluations were conducted at three external laboratory sites located in the mid-west United States and one in-house virology laboratory. Three specimens from site 4 were not evaluated due to bacterial contamination of the monolayers, leaving 527 for analysis. Each of the study sites tested different legally marketed HSV kits as comparison devices.

Percent Agreement between the D³ DFA HSV and Comparison Devices was calculated and tabulated for all the tested specimens, and is presented in the Table below. Data are represented as positive and negative percent agreement between D³ DFA HSV Kit and a comparative device (statistically calculating the 95 percent confidence interval for each positive and negative percent agreement by the exact method).

Results from Testing All Specimens:

D³ DFA HSV Kit and a Legally Marketed Device (Comparison Device)

		Comparison Device	
		+	-
D ³ DFA HSV	+	200	1
	-	1	325

		95% CI
Positive Percent Agreement (PPA) =	99.5%	97.3 to 100%
Negative Percent Agreement (NPA) =	99.7%	98.3% - 100%

Results from Individual Study Sites:

Study Site 1 Results (Tube cultures):

		Comparison Device	
		+	-
D ³ DFA HSV	+	63	0
	-	0	44
		95% CI	
PPA =	100%	94.2% - 100%	
NPA =	100%	91.9% - 100%	

Study Site 2 Results (Shell vial culture):

		Comparison Device	
		+	-
D ³ DFA HSV	+	50	0
	-	0	79
		95% CI	
PPA =	100%	92.8% - 100%	
NPA =	100%	95.3% - 100%	

Study Site 3 Results (Tube culture):

		Comparison Device	
		+	-
D ³ DFA HSV	+	47	1
	-	1	101
		95% CI	
PPA =	97.9%	88.9 to 99.9%	
NPA =	99.1%	94.7% -100.0%	

Study Site 4 Results (Multi-well plate culture):

		Comparison Device	
		+	-
D ³ DFA HSV	+	40	0
	-	0	101
		95% CI	
PPA =	100%	91.1% - 100%	
NPA =	100%	96.3% - 100%	

b. *Matrix comparison*: Not applicable.

3. Clinical studies: Testing was performed with clinically collected specimens submitted to the four different laboratories for HSV testing. Results were compared with a legally marketed HSV device(s).

a. *Clinical Sensitivity*: Not applicable.

b. *Clinical specificity*: Not applicable.

c. Other clinical supportive data: Not applicable.

4. Clinical cut-off: Not applicable.

5. Expected values/Reference range: Not applicable.

N. Proposed Labeling: The labeling is considered adequate and satisfies the requirements of 21 CFR Part 809.10 for safety and effectiveness and substantial equivalence to a legally marketed device.

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