

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

A. 510(k) Number: K063675

B. Purpose for Submission: The 510(k) holder would like to introduce D³ DFA Chlamydiae Culture Confirmation Kit into interstate commerce.

C. Measurand: Chlamydiae lipopolysaccharide antigen

D. Type of Test: Determination of the presence of Chlamydiae amplified in cell culture by immunofluorescence using fluoresceinated monoclonal antibodies

E. Applicant: Diagnostic Hybrids, Inc.

F. Proprietary and Established Names: Diagnostic Hybrids' D³ DFA Chlamydiae Culture Confirmation Kit

G. Regulatory Information:

1. Regulation section:
21CFR 866.3120, Chlamydia serological reagents
2. Classification:
Class I
3. Product code:
LJP – Antiserum, fluorescent, Chlamydia trachomatis
4. Panel:
Microbiology (83)

Product Code	Classification	Regulation Section	Panel
LJP	Class I	21CFR 866.3120	Microbiology (83)

H. Intended Use:

1. Intended use(s): The Diagnostic Hybrids' D³ DFA Chlamydiae Culture Confirmation Kit is intended for the qualitative detection of Chlamydiae lipopolysaccharide (LPS) in inoculated cell cultures by immunofluorescence using fluoresceinated monoclonal antibodies (MAbs).
Performance has not been established with direct patient specimens.
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' D³ DFA Chlamydiae Culture Confirmation Kit includes a Chlamydiae DFA Reagent that contains a blend of two murine MAbs directed against epitopes on the lipopolysaccharide of Chlamydiae. The kit is used for Chlamydiae identification in cell cultures inoculated with patient specimens.

J. Substantial Equivalence Information:

1. Predicate device names:

- a. American Microscan (distributed by Trinity Biotech) DFA Chlamydia Detection Kit
- b. Kallestad (distributed by BioRad) Pathfinder[®] Chlamydia Culture Confirmation Kit/System
- c. Diagnostic Products, Corp. (distributed by Remel) PathoDx[®] Chlamydia Culture Confirmation Kit

2. Predicate K numbers:

- a. K864389
- b. K864663
- c. K895839

3. Comparison with predicate:

The similarities (Table 1) to the predicate devices are in Intended Use, operating principle, basic design, materials and formulation. All kits use standard immunofluorescence assay techniques for staining fixed, cultured cell monolayers following incubation for Chlamydiae isolation. The kits employ MAbs specific for the lipopolysaccharide of Chlamydiae as the detector antibodies and utilize fluorescein as the fluorophore enabling visualization of the infected cells.

Table 1 Similarities to Comparison Devices

Similarities		
Item	Device	Comparison Device
Intended Use	For the qualitative detection of Chlamydiae lipopolysaccharide (LPS) in inoculated cell cultures by immunofluorescence using fluoresceinated monoclonal antibodies (MAbs). Performance has not been established with direct patient specimens.	a. American Microscan b. Kallestad c. Diagnostic Products, Corp.
Basic principle	Direct Fluorescent Antibody (DFA) test -Immunofluorescence using fluoresceinated MAbs	a. American Microscan b. Kallestad c. Diagnostic Products, Corp.
Antibody Antigen	Murine monoclonal antibodies against epitopes of the lipopolysaccharide of Chlamydiae Lipopolysaccharide	a. American Microscan b. Kallestad c. Diagnostic Products, Corp.

Similarities		
Item	Device	Comparison Device
Instrumentation (required but not provided)	Fluorescence microscope with filter combination for fluorescein (excitation peak = 490 nm, emission peak = 520nm).	a. American Microscan b. Kallestad c. Diagnostic Products, Corp.
Sample type	Swab in Transport Medium	a. American Microscan b. Kallestad c. Diagnostic Products, Corp.

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

L. Test Principle:

The test kit uses Chlamydiae antigen-specific murine monoclonal antibodies (MAbs) that are directly labeled with fluorescein for rapid detection of Chlamydiae which are directed against epitopes on the lipopolysaccharide of Chlamydiae.

The cell cultures to be tested are fixed in acetone. The Chlamydiae DFA Reagent is added to the cells to determine the presence of chlamydial antigens. After incubating at 35°C to 37°C for 48 to 72 hours, the infected cells are stained with a fluorescein conjugated MAb while uninfected cells are counterstained with an Evan's Blue dye. The stained cells are rinsed with the diluted PBS Concentrate, a drop of the supplied Mounting Fluid is added and a cover slip is placed on the prepared cells. The cells are examined using a fluorescence microscope.

Interpretation of results: It is recommended that controls be examined first to ensure proper test performance before examination of the specimens. A result is considered positive when bright, apple-green fluorescence is observed in the infected cells. If fluorescent cells are found, indicating a Chlamydia-positive specimen, it should be reported as, "Chlamydia isolated by cell culture." Uninfected cells will stain dull red due to the Evan's Blue counter-stain included in this device. The entire monolayer of cells must be examined for Chlamydiae-infected, fluorescent cells. If no fluorescent cells are found, the results of testing of the specimen should be reported as, "No Chlamydia detected."

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. *Analytical Sensitivity:*

Analytical sensitivity was determined by inoculating two 96-well cell culture plates with *Chlamydia trachomatis* diluted to one (1) TCID₅₀ per well for the entire plate. The plates were incubated at 37°C for 48 hours and then stained with either the Subject MABs or the Predicate, Chlamydia CC FA Kit (Trinity). Both plates were stained using the procedure in the respective product insert. This assay was performed 3 times with an average of 36 positive wells detected with the subject MABs and an average of 37 positive wells with the predicate. These results are not statistically different by a paired t-test.

C. trachomatis was diluted to a value of ~350 TCID₅₀ and serial 2-fold dilutions were then made to a final value of ~0.7 TCID₅₀. Each dilution of *C. trachomatis* was inoculated into 6 McCoy shell vials, centrifuged at 700xg for 60 minutes and incubated at 35-37°C for 48 hours. The subject MABs or the predicate, Chlamydia CC FA Kit (Trinity), was used to stain 3 shell vials of each *C. trachomatis* dilution according to the product inserts. The sensitivity of both fluorescent antibody stains is substantially equivalent, with ~ 0.7 – 1.4 TCID₅₀ as the minimum *C. trachomatis* dilution detected, as indicated by at least one cover slip having no detectable infection.

e. *Analytical specificity:*

The Chlamydiae DFA Reagent was tested for its ability to detect in cell culture the 15 known serovars of *Chlamydia trachomatis* as well as the *C. psittaci* and *C. pneumoniae*. Cell cultures (McCoy, BGMK, or HEp-2) were inoculated with approximately 1.5 x 10⁶ infective units of *Chlamydia trachomatis*, *Chlamydophila psittaci*, or *Chlamydophila pneumoniae*, and incubated for 2 days to yield a 3+ to 4+ infection. Cultures were processed and stained with the Chlamydia DFA Reagent or specially prepared DFA reagents containing only one of the Chlamydia MABs. The MABs were found to react with all organisms tested, as presented in Table 2

Table 2 Chlamydiae Tested for Reactivity with D³ DFA Chlamydiae Culture Confirmation Kit

<u>CHLAMYDIAE</u>	Serovar	Inoculum (Infective units per culture)	Result (Reactive+) (Negative -)
<i>Chlamydia trachomatis</i>	A	1.5 x 10 ⁶	+
	B	1.5 x 10 ⁶	+
	C	1.5 x 10 ⁶	+
	D	1.5 x 10 ⁶	+
	E	1.5 x 10 ⁶	+
	F	1.5 x 10 ⁶	+
	G	1.5 x 10 ⁶	+
	H	1.5 x 10 ⁶	+
	I	1.5 x 10 ⁶	+
	J	1.5 x 10 ⁶	+

	K	1.5 x 10 ⁶	+
	L1	1.5 x 10 ⁶	+
	L2	1.5 x 10 ⁶	+
	L3	1.5 x 10 ⁶	+
	Ba	1.5 x 10 ⁶	+
<i>Chlamydomonas reinhardtii</i>		1.5 x 10 ⁶	+
<i>Chlamydomonas reinhardtii</i>		1.5 x 10 ⁶	+

The Chlamydiae DFA Reagent was also tested for cross-reactivity against 57 virus strains (cultured and processed for staining), 20 host culture cell types, one yeast, one protozoa, and 28 bacterial cultures including *Staphylococcus aureus*, a protein-A-producing bacterium. The DFAs were prepared at 1.5X the concentration that is provided in the kit. Each of the tested viruses was prepared as infected cell monolayers, 150 to 2100 TCID₅₀. Bacterial strains were cultured, processed as suspensions, then spotted on microscope slides at concentrations ranging from 6.4x10⁴ to 2.93x10⁷ cfu per well in a 10 µL dot, then stained with the 1.5X DFAs according to the procedure in the product insert. Cell cultures were stained as confluent monolayers. No cross-reactivity was observed with all tested microorganisms and cell cultures except the *S. aureus*, which generated small points of fluorescence. Protein A specifically binds 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.

Table 3 Cell Lines and Viruses Cross-reactivity Testing

Organism	Strain or Type	Lot Number	Chlamydiae DFA Reagent	TCID ₅₀ or CFU
Cell Line	A-549	C560316	-	n/a
Cell Line	BGMK	C530316	-	n/a
Cell Line	HEp-2	C570316	-	n/a
Cell Line	HFF (Hs27)	C870315	-	n/a
Cell Line	LLC-MK2	C860316S	-	n/a
Cell Line	McCoy	C540316	-	n/a
Cell Line	MDCK	C830316S	-	n/a
Cell Line	MRC-5	C510315A	-	n/a
Cell Line	MRHF	C440314	-	n/a
Cell Line	Mv1Lu	C580317S	-	n/a
Cell Line	NCI-H292	C590313S	-	n/a
Cell Line	pCMK	470309	-	n/a
Cell Line	pRhMK	A490310	-	n/a
Cell Line	RD	C760317S	-	n/a
Cell Line	RhMK II	490311YS	-	n/a

Cell Line	RK (passage 1)	C480314PS	-	n/a
Cell Line	R-Mix	C960314S	-	n/a
Cell Line	Vero	C840331S	-	n/a
Cell Line	Vero 76	C670329S	-	n/a
Cell Line	WI-38	850318W	-	n/a
VIRUS				
Adenovirus	Type 1, ATCC VR-1	061704J	-	725
Adenovirus	Type 3, ATCC VR-3	112701A	-	725
Adenovirus	Type 6, ATCC VR-1083	102904	-	725
Adenovirus	Type 7, ATCC VR-7	112701C	-	725
Adenovirus	Type 8, ATCC VR-1368	111201D	-	725
Adenovirus	Type 10, ATCC VR-1087	112701D	-	725
Adenovirus	Type 13, ATCC VR-14	112701E	-	725
Adenovirus	Type 14, ATCC VR-15	033104	-	725
Adenovirus	Type 18, ATCC VR-19	011702B	-	725
Adenovirus	Type 31, ATCC VR-1109	011702	-	725
Adenovirus	Type 40, ATCC VR-931	012802A	-	725
Adenovirus	Type 41, ATCC VR-930	012802	-	725
Influenza A	Aichi, ATCC VR-547	022604A	-	2.1e3
Influenza A	Malaya, ATCC VR-98	022604M	-	2.1e3
Influenza A	Hong Kong, ATCC VR-544	040104	-	2.1e3
Influenza A	Denver, ATCC VR-546	022604D	-	2.1e3
Influenza A	Port Chalmers, ATCC VR-810	040104	-	2.1e3
Influenza A	PR, ATCC VR-1469	022604P	-	2.1e3
Influenza A	Victoria, ATCC VR-822	042105	-	2.1e3
Influenza B	Hong Kong, ATCC VR-823	091704	-	2.1e3
Influenza B	Maryland, ATCC VR-296	091704	-	2.1e3
Influenza B	Mass, ATCC VR-523	061704	-	2.1e3
Influenza B	Taiwan, ATCC VR-295	061704E	-	2.1e3
Influenza B	GL, ATCC VR-103	061704F	-	2.1e3
Influenza B	Russia, ATCC VR-790	091704	-	2.1e3
RSV	Long, ATCC VR-26	042204L	-	2.1e3
RSV	Wash, ATCC VR-1401	061704G	-	2.1e3
RSV	9320, ATCC VR-955	061704I	-	2.1e3
Parainfluenza 1	C-35, ATCC VR-94	020105	-	Control Slide*
Parainfluenza 2	Greer, ATCC VR-92	020105	-	Control Slide*
Parainfluenza 3	C 243, ATCC VR-93	020105	-	Control Slide*
HSV-1	1F, ATCC VR-733	092704	-	150
HSV-1	MacIntyre, ATCC VR-539	092804A	-	150

HSV-2	MS, ATCC VR-504	112701Y	-	150
HSV-2	Strain G, ATCC VR-734	092804B	-	150
CMV	Towne, ATCC VR-977	011503	-	700
CMV	AD169, ATCC VR-538	062304A	-	700
CMV	Davis, ATCC VR- 807	052605	-	700
VZV	Ellen, ATCC VR-1367	032804E	-	500
Echovirus	4, Bion Enterprises	QEC-0007	-	Control Slide*
Echovirus	6, Bion Enterprises	QEC-0007	-	Control Slide*
Echovirus	9, Bion Enterprises	QEC-0007	-	Control Slide*
Echovirus	11, Bion Enterprises	QEC-0007	-	Control Slide*
Echovirus	30, Bion Enterprises	QEC-0007	-	Control Slide*
Echovirus	34, Bion Enterprises	QEC-0007	-	Control Slide*
Coxsackievirus	B1, Bion Enterprises	QCB-0011	-	Control Slide*
Coxsackievirus	B2, Bion Enterprises	QCB-0011	-	Control Slide*
Coxsackievirus	B3, Bion Enterprises	QCB-0011	-	Control Slide*
Coxsackievirus	B4, Bion Enterprises	QCB-0011	-	Control Slide*
Coxsackievirus	B5, Bion Enterprises	QCB-0011	-	Control Slide*
Coxsackievirus	B6, Bion Enterprises	QCB-0011	-	Control Slide*
Mumps	Bion Enterprises	QMU-0298	-	Control Slide*
Measles (Rubeola)	Bion Enterprises	QME-0424	-	Control Slide*
Poliovirus	Type 1, Bion Enterprises	QPV-0009	-	Control Slide*
Poliovirus	Type 2, Bion Enterprises	QPV-0009	-	Control Slide*
Poliovirus	Type 3, Bion Enterprises	QPV-0009	-	Control Slide*
Epstein-Barr	Bion Enterprises	EAR/D-0090	-	Control Slide*

Table 4 Bacteria, Yeast, and Protozoa Cross Reactivity Testing

Organism	Strain or Type	Lot Number	Chlamydiae DFA Reagent	TCID₅₀ or CFU
Bacteria	<i>Acholeplasma laidlawi</i>	031404	-	~1.0e7
Bacteria	<i>Acinetobacter calcoaceticus</i>	934332	-	9.7e5
Bacteria	<i>Bordetella bronchiseptica</i>	031404	-	1.8e5
Bacteria	<i>Bordetella pertussis</i>	031404	-	4.7e6
Bacteria	<i>Chlamydiophila pneumoniae</i>	CP-0176	+	Control Slide*
Bacteria	<i>Chlamydiophila psittaci</i>	FP-12-050218	+	Control Slide*
Bacteria	<i>Chlamydia trachomatis</i>	052705	+	Control Slide*
Bacteria	<i>Corynebacterium diphtheriae</i>	031404	-	2.5e6

Bacteria	<i>Escherichia coli</i>	335472	-	2.6e5
Bacteria	<i>Gardnerella vaginalis</i>	3457511	-	5.0e5
Bacteria	<i>Haemophilis influenzae</i> type A	031404	-	9.3e5
Bacteria	<i>Klebsiella pneumoniae</i>	031404	-	6.4e6
Bacteria	<i>Legionella pneumophila</i>	031404	-	6.5e4
Bacteria	<i>Moraxella cartarrhalis</i>	031404	-	6.4e4
Bacteria	<i>Mycoplasma hominis</i>	031404	-	~1.0e4
Bacteria	<i>Mycoplasma orale</i>	031404	-	~1.0e4
Bacteria	<i>Mycoplasma pneumoniae</i>	031404	-	~1.0e4
Bacteria	<i>Mycoplasma salivarium</i>	031404	-	~1.0e7
Bacteria	<i>Neisseria gonorrhoeae</i>	060805	-	1.3e6
Bacteria	<i>Proteus mirabilis</i>	440498	-	2.1e6
Bacteria	<i>Pseudomonas aeruginosa</i>	031404	-	1.0e7
Bacteria	<i>Salmonella enteritidis</i>	3457511	-	2.5e6
Bacteria	<i>Salmonella typhimurium</i>	363162	-	1.8e6
Bacteria	<i>Staphylococcus aureus</i>	081100	+	1.0e7
Bacteria	<i>Streptococcus agalactiae</i>	370784	-	9.6e6
Bacteria	<i>Streptococcus pneumoniae</i>	031404	-	8.0e5
Bacteria	<i>Streptococcus pyogenes</i>	031404	-	2.9e7
Bacteria	<i>Ureaplasma urealyticum</i>	031404	-	~1.0e4
Yeast	<i>Candida glabrata</i>	992206	-	8.7e6
Protozoa	<i>Trichomonas vaginalis</i>	410721	-	

f. Assay cut-off: Not applicable

2. Comparison studies:

a. *Method comparison with predicate device:*

This study included nine hundred and ninety four (994) original specimens evaluated for the presence of Chlamydiae by this product (“Subject” test) and three currently marketed Culture Confirmation Kits (“Comparison” tests). These evaluations were conducted at three external laboratory sites using one Comparison Device (American Microscan DFA Chlamydia Detection Kit) and one in-house laboratory where the two other Comparison Devices were used: (1) A reference laboratory in the southeastern United States; (2) A hospital laboratory in the mid-west United States; (3) A hospital laboratory in the southwestern United States; and (4) Diagnostic Hybrids’ in-house virology laboratory.

Table 5 A Summary of the Specimens by Site.

	Fresh prospective	Frozen prospective	Archived (frozen)	Site Total
1 - FL	156	240		396
2 - MO	90	84	26	200
3 - TX	23	68	187	278
4 - OH	0	120		120

b. *Matrix comparison: Not applicable*

3. Clinical studies:

A subset of 661 prospectively collected and contiguous specimens, from study sites 1, 2, and 3, were tested using the same predicate device as comparator. Percent Agreement and 95% Confidence Interval between the Subject Device and Comparison test for the three external laboratory sites was calculated and is presented in Table 6. Results from both fresh and frozen specimens are included. PPA = positive percent agreement, NPA = negative percent agreement.

Table 6 Device Performance with Prospective Specimens

Subject Device	Combined Prospective Specimens		Fresh Prospective		Frozen Prospective	
	Comparison Device		Comparison Device		Comparison Device	
	+	-	+	-	+	-
+	42	4	11	0	21	4
-	2	613	0	258	2	355
PPA	95.5%		100%		97.0%	
95% CI – PPA	84.5% - 99.4%		71.5% - 100%		72.0% - 98.9%	
NPA	99.4%		100%		98.9%	
95% CI – NPA	98.3% - 99.8%		98.6% - 100%		97.2%-99.7%	

Results from the remaining specimens were not included in these calculations and are presented separately in section c below since (1) Sites 2 and 3 included a total of 213 specimens which had been frozen and archived in order to show performance of the Subject Device on a larger number of Chlamydiae-positive specimens, and (2) Site 4 tested the Subject Device against two Comparison Devices using 120 frozen specimens (prospectively collected, and contiguous).

a. *Clinical Sensitivity: Not applicable*

b. *Clinical specificity: Not applicable*

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

Due to low prevalence of Chlamydiae in the populations of specimens, investigators evaluated additional archived (frozen) specimens that had originally been identified as containing Chlamydiae. The combined results of testing of archived specimens at Sites 2 and 3 are presented in Table 7 below.

Table 7 Device Performance with Archival Specimens

Percent Agreement, Archival Specimens

		Comparison Device	
		+	-
Subject Device	+	159	0
	-	0	54

PPA 100%

95% CI - PPA 97.3-100%

NPA 100%

95% CI - NPA 93.3-100%

Site 4 Study: A total of 120 frozen specimens were tested for the presence of Chlamydiae against two different comparison devices:

Comparison Device PathoDx results Compared to those of Diagnostic Hybrids' D³ DFA Chlamydiae Device are presented in Table 8A

Table 8A Device Performance Compared to PathoDx

Frozen

		Comparison Device	
		+	-
Subject Device Diagnostic Hybrids	+	24	0
	-	0	96

PPA 100%

95% CI - PPA 85.8% - 100%

NPA 100%

95% CI - NPA 96.2% - 100%

Comparison Device Pathfinder results Compared to those of Diagnostic Hybrids' D³ DFA Chlamydiae Device are presented in Table 8B

Table 8 B Device Performance Compared to Pathfinder

		Frozen Comparison Device	
		+	-
Subject Device Diagnostic Hybrids	+	23	1
	-	0	96
PPA		100%	
95% CI – PPA		85.2% - 100%	
NPA		99.0%	
95% CI – NPA		94.4% - 100%	

4. Clinical cut-off: Not applicable

5. Expected values/Reference range:

Clinical studies included patient specimens from a reference laboratory in the Southeastern United States, and hospital laboratories in Midwestern and Southwestern United States. Patient demographics for the specimens tested are presented below (Table 9A).

Table 9A Clinical Study Specimens: Patient Age and Gender

Values are # pos / Total	Site 1		Site 2		Site 3		Site 4	
	F	M	F	M	F	M	F	M
TOTALS	362	32	170	30	215	63	98	22
<1m	0/1	0/3	0/2	2/4	9/15	14/19	0	0
1m to 2y	0	0	0/4	0/2	4/28	11/22	0	0
2y to 12y	0/2	0	1/28	1/13	6/13	0	0	0
12y to 18y	0/17	0	31/117	5/10	79/143	14/20	0/7	0
18y to 21y	6/56	1/4	4/19	1/1	4/13	0/2	9/30	3/7
>21y	9/283	3/25	0	0	0/3	0	8/61	4/15
Age not reported	1/3	0	0	0	0	0	0	0
Age/gender not reported	0/2		0		0		0	

The clinical studies were comprised of specimens collected and cultured for the presence of Chlamydiae. The specimen sources are described in Table 9B below.

Table 9B Clinical Study Specimens: Specimen Types

Site	Total	Unknown or Not Indicated	genital	penis	vaginal	cervical	rectal	perineum*	eye	urethral	mouth**	respiratory [†]	intra abdominal Aspirate
1	396	9	155	5	35	177		2	5	6	1	1	
2	200			13	55	83	34		6	3	2	4	
3	278			13	146	32	5	2	64	7	2	6	1
4	120		120										

*perineum: groin, pubic, perianal, pelvic, endocervix
 **mouth: mouth, throat
[†]respiratory: nasopharyngeal, nasal aspirate, tracheal aspirate, sputum

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

1. The submitted information in this premarket notification is complete and supports a substantial equivalence decision.