

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

A. 510(k) Number: K070265

B. Purpose for Submission: Premarket notification 510(k)

C. Measurand: Herpes simplex virus type 1 and type 2 (HSV-1 and HSV-2)

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 and Typing Kit

Common Name: Fluorescent antibody test for ID and typing HSV-1 and HSV-2 from cell culture

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): The Diagnostic Hybrids D³ DFA Herpes Simplex Virus Identification and Typing Kit is intended for use in the qualitative detection and typing of human herpes simplex virus type 1 and type 2 (HSV-1 and HSV-2) 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:

The HSV-1 MAbs were developed using HSV-1(f) cell lysate as immunogen – one has been determined to be directed against HSV-1 glycoprotein C1, the antigen to the other is undetermined. The HSV-2 MAbs were developed using a HSV-2 recombinant glycoprotein G immunogen.

1. HSV-1 DFA Reagent – One dropper bottle containing a blend of two fluorescein labeled murine monoclonal antibodies directed against HSV-1 specific glycoproteins. The HSV-1 MAbs were developed using HSV-1(f) cell lysate as immunogen – one MAb has been determined to be directed against HSV-1 glycoprotein G1 (gG1), the antigen to the other is undetermined. The buffered, stabilized, aqueous solution contains Evan's Blue as a counter-stain and 0.1% sodium azide as preservative.
2. HSV-2 DFA Reagent – One dropper bottle containing a blend of two fluorescein labeled murine monoclonal antibodies directed against HSV-2 specific glycoproteins. The HSV-2 MAbs were developed using a HSV-2 recombinant glycoprotein C immunogen. The buffered, stabilized, aqueous solution contains Evan's Blue as a counter-stain and 0.1% sodium azide as preservative.
3. HSV-1/HSV-2 Antigen Control Slides - Individually packaged control slides containing wells with cell culture derived positive and negative control cells. Each slide consists of four wells containing acetone fixed cells; two wells of uninfected cells and one well each of HSV-1 infected cells and HSV-2 infected cells. Each slide is intended to be stained only one time.
4. 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%).
5. Mounting Fluid - An aqueous, buffered, stabilized solution of glycerol and 0.1% sodium azide.

J. Substantial Equivalence Information:

1. Predicate device name(s): Pathodx[®] Herpes Typing Kit
2. Predicate K number(s): K904167

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Intended Use	For the qualitative detection of herpes simplex virus (HSV) types HSV-1 or HSV-2 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.	Remel PathoDx Herpes Typing Kit is an immunofluorescence test designed for the detection and typing of herpes simplex virus types I and II (HSV-1 and HSV-11) in direct clinical specimens and following growth in tissue culture. Culture confirmation slides and shell vial assays may undergo detection and typing with a bivalent staining procedure.
Basic principle	Direct Fluorescent Antibody (DFA) test - Immunofluorescence using fluoresceinated MAbs.	DFA (Direct Fluorescent Antibody) test - Immunofluorescence using fluoresceinated MAbs.
Antibody	A blend of two fluorescein labeled murine monoclonal antibodies against HSV-1 and two against HSV-1. Specific glycoproteins with were developed using HSV-1(f) cell lysate as immunogen – one MAb has been determined to be directed against HSV-1 glycoprotein C1; the antigen to the other is undetermined. The HSV-2 MAbs were developed using two fluorescein labeled murine HSV-2 recombinant glycoprotein G immunogens.	The HSV-I antibody is a blend of two monoclonal antibodies which react with polypeptides having 85K and 142K dalton molecular weights. The HSV-II typing reagent contains two monoclonal antibodies, one that recognizes a 79K dalton polypeptide and the other a 41K dalton polypeptide molecule.
Instrumentation (required but not provided)	Fluorescence microscope with the correct filter combination for FITC (excitation peak = 490 nm, emission peak = 520 nm).	Fluorescence microscope with the correct filter combination for FITC (excitation peak = 490 nm, emission peak = 520 nm).
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. All direct clinical specimens which are negative or have inadequate numbers of cells must be re-evaluated by cell culture.

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

L. Test Principle: A suspected specimen is inoculated into tube culture, shell vials or multi-well plates coated containing a monolayer of cells known to be sensitive to HSV. When infected with HSV, a characteristic deterioration of cells, termed cytopathic effect (CPE), can be observed. Tube culture can take several days before CPE is evident. However, the rate of isolation may be enhanced including a decreased time for identification and typing of inoculated specimens by centrifugation in shell vials or multi-well plates. Monolayers are fixed in acetone with a slide(s) prepared from tube culture scrapings or from shell vials or multi-well plates.

The D³ DFA HSV-1 or HSV-2 Reagents are added to separate monolayers. After incubating, the stained cells are washed and, covered with a supplied Mounting Fluid. The fixed monolayers are examined using a fluorescence microscope. The HSV-1 and HSV-2 DFA reagents contain Evan's Blue as a counter-stain. HSV-1 or HSV-2 infected cells will both stain with a characteristic bright apple-green fluorescence distinguished from the counter-stained uninfected cells by a dull red fluorescence.

M. Performance Characteristics (if/when applicable):

2. 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. Analytical specificity was evaluated by staining cultures infected with a number of ATCC reference HSV-1 and HSV-2 strains and found to react with all of them. The D³ HSV-1 and HSV-2 DFA reagents were tested for cross-reactivity against a wide variety of other microorganisms and cells. No cross-reactivity was observed for 59 virus strains (cultured and processed for staining) or for 17 host culture cell types. Conditions for cross-reactivity testing were achieved by using the D³ HSV-1 or HSV-2 DFA Reagent along with relatively high titers of microorganisms. The DFA Reagents were prepared at 1.5X the concentration that is provided in the kit.
1. Depending on the particular virus, 500 to 715 TCID₅₀ viruses were inoculated into multi-well plate cultures and incubated for 24 hours, processed and stained with the 1.5X DFAs according to the procedure detailed in the product insert. Stained cells were examined at 200x magnification. Cell cultures were stained as confluent monolayers. Bacteria and yeast were cultured, processed as suspensions, then spotted on microscope slides (at CFUs ranging from 6.4×10^4 to 2.93×10^7 /well in a 10 μ L dot, depending on the bacterium), then stained with the 1.5X DFAs according to the procedure in the product insert.
 2. 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. [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.]

Results from previously described cross-reactivity testing for microbiological organisms are listed below:

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. ¹			
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				

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

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

<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 enteritidis</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.

3. Comparison studies:

- a. *Method comparison with predicate device:* This study included four hundred and one (401) prospectively collected specimens submitted for Herpes simplex culture. Each specimen was evaluated by the Diagnostic Hybrids D³ DFA Herpes Simplex Virus Identification and Typing Kit (“D³ DFA HSV ID & Typing Kit”) and compared to a currently marketed HSV identification kit (“Comparison Device”). A combination of fresh (154) and frozen (247) specimens were tested. Three specimens from site 3 were not evaluated due to bacterial contamination of the monolayers, leaving 398 for analysis. These studies were conducted at two external laboratory sites located in the mid-west United States and one in-house virology laboratory.

Percent Agreement between the D³ DFA HSV ID & Typing Kit and the Comparison Devices was calculated and tabulated for all the tested specimens,

and is presented below. The term, “95% CI” refers to 95% Confidence Intervals, which were calculated according to Exact method (Clopper, C. and S. Pearson, Biometrika 26:404-413, 1934).

Results from Testing of All Specimens:

Total results - D³ DFA HSV ID & Typing Kit and comparison device using various culture methodologies.

		<u>HSV-1</u>		<u>HSV-2</u>	
		Comparison Device		Comparison Device	
		+	–	+	–
D ³ DFA HSV ID & Typing Kit	+	73	0	76	1
	–	1	324	0	321
		95% CI		95% CI	
Positive Percent Agreement (PPA) =	73/74 98.6%	92.7% - 100%		76/76 100%	95.3% - 100%
Negative Percent Agreement (NPA) =	324/324 100%	98.9% - 100%		321/322 99.7%	98.3% - 100%

Results from Individual Study Sites:

- i. **Study Site 1:** A total of 107 specimens (4 fresh and 103 frozen) were tested using the D³ DFA HSV ID & Typing Kit. An aliquot from each specimen was inoculated into MRC-5 and A549 tubes, one tube for each HSV type. The inoculated cells were incubated at 35° to 37°C and examined daily for CPE for seven days. Tube cultures exhibiting no CPE after seven days of incubation were scraped and cell spots made on multi-well slides. The cell spots were fixed with acetone and in accordance with the respective product insert (subject and predicate devices). All calculations for confidence intervals were done according to the Exact Method. The results of comparison testing are summarized below (Table 6).

		<u>Table 6a – HSV-1</u>		<u>Table 6b – HSV-2</u>	
		Comparison Device		Comparison Device	
		+	–	+	–
D ³ DFA HSV ID & Typing Kit	+	35	0	28	0
	–	0	72	0	79
		95% CI		95% CI	
PPA =	35/35 100%	90.0% - 100%		28/28 100%	87.7% - 100%
NPA =	72/72 100%	95.0% - 100%		79/79 100%	95.4% - 100%

- ii. **Study Site 2:** A total of 150 fresh specimens were cultured for HSV. Briefly, an aliquot from each specimen was inoculated into MRC-5 tubes, one tube for each HSV type. The inoculated cells were incubated at 35° to 37°C and examined daily for CPE. Tube cultures exhibiting CPE were scraped and cell spots made on multi-well slides according to the D³ DFA HSV ID & Typing Kit and Comparison Device product insert procedures. Tube cultures exhibiting no CPE at 7-days were also scraped and cell spots made to confirm the absence of HSV. All 150 specimens were tested for the presence of HSV. All calculations for confidence intervals were done according to the Exact Method. The results of this testing site are summarized below (Table 7).

Table 7a – HSV-1				Table 7b – HSV-2			
		Comparison Device				Comparison Device	
		+	–			+	–
D ³ DFA HSV ID & Typing Kit	+	21	0		+	25	1
	–	1	128		–	0	124
		95% CI				95% CI	
PPA =	21/22 95.5%	77.2% - 99.9%			25/25 100%	86.3% - 100%	
NPA =	128/128 100%	97.2% - 100%			124/125 99.2%	95.6% - 100%	

- iii. **Study Site 3:** The study was conducted at a Diagnostic Hybrids in-house virology laboratory. A total of 141 frozen specimens provided by an independent laboratory were processed. An aliquot from each specimen was inoculated into duplicate wells of DHI H&V-Mix™ multi-well plates. The inoculated cells were centrifuged, incubated up to 24-hours then stained in accordance with the respective product insert. All calculations for confidence intervals were done according to the Exact Method. The results of this testing are summarized below (Table 8).

Table 8a – HSV-1				Table 8b – HSV-2			
		Comparison Device				Comparison Device	
		+	–			+	–
D ³ DFA HSV ID & Typing Kit	+	17	0		+	23	0
	–	0	124		–	0	118
		95% CI				95% CI	
PPA =	17/17 100%	80.5% - 100%			23/23 100%	85.2% - 100%	
NPA =	124/124 100%	97.1% - 100%			118/118 100%	96.9% - 100%	

- b. *Matrix comparison:* Not applicable
4. **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.
- 5. Clinical cut-off: Not applicable.
 - 6. 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.