CE

D^3 FastPoint®

RSV/MPV IDENTIFICATION KIT

For the qualitative identification of respiratory syncytial virus and human metapneumovirus in nasal and nasopharyngeal swabs and aspirate/wash specimens.

FOR *IN VITRO* DIAGNOSTIC USE

Rx ONLY

INTENDED USE

The D^3 FastPoint L-DFA RSV/MPV Identification Kit is intended for the qualitative identification of respiratory syncytial virus and human metapneumovirus 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 respiratory syncytial virus after examination of the direct specimen result be confirmed by cell culture. Specimens found to be negative for human metapneumovirus after examination of the direct specimen results should be confirmed by an FDA-cleared human metapneumovirus molecular assay. Negative results do not preclude human metapneumovirus or respiratory syncytial virus infection and should not be used as the sole basis for diagnosis, treatment or other management decisions.

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.

Respiratory Syncytial Virus (RSV)

RSV (family *Paramyxoviridae*) is an enveloped virus with a single, negative strand RNA genome. RSV infections cause viral bronchiolitis and pneumonia in infants and the common cold in adults.[2] RSV is the primary viral cause of lower respiratory disease in infants and young children with peak mortality due to RSV in 3-4 month old infants. RSV is usually a seasonal (winter and early spring) infection with epidemics lasting up to 5 months. There are two major subtypes, A and B: subtype B is characterized as the asymptomatic strain that the majority of the population experiences. More severe clinical illness involves subtype A strains which tend to predominate in most outbreaks.[3] Re-infections do occur but tend to be limited to minor upper respiratory infections.[4] RSV is also recognized as a significant problem in certain adult populations including the elderly, individuals with cardiopulmonary diseases, and immunocompromised hosts.[5]

RSV is commonly detected directly in cells from the nasopharyngeal epithelium by staining with immunofluorescent reagents.[3]

Human Metapneumovirus (hMPV)

hMPV is a respiratory viral pathogen that causes a spectrum of illnesses ranging from asymptomatic infection to severe bronchiolitis. hMPV was first described in 2001 by researchers at the Erasmus Medical Center at Erasmus University in Rotterdam, The Netherlands.[6] This newly recognized human viral pathogen was isolated from respiratory samples submitted for viral culture during the winter season. Half of the initial 28 hMPV isolates were cultured from patients younger than 1 year, and 96% were isolated from children younger than 6 years. Seroprevalence studies revealed that of all children aged 6-12 months who were tested in the 2001 study, 25% had detectable antibodies to hMPV; by age 5 years, 100% of patients showed evidence of past infection. A separate report from Australia[7] describing three additional cases of hMPV infection supports the contention that this newly discovered virus is ubiquitous and additional information relating to pathogenesis and epidemiology continues to become available.[8]

Diagnosis of hMPV infections has relied primarily on detection by real-time reverse transcriptase polymerase chain reaction (RT-PCR).[9,10,11] Development of specific MAbs directed against the four known subtypes of hMPV (A1, A2, B1, B2) has been described.[12] The use of MAbs in immunofluorescence applications in the clinical laboratory to diagnose hMPV infections by direct detection of infected cells of patients has been reported.[13,14,15,16,17]

PRINCIPLE OF THE PROCEDURE

The D³ FastPoint L-DFA RSV/MPV 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) (respiratory syncytial virus) or fluorescein isothiocyanate (FITC) (human metapneumovirus) for the rapid identification of respiratory syncytial virus and human metapneumovirus in nasal and nasopharyngeal (NP) swabs and aspirates 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 35°C to 37°C 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 RSV will exhibit golden-yellow fluorescence due to the PE. Cells infected with hMPV will exhibit apple-green fluorescence due to the FITC. Non-infected cells will exhibit 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 respiratory syncytial virus after examination of the direct specimen result be confirmed by cell culture. Specimens found to be negative for human metapneumovirus after examination of the direct specimen results should be confirmed by an FDA-cleared human metapneumovirus molecular assay.

REAGENTS AND MATERIALS PROVIDED

The D³ FastPoint L-DFA RSV/MPV Identification Kit contains the following:

D³ FastPoint L-DFA RSV/MPV Reagent **5 mL**

One dropper bottle containing a mixture of PE-labeled murine monoclonal antibodies directed against respiratory syncytial virus antigens and FITC-labeled murine monoclonal antibodies directed against human metapneumovirus antigens. The buffered, stabilized, aqueous solution contains Evans Blue and propidium iodide as counter-stains and 0.1% sodium azide as preservative.

Re-suspension Buffer **6 mL**

One bottle of a buffered glycerol solution and 0.1% sodium azide.

D³ FastPoint L-DFA RSV/MPV Antigen Control Slides **5 Slides**

Five individually packaged control slides containing 2 wells with cell culture-derived positive and negative control cells. Each positive well contains cells infected with both respiratory syncytial virus and human metapneumovirus. The negative wells contain non-infected cells. Each slide is intended to be stained only one time.

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).

D³ FastPoint L-DFA Specimen Slides **50 slides**

Fifty (50) 3-channel specimen slides. Each slide is intended to be used only one time.

A Material Safety Data Sheet for sodium azide or for other Quidel reagents is available by contacting Quidel Technical Services.

MATERIALS REQUIRED BUT NOT PROVIDED

- Fluorescence microscope with the correct filter combination for FITC (excitation peak = 490 nm, emission peak = 520 nm) and for R-PE; magnification 200 to 400X
- Fine-tip, disposable transfer pipettes
- Cover slips (22 x 50mm) for Antigen Control Slides
- Adjustable pipettes (20 to 200 µL and 200 to 1000 µL)
- Pipette tips (20 to 200 µL and 200 to 1000 µL)
- Wash bottle, 200 mL
- 1.7 mL centrifuge vials
- 15 mL conical centrifuge tube
- Sodium hypochlorite solution (1:10 final dilution of household bleach)
- Humidified chamber (e.g., covered Petri dish with a damp paper towel placed in the bottom) or humidified incubator
- Incubator, 35°C to 37°C (CO_2 or non-CO_2, depending on the cell culture format used)
- Centrifuge with free-swinging bucket rotor
- De-mineralized water for dilution of 40X PBS Concentrate
- Stat-Spin Centrifuge (or benchtop centrifuge capable of 2 minutes at 2000xg)

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use only
- For professional use only
- For more information, consult the Safety Data Sheet (SDS) available on quidel.com
- Follow Universal Precautions when handling the contents of this kit and patient samples.
- 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.
  - Conduct all procedures in accordance with the OSHA Standard on Blood-borne Pathogens[18]; 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".[19]
    - Follow Biosafety Level 2 or other appropriate biosafety practices
    - Decontaminate specimens using a 1:10 final dilution of household bleach.
    - 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.
- Do not pipette reagents or clinical samples by mouth. Protect broken skin from contact with clinical samples.
- Avoid splashing and the generation of aerosols with clinical samples.
- Sodium azide is included in the 40X PBS Concentrate at 4%, and in the other solutions in this kit at 0.1%.
    - Sodium azide is considered poisonous. If the 40X PBS Concentrate is swallowed, seek medical advice immediately.
    - Aqueous solutions of sodium azide, when mixed with acids, may liberate toxic gas.
    - Avoid disposal of these solutions down sanitary or industrial plumbing systems. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides.
    - Avoid release to the environment.
- Evans Blue counter-stain is a potential carcinogen. If eye or skin contact occurs, immediately flush with copious amounts of water.
- Propidium iodide counter-stain is a potential carcinogen and mutagen. If skin contact occurs, flush with water immediately.
- The L-DFA Reagent is supplied at working strength. Any dilution of the reagent will decrease sensitivity.
- Reagents should be used prior to their expiration date.
- Each Antigen Control Slide should be used only once. Do not re-use a control slide.
- Microbial contamination of the L-DFA Reagent may cause a decrease in sensitivity.
- Store 1X PBS in a clean container to prevent contamination.
- Reusable glassware must be washed and thoroughly rinsed free of all detergents.
- Do not expose the L-DFA Reagents to bright light during staining or storage.
- Use of reagents other than those specified with the components of this kit may lead to erroneous results.
- Testing should be performed in an area with adequate ventilation.
- Dispose of containers and unused contents in accordance with Federal, State, and Local regulatory requirements.
- Wear suitable protective clothing, gloves, and eye/face protection when handling the contents of this kit.
- Wash hands thoroughly after handling.
- For additional information on hazard symbols, safety, handling and disposal of the components within this kit, please refer to the Safety Data Sheet (SDS) located at quidel.com.

Preparation of 1X PBS Solution

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

Storage

Table 1. Reagent Storage Conditions

<table>
<tr><td rowspan="2">Kit Box 1</td><td>D³ FastPoint L-DFA RSV/MPV Reagent</td><td rowspan="2">Store at room temperature (20°C to 25°C) in the dark.</td></tr>
<tr><td>D³ FastPoint L-DFA Specimen Slides</td></tr>
<tr><td rowspan="4">Kit Box 2</td><td>Re-suspension Buffer</td><td rowspan="2">Store at 2°C to 8°C.</td></tr>
<tr><td>D³ FastPoint L-DFA RSV/MPV Antigen Control Slides</td></tr>
<tr><td>40X PBS Concentrate
NOTE: The Concentrate may crystallize when stored at 2°C to 8°C. The crystals will dissolve when the Concentrate is warmed to room temperature.</td><td>Store liquid at 2°C to 8°C prior to dilution.</td></tr>
<tr><td>1X PBS</td><td>Store at ambient temperature (20°C to 25°C).</td></tr>
</table>

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.

SPECIMEN HANDLING AND PREPARATION

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.[20]

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°C 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°C 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.

PROCEDURE

Preliminary Comments and Precautions

- Adhere to the recommended volumes and times stated in the following procedure to ensure that accurate results are obtained.
- 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.

- Bring the Re-suspension Buffer to room temperature prior to use, and immediately return to refrigerator after use for storage at 2°C to 8°C.

Regarding Immunofluorescence Microscopy

- 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.
- Three aspects of the fluorescence microscope must be functioning properly and optimally to achieve maximum brightness of fluorescence:
  - 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.
  - The light source is focused by a number of lenses and mirror(s). For maximum intensity, these must be properly aligned.
  - The filters used in the light path must be appropriate for fluorescein.
- Fluorescent artifacts may be observed during examination of the stained cells.
  - 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.
  - 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.
  - Inadequate removal of mucus from direct specimen material can lead to nonspecific staining when conducting the test.
  - Generalized, low-grade fluorescence may be seen particularly in areas that have clumped cells.
  - On direct specimens, leukocytes and monocytes may trap fluorescence or RBC may leave a green haze on the sample.
- Protect stained slides and monolayers from light as much as possible during testing.
  - Bleaching or fading of the fluorescence of stained cells may occur on exposure to light, particularly light of high intensity.
  - This bleaching can occur when a stained cell is viewed in a fluorescence microscope for an extended period.

Specimen Preparation

For cell suspension preparation recommendations, refer to CLSI Approved Viral Culture Guidelines.[21]

Cell Suspension Permeabilizing and Staining

1. Remove Re-suspension Buffer from the refrigerator and allow it to warm to room temperature for 15 to 30 minutes prior to use.
2. Label a 1.7 mL Centrifuge vial: RSV/MPV (R/M).
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 the centrifuge vial from step 2 above.
5. Add 2 drops of D^3 FastPoint L-DFA RSV/MPV Reagent to the labeled vial.
6. Mix by vortex for 1 to 2 seconds.
7. Incubate vials at 35°C to 37°C for 5 minutes.
8. Add approximately 1.5 mL of 1X PBS to each vial using the squeeze bottle.
9. Centrifuge the vials for 2 minutes at 2000xg.
10. Decant the PBS gently from each 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 to 10 times with a 20 µL pipette, changing tips after each vial.
14. Label a 3-channel 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 fluorescent microscope.
17. Refer to *INTERPRETATION OF RESULTS*.

RSV/MPV Antigen Control Slide Staining Procedure

1. Remove D³ FastPoint L-DFA RSV/MPV Antigen Control Slide from the refrigerator and allow to warm to room temperature for 15 to 30 minutes prior to use.
2. To each of the wells of a fresh D³ FastPoint L-DFA RSV/MPV Antigen Control Slide, add one drop D³ FastPoint L-DFA RSV/MPV Reagent.
3. Place the slide at 35°C 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 *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.

QUALITY CONTROL

- A fresh D³ FastPoint L-DFA RSV/MPV Antigen Control Slide should be stained each time the staining procedure is performed to ensure proper test performance.
- The wells containing infected cells will fluoresce either golden-yellow or apple-green, depending on the infecting virus, while non-infected cells stain a dull red due to the Evans Blue counter-stain. The nuclei of all cells will stain red due to the propidium iodide.
- The negative well will show only non-infected cells staining a dull red. The nuclei of all cells will stain red due to the propidium iodide.
- 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.

INTERPRETATION OF RESULTS

Examination of Samples and Controls

- Examine controls first to ensure proper test performance before examining patient specimens.
- Do not report results for patient samples unless controls perform as expected.
- Examine the entire cell spot before reporting final results.
- Evaluation of sample suitability
  - **Each stained patient specimen** should be reviewed 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.
  - **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.

- **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.

- **Interpretation of D³ FastPoint L-DFA RSV/MPV Reagent**
  - **RSV**: The golden-yellow fluorescence is cytoplasmic. Staining is often bright cytoplasmic and sometimes punctate with inclusions in the syncytia. Stained cells are usually round in appearance and sometimes larger than non-infected cells.
  - **MPV**: The apple-green fluorescence is cytoplasmic. Staining is cytoplasmic and sometimes punctate with inclusions in the syncytia. Stained cells are usually round in appearance and sometimes larger than non-infected cells. **NOTE: The ciliated tops of nasopharyngeal cells may trap the FITC-labeled hMPV antibodies. This staining is substantially duller than that of typical positive cells and should be interpreted as negative.**

Reporting Results of Direct Specimen Staining

- The entire cell suspension 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 respiratory syncytial virus or metapneumovirus viral antigens detected by direct specimen testing." Such negative results, however, should be confirmed by cell culture, and using an FDA-cleared hMPV molecular assay.
- If golden-yellow fluorescent cells are found, it should be reported as "respiratory syncytial virus viral antigens detected by direct specimen testing."
- If apple-green fluorescent cells are found, it should be reported as "metapneumovirus viral antigens detected by direct specimen testing."
- If co-infection with multiple viruses is detected it should be reported as "respiratory syncytial virus and metapneumovirus viral antigens detected by direct specimen testing."

LIMITATIONS OF THE PROCEDURE

- Inappropriate specimen collection, storage, and transport may lead to incorrect results.[22]
- 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 RSV/MPV Identification Kit for direct testing of other respiratory specimens is limited and it is the user's responsibility to establish assay performance parameters.
- Incubation times or temperatures other than those cited in the test instructions may give erroneous results.
- Detection of RSV and hMPV 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.
- The effects of antiviral therapy on the performance of this kit have not been established.
- 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.
- 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.
- The monoclonal antibodies used in this kit are not group-specific and therefore cannot be used to differentiate among the different sub-types of RSV and hMPV.
- The viral antigens detected in some direct specimens may be from non-viable virus and cannot be isolated by culture. This is particularly true for RSV which is known for its instability and loss of viability.

- A negative result on a direct specimen does not rule out the presence of virus. 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. A negative direct specimen should also be tested for hMPV using an FDA-cleared hMPV molecular assay.
- Performance of the kit can be assured only when components used in the assay are those supplied by Quidel.
- Prolonged storage of the D³ FastPoint L-DFA RSV/MPV 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.
- 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.

EXPECTED VALUES

Clinical studies were performed at 4 study sites in the United States in the winter of 2009 (January 2009 through March 2009). Prevalence of the respiratory syncytial virus and human metapneumovirus within this population as determined by the D³ FastPoint L-DFA RSV/MPV Identification Kit direct specimen testing is noted in Table 2 below.

Table 2. RSV/hMPV Prevalence*

Age	Total Specimens Evaluated	RSV	hMPV
		# positive (prevalence)	# positive prevalence
0 to 1 month	55	15 (27.3%)	2 (3.6%)
> 1 month to 2 years	577	154 (26.7%)	41 (7.1%)
> 2 years to 12 years	391	25 (6.4%)	17 (4.3%)
> 12 years to 21 years	173	4 (2.3%)	3 (1.7%)
22 years to 30 years	57	0	1 (1.8%)
31 years to 40 years	71	1 (1.4%)	3 (4.2%)
41 years to 50 years	52	0	1 (1.9%)
51 years to 60 years	46	1 (2.2%)	3 (6.5%)
61 years to 70 years	33	1 (3.0%)	1 (3.0%)
71 years to 80 years	16	1 (6.3%)	4 (25.0%)
81 years and above	7	1 (14.3%)	0
Age Not Reported	41	0	1 (2.4%)
Total	1519	203 (13.4%)	77 (5.1%)

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

SPECIFIC PERFORMANCE CHARACTERISTICS

Clinical Performance Studies

Performance of the D³ FastPoint L-DFA RSV/MPV Identification Kit testing direct respiratory specimens was established during prospective studies at 4 geographically diverse U.S. clinical laboratories during the 2009 respiratory virus season (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 de-linked 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 RSV/MPV Identification Kit was assessed and compared to a predetermined algorithm that used composite comparator methods. The composite comparator methods for respiratory syncytial virus 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). For human metapneumovirus the composite comparator methods consisted of DSFA with an FDA-cleared device, and confirmation of all negative specimens (as determined by the comparator DSFA test) using a validated[1] hMPV real-time RT-PCR followed by bi-directional sequencing analysis comparator assay. The hMPV real-time RT-PCR comparator assay targets the hMPV Nucleocapsid gene. “True” positive was defined as any sample that either tested positive by the comparator DSFA test or viral culture, or had bi-directional sequencing data meeting pre-defined quality acceptance criteria that matched hMPV sequences deposited in the National Center for Biotechnology Information (NCBI) GenBank database (www.ncbi.nlm.nih.gov), with acceptable E-values.[2] “True” negative was defined as any sample that tested negative by both the comparator DSFA test and either viral culture or the hMPV real-time RT-PCR comparator assay.

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 from 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 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

[1] Analytical validation of the real-time hMPV RT-PCR followed by bi-directional sequencing analysis comparator assay included analytical sensitivity and reactivity study, analytical specificity study, and extraction efficiency study. The analytical sensitivity (limit of detection or LOD) of the real-time hMPV RT-PCR followed by bi-directional sequencing analysis comparator assay was determined using quantified ($TCID_{50}$/mL) stocks of the 4 hMPV (subtypes A1, A2, B1 and B2) strains diluted in hMPV negative nasopharyngeal clinical matrix, and ranged from 10-50 $TCID_{50}$/mL.

[2] The E-values generated from the clinical trials range from a low of 5e-78 to a high of 1e-20. The E-Value from NCBI BLAST Alignment indicates the statistical significance of a given pair-wise alignment and reflects the size of the database and the scoring system used. The lower the E-Value, the more significant the hit. A sequence alignment that has an E-Value of 1e-3 means that this similarity has a 1 in 1000 chance of occurring by chance alone. (http://www.ncbi.nlm.nih.gov/books/bv.fcgi?rid=handbook.section.614).

Of the 323 fresh respiratory specimens tested, all were nasal wash/nasopharyngeal aspirate specimens. Of the 323 fresh nasal wash/nasopharyngeal aspirate specimens tested, 2 nasal wash/nasopharyngeal aspirate specimens were excluded from the performance analysis due to insufficient sample volume for the investigational device testing (0.62%). Thirty-seven (37) specimens for RSV and 3 specimens for hMPV were also excluded from the respective performance analysis due to insufficient sample volume for the comparator culture or real-time RT-PCR methods, respectively, resulting in a total of 284 fresh nasal wash/nasopharyngeal aspirate specimens for RSV and 318 fresh nasal wash/nasopharyngeal aspirate specimens for hMPV. Tables 4 and 5 below show the study results of the claimed specimen type at Study Site 1.

Table 4. Respiratory Syncytial Virus

Fresh nasal/nasopharyngeal wash/aspirate	**Comparator DSFA (negatives followed by culture with DFA)**		
DSFA	Positive	Negative	Total
Positive	137	1	138
Negative	1	145	146
Total	138	146	284
			95% CI
Sensitivity	137/138	99.3%	96.0% to 100%
Specificity	145/146	99.3%	96.2% to 100%

Table 5. Human Metapneumovirus

Fresh nasal/nasopharyngeal wash/aspirate	**Comparator DSFA (negatives confirmed by a validated hMPV real-time RT-PCR followed by bi-directional sequencing analysis comparator assay)**		
DSFA	Positive	Negative	Total
Positive	22	0	22
Negative	8	288	296
Total	30	288	318
			95% CI
Sensitivity	22/30	73.3%	54.1% to 87.7%
Specificity	288/288	100%	98.7% to 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 from 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 6 shows the age 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

Sex	F	M
Total	**48**	**57**
Age		
> 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

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 RSV/MPV Identification Kit, 19 other types of respiratory specimens were removed from performance analysis. None of the nasal wash/nasopharyngeal aspirate samples for RSV were excluded from the respective performance analysis due to insufficient sample volume for the comparator culture method, resulting in a total of 86 fresh nasal wash/nasopharyngeal aspirate specimens for RSV to be included in the respective performance analysis. Ten (10) specimens for hMPV were also excluded from the respective performance analysis due to insufficient sample volume for the comparator methods, resulting in a total of 76 fresh nasal wash/nasopharyngeal aspirate specimens for hMPV to be included in the respective performance analysis. Tables 7 and 8 below show the study results of the claimed specimen type at Study Site 2.

Table 7. Respiratory Syncytial Virus

Fresh nasal/nasopharyngeal wash/aspirate	**Comparator DSFA (negatives followed by culture with DFA)**		
DSFA	Positive	Negative	Total
Positive	18	0	18
Negative	0	68	68
Total	18	68	86
			95% CI
Sensitivity	18/18	100.0%	81.5% to 100%
Specificity	68/68	100.0%	94.7% to 100%

Table 8. Human Metapneumovirus

Fresh nasal/nasopharyngeal wash/aspirate	Comparator DSFA (negatives confirmed by a validated hMPV real-time RT-PCR followed by bi-directional sequencing analysis comparator assay)		
DSFA	Positive	Negative	Total
Positive	5	0	5
Negative	2	69	71
Total	7	69	76
			95% CI
Sensitivity	5/7	71.4%	29.0% to 96.3%
Specificity	69/69	100%	94.8% to 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 from 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 9 shows the age 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

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 RSV/MPV Identification Kit, 2 other types of respiratory specimens were further removed from performance analysis. None of the remaining nasal wash/nasopharyngeal aspirate samples for RSV and hMPV was excluded from the respective performance analysis due to insufficient sample volume for the comparator culture method, resulting in a total of 300 fresh nasal wash/nasopharyngeal aspirate specimens for RSV and hMPV to be included in the respective performance analysis. None of the nasal/nasopharyngeal swab specimens for RSV was excluded from the respective performance analysis due to insufficient sample volume for the comparator methods, resulting in a total of 140 nasal/nasopharyngeal swab

specimens for RSV to be included in the respective performance analysis. One (1) nasal/nasopharyngeal swab specimen for hMPV was also excluded from the performance analysis due to insufficient sample volume for the comparator methods, resulting in a total of 139 fresh nasal wash/nasopharyngeal aspirate specimens for hMPV to be included in the respective performance analysis. Tables 10 through 13 below show the study results of the claimed specimen types at Study Site 3.

Table 10. Respiratory Syncytial Virus

Fresh nasal/nasopharyngeal wash/aspirate	**Comparator DSFA (negatives followed by culture with DFA)**		
DSFA	Positive	Negative	Total
Positive	49	0	49
Negative	2	249	251
Total	51	249	300
			95% CI
Sensitivity	49/51	96.1%	86.5% to 99.5%
Specificity	249/249	100.0%	98.5% to 100%

Table 11. Human Metapneumovirus Predicate DSFA (Negatives Followed by Culture with DFA)

Fresh nasal/nasopharyngeal wash/aspirate	**Comparator DSFA (negatives confirmed by a validated hMPV real-time RT-PCR followed by bi-directional sequencing analysis comparator assay)**		
DSFA	Positive	Negative	Total
Positive	28	0	28
Negative	15	257	272
Total	43	257	300
			95% CI
Sensitivity	28/43	65.1%	49.1% to 79.0%
Specificity	257/257	100%	98.6% to 100%

Table 12. Respiratory Syncytial Virus

Fresh nasal/nasopharyngeal swab	**Comparator DSFA (negatives followed by culture with DFA)**		
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% to 99.8%
Specificity	129/129	100.0%	97.2% to 100%

Table 13. Human Metapneumovirus Predicate DSFA (Negatives Followed by Culture with DFA)

Fresh nasal/nasopharyngeal swab	Comparator DSFA (negatives confirmed by a validated hMPV real-time RT-PCR followed by bi-directional sequencing analysis comparator assay)		
DSFA	Positive	Negative	Total
Positive	9	0	9
Negative	8	122	130
Total	17	122	139
			95% CI
Sensitivity	9/17	52.9%	27.8% to 77.0%
Specificity	122/122	100%	97.0% to 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 Quidel within 48 hours of collection. Slides were prepared from 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 14 shows the age distribution for individuals studied at Study Site 4.

Table 14. Study Site 4 – Age and 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

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-six (96) samples for RSV and 106 samples for hMPV were also excluded from the respective performance analysis due to insufficient sample volume for the

comparator culture or real-time RT-PCR methods, respectively, resulting in a total of 547 fresh nasal wash/nasopharyngeal aspirate specimens for RSV and 537 fresh nasal wash/nasopharyngeal aspirate specimens for hMPV to be included in the respective performance analysis. Tables 15 and 16 show the study results of the claimed specimen type at Study Site 4.

Table 15. Respiratory syncytial virus

Fresh nasal/nasopharyngeal swab	**Comparator DSFA (negatives followed by culture with DFA)**		
DSFA	Positive	Negative	Total
Positive	29	0	29
Negative	0	518	518
Total	29	518	547
			95% CI
Sensitivity	29/29	100.0%	88.1% to 100%
Specificity	518/518	100.0%	99.3% to 100%

Table 16. Human Metapneumovirus

Fresh nasal/nasopharyngeal swab	**Comparator DSFA (negatives confirmed by a validated hMPV real-time RT-PCR followed by bi-directional sequencing analysis comparator assay)**		
DSFA	Positive	Negative	Total
Positive	15	0	15
Negative	12	510	522
Total	27	510	537
			95% CI
Sensitivity	15/27	55.6%	35.3% to 74.5%
Specificity	510/510	100%	99.3% to 100%

All Study Sites Combined

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

Table 17. Respiratory Syncytial Virus

Fresh nasal/nasopharyngeal wash/aspirate	**Comparator DSFA (negatives followed by culture with DFA)**		
DSFA	Positive	Negative	Total
Positive	204	1	205
Negative	3	462	465
Total	207	463	670
			95% CI
Sensitivity	204/207	98.6%	95.8% to 99.7%
Specificity	462/463	99.8%	98.8% to 100%

Table 18. Human Metapneumovirus

Fresh nasal/nasopharyngeal wash/aspirate	Comparator DSFA (negatives confirmed by a validated hMPV real-time RT-PCR followed by bi-directional sequencing analysis comparator assay)		
DSFA	Positive	Negative	Total
Positive	55	0	55
Negative	25	614	639
Total	80	614	694
			95% CI
Sensitivity	55/80	68.8%	57.4% to 78.7%
Specificity	614/614	100.0%	99.4% to 100%

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

Table 19. Respiratory Syncytial Virus

Fresh nasal/nasopharyngeal swab	Comparator DSFA (negatives followed by culture with DFA)		
DSFA	Positive	Negative	Total
Positive	39	0	39
Negative	1	647	648
Total	40	647	687
			95% CI
Sensitivity	39/40	97.5%	86.8% to 99.9%
Specificity	647/647	100.0%	99.4% to 100%

Table 20. Human Metapneumovirus

Fresh nasal/nasopharyngeal swab	Comparator DSFA (negatives confirmed by a validated hMPV real-time RT-PCR followed by bi-directional sequencing analysis comparator assay)		
DSFA	Positive	Negative	Total
Positive	24	0	24
Negative	20	632	652
Total	44	632	676
			95% CI
Sensitivity	24/44	54.5%	38.8% to 69.9%
Specificity	632/632	100.0%	99.4% to 100%

Analytical Performance Studies

Analytical Sensitivity (Limit of Detection)

Analytical Limits of Detection (LODs) of the D³ FastPoint L-DFA RSV/MPV Reagent was addressed using dilution series of infected model cells. Model cells for respiratory syncytial virus (ATCC Washington strain), and human metapneumovirus subtype A1 (clinical 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. 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 of the D³ FastPoint L-DFA Respiratory Virus Identification Kit

Virus Strain	Infected cells/mL	Number of replicates with positive cells	LOD determination
RSV (ATCC Washington strain)	1000	10/10	100 infected cells/mL
	200	10/10	
	100	**10/10**	
	50	7/10	
	25	7/10	
	12.5	6/10	
	6	1/10	
	3	0/10	
	1.5	0/10	
	0.8	0/10	
hMPV A1 (Clinical strain)	2000	10/10	100 infected cells/mL
	400	10/10	
	200	10/10	
	100	**10/10**	
	50	6/10	
	25	2/10	
	12.5	0/10	
	6	0/10	
	3	0/10	
	1.5	0/10	

Analytical Reactivity (Inclusivity)

Analytical reactivity (inclusivity) of the D³ FastPoint L-DFA RSV/MPV Reagent was evaluated using 3 RSV virus and 4 hMPV 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 RSV/MPV Kit.

Table 22. Analytical Reactivity (inclusivity) of the D³ FastPoint L-DFA RSV/MPV Kit on Various RSV Virus and hMPV Virus Strains

RSV and hMPV Strains	Infected Cell Concentration (as multiples of the respective established LOD concentration)	D³ FastPoint L-DFA RSV/MPV Kit Results
RSV 9320	10x LOD	22 Golden-yellow fluorescent cells
RSV Washington	10x LOD	22 Golden-yellow fluorescent cells
RSV Long	10x LOD	32 Golden-yellow fluorescent cells
hMPV A1	10x LOD	25 Apple-green fluorescent cells
hMPV A2	10x LOD	25 Apple-green fluorescent cells
hMPV B1	10x LOD	25 Apple-green fluorescent cells
hMPV B2	10x LOD	37 Apple-green fluorescent cells

Analytical Specificity

D³ FastPoint L-DFA RSV/MPV 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 RSV/MPV Kit, all other microorganisms tested negative.

Staining of *S. aureus* appeared as small points of fluorescence (see LIMITATIONS OF PROCEDURE**).**

Fifty-nine (59) virus strains were tested for cross-reactivity. Depending on the particular virus, 1.4×10^4 to 1.4×10^5 $TCID_{50}$ 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 (UTM)). Each cell suspension of infected Model Cells was processed according to the D³ FastPoint L-DFA RSV/MPV 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 RSV/MPV Kit

Organism	Strain or Type	D³ FastPoint L-DFA RSV/MPV Reagent Results	Inoculum ($TCID_{50}$)
Adenovirus	Type 1	Negative	1.4×10^4
	Type 3	Negative	1.4×10^4
	Type 5	Negative	1.4×10^4
	Type 7	Negative	1.4×10^4
	Type 10	Negative	1.4×10^4
	Type 16	Negative	1.4×10^4
	Type 17	Negative	1.4×10^4
Metapneumovirus (hMPV)	Subtype A1	Apple-Green Fluorescence	1.4×10^4
	Subtype A2	Apple-Green Fluorescence	1.4×10^4
	Subtype B1	Apple-Green Fluorescence	1.4×10^4
	Subtype B2	Apple-Green Fluorescence	1.4×10^4
Influenza A	Aichi (H3N2)	Negative	1.4×10^4

Organism	Strain or Type	D^3 FastPoint L-DFA RSV/MPV Reagent Results	Inoculum ($TCID_{50}$)
	Mal (H1N1)	Negative	1.4 x 10^4
	Hong Kong (H3N2)	Negative	1.4 x 10^4
	Denver (H1N1)	Negative	1.4 x 10^4
	Port Chalmers (H3N2)	Negative	1.4 x 10^4
	Victoria (H3N2)	Negative	1.4 x 10^4
	New Jersey (HSWN1)	Negative	1.4 x 10^4
	WS (H1N1)	Negative	1.4 x 10^4
	PR (H1N1)	Negative	1.4 x 10^4
	Wisconsin (H3N2)	Negative	1.4 x 10^4
	A/NWS/33 (H1N1)	Negative	1.4 x 10^4
	A Mexico/4108/2009 (H1N1)	Negative	1.4 x 10^4
	A California/07/2009 (H1N1)	Negative	1.4 x 10^4
Influenza B	Hong Kong	Negative	1.4 x 10^4
	Maryland	Negative	1.4 x 10^4
	Mass	Negative	1.4 x 10^4
	GL	Negative	1.4 x 10^4
	Taiwan	Negative	1.4 x 10^4
	B/Lee/40	Negative	1.4 x 10^4
	Russia	Negative	1.4 x 10^4
RSV	Long	Golden-Yellow Fluorescence	1.4 x 10^4
	Wash	Golden-Yellow Fluorescence	1.4 x 10^4
	9320	Golden-Yellow Fluorescence	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
	7	Negative	1.4 x 10^5
	22	Negative	1.4 x 10^5
Coxsackievirus	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

Organism	Strain or Type	D³ FastPoint L-DFA RSV/MPV Reagent Results	Inoculum ($TCID_{50}$)
Rhinovirus	209 Picornavirus	Negative	1.4×10^5
Enterovirus 70	VR-836	Negative	1.4×10^5
Enterovirus 71	VR-1432	Negative	1.4×10^5

Twenty four (24) microorganisms, including 22 bacteria, 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 (in colony-forming units (CFUs)) ranging from 1.6×10^9 to 3.5×10^{10} CFUs depending on the bacterium. These suspensions of Model Cells with bacteria were then processed according to the D³ FastPoint L-DFA RSV/MPV Kit protocol, using 2X MAb reagents. Except for *Staphylococcus aureus*, which was cross-reactive with the D³ FastPoint L-DFA RSV/MPV 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 the D³ FastPoint L-DFA RSV/MPV Kit

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

*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 consisting of 5 randomized panel members.

The RSV/hMPV panel consisted of the following:

- Low level RSV (Washington strain) infected cells.
- Low level hMPV (A1 subtype) infected cells.
- Low level RSV (Washington strain) infected cells mixed with mid-level hMPV (A1 subtype) infected cells.
- Low level hMPV (A1 subtype) infected cells mixed with mid-level RSV (Washington strain) infected cells.
- 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 2.5×10^5 to 3.5×10^5 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:

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

For the D^3 FastPoint L-DFA RSV/MPV Reagent, the combined data from the four Study Sites demonstrated reproducible detection of RSV by the R-PE labeled MAbs and reproducible detection of hMPV by the FITC-labeled MAbs. The presence of RSV infected cells was reported in 100% (120/120) of the wells in which the infected cells were expected. The presence of hMPV 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 100% (40/40) of the wells in which infected cells were not present. The total percent agreement for the D^3 FastPoint L-DFA RSV/MPV Reagent was 100% (280/280).

Table 25. Reproducibility Study Results Using the D^3 FastPoint L-DFA RSV/MPV Kit

Sites	Panel Member	Negative	RSV Low Level	hMPV Low Level	Mixed Infection		Mixed Infection		Total % Agreement
					RSV Mid-level	hMPV Low Level	RSV Low Level	hMPV Mid-level	
Site 1	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 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		40/40 (100%)	40/40 (100%)	40/40 (100%)	40/40 (100%)	40/40 (100%)	40/40 (100%)	40/40 (100%)	**280/280 (100%)**
95% CI		91.2-100%	91.2% to 100%	91.2% to 100%	91.2% to 100%	91.2% to 100%	91.25 to 100%	91.2% to 100%	**98.7% to 100%**

ASSISTANCE

To place an order or for technical support, please contact a Quidel Representative at 800.874.1517 (in the U.S.) or 858.552.1100 (outside the U.S.), Monday through Friday, from 8:00 a.m. to 5:00 p.m., Eastern Time. Orders may also be placed by fax at (740) 592-9820. For e-mail support contact customerservice@quidel.com or technicalsupport@quidel.com.

For services outside the U.S.A., please contact your local distributor. Additional information about Quidel, our products, and our distributors can be found on our website quidel.com.

REFERENCES

1. Englund, J.A., (2002). Antiviral therapy of influenza. *Semin. Pediatr. Infect. Dis.*, 13(2):120-128.
2. Fete, T.J., Noyes, B. (1996). Common (but not always considered) viral infections of the lower respiratory tract. *Pediatr. Ann.,* 25:10), 577-584.
3. Hall, C.B. (1981). Respiratory Syncytial Virus. In: Feigin, R. D., Cherry, J.D., eds. *Textbook of Pediatric Infectious Diseases*, Phila., W.B. Saunders, 1247-1267.
4. Hall, C.B., Hall, W.J., Gala, C.L., MaGill, F.B., Leddy, J.P. (1984). Longterm prospective study in children after Respiratory Syncytial Virus infection. *J. Pediatr*., 105:358-364.
5. Falsey, Ann R. and Walsh, E.E. (2000). Respiratory Syncytial Virus Infection in Adults. Clinical Microbiology Reviews 13(3):371-384.
6. van den Hoogen BG, de Jong JC, Groen J, Kuiken T, de Groot R, Fouchier RA, Osterhaus AD. A newly discovered human pneumovirus isolated from young children with respiratory tract disease. 2001 Nat Med 7:719-24.
7. Nissen MD, Siebert DJ, Mackay LM, Sloots TP, Withers SJ. Evidence of human metapneumovirus in Australian children. 2002 Med J Aust. 176:188.
8. Kahn, Jeffrey S. Epidemiology of human metapneumovirus. 2006 Clin Microbiol Rev. 19(3):546-557.
9. Esper, Frank, Richard A. Martinello, Derek Boucher, Carla Weibel, David Ferguson, Marie L. Landry, and Jeffrey S. Kahn, A 1-year experience with human metapneumovirus in children aged <5 years. 2004 J Infect Dis. 189:1388-1396.
10. Falsey, Ann R., Mary C. Criddle, Edward E. Walsh, Detection of respiratory syncytial virus and human metapneumovirus by reverse transcription polymerase chain reaction in adults with and without respiratory illness. 2006 J Clin Virol. 35:46-50.
11. Maertzdorf, J., C.K. Wang, J.B. Brown, J.D. Quinto, M. Chu, M. de Graff, B.G. van den Hoogen, R. Spaete, A.D.M.E. Osterhaus, and R.A.M. Fouchier. Real-time reverse transcriptase PCR assay for detection of human metapneumoviruses from all known lineages. 2004 J Clin Microbiol. 42:981-986.
12. Percivalle E, Sarasini A, Visia L, Revello G, Gerna G. Rapid Detection of Human Metapneumovirus Strains in Nasopharyngeal Aspirates and Shell Vial Cultures by Monoclonal Antibodies. 2005 J Clin Microbiol. 43.7.3443-3446
13. Landry, Marie L., David Ferguson, Sandra Cohen, Teresa C.T. Peret, and Dean D. Erdman, Detection of human metapneumovirus in clinical samples by immunofluorescence staining of shell vial centrifugation cultures prepared from three different cell lines. 2005 J Clin Micro 43(4):1950-1952.
14. Ebihara, Takashi, Rika Endo, Xiaoming Ma, Nobuhisa Ishiguro, and Hideaki Kikuta, Detection of human metapneumovirus antigens in nasopharyngeal secretions by an immunofluorescent-antibody test. 2005 J Clin Micro 43(3):1138-1141.
15. Kyung Ran Jun, Young Dae Woo, Heungsup Sung, and Mi-Na Kim, Detection of human metapneumovirus by direct antigen test and shell vial cultures using immunofluorescent antibody staining. 2008 J Virol Meth. 152:109-111.

16. Manoha, C., J.B. Bour, C. Pitoiset, M. Darniot, S. Aho, and P. Pothier, Rapid and sensitive detection of metapneumovirus in clinical specimens by indirect fluorescence assay using a monoclonal antibody. 2008 J Med Virol. 80:154-158.
17. Gerna, Giuseppe, Antonella Sarasini, Elena Percivalle, Giulia Campanini, Francesca Rovida, Antonietta Marchi, Fausto Baldanti, Prospective study of human metapneumovirus infection: Diagnosis, typing and virus quantification in nasopharyngeal secretions from pediatric patients. 2007 J Clin Virol. 40:236-240
18. US Department of Labor, Occupational Safety and Health Administration, 29 CFR Part 1910.1030, Occupational safety and health standards, bloodborne pathogens.
19. CLSI/NCCLS M29A3 Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline, David L. Sewell, Third Edition, Clinical and Laboratory Standards Institute, 01Mar2005.
20. Clinical and Laboratory Standards Institute. *Viral Culture; Approved Guidelines.* CLSI document M41-A [ISBN 1562386239]. Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898, USA 2006.
21. Clinical and Laboratory Standards Institute. *Viral Culture; Approved Guidelines.* CLSI document M41-A [ISBN 1562386239]. Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898, USA 2006,
22. Leland, Diane S. (1996). *Clinical Virology,* published by W.B. Saunders, Philadelphia, PA.

REF 01-120002 – D³ FastPoint L-DFA RSV/MPV Identification Kit

IVD

EC REP

MDSS GmbH
Schiffgraben 41
30175 Hannover,
Germany

Diagnostic Hybrids, Inc. a subsidiary of Quidel Corporation
2005 East State Street, Suite 100
Athens, OH 45701 USA
quidel.com

PI3162000EN00 (09/18)

REF

Catalogue number

CE mark of conformity

EC REP

Authorized Representative
in the European Community

LOT

Batch code

Use by

Manufacturer

Temperature limitation

Intended use

Consult e-labeling
instructions for use

Do not reuse

IVD

For *In Vitro* diagnostic use

CONT | NaN_3

Contents/Contains

NaN_3 | 4%

Contents/Contains