

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY**

A. 510(k) Number:

K081527

B. Purpose for Submission:

New Device

C. Measurand:

Herpes simplex virus type 1 and type 2 (HSV-1 and HSV-2) antigen

D. Type of Test:

Direct Immunofluorescence Assay and cell culture confirmation to detect the presence of HSV1 and/ or HSV2 antigens using fluorescein-labeled monoclonal antibodies

E. Applicant:

Millipore Corporation

F. Proprietary and Established Names:

*Light Diagnostics*TM HSV 1/2 Typing DFA Kit

G. Regulatory Information:

1. Regulation section:

21CFR 866.3305 Herpes simplex virus serological reagent

2. Classification:

Class II (Special controls)

3. Product code:

GQL: Antisera, fluorescent, herpesvirus hominis 1,2

4. Panel:

Microbiology

H. Intended Use:

1. Intended use(s):

The Light Diagnostics™ HSV 1/2 Typing DFA Kit is an in vitro diagnostic test for the qualitative detection and identification of herpes simplex virus type 1 and/or type 2 in direct specimens from patients with vesicular lesions and symptoms consistent with herpes infection and for culture confirmation by immunofluorescence. Negative results do not preclude an infection and should not be used as the sole basis for diagnosis, treatment or other management decisions. Specimens found negative on direct specimen detection should be confirmed by culture.

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 100 watt mercury or halogen lamp, appropriate filter combination for FITC (excitation peak = 490 nm, emission peak = 520 nm), 100x, 200x and 400x magnification (dry objective)

I. Device Description:

HSV1 Typing reagent consists of two fluorescein-labeled monoclonal antibodies specific for HSV 1 glycoprotein C and ICP35 respectively. HSV 2 Typing Reagent consists of two fluorescein-labeled monoclonal antibodies that specifically bind HSV 2 polypeptides. In Western blots these appear as two major bands with molecular weights of between 110-120 kD and between 78-82 kD and are consistent with the monoclonal antibodies recognizing epitopes within glycoprotein G of HSV 2.

Kit Components

1. HSV 1 Typing Reagent - (Catalog No. 5233). One 2 mL dropper vial containing a fluorescein-labeled monoclonal antibodies specific for HSV 1, protein stabilizer, Evans blue and 0.1% sodium azide (preservative).
2. HSV 2 Typing Reagent - (Catalog No. 5234). One 2 mL dropper vial containing a fluorescein-labeled monoclonal antibodies specific for HSV 2, protein stabilizer, Evans blue and 0.1% sodium azide (preservative).
3. HSV Control Slides - (Catalog No. 5093). Two slides containing one well of HSV type 1-infected cells, one well of HSV type 2 infected cells and one well of uninfected cells.

4. Phosphate-Buffered Saline (PBS) - (Catalog No. 5087). One packet of phosphate-buffered saline salts.
5. Tween 20/Sodium Azide Solution (100X) - (Catalog No. 5037). One 10 mL vial containing Tween 20 /sodium azide concentrate.
6. Mounting Fluid - (Catalog No. 5013). One 10 mL dropper vial containing Tris-buffered glycerin, a fluorescence enhancer and 0.1% sodium azide (preservative)

J. Substantial Equivalence Information:

1. Predicate device name(s):

1. Cell Culture is used as a reference method to compare the performance of the direct specimen claim. An FDA cleared DFA test for the detection and identification of HSV-1 and HSV-2 following amplification in cell culture was used.
2. For the cell culture claim the same FDA cleared, DFA test for HSV-1 and HSV-2 mentioned in (1) above was used as a predicate.

2. Predicate K number(s):

K991880

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Intended use	In vitro diagnostic test for the qualitative detection and identification of herpes simplex virus type 1 and/or type 2 in direct specimens from patients with vesicular lesions and symptoms consistent with herpes infection and for culture confirmation by immunofluorescence. Negative results do not preclude an infection and should not be used as the sole basis for diagnosis, treatment or other management decisions. Specimens found negative on direct specimen	In vitro diagnostic test for direct immunofluorescence test intended for the detection and identification of herpes simplex virus type 1 (HSV-1) or herpes simplex virus type 2 (HSV-2) following amplification in cell culture or by direct examination of clinical specimens prepared by cytospin. Specimens found to be negative on direct specimen examination should be tested by cell culture.

Similarities		
Item	Device	Predicate
	detection should be confirmed by culture.	
Basic Principle	Directly labeled fluorescent antibodies specific to HSV-1 and HSV-2 to identify the presence of these viruses in patient specimens.	Directly labeled fluorescent antibodies specific to HSV-1 and HSV-2 to identify the presence of these viruses in patient specimens.
Sample Type	Vesicular fluid samples, from vesicular lesions	Vesicular fluid samples, from vesicular lesions
HSV1 antibody	The HSV-1 Typing Reagent consists of two fluorescein-labeled monoclonal antibodies specific for HSV-1 glycoprotein C and ICP35 respectively.	The primary component specific to HSV-1 will bind to the glycoprotein C and a capsid-associated protein in HSV-1 infected cells
HSV2 antibody	The HSV-2 Typing Reagent consists of two fluorescein-labeled monoclonal antibodies that specifically bind HSV-2 polypeptides. In Western blots these appear as two major bands with molecular weights of between 110-120 kD and between 78-82 kD and are consistent with the monoclonal antibodies recognizing epitopes within glycoprotein G of HSV-2.	The secondary component, specific for HSV-2, will bind to the glycoprotein G in HSV-2 infected cells
Instrumentation (required but not provided)	Fluorescence microscope with 100 watt mercury or halogen lamp, appropriate filter combination for FITC (excitation peak = 490 nm, emission peak = 520 nm), 100x, 200x and 400x magnification (dry objective)	Fluorescence microscope with 100 watt mercury or halogen lamp, appropriate filter combination for FITC (excitation peak = 490 nm, emission peak = 520 nm), 100x, 200x and 400x magnification (dry objective)

Differences		
Item	Device	Predicate
Labeling method	Two separate reagents, each of which contains FITC-labeled monoclonal antibodies directed against either HSV-1 or HSV-2. Two separate wells are necessary to detect and identify both viruses in one sample. Illumination with ultraviolet light allows visualization of the antigen-antibody complexes by fluorescence microscopy. HSV-infected cells will exhibit apple-green fluorescence with the specific reagent while cells stain a dull red due to the presence of Evans blue in the typing reagents.	One reagent contains specific monoclonal antibodies directed against HSV-1 and HSV-2 and tagged with two different fluorescent labels. This allows simultaneous visualization and identification of both HSV-1 and HSV-2-infected cells in one well. When an FITC filter set is used, HSV-1 infected cells will exhibit apple-green fluorescence and HSV-2 infected cells will exhibit yellow-gold fluorescence. The uninfected cells will stain a dull red due to the presence of Evans blue in the reagent.

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

L. Test Principle:

Light Diagnostics™ HSV 1/2 Typing DFA Kit utilizes specific reagents for the detection and identification of HSV 1 and HSV 2. The HSV 1 Typing Reagent consists of two fluorescein-labeled monoclonal antibodies specific for HSV 1 glycoprotein C and ICP35 respectively. HSV 2 Typing Reagent consists of two fluorescein-labeled monoclonal antibodies that specifically bind HSV 2 polypeptides. In Western blots these appear as two major bands with molecular weights of between 110-120 kD and between 78-82 kD and are consistent with the monoclonal antibodies recognizing epitopes within glycoprotein G of HSV 2. The typing reagents will bind to HSV 1 or HSV 2 infected cells fixed on microscope slides specifically. Separate cell spots on slides should be prepared for use with each reagent. Unbound reagent is removed by rinsing with

phosphate-buffered saline (PBS). Illumination with ultraviolet light allows visualization of the antigen-antibody complexes by fluorescence microscopy. HSV-infected cells will exhibit apple-green fluorescence with the specific reagent while cells stain a dull red due to the presence of Evans blue in the typing reagents.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

Internal and external precision studies were conducted at 3 sites using multiple operators, multiple runs per day and multiple viral concentrations. Results were 100% in accordance with expected results. There were no invalid or equivocal results.

Number of Operators	Number of Sites	Number of Runs	Viral control level	Results	% Accordance with expected results
6	3	40	High	Positive	100%
6	3	116	Low	Positive	100%
6	3	116	Uninfected	Negative	100%

b. *Linearity/assay reportable range:*

N/A

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*

N/A

d. *Detection limit:*

The limit of detection for each reagent was tested with five (ten-fold) dilutions made from viral seed stocks. Positive results were obtained using HSV-1 reagent on HSV-1 infected cells down to 80 PFU/ml of inoculum. Positive results were obtained using HSV-2 reagent on HSV-2 infected cells down to 140 PFU/ml of inoculum. In these tests the limit of detection was defined as the lowest concentration at which $\geq 95\%$ of replicates are positive

e. *Analytical specificity:*

The analytical specificity of the HSV 1 and HSV 2 Typing Reagents has been examined on slides prepared from reference strains as well as previously typed clinical isolates after cell culture at 1×10^6 - 8×10^6 cells/mL. The HSV 1 and HSV 2 Typing Reagents were tested using the same formulation as the

reagents included in this kit and did not show any cross-reactivity. The Typing Reagents have been tested on slides prepared from a wide range of other viruses, bacteria, and cell lines. Depending on the particular organism, concentrations between $\geq 1 \times 10^6$ cells/mL were tested and showed no cross-reactivity.

Organism	HSV-1 Reagent Result	HSV-2 Reagent Result
Herpes Viruses		
Herpes simplex virus type 1; ATCC VR733/735 - Clinical isolates (8)	+	-
Herpes simplex virus type 2; ATCC VR734 - Clinical isolates(7)	-	+
Varicella zoster virus; Oka strain	-	-
Cytomegalovirus; Clinical isolate 70-35	-	-
Human herpes virus 6; strain Z-29	-	-
Epstein-Barr virus; Human Lymph. P3HR1	-	-
Other viruses		
Adenovirus; CDC strains V5002	-	-
Influenza A; Clinical isolate	-	-
Influenza B Clinical isolate	-	-
Mumps; CDC V5004	-	-
Parainfluenza 1; CDC V6004	-	-
Parainfluenza 2; CDC V7003	-	-
Parainfluenza 3; CDC V5003	-	-
Parainfluenza 4; ATCC strain VR-1378	-	-
Respiratory syncytial virus; CDC strain A2	-	-
Rubella; VR315 strain M-33	-	-
Bacteria		
<i>Bordetella bronchiseptica</i>	-	-
<i>Bordetella pertussis</i>	-	-
<i>Branhamella catarrhalis</i>	-	-
<i>Candida albicans</i>	-	-
<i>Chlamydia pneumonia</i>	-	-
<i>Chlamydia trachomatis</i>	-	-
<i>Corynebacterium diphtheriae</i>	-	-
<i>E. coli</i>	-	-
<i>Legionella micdadei</i>	-	-
<i>Legionella pneumophila</i>	-	-
<i>Mycobacterium tuberculosis</i>	-	-
<i>Mycoplasma hominis</i>	-	-
<i>Mycoplasma pneumoniae</i>	-	-
<i>Neisseria Meningitidis</i>	-	-
<i>Pneumocystis carinii pneumonia</i>	-	-
<i>Staphylococcus aureus</i>	-	-

Organism	HSV-1 Reagent Result	HSV-2 Reagent Result
<i>Staphylococcus epidermidis</i>	-	-
<i>Streptococcus pneumonia</i>	-	-
<i>Streptococcus pyogenes</i>	-	-
<i>Trichomonas vaginalis</i>	-	-
Cell Lines		
MRC-5	-	-
A549	-	-
Vero	-	-
LLC-MK2	-	-
Hep-2	-	-

f. *Assay cut-off:*

N/A

2. Comparison studies:

a. *Method comparison with predicate device:*

A total of 454 specimens collected from 3 clinical sites were included in this study, 258 samples were from the Eastern region of the United States and 196 from the Southeastern region of the US. The specimens include fresh vesicular fluid from herpetic lesions collected from patients with lesions and exhibiting symptoms of HSV 1 or HSV 2 infections.

Clinical samples were submitted to each laboratory in viral transport media. Cells were washed in PBS, dropped onto slides, and fixed in acetone. Slides were stained with the Light Diagnostics™ HSV 1/2 Typing DFA Kit reagents, and another HSV typing reagent for reference, and examined using fluorescence microscopy. All specimens were placed in MRC-5 and/or HNF standard tubes for cell culture. Slides from positive cultures were stained with the Light Diagnostics™ HSV 1/2 Typing DFA Kit reagents, and reference HSV typing reagent and examined using fluorescence microscopy.

Sixteen specimens were excluded from HSV-1 direct specimen analysis and 18 specimens were excluded from HSV-2 direct specimen analysis, because of insufficient numbers of cells on the direct specimen slides. The results of the remaining direct specimen slides were compared to the results of culture isolation.

Culture results were not recorded for three specimens. Analysis was performed on the remaining specimens.

Data from all clinical sites was combined and summarized below.

Table: Detection of HSV-1 in Direct Specimens using Light Diagnostics™ HSV 1/2 Typing DFA Kit vs. Culture Confirmation with HSV typing reagent.

DETECTING HSV-1		Culture Confirmation with HSV typing reagent		Total	Comments
		Positive	Negative		
Light Diagnostics™ HSV 1/2 Typing DFA Kit on Direct Specimens	Positive	87	3	90	Sensitivity 84% (87/104) (75-90%) 95% CI
	Negative	17	331	348	Specificity 99% (331/334) (97-99%) 95% CI
	Total	104	334	438	

Table: Detection of HSV-2 in Direct Specimens using Light Diagnostics™ HSV 1/2 Typing DFA Kit vs. Culture Confirmation with HSV typing reagent.

DETECTING HSV-2		Culture Confirmation with HSV typing reagent		Total	Comments
		Positive	Negative		
Light Diagnostics™ HSV 1/2 Typing DFA Kit on Direct Specimens	Positive	57	2	59	Sensitivity 85% (57/67) (75-92%) 95% CI
	Negative	10	367	377	Specificity 99% (367/369) (98-99%) 95% CI
	Total	67	369	436	

Culture testing results combined from all sites

Table: Detection of HSV-1 using Light Diagnostics™ HSV 1/2 Typing DFA Kit vs. HSV typing reagent in culture amplified specimens.

DETECTING HSV-1		HSV typing reagent		Total	Comments
		Positive	Negative		
Light Diagnostics™ HSV 1/2 Typing DFA Kit on Culture Specimens	Positive	105	0	105	Sensitivity 100% (105/105) (97-100%) 95% CI
	Negative	0	71	71	Specificity 100% (71/71) (95-100%) 95% CI
	Total	105	71	176	

Table: Detection of HSV-2 using Light Diagnostics™ HSV 1/2 Typing DFA Kit vs. HSV typing reagent in culture amplified specimens

DETECTING HSV-2		HSV typing reagent		Total	Comments
		Positive	Negative		
Light Diagnostics™ HSV 1/2 Typing DFA Kit on Culture Specimens	Positive	70	0	70	Sensitivity 100% (70/70) (95-100%) 95% CI
	Negative	0	106	106	Specificity 100% (106/106) (97-100%) 95% CI
Total		70	106	176	

b. *Matrix comparison:*

N/A

3. Clinical studies:

a. *Clinical Sensitivity:*

N/A

b. *Clinical specificity:*

N/A

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

N/A

4. Clinical cut-off:

N/A

5. Expected values/Reference range:

A total of 454 specimens were included in this study, 258 samples from the Eastern region of the United States and 196 from the Southeastern region of the US. HSV-1 was identified in 105 specimens for an overall prevalence of 23.1%, while HSV-2 was identified in 70 specimens for a prevalence of 15.4%. The relative prevalence for of HSV-1 and HSV-2 from each site is indicated in the table below.

Table: Prevalence and Expected Value per Clinical Site

	HSV-1	HSV-2
Eastern U.S.	24.4%	10.8%
Southwestern U.S.	19.4%	19.4%
Southeastern U.S.	46.6%	46.6%
Overall	23.1%	15.4%
Direct Specimen Testing Positive Predictive Value	97% (91-99%) 95% CI	97% (89-99%) 95% CI
Direct Specimen Testing Negative Predictive Value	95% (92-97%) 95% CI	97% (95-99%) 95% CI
Culture Confirmation Positive Predictive Value	100% (97-100%) 95% CI	100% (95-100%) 95% CI
Culture Confirmation Negative Predictive Value	100% (95-100%) 95% CI	100% (97-100%) 95% CI

Specimens submitted for evaluation in this study included lesions from genital, oral, dermatological and other locations. The relative prevalence of HSV-1 and HSV-2 in these specimens is summarized in the table below.

	Genital¹ (n=228)	Oral² (n=104)	Skin³ (n=116)	Other (n=6)
HSV-1	20.7% (47)	37.1% (39)	16.4% (19)	0%
HSV-2	26.4% (60)	2.9% (3)	5.2% (6)	16.7% (1)
Total Positive	47.1% (107)	40% (42)	21.5% (25)	16.7% (1)

1. Genital – anal, vaginal, penis, etc. 3. Skin – forehead, back, finger, etc.
 2. Oral = mouth, lips, etc. 4. Other = ocular, unspecified anatomical locations

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.