D³ FastPoint™

L-DFA™ Influenza A/Influenza B Virus Identification Kit

REF: 01-120001

For *in vitro* Diagnostic Use

Please contact Diagnostic HYBRIDS Technical Services for technical assistance regarding this procedure.

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US Patent Nos.: 6,168,915; 6,280,928; 6,306,582; 6,376,172; 6,406,842; 6,946,291 and patents pending.

Symbols Lexicon/Glossary

IVD	In Vitro Diagnostic Medical Device	LOT	Batch code/lot number
	Temperature limit	REF	Catalog number
	Consult instructions for use		Use by YYYY-MON-DD
	Do not reuse		Manufacturer
	Patent Numbers		
CONT NaN_3	Contains sodium azide	EC REP	Authorized representative in the European Community
NaN_3 4%	Contains 4% sodium azide when undiluted	CE	CE mark of conformity (Conformité Européen)

I. INTENDED USE

The Diagnostic Hybrids, Inc. device, D³ FastPoint™ L-DFA™ Influenza A/ Influenza B Virus Identification Kit is intended for the qualitative identification of influenza A virus and influenza B virus in nasal and nasopharyngeal swabs and aspirates/washes specimens from patients with signs and symptoms of respiratory infection by direct detection of immunofluorescence using monoclonal antibodies (MAbs).

It is recommended that specimens found to be negative for influenza A or influenza B virus after examination of the direct specimen result be confirmed by cell culture. Negative results do not preclude influenza A or influenza B virus infection and should not be used as the sole basis for diagnosis, treatment or other management decisions.

Performance characteristics for influenza A virus detection and identification were established when influenza A (H3N2) and influenza A (H1N1) were the predominant influenza A strains circulating in the United States. Since influenza strains display antigenic drift and shift from year to year, performance characteristics may vary. If infection with a novel influenza A virus is suspected, based on clinical and epidemiological screening criteria communicated by public health authorities, collect specimens following appropriate infection control precautions and submit to state or local health departments, for testing. Viral culture should not be attempted in these cases unless a BSL 3+ facility[1] is available to receive and culture specimens.[2]

II. SUMMARY AND EXPLANATION OF THE TEST

With the development of drug treatments for influenza[1], rapid and sensitive laboratory tests for virus identification can impact the choice of specific therapy, eliminating the inappropriate use of antibiotics and other agents. Virus identification using specific, fluorescent MAbs for direct antigen detection in respiratory specimens continues to be a diagnostic procedure used in clinical virology laboratories.

Influenza Types A and B

Influenza viruses (family *Orthomyxoviridae*) contain a single-stranded RNA genome which is present in eight separate segments of ribonucleoprotein. Segmentation of the genome is rare among viruses and contributes to the development of new influenza strains through interchange of gene segments when two different influenza strains infect the same cell. There are three influenza types: A, B and C. Type A has counterparts in birds, horses, sea mammals and pigs as well as in humans, while types B and C are primarily known in humans.

With the potential for an additional influenza A pandemic such as occurred in 1918 when 25-35 million people died worldwide, the Centers for Disease Control (CDC) and the World Health Organization (WHO) maintain surveillance of emerging influenza strains and make recommendations for suitable strains for vaccine production.

Influenza infects an estimated 120 million people in the US, Europe and Japan each year, and it is estimated there are 75,000 deaths annually in the US from pneumonia caused by influenza infection.[2] Primary viral pneumonia or pneumonia from secondary bacterial infections are the primary causes of morbidity associated with influenza infection.[2] Complications tend to occur in the young, the elderly and persons with chronic cardio-pulmonary diseases.

Pandemics of influenza A occur about every 10 to 30 years while annual epidemics are usually of either influenza A or B; however, both types may circulate concurrently. Infections are seasonal, typically extending from November to April in the northern hemisphere. Disease incubation is 1-3 days with rapid transmission through aerosolized droplets and fomites. The disease is characterized by sudden onset, fever, myalgia, headache and pharyngitis.

III. PRINCIPLE OF THE PROCEDURE

The D³ FastPoint L-DFA Influenza A/Influenza B Virus Identification Kit uses a blend (called a "L-DFA Reagent") of viral antigen-specific murine monoclonal antibodies that are directly labeled with either R-phycoerythin (PE) (influenza A virus) or fluorescein isothiocyanate (FITC) (influenza B virus) for the rapid identification of influenza A virus and influenza B virus in nasal and nasopharyngeal (NP) swabs and aspirates/washes specimens from patients with signs and symptoms of respiratory infection.

The cells to be tested are derived from respiratory specimens from patients with signs and symptoms of respiratory infection. The cells are permeabilized and stained concurrently in a liquid suspension format with the L-DFA Reagent. After incubating at 35º to 37ºC for 5-minutes, the stained cell suspensions are rinsed with 1X PBS. The rinsed cells are pelleted by centrifugation and then re-suspended with the Re-suspension Buffer and loaded onto a specimen slide channel. The cells are examined using a fluorescence microscope. Cells infected with influenza A virus will exhibit golden-yellow fluorescence due to the PE. Cells infected with influenza B virus will exhibit apple-green fluorescence due to the FITC. Non-infected cells will exhibit dull red fluorescence due to the Evans Blue counter-stain. Nuclei of intact cells will exhibit orange-red fluorescence due to the propidium iodide.

It is recommended that specimens found to be negative for influenza A virus or influenza B virus after examination of the direct specimen result be confirmed by cell culture.

IV. REAGENTS

A. Kit Components[3]

1. **D³ FastPoint L-DFA Influenza A/Influenza B Reagent**, 5.0-mL. One dropper bottle containing a mixture of PE-labeled murine monoclonal antibodies directed against influenza A virus antigens and FITC-labeled murine monoclonal antibodies directed against influenza B virus antigens. The buffered, stabilized, aqueous solution contains Evans Blue and propidium iodide as counter-stains and 0.1% sodium azide as preservative.
2. **40X PBS Concentrate,** 25-mL. One bottle of 40X PBS concentrate containing 4% sodium azide (0.1% sodium azide after dilution to 1X using de-mineralized water).
3. **Re-suspension Buffer**, 6.0-mL. One bottle of a buffered glycerol solution and 0.1% sodium azide.
4. **D³ FastPoint L-DFA Influenza A/Influenza B Antigen Control Slides**, 5-slides. Five (5) individually packaged control slides containing 2 wells with cell culture-derived positive and negative control cells. Each positive well contains cells infected with both influenza A virus and influenza B virus. The negative wells contain non-infected cells. Each slide is intended to be stained only one time.
5. **D³ FastPoint L-DFA Specimen Slides**, 50-slides. Fifty (50) 3-channel specimen slides with coverslips. Each slide is intended to be used only one time.

[1] www.cdc.gov

[2] FDA Guidance Document: In Vitro Diagnostic Devices to Detect Influenza A Viruses: Labeling and Regulatory Path; Issued 4/10/2006

[3] A Material Safety Data Sheet for sodium azide or for other Diagnostic Hybrids, Inc (DHI) reagents is available by contacting a DHI Technical Services.

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B. Warnings and Precautions

For *in vitro* diagnostic use.

1. Consider all human specimens, blood derivatives, reagents and materials used for processing as capable of transmitting infectious diseases and handle them in a manner which prevents infection of laboratory personnel. No known test method can offer complete assurance that infectious agents are absent.
   a. Conduct all procedures in accordance with the OSHA Standard on Blood-borne Pathogens[3]; the manual "Biosafety in Microbiological and Biomedical Laboratories", CDC, 5th edition, 2007; and, the standard, CLSI/NCCLS Approved Guideline, M29-A3, "Protection of Laboratory Workers from Occupationally Acquired Infections".[4]
   b. Follow Biosafety Level 2 or other appropriate biosafety practices.
   c. Decontaminate specimens using a 1:10 dilution of household bleach.
   d. Although Antigen Control Slides have been shown to be non-infectious, the same precautions taken in handling and disposing of other infectious materials should be employed in their use.
2. Do not pipette reagents or clinical samples by mouth. Protect broken skin from contact with clinical samples.
3. Avoid splashing and the generation of aerosols with clinical samples.
4. Sodium azide is included in the 40X PBS Concentrate at a concentration of 4% (w/v), and in the other solutions in this kit at 0.1% concentration.

T: Sodium azide at very low levels causes damage to health.

R28 Very toxic if swallowed.
R32 Contact with acids liberates very toxic gas.
S28 After contact with skin, wash immediately with plenty of water.
S45 In case of accident, or if you feel unwell, seek medical advice immediately.
S60 This material and its container must be disposed of as hazardous waste.

N: Sodium azide may present an immediate or delayed danger to one or more components of the environment.

R51/53 Toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment.
S61 Avoid release to the environment. Refer to special instructions/safety data sheets.

   a. Reagents containing sodium azide should be considered poisons. If products containing sodium azide are swallowed, seek medical advice immediately and show product container, label, or MSDS to medical personnel. (Refer to NIOSH, National Institute for Occupational Safety and Health; CAS#: 2628-22-8; EC# 247-852-1; and also to GHS, The Globally Harmonized System of Classification and Labeling of Chemicals.)
   b. Evaluate reagents containing sodium azide for proper use and disposal. When mixed with acids, aqueous solutions of sodium azide may liberate toxic gas. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. If products containing sodium azide are discarded into a drain, flush with a large volume of water to prevent azide build-up. Check with local regulatory agencies to determine the concentration of sodium azide that may require regulation as hazardous waste.
5. Evans Blue counter-stain is a potential carcinogen. If skin contact occurs, flush with water immediately.
6. Propidium iodide counter-stain is a potential carcinogen and mutagen. If skin contact occurs, flush with water immediately.
7. The L-DFA Reagent is supplied at working strength. Any dilution of the reagent will decrease sensitivity.
8. Reagents should be used prior to their expiration date.
9. Each Influenza A/Influenza B Antigen Control Slide should be used only once. Do not re-use a control slide.
10. Microbial contamination of the L-DFA Reagent may cause a decrease in sensitivity.
11. Store 1X PBS in a clean container to prevent contamination.
12. Reusable glassware must be washed and thoroughly rinsed free of all detergents.
13. Do not expose the L-DFA Reagents to bright light during staining or storage.
14. Use of reagents other than those specified with the components of this kit may lead to erroneous results.

C. Preparation of 1X PBS Solution

1. After storage at 2° to 8°C, some salts in the 40X PBS Concentrate may have crystallized. Warm the solution to ambient temperature (20° to 25°C) to re-dissolve the crystals, then mix.
2. Add contents of the fully dissolved 25-mL 40X PBS Concentrate to 975-mL of de-mineralized water.
3. Label the 1X PBS with a sixty (60) day expiration date after reconstitution, and store at ambient temperature.

D. Reagent Storage Instructions

TABLE 1: Reagent Storage Conditions	
Kit Box #1	
1. D³ FastPoint L-DFA Influenza A/Influenza B Reagent	Store in the dark at ambient temperature (20° to 25°C)
2. D³ FastPoint L-DFA Specimen Slides	
Kit Box #2	
3. Re-suspension Buffer	Store at 2° to 8°C
4. D³ FastPoint L-DFA Influenza A/Influenza B Virus Antigen Control Slides	
5. 40X PBS Concentrate NOTE: The Concentrate may crystallize when stored at 2° to 8°C. The crystals will dissolve when the Concentrate is warmed to ambient temperature.	Store liquid at 2° to 8°C prior to dilution
6. 1X PBS	Store at ambient temperature (20° to 25°C)

E. Stability

Reagents and components will retain their full potency through the expiration date shown on the label of each bottle when stored under recommended conditions. Light exposure of the L-DFA Reagent should be kept to a minimum.

Discard 1X PBS solution if it becomes cloudy.

V. SPECIMEN COLLECTION, TRANSPORT, AND STORAGE

Proper collection and handling of the patient specimen are the most important factors in successful respiratory virus detection. Specimen collection, specimen processing, and cell culture isolation of viruses should be attempted only by personnel trained in performing such procedures. Care should be taken during all specimen collection and handling to avoid generation of aerosols.

For specimen collection and processing recommendations, refer to CLSI Approved Viral Culture Guidelines.[5]

A. Specimen Transport and Storage

All potentially infectious agents should be transported according to International Air Transport Association (IATA), International Civil Aviation Organization, (ICAO), Titles 42 and 49 of the US Code of Federal Regulations, or other regulatory requirements, as may be applicable.

Specimens should be transported to the laboratory at 2° to 8°C. These temperatures can be attained using cold packs, wet ice, foam refrigerant, or other coolants. Specimens should be processed and tested as soon as possible but may be stored at 2° to 8°C for up to 72-hours before being tested. If longer storage is required, the specimens should be frozen at –70°C or lower.

VI. PROCEDURE

A. Materials Provided

1. D³ FastPoint L-DFA Influenza A/Influenza B Reagent
2. Re-suspension Buffer
3. D³ FastPoint L-DFA Influenza A/Influenza B Antigen Control Slides
4. 40X PBS Concentrate
5. D³ FastPoint L-DFA Specimen Slides

B. Materials Required But Not Provided

1. Fluorescence microscope with the correct filter combination for FITC (excitation peak = 490 nm, emission peak = 520nm) and for R-PE; magnification 200 to 400X.
2. Cover slips (22 x 50mm) for Antigen Control Slides.
3. Fine-tip, disposable transfer pipettes.
4. Adjustable pipettes (20 to 200-µL and 200 to1000-µL).
5. Pipette tips (20 to 200-µL and 200 to1000-µL)
6. 200-mL wash bottle.
7. 1.7-mL centrifuge vials.
8. 15-mL conical centrifuge tube.
9. Sodium hypochlorite solution (1:10 final dilution of household bleach).
10. Humidified chamber (e.g., covered Petri dish with a damp paper towel placed in the bottom) or humidified incubator.
11. Incubator, 35° to 37°C (CO_2 or non-CO_2, depending on the cell culture format used).
12. Centrifuge with free-swinging bucket rotor.
13. De-mineralized water for dilution of 40X PBS Concentrate.
14. Stat-Spin Centrifuge (or bench top centrifuge capable of 2-minutes at 2000xg).

C. Comments and Precautions

1. Adhere to the recommended volumes and times stated in the following procedure to ensure that accurate results are obtained.
2. When staining with fluorescent reagents and examining cells microscopically for fluorescence, include both positive and negative controls, to monitor the procedure and performance of the reagents. Run controls with each batch of patient specimens.
3. Following use of the L-DFA Reagent, return to ambient temperature storage in the dark.

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4. Examine the positive and negative controls before examining the test specimens. If a control fails to perform as expected, review the steps and conditions under which the test was performed to determine the root cause(s) of failure. Do not report results for patient samples unless controls perform as expected.
5. Three aspects of the fluorescence microscope must be functioning properly and optimally to achieve maximum brightness of fluorescence:
   a. The activation light source has a finite life. As the light source ages, its output decreases, resulting in lower fluorescence intensity from the L-DFA Reagent. Change the fluorescent bulb according to the manufacturer's recommendations.
   b. The light source is focused by a number of lenses and mirror(s). For maximum intensity, these must be properly aligned.
   c. The filters used in the light path must be appropriate for fluorescein.
6. Fluorescent artifacts may be observed during examination of the stained cells.
   a. Morphologically, staining artifacts do not have the appearance of a complete cell and typically do not appear to be on the plane of the monolayer. Cell debris, lint, etc. can non-specifically adsorb the L-DFA Reagent, resulting in highly intense fluorescence.
   b. Intense fluorescence around the periphery of slide wells indicates drying of the L-DFA Reagent, suggesting that incubation was too long or the humidity was not controlled.
   c. Inadequate removal of mucus from direct specimen material can lead to non-specific staining when conducting the test on direct specimens.
   d. Generalized, low-grade fluorescence may be seen particularly in areas that have clumped cells. Diffusion of the trapped fluorescent stain is impeded during the washing steps resulting in non-specific fluorescence.
   e. On direct specimens, leukocytes and monocytes may trap fluorescence or RBC may leave a green haze on the sample.
7. Protect stained slides and monolayers from light as much as possible during testing.
   a. Bleaching or fading of the fluorescence of stained cells may occur on exposure to light, particularly light of high intensity.
   b. This bleaching can occur when a stained cell is viewed in a fluorescence microscope for an extended period.

D. Specimen Preparation

For cell suspension preparation recommendations, refer to CLSI Approved Viral Culture Guidelines.[6]

E. Cell Suspension Permeabilizing and Staining Procedure

1. Remove Re-suspension Buffer from the refrigerator and allow it to warm to ambient temperature for 15- to 30-minutes prior to use.
2. Label a 1.7-mL centrifuge vial, influenza A/influenza B (A/B).
3. Vortex cell suspension for 5- to 10-seconds.
4. Using a fine tip transfer pipette, add 3-drops (~70-µL) of the cell suspension to centrifuge vial from step 2 above.
5. Add 2-drops of D³ FastPoint L-DFA Influenza A/Influenza B Reagent to the labeled vial.
6. Mix by vortex for 1- to 2-seconds.
7. Incubate vial at 35° to 37°C for 5-minutes.
8. Add approximately 1.5-mL of 1X PBS to the vial using the wash bottle.
9. Centrifuge the vial for 2-minutes at 2000xg.
10. Decant the PBS gently from the vial.
11. Blot excess PBS from the vial onto an absorbent paper towel by lightly tapping the vial.
12. Add 1-drop (~20-µL) of the Re-suspension Buffer to the vial.
13. Break up the cell pellet by pipetting up and down 5- to10-times with a 20-µL pipette, changing tips after each vial.
14. Label a specimen slide with the specimen identifier.
15. Using the fixed volume pipette, add 20-µL from each vial to the appropriate labeled channel, changing tips after each vial.
16. Examine each channel for the presence of fluorescent cells at 200X magnification with a fluorescence microscope.
17. Refer to Section VIII, 'Interpretation of Results'.

F. Respiratory Virus Antigen Control Slide Staining Procedure

1. Remove D³ FastPoint L-DFA Influenza A/Influenza B Antigen Control Slide from the refrigerator and allow to warm to ambient temperature for 15- to 30-minutes prior to use.
2. To each of the wells of a fresh D³ FastPoint L-DFA Influenza A/Influenza B Antigen Control Slide, add one drop D³ FastPoint L-DFA Influenza A/Influenza B Reagent.
3. Place the slide at 35° to 37°C for 5-minutes in a humidified chamber.
4. Rinse the stained cells using a wash bottle of 1X PBS; direct the wash stream above each row of wells.
5. Blot the excess 1X PBS, add a small drop of Re-suspension Buffer to each cell-containing well and cover the wells with a coverslip.
6. Examine each well for the presence of fluorescent cells at 200X magnification with a fluorescence microscope.
7. Refer to Section VIII, 'Interpretation of Results'.
8. The Antigen Control Slide should be stained only once, as it contains individual wells of viral infected cells and non-infected cells.

VII. QUALITY CONTROL

1. A fresh D³ FastPoint L-DFA Influenza A/Influenza B Antigen Control Slide should be stained each time the staining procedure is performed to ensure proper test performance.
2. Within the positive control well, the infected cells will fluoresce either golden-yellow or apple-green, depending on the infecting virus, while non-infected cells will stain a dull red due to the Evans Blue counter-stain. The nuclei of all cells will stain orange-red due to the propidium iodide.
3. The negative well will show only non-infected cells staining a dull red. The nuclei of all cells will stain orange-red due to the propidium iodide.
4. Positive and negative controls must demonstrate appropriate fluorescence for specimen results to be considered valid. Antigen Control Slides may also aid in the interpretation of patient specimens.

VIII. INTERPRETATION OF RESULTS

A. Examination of Samples and Controls

1. Examine controls first to ensure proper test performance before examining patient specimens.
2. Examine the entire cell spot before reporting final results.
3. Do not report results for patient samples unless controls perform as expected.
4. Evaluation of sample suitability:
   a. **Each stained patient specimen** should be examined for the presence of columnar epithelial cells (cells that are taller than they are wide). The quality of the specimen with respect to the number of epithelial cells in the sample can be assessed by examining different fields at a magnification of 200X.
   b. **A satisfactory specimen** should have at least 2-columnar epithelial cells per field. A negative result is indicated by the absence of fluorescence in a minimum sampling of 20-columnar epithelial cells**.**
   c. **An inadequate specimen** is indicated by fewer than 20-columnar epithelial cells present in the sample, in which case the test is considered invalid. A new specimen should be obtained and tested or cell culture for virus isolation should be initiated from the remaining specimen.
5. Interpretation of D³ FastPoint L-DFA Influenza A/ Influenza B Reagent:
   a. **Influenza A**: The golden-yellow fluorescence is cytoplasmic, nuclear or both. Cytoplasmic staining is often punctate with large inclusions while nuclear staining is uniformly bright. Stained cells are usually round in appearance and sometimes larger than non-infected cells.
   b. **Influenza B**: The apple-green fluorescence is cytoplasmic, nuclear or both. Cytoplasmic staining is often punctate with large inclusions while nuclear staining is uniformly bright. Stained cells are usually round in appearance and sometimes larger than non-infected cells.

B. Reporting Results of Direct Specimen Staining

1. The entire cell spots from each channel must be examined for virus-infected, golden-yellow or apple-green fluorescent cells. A satisfactory specimen with no fluorescent cells observed should be reported as "No influenza A virus or influenza B virus viral antigens detected by direct specimen testing" Such negative results, however, should be confirmed by cell culture.
2. If golden-yellow fluorescent cells are found, it should be reported as "influenza A virus viral antigens detected by direct specimen testing."
3. If apple-green fluorescent cells are found, it should be reported as "influenza B virus viral antigens detected by direct specimen testing."
4. If co-infection with both viruses is detected it should be reported as "influenza A virus *and* influenza B virus viral antigens detected by direct specimen testing."

IX. LIMITATIONS OF PROCEDURE

1. Inappropriate specimen collection, storage, and transport may lead to incorrect results[7].
2. Assay performance characteristics have not been established for direct specimen staining on respiratory specimens other than nasal/nasopharyngeal swabs and aspirates/washes. Data supporting the use of the D³ FastPoint L-DFA Influenza A/Influenza B Virus Identification Kit for direct testing of other respiratory specimens is limited and it is the user's responsibility to establish assay performance parameters.
3. Incubation times or temperatures other than those cited in the test instructions may give erroneous results.
4. Detection of influenza A virus and influenza B virus will vary greatly depending upon the specimen quality and subsequent handling. A negative result does not exclude the possibility of virus infection. Results of the test should be interpreted in conjunction with information available from epidemiological studies, clinical evaluation of the patient and other diagnostic procedures.
5. The effects of antiviral therapy on the performance of this kit have not been established.
6. The monoclonal antibodies used in this kit are from hybridomas created using viral infected cells as the immunogen. The specific viral epitopes detected by the antibodies are undetermined.
7. Since the monoclonal antibodies have been prepared using defined virus strains, they may not detect all antigenic variants or new strains of the viruses, should they arise. Monoclonal antibodies may fail to detect strains of viruses which have undergone minor amino acid changes in the target epitope region.
8. The monoclonal antibodies used in this kit are not group-specific and therefore cannot be used to differentiate among the different sub-types of influenza A virus.

9. The viral antigens detected in some direct specimens may be from non-viable virus and cannot be isolated by culture.
10. A negative direct specimen should be inoculated into appropriate cell culture(s) and incubated to isolate and identify any respiratory viruses that may be present in the specimen.
11. A negative result on a direct or cultured specimen does not rule out the presence of virus.
12. Performance of the kit can be assured only when components used in the assay are those supplied by DHI.
13. Prolonged storage of the D³ FastPoint L-DFA Influenza A/Influenza B Virus Identification Kit Reagent under bright light will decrease the staining intensity. Stained slides left under UV light will lose their fluorescence. Remove slide from fluorescence microscope stage as soon as possible.
14. Light background staining may occur with specimens contaminated with *Staphylococcus aureus* strains containing large amounts of protein A. Protein A will 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 testing direct specimens with bacterial contamination must be interpreted with caution.

X. EXPECTED VALUES

Clinical studies were performed at 4 study sites in the United States in the winter of 2009 (January 2008 through March 2009). Prevalence of Influenza A/B viruses within this population as determined by the D³ FastPoint L-DFA Influenza A/Influenza B Virus Identification Kit direct specimen testing is noted in Table 2 below:

TABLE 2: Influenza A/ Influenza B Virus Prevalence

Age	Total Specimens Evaluated	Influenza A # positive (prevalence)	Influenza B # positive (prevalence)
0 to 1 month	55	0	0
> 1 month to 2 years	577	27 (4.7%)	20 (3.5%)
> 2 years to 12 years	391	43 (11.0%)	104 (26.6%)
> 12 years to 21 years	173	19 (11.0%)	41 (23.7%)
22 years to 30 years	57	3 (5.3%)	14 (24.6%)
31 years to 40 years	71	9 (12.7%)	9 (12.7%)
41 years to 50 years	52	5 (9.6%)	5 (9.6%)
51 years to 60 years	46	3 (6.5%)	3 (6.5%)
61 years to 70 years	33	2 (6.1%)	2 (6.1%)
71 years to 80 years	16	2 (12.5%)	1 (6.3%)
81 years and above	7	0	0
Age Not Reported	41	2 (4.9%)	14 (34.1%)
Total	1519	115 (7.6%)	213 (14.0%)

***** There were two (2) respiratory syncytial virus + metapneumovirus co-infections detected.

XI. PERFORMANCE CHARACTERISTICS

A. CLINICAL PERFORMANCE STUDIES

Performance of the D³ FastPoint L-DFA Influenza A/Influenza B Virus Identification Kit testing direct respiratory specimens was established during prospective studies at 4 geographically diverse U.S. clinical laboratories during the 2009 respiratory virus seasons (January 2009 through March 2009). All specimens used in the studies meeting the inclusion and exclusion criteria represented excess, remnants of respiratory specimens that were prospectively collected from symptomatic individuals suspected of respiratory infection, and were submitted for routine care or analysis by each site, and that otherwise would have been discarded. Individual specimens were delinked from all patient identifiers and given a study sample code. All clinical sites were granted waivers of informed consent by their IRBs for this study.

Performance of the D³ FastPoint L-DFA Influenza A/Influenza B Virus Identification Kit was assessed and compared to a predetermined algorithm that used composite comparator methods. The composite comparator methods consisted of Direct Specimen Fluorescent Antibody (DSFA) test with an FDA-cleared device and viral culture confirmation of all the negatives (as determined by the comparator DSFA test). "True" positive was defined as any sample that either tested positive by the comparator DSFA test or viral culture. "True" negative was defined as any sample that tested negative by both the comparator DSFA test and viral culture.

Study Site 1

The study at Study Site 1 consisted of a total of 323 fresh respiratory specimens submitted, January through March, 2009, to the laboratory for respiratory virus testing. Slides were prepared using Phosphate Buffered Saline (PBS)-washed cells from the fresh specimens and processed according to the prescribed protocols. The specimens were stained in accordance with the procedure defined in this document.

Table 3 shows the age and gender distribution for individuals studied at Study Site 1:

TABLE 3: Study Site 1 – Age and Gender Distribution

Sex	F	M
Total	**150**	**173**
Age		
0 to 1 month	13	7
> 1 month to 2 years	100	131
> 2 years to 12 years	35	35
> 12 years to 21 years	2	0
22 years to 30 years	0	0
31 years to 40 years	0	0
41 years to 50 years	0	0
51 years to 60 years	0	0
61 years to 70 years	0	0
71 years to 80 years	0	0
81 years and above	0	0
Age Not Reported	0	0
Total	150	173

Of the 323 fresh respiratory specimens tested, all were nasal wash/nasopharyngeal aspirate specimens. Two (2) specimens were excluded from the performance analysis due to insufficient sample volume for the investigational device testing (0.62%). Seventy (70) specimens for influenza A, 79 specimens for influenza B, were also excluded from the respective performance analysis due to insufficient sample volume for the comparator culture method, Tables 4 and 5 below show the study results of the claimed specimen type at Study Site 1:

TABLE 4: Influenza A

Fresh nasal/nasopharyngeal wash/aspirate	Comparator DSFA (negatives followed by culture with DFA)		
DHI DSFA	**Positive**	**Negative**	**Total**
Positive	21	1	22
Negative	4	225	229
Total	25	226	251
			95% CI
Sensitivity	21/25	84%	63.9-95.5%
Specificity	225/226	99.6%	97.6-100%

TABLE 5: Influenza B

Fresh nasal/nasopharyngeal wash/aspirate	Comparator DSFA (negatives followed by culture with DFA)		
DHI DSFA	**Positive**	**Negative**	**Total**
Positive	2	0	2
Negative	0	240	240
Total	2	240	242
			95% CI
Sensitivity	2/2	100%	15.8-100%
Specificity	240/240	100%	98.5-100%

Study Site 2

The study at Study Site 2 consisted of a total of 105 fresh respiratory specimens submitted, February through March 2009, to the laboratory for respiratory virus testing. Slides were prepared using PBS-washed cells from the fresh specimens and processed according to the prescribed protocols. The specimens were stained in accordance with the procedure defined in this document.

Table 6 shows the age and gender distribution for individuals studied at Study Site 2:

TABLE 6: Study Site 2 – Age and Gender Distribution

Sex	F	M
Total	**48**	**57**
Age		
0 to 1 month	2	4
> 1 month to 2 years	15	17
> 2 years to 12 years	6	5
> 12 years to 21 years	4	6
22 years to 30 years	2	2
31 years to 40 years	4	6
41 years to 50 years	1	4
51 years to 60 years	6	5
61 years to 70 years	3	6
71 years to 80 years	3	2
81 years and above	2	0
Age Not Reported	0	0
Total	48	57

Of the 105 fresh respiratory specimens tested, 86 were nasal wash/nasopharyngeal aspirate specimens. Due to insufficient sample numbers to establish performance of the D³ FastPoint L-DFA Influenza A/Influenza B Virus Identification Kit, 19 other types of respiratory specimens were removed from performance analysis. Tables 7 and 8 below show the study results of the claimed specimen type at Study Site 2:

TABLE 7: Influenza A			
Fresh nasal/nasopharyngeal wash/aspirate	Comparator DSFA (negatives followed by culture with DFA)		
DHI DSFA	Positive	Negative	Total
Positive	6	2	8
Negative	0	78	78
Total	6	80	86
			95% CI
Sensitivity	6/6	100%	54.1-100%
Specificity	78/80	97.5%	91.3-99.7%

TABLE 8: Influenza B			
Fresh nasal/nasopharyngeal wash/aspirate	Comparator DSFA (negatives followed by culture with DFA)		
DHI DSFA	Positive	Negative	Total
Positive	4	0	4
Negative	1	81	82
Total	5	81	86
			95% CI
Sensitivity	4/5	80%	28.4-99.5%
Specificity	81/81	100%	95.5-100%

Study Site 3

The study at Study Site 3 consisted of a total of 443 fresh respiratory specimens submitted, February through March 2009, to the laboratory for respiratory virus testing. Slides were prepared using PBS-washed cells from the fresh specimens and processed according to the prescribed protocols. The specimens were stained in accordance with the procedure defined in this document.

Table 9 shows the age and gender distribution for individuals studied at Study Site 3:

TABLE 9: Study Site 3 – Age and Gender Distribution			
Sex	F	M	Sex Not Reported
Total	231	209	3
Age			
0 to 1 month	17	10	1
> 1 month to 2 years	116	132	2
> 2 years to 12 years	48	39	0
> 12 years to 21 years	8	15	0
22 years to 30 years	5	2	0
31 years to 40 years	9	4	0
41 years to 50 years	8	4	0
51 years to 60 years	5	1	0
61 years to 70 years	6	1	0
71 years to 80 years	6	0	0
81 years and above	2	0	0
Age Not Reported	1	1	0
Total	231	209	3

Of the 443 fresh respiratory specimens tested, 301 were nasal wash/ nasopharyngeal aspirate specimens, and 140 were nasal/nasopharyngeal swab specimens. One (1) nasal wash/nasopharyngeal aspirate specimen was excluded from the performance analysis due to the fact that the sample was tested by the investigational device greater than 48-hours post sample collection. Due to insufficient sample numbers to establish performance of the D³ FastPoint L-DFA Influenza A/Influenza B Virus Identification Kit, 2 other types of respiratory specimens were further removed from performance analysis. Tables 10 through 13 below show the study results of the claimed specimen types at Study Site 3:

TABLE 10: Influenza A			
Fresh nasal/nasopharyngeal wash/aspirate	Comparator DSFA (negatives followed by culture with DFA)		
DHI DSFA	Positive	Negative	Total
Positive	29	0	29
Negative	6	265	271
Total	35	265	300
			95% CI
Sensitivity	29/35	82.9%	66.4-93.4%
Specificity	265/265	100%	98.6-100%

TABLE 11: Influenza B			
Fresh nasal/nasopharyngeal wash/aspirate	Comparator DSFA (negatives followed by culture with DFA)		
DHI DSFA	Positive	Negative	Total
Positive	3	0	3
Negative	1	296	297
Total	4	296	300
			95% CI
Sensitivity	3/4	75%	19.4-99.4%
Specificity	296/296	100%	98.8-100%

TABLE 12: Influenza A			
Fresh nasal/nasopharyngeal swab	Comparator DSFA (negatives followed by culture with DFA)		
DHI DSFA	Positive	Negative	Total
Positive	10	0	10
Negative	1	129	130
Total	11	129	140
			95% CI
Sensitivity	10/11	90.9%	58.7-99.8%
Specificity	129/129	100%	97.2-100%

TABLE 13: Influenza B			
Fresh nasal/nasopharyngeal swab	Comparator DSFA (negatives followed by culture with DFA)		
DHI DSFA	Positive	Negative	Total
Positive	2	0	2
Negative	1	137	138
Total	3	137	140
			95% CI
Sensitivity	2/3	66.7%	9.4-99.2%
Specificity	137/137	100.0%	97.3-100%

Study Site 4

The study at Study Site 4 consisted of a total of 648 fresh specimens submitted, February through March 2009, to the laboratory for respiratory virus testing. Specimens were received and processed by DHI within 48-hours of collection. Slides were prepared using PBS-washed cells from the fresh specimens and processed according to the prescribed protocols. The specimens were stained in accordance with the procedure defined in this document.

Table 14 shows the age and gender distribution for individuals studied at Study Site 4:

TABLE 14: Study Site 4 – Age and Gender Distribution			
Sex	F	M	Sex Not Reported
Total	331	279	38
Age			
0 to 1 month	0	1	0
> 1 month to 2 years	29	35	0
> 2 years to 12 years	109	114	0
> 12 years to 21 years	77	61	0
22 years to 30 years	31	15	0
31 years to 40 years	27	21	0
41 years to 50 years	21	14	0
51 years to 60 years	21	8	0
61 years to 70 years	9	8	0
71 years to 80 years	4	1	0
81 years and above	2	1	0
Age Not Reported	1	0	38
Total	331	279	38

Of the 648 fresh respiratory specimens tested, all were nasal/nasopharyngeal swab specimens. Three (3) nasal/nasopharyngeal swab specimens were excluded from the performance analysis due to insufficient sample volume for both the investigational device and the comparator DSFA device testing (0.46%). One (1) additional nasal/nasopharyngeal swab specimen was excluded from the performance analysis due to insufficient sample volume for the investigational device testing (0.15%). One (1) nasal/nasopharyngeal swab specimen was also excluded from the performance analysis due to un-interpretable result generated by the investigational device because of high background. Ninety-three (93) samples for influenza A and 72 samples for influenza B were also excluded from the respective performance analysis due to insufficient sample volume for the comparator culture method. Tables 15 and 16 below show the study results of the claimed specimen type at Study Site 4:

TABLE 15: Influenza A			
Fresh nasal/nasopharyngeal swab	Comparator DSFA (negatives followed by culture with DFA)		
DHI DSFA	Positive	Negative	Total
Positive	47	1	48
Negative	7	495	502
Total	54	496	550
			95% CI
Sensitivity	47/54	87%	75.1-94.6%
Specificity	495/496	99.8%	98.9-100%

TABLE 16: Influenza B			
Fresh nasal/nasopharyngeal swab	Comparator DSFA (negatives followed by culture with DFA)		
DHI DSFA	Positive	Negative	Total
Positive	201	1	202
Negative	27	342	369
Total	228	343	571
			95% CI
Sensitivity	201/228	88.2%	84-92.4%
Specificity	342/343	99.7%	98.4-100%

All Study Sites Combined
Tables 17 and 18 below show the study results of the NP wash/aspirate specimen type (Sites 1, 2, and 3 combined):

TABLE 17: Influenza A			
Fresh nasal/nasopharyngeal wash/aspirate	Comparator DSFA (negatives followed by culture with DFA)		
DHI DSFA	Positive	Negative	Total
Positive	56	3	59
Negative	10	568	578
Total	66	571	637
			95% CI
Sensitivity	56/66	84.8%	73.9-92.5%
Specificity	568/571	99.5%	98.5-99.9%

TABLE 18: Influenza B			
Fresh nasal/nasopharyngeal wash/aspirate	Comparator DSFA (negatives followed by culture with DFA)		
DHI DSFA	Positive	Negative	Total
Positive	9	0	9
Negative	2	617	619
Total	11	617	628
			95% CI
Sensitivity	9/11	81.8%	48.2-97.7%
Specificity	617/617	100%	99.4-100%

Tables 19 and 20 below show the study results of the NP swab specimen type (Sites 3 and 4 combined):

TABLE 19: Influenza A			
Fresh nasal/nasopharyngeal swab	Comparator DSFA (negatives followed by culture with DFA)		
DHI DSFA	Positive	Negative	Total
Positive	57	1	58
Negative	8	624	632
Total	65	625	690
			95% CI
Sensitivity	57/65	87.7%	77.2-94.5%
Specificity	624/625	99.8%	99.1-100%

TABLE 20: Influenza B			
Fresh nasal/nasopharyngeal swab	Comparator DSFA (negatives followed by culture with DFA)		
DHI DSFA	Positive	Negative	Total
Positive	203	1	204
Negative	28	479	507
Total	231	480	711
			95% CI
Sensitivity	203/231	87.9%	83.7-92.1%
Specificity	479/480	99.8%	98.8-100%

B. ANALYTICAL PERFORMANCE STUDIES

Analytical Sensitivity (Limit of Detection)
Analytical Limit of Detection (LoD) of the D³ FastPoint L-DFA Influenza A/Influenza B Virus Reagent was addressed using dilution series of infected model cells. Model cells for influenza A virus (ATCC Victoria strain), influenza B virus (ATCC Taiwan strain) were diluted with non-infected cells to produce a suspension equivalent to 1,000 infected cells per milliliter. This level theoretically yields approximately 25 infected cells per 25-µL of suspension. This suspension was then serially diluted to a theoretical level of less than 1 cell per milliliter. (NOTE: This level was the target to begin with a low positive level. Actual starting levels vary, however, and are within 1 dilution of the 25 infected cell target level). Twenty-five (25)-µL aliquots from each dilution level were spotted onto 10 replicate microscope slides, and then stained according to the instructions for use described in this product insert. Each cell spot was examined at 200X magnification. Results were reported as numbers of positive replicates for each set of 10. Analytical detection limits for each of the 2 analytes were defined as the lowest dilutions at which at least 9 out of 10 replicates were detected. LoD study results are summarized in Table 21 below:

TABLE 21: Limit of Detections for D³ FastPoint L-DFA Influenza A/ Influenza B Reagent			
Virus Strain	Infected cells/mL	Number of replicates with positive cells	LOD determination
Influenza A (ATCC Victoria strain)	500	10/10	50 infected cells/mL
	100	10/10	
	50	**10/10**	
	25	5/10	
	12.5	3/10	
	6	2/10	
	3	0/10	
	1.5	2/10	
	0.8	0/10	
	0.4	0/10	
Influenza B (ATCC Taiwan strain)	2000	10/10	50 infected cells/mL
	400	10/10	
	200	10/10	

TABLE 21: Limit of Detections for D³ FastPoint L-DFA Influenza A/ Influenza B Reagent			
Virus Strain	Infected cells/mL	Number of replicates with positive cells	LOD determination
	100	10/10	
	50	**10/10**	
	25	7/10	
	12.5	4/10	
	6	2/10	
	3	0/10	
	1.5	0/10	

Analytical Reactivity (Inclusivity)
Analytical reactivity (inclusivity) of the D³FastPoint L-DFA Influenza A/Influenza B Reagent was evaluated using 13 influenza A virus and 7 influenza B virus strains. Low concentration infected cell suspensions (approximately 4% cells infected, 25-50 infected cells) were prepared for each viral strain. The suspensions were stained with the D³ FastPoint L-DFA Influenza A/Influenza B Reagent.

TABLE 22: Analytical Reactivity (inclusivity) of the D³ FastPoint L-DFA Influenza A/Influenza B Reagent on various influenza A virus and influenza B virus strains		
Influenza Strains	Infected Cell Concentration (as multiples of the respective established LoD concentration)	D³ FastPoint L-DFA Influenza A /Influenza B Reagent Results
Influenza A Mexico/4108/2009 (H1N1) from CDC*	20x LoD	19 Golden-yellow fluorescent cells
Influenza A California/07/2009 (H1N1) from CDC*	20x LoD	26 Golden-yellow fluorescent cells
Influenza A Wisconsin/56/2005 (H3N2)	20x LoD	39 Golden-yellow fluorescent cells
Influenza A WS, VR-1520 (H1N1)	20x LoD	67 Golden-yellow fluorescent cells
Influenza A Hong Kong, VR-544 (H3N2)	20x LoD	13 Golden-yellow fluorescent cells
Influenza A New Jersey, VR-897 (H1N1)	20x LoD	15 Golden-yellow fluorescent cells
Influenza A A/NWS/33 (H1N1)	20x LoD	10 Golden-yellow fluorescent cells
Influenza A Victoria, VR-822 (H3N2)	20x LoD	10 Golden-yellow fluorescent cells
Influenza A PR, VR-95 (H1N1)	20x LoD	20 Golden-yellow fluorescent cells
Influenza A Port Chalmers, VR-810 (H3N2)	20x LoD	8 Golden-yellow fluorescent cells
Influenza A Aichi, VR-547 (H3N2)	20x LoD	28 Golden-yellow fluorescent cells
Influenza A Denver, VR-546 (H1N1)	20x LoD	30 Golden-yellow fluorescent cells
Influenza A Mal, VR-98 (H1N1)	20x LoD	21 Golden-yellow fluorescent cells
Influenza B GL/1739/54, VR-103	20x LoD	13 Apple-green fluorescent cells
Influenza B Taiwan/2/62, VR-295	20x LoD	44 Apple-green fluorescent cells
Influenza B Hong Kong/5/72, VR-823	20x LoD	21 Apple-green fluorescent cells
Influenza B Maryland/1/59, VR-296	20x LoD	22 Apple-green fluorescent cells
Influenza B Russia, VR-790	20x LoD	36 Apple-green fluorescent cells
Influenza B B/Lee/40	20x LoD	41 Apple-green fluorescent cells
Influenza B Massachusetts, VR-523	20x LoD	67 Apple-green fluorescent cells
* Although the D³ FastPoint L-DFA Influenza A/Influenza B Reagent has been shown to detect the 2009 H1N1 influenza virus in two culture isolates, the performance characteristics of this device with clinical specimens that are positive for the 2009 H1N1 influenza virus have not been established. The D³ FastPoint L-DFA Influenza A/Influenza B Reagent can distinguish between influenza A and B viruses, but it cannot differentiate influenza subtypes.		

Analytical Specificity
D³ FastPoint L-DFA Influenza A/Influenza B Virus Identification Kit was tested for cross-reactivity against a variety of microorganisms. Stringent conditions for cross-reactivity testing were achieved by using both the 1.5 X concentration of MAbs and relatively high titers of microorganisms. No cross-reactivity was observed for 59 virus strains. Twenty-two (22) bacterial strains, one yeast, and one *Chlamydia spp.* were also evaluated for cross-reactivity, including *Staphylococcus aureus,* a protein-A-producing bacterium. Except for *Staphylococcus aureus*, which was cross-reactive with the D³ FastPoint L-DFA Influenza A/Influenza B Virus Identification Kit, all other microorganisms tested negative.

Staining of *S. aureus* appeared as small points of fluorescence (Limitations of Procedure, Section VIII).

- Fifty-nine (59) virus strains were tested for cross-reactivity. Depending on the particular virus, 1.4 x 10^4 to 1.4 x 10^5 $TCID_{50}$ viruses were inoculated into multi-well plate cultures and incubated for 24- to 72-hours to yield a 1+ to 4+ cytopathic effect. For each virus, a confirmation stain was done with the appropriate MAb to ensure the desired titer was achieved. These cells were then prepared as Model Cells (scraped and re-suspended in Universal Transport Medium). Each cell suspension of infected Model Cells was processed according to the D³ FastPoint L-DFA Kit protocol, using 2X MAb and was examined at 200X magnification. No cross-reactivity was observed for the viruses listed below:

TABLE 23: Virus Strains Tested for Cross-Reactivity with the D³ FastPoint L-DFA Influenza A/Influenza B Virus Reagent

Organism	Strain or Type	D³ FastPoint L-DFA Influenza A /Influenza B Reagent Results	Inoculum ($TCID_{50}$)
Virus			
Adenovirus	Type 1	Negative	1.4 x 10^4
	Type 3	Negative	1.4 x 10^4
	Type 5	Negative	1.4 x 10^4
	Type 7	Negative	1.4 x 10^4
	Type 10	Negative	1.4 x 10^4
	Type 16	Negative	1.4 x 10^4
	Type 17	Negative	1.4 x 10^4
Metapneumovirus (hMPV)	Subtype A1	Negative	1.4 x 10^4
	Subtype A2	Negative	1.4 x 10^4
	Subtype B1	Negative	1.4 x 10^4
	Subtype B2	Negative	1.4 x 10^4
Influenza A	Aichi (H3N2)	Golden-Yellow Fluorescence	1.4 x 10^4
	Mal (H1N1)	Golden-Yellow Fluorescence	1.4 x 10^4
	Hong Kong (H3N2)	Golden-Yellow Fluorescence	1.4 x 10^4
	Denver (H1N1)	Golden-Yellow Fluorescence	1.4 x 10^4
	Port Chalmers (H3N2)	Golden-Yellow Fluorescence	1.4 x 10^4
	Victoria (H3N2)	Golden-Yellow Fluorescence	1.4 x 10^4
	New Jersey (HSWN1)	Golden-Yellow Fluorescence	1.4 x 10^4
	WS (H1N1)	Golden-Yellow Fluorescence	1.4 x 10^4
	PR (H1N1)	Golden-Yellow Fluorescence	1.4 x 10^4
	Wisconsin (H3N2)	Golden-Yellow Fluorescence	1.4 x 10^4
	A/NWS/33 (H1N1)	Golden-Yellow Fluorescence	1.4 x 10^4
	A Mexico/4108/2009 (H1N1)	Golden-Yellow Fluorescence	1.4 x 10^4
	A California/07/2009 (H1N1)	Golden-Yellow Fluorescence	1.4 x 10^4
Influenza B	Hong Kong	Apple-Green Fluorescence	1.4 x 10^4
	Maryland	Apple-Green Fluorescence	1.4 x 10^4
	Mass	Apple-Green Fluorescence	1.4 x 10^4
	GL	Apple-Green Fluorescence	1.4 x 10^4
	Taiwan	Apple-Green Fluorescence	1.4 x 10^4
	B/Lee/40	Apple-Green Fluorescence	1.4 x 10^4
	Russia	Apple-Green Fluorescence	1.4 x 10^4
RSV	Long	Negative	1.4 x 10^4
	Wash	Negative	1.4 x 10^4
	9320	Negative	1.4 x 10^4
Parainfluenza 1	C-35	Negative	1.4 x 10^4
Parainfluenza 2	Greer	Negative	1.4 x 10^4
Parainfluenza 3	C-243	Negative	1.4 x 10^4
Parainfluenza 4	M-25	Negative	1.4 x 10^5
Parainfluenza 4b	CH-19503	Negative	1.4 x 10^5
HSV-1	1(f)	Negative	1.4 x 10^5
	MacIntyre	Negative	1.4 x 10^5
HSV-2	Clinical Isolate CWOH-0011	Negative	1.4 x 10^5
	Strain G	Negative	1.4 x 10^5
CMV	Towne	Negative	1.4 x 10^5
	AD169	Negative	1.4 x 10^5
Varicella-zoster	AV92-3	Negative	1.4 x 10^5
Echovirus	4	Negative	1.4 x 10^5
	6	Negative	1.4 x 10^5
Coxsackievirus	7	Negative	1.4 x 10^5
	22	Negative	1.4 x 10^5
	A9	Negative	1.4 x 10^5
	B1	Negative	1.4 x 10^5
	B3	Negative	1.4 x 10^5
	B4	Negative	1.4 x 10^5
Coronavirus	229E	Negative	1.4 x 10^5
	OC43	Negative	1.4 x 10^5
Rhinovirus	209 Picornavirus	Negative	1.4 x 10^5
Enterovirus 70	VR-836	Negative	1.4 x 10^5
Enterovirus 71	VR-1432	Negative	1.4 x 10^5

- Twenty four (24) microorganisms, including 22 bacterial strains, 1 yeast, and 1 *Chlamydia spp.* were tested for cross-reactivity. Bacteria were cultured, processed as suspensions, then spiked into non-infected Model Cells suspensions at levels (as CFUs, colony-forming units) ranging from 1.6 x 10^9 to 3.5 x 10^{10} CFUs depending on the bacterium. These suspensions of Model Cells with bacteria were then processed according to the D³ FastPoint L-DFA Influenza A/Influenza B Kit protocol, using 2X MAb reagents. Except for *Staphylococcus aureus*, which was cross-reactive with the D³ FastPoint L-DFA Influenza A/Influenza B Virus Identification Kit, all other microorganisms tested negative. Reactivity with *Staphylococcus aureus* is more than likely due to binding the protein A produced by *Staphylococcus aureus*. Microorganisms tested are listed in the table below:

TABLE 24: Microorganisms Tested for Cross-Reactivity with D³ FastPoint L-DFA Respiratory Virus Identification Kit

Organism	D³ FastPoint L-DFA Influenza A /Influenza B Reagent Results	CFU tested
Bacteria		
Acholeplasma laidlawii	Negative	Control Slide
Acinetobacter calcoaceticus	Negative	3.6 x 10^9
Bordetella bronchiseptica	Negative	1.1 x 10^{10}
Bordetella pertussis	Negative	4.3 x 10^9
Chlamydia trachomatis (Apache-2)	Negative	LGV-II/Control Slide
Corynebacterium diphtheriae	Negative	5.7 x 10^7
Escherichia coli	Negative	7.5 x 10^8
Gardnerella vaginalis	Negative	Control Slide
Haemophilis influenzae type A	Negative	4.1 x 10^9
Klebsiella pneumoniae	Negative	1.2 x 10^9
Moraxella cartarrhalis	Negative	1.2 x 10^{10}
Mycoplasma hominis	Negative	3.5 x 10^{10}
Mycoplasma orale	Negative	6.6 x 10^9
Mycoplasma pneumoniae	Negative	7.9 x 10^9
Mycoplasma salivarium	Negative	7.7 x 10^8
Proteus mirabilis	Negative	3.6 x 10^9
Pseudomonas aeruginosa	Negative	1.0 x 10^8
Salmonella enteriditis	Negative	8.7 x 10^9
Salmonella typhimurium	Negative	7.5 x 10^9
*Staphylococcus aureus**	Positive	6.3 x 10^9
Streptococcus agalactiae	Negative	5.5 x 10^8
Streptococcus pneumoniae	Negative	6.7 x 10^9
Streptococcus pyogenes	Negative	6.9 x 10^9
Yeast		
Candida glabrata	Negative	1.6 x 10^6

***** Reactivity with *Staphylococcus aureus* is more than likely due to binding the protein A produced by *Staphylococcus aureus*.

Reproducibility

Assay precision, intra-assay variability and inter-assay variability were assessed with a reproducibility panel of proficiency-level antigen control slides. The reproducibility panel consisted of 5 randomized panel members.

The Influenza A/B panel consisted of the following:

a. Low level influenza A (Victoria strain) infected cells.
b. Low level influenza B (Taiwan strain) infected cells.
c. Low level influenza A (Victoria strain) infected cells mixed with mid level influenza B (Taiwan strain) infected cells.
d. Low level influenza B (Taiwan strain) infected cells mixed with mid level influenza A (Victoria strain) infected cells.
e. Mid level non-infected (negative) cells.

The low level is estimated to contain between 4 to 10% infected cells in the sample. The mid level is estimated to contain between 20 to 25% infected cells in the sample. Each sample contains 2.5 x10^5 to 3.5 x10^5 total cells.

Each panel was tested daily in two separate runs for 5-days by four different laboratories (40 total runs). The following results were recorded:

a. Presence or absence of golden-yellow fluorescence.

b. Percent of cells exhibiting golden-yellow fluorescence.
c. Presence or absence of apple-green fluorescence.
d. Percent of cells exhibiting apple-green fluorescence.

For the D³ FastPoint L-DFA Influenza A/Influenza B Reagent, the combined data from the four Study Sites demonstrated reproducible detection of influenza A virus by the R-PE labeled MAbs and reproducible detection of influenza B virus by the FITC-labeled MAbs. The presence of influenza A virus infected cells was reported in 100% (120/120) of the wells in which the infected cells were expected. The presence of influenza B virus infected cells was reported in 100% (120/120) of the wells in which the infected cells were expected. The absence of infected cells was reported in 95% (38/40) of the wells in which infected cells were not present. The total percent agreement for the D³ FastPoint L-DFA Influenza A/Influenza B Reagent was 99.3% (278/280):

TABLE 25: Reproducibility Study Results using the D³ FastPoint L-DFA Influenza A/Influenza B Reagent

Sites	Panel Member	Negative	Flu A Low Level	Flu B Low Level	Mixed Infection		Mixed Infection		Total % Agreement
					Flu A Mid Level	Flu B Low Level	Flu A Low Level	Flu B Mid Level	
	Concentration	No infected cells	4 to 10% infected cells	4 to 10% infected cells	20 to 30% infected cells	4 to 10% infected cells	4 to 10% infected cells	20 to 30% infected cells	
Site 1	Agreement with Expected result	8/10 (80%)	10/10 (100%)	10/10 (100%)	10/10 (100%)	10/10 (100%)	10/10 (100%)	10/10 (100%)	**68/70 (97.1%)**
Site 2	Agreement with Expected result	10/10 (100%)	10/10 (100%)	10/10 (100%)	10/10 (100%)	10/10 (100%)	10/10 (100%)	10/10 (100%)	**70/70 (100%)**
Site 3	Agreement with Expected result	10/10 (100%)	10/10 (100%)	10/10 (100%)	10/10 (100%)	10/10 (100%)	10/10 (100%)	10/10 (100%)	**70/70 (100%)**
Site 4	Agreement with Expected result	10/10 (100%)	10/10 (100%)	10/10 (100%)	10/10 (100%)	10/10 (100%)	10/10 (100%)	10/10 (100%)	**70/70 (100%)**
Total Agreement with Expected result		38/40 (95%)	40/40 (100%)	40/40 (100%)	40/40 (100%)	40/40 (100%)	40/40 (100%)	40/40 (100%)	**278/280 (99.3%)**
95% CI		83.1 – 99.4%	91.2 – 100%	91.2 – 100%	91.2 – 100%	91.2 – 100%	91.2 – 100%	91.2 – 100%	**97.4 – 99.9%**

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WARRANTY STATEMENT

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