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QUIDEL | D3 DFA Metapneumovirus IDENTIFICATION KIT

For the qualitative detection and identification of human metapneumovirus (hMPV) in nasal and nasopharyngeal swabs and aspirates/washes or cell

FOR *IN VITRO* DIAGNOSTIC USE

℞ ONLY

INTENDED USE

The Diagnostic Hybrids, Inc. D^3 DFA Metapneumovirus Identification Kit, is intended for the qualitative detection and identification of human metapneumovirus (hMPV) in nasal and nasopharyngeal swabs and aspirates/washes or cell culture. The assay detects hMPV antigens by immunofluorescence using a blend of three monoclonal antibodies (MAbs), from patients with signs and symptoms of acute respiratory infection. This assay detects but is not intended to differentiate the four-recognized genetic sub-lineages of hMPV.

Negative results do not preclude hMPV infection and should not be used as the sole basis for diagnosis, treatment or other management decisions. It is recommended that specimens found to be negative after examination of the direct specimen results be confirmed by an FDA-cleared hMPV molecular assay.

SUMMARY AND EXPLANATION OF THE TEST

Human metapneumovirus is a respiratory viral pathogen that causes a spectrum of illnesses ranging from asymptomatic infection to severe bronchiolitis. Human metapneumovirus was first described in 2001 by researchers at the Erasmus Medical Center at Erasmus University in Rotterdam, The Netherlands.[1] 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[2] 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.[3]

Diagnosis of hMPV infections has relied primarily on detection by real-time reverse transcriptase polymerase chain reaction (RT-PCR).[4,5,6] Development of specific MAbs directed against the four known subtypes of hMPV has been described.[7] The use of MAbs in immunofluorescence applications in the clinical laboratory will greatly improve the ability to diagnose hMPV infections by allowing for detection directly from infected cells of patients and detection of viable virus through cell culture.[8,9,10,11,12]

PRINCIPLE OF THE PROCEDURE

The D^3 DFA Metapneumovirus Identification Kit uses a blend of three hMPV antigen-specific murine MAbs that are directly labeled with fluorescein for detection of hMPV antigens. The Metapneumovirus DFA Reagent

detects, but does not differentiate, between the four recognized subtypes of hMPV (subtypes A1, A2, B1, and B2).

The cells to be tested, derived from a clinical specimen or cell culture, are placed onto a glass slide, allowed to air dry and are fixed in acetone. The Metapneumovirus DFA Reagent is added to the cells which are then incubated for 15 to 30 minutes at 35°C to 37°C in a humidified chamber or humidified incubator. The stained cells are then washed with the diluted Phosphate Buffered Saline (1X PBS), a drop of the supplied Mounting Fluid is added and a coverslip is placed on the prepared cells. The cells are examined using a fluorescence microscope. The hMPV-infected cells will fluoresce apple-green. Non-infected cells will contain no fluorescence but will be stained red by the Evans Blue counter-stain.

It is recommended that specimens found to contain no fluorescent cells after examination of the direct specimen be confirmed by an FDA-cleared hMPV molecular assay.

REAGENTS AND MATERIALS PROVIDED

The D^3 DFA Metapneumovirus Identification Kit contains the following:

Metapneumovirus DFA Reagent **5 mL**

One dropper bottle containing a blend of fluorescein-labeled murine monoclonal antibodies directed against hMPV antigens. The buffered, stabilized, aqueous solution contains Evans Blue as a counter-stain and 0.1% sodium azide as a preservative.

hMPV Antigen Control Slides **5 slides**

Five (5) individually packaged control slides, each with two wells, one well containing cell culture-derived hMPV positive cells and one well containing cell culture-derived negative cells. Each slide is intended for staining only one time. Control material has been treated to be non-infectious; however, biohazard safety precautions for handling infectious materials are required.

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).

Mounting Fluid **7 mL**

One dropper bottle containing an aqueous, buffered, stabilized solution of glycerol with 0.1% sodium azide.

MATERIALS REQUIRED BUT NOT PROVIDED

- Fluorescence microscope with the appropriate filter combination for FITC (excitation peak = 490 nm, emission peak = 520 nm); magnification 200X to 400X.
- Cell culture for hMPV isolation. Suggested cell lines[7] include LLC-MK2, HEp-2, A549 cells; R-Mix™ and R-Mix Too™ MixedCells™; and primary Rhesus monkey kidney cells. All available from Quidel.
- Live control viruses for positive culture controls: Known strains of hMPV for use in monitoring cell culture and staining procedures. Such control virus strains can be obtained from Quidel.
- Coverslips (22 x 50 mm) for Antigen Control Slides and for specimen slides.
- Universal Transport Medium. Available from Quidel.
- R-Mix Refeed Medium (for use with R-Mix and R-Mix Too MixedCells) or other standard refeed medium. Available from Quidel.
- Reagent grade acetone (> 99% pure) chilled at 2°C to 8°C for fixing direct specimen slides, shell-vials, and cultured cell preparations.

  NOTES:

  - Keep the reagent-grade acetone container tightly sealed to avoid hygroscopic absorption of water which may cause a hazy, non-specific, background fluorescence.

- A mixture of 80% acetone / 20% de-mineralized water is used for fixing cells in plastic multi-well plates. Store at ambient temperature (20°C to 25°C).

- Sterile graduated pipettes: 10 mL, 5 mL, and 1 mL.
- Sterile Pasteur pipettes or other transfer pipettes.
  Caution: One should not use solvents such as acetone with polyethylene transfer pipettes.
- Fine-tipped forceps.
- Wash bottle, 200 mL.
- Bent-tip teasing needle (for removal of coverslip from a shell-vial): Fashion the teasing needle by bending the tip of a syringe needle or similar object (e.g., mycology teasing needle) against a benchtop or with a pair of forceps taking care to avoid injury.
- Sodium hypochlorite solution (1:10 final dilution of household bleach).
- Humid chamber (e.g., covered Petri dish with a damp paper towel placed in the bottom).
- Glass microscope slides.
- Acetone-cleaned glass microscope slides.
- Sterile, nylon flocked swabs or polyester swabs, which are non-inhibitory to viruses and cell cultures.
- Incubator, 35°C to 37°C (5% 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* (see *REAGENT PREPARATION*) and for dilution of the reagent grade acetone when fixing cells in polystyrene multi-well plates (see *MATERIALS REQUIRED BUT NOT PROVIDED*).
- Aspirator Set-up: Vacuum aspirator with disinfectant trap containing sufficient household bleach (5% sodium hypochlorite) that the concentration is not decreased by more than 10-fold as it is diluted with discarded fluids.
- Wash Container: Beaker, wash bottle, or Coplin jar for washing slides.
- Fixing Container: Coplin jar, slide dish, or polyethylene holder for fixing cells on slides.
- Inverted Light Microscope with 40X to 100X magnification capability: Used for examining cell monolayers for toxicity and confluency prior to inoculation.

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use.
- 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 Bloodborne Pathogens[13]; 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".[14]
  - Cell cultures may have potential as biohazards. Personnel working with cultures must be properly trained in safe handling techniques[15] and have proficiency with cell culture and aseptic techniques before attempting this procedure.
  - Follow Biosafety Level 2 or other appropriate biosafety practices.
  - Decontaminate specimens and cultures using a 1:10 final dilution of household bleach.
  - Although Antigen Control Slides have been shown to be non-infectious, practice biohazard safety precautions when handling and disposing of these materials.
- Never pipette reagents or clinical samples by mouth; avoid all contact of clinical samples with broken skin.
- Avoid splashing and the generation of aerosols with clinical samples.
- Use aseptic technique and sterile equipment and materials for all tissue culture procedures.
- Acetone, a reagent that is required for the test but not provided in the kit, is a flammable, volatile organic solvent. Use it in a well-ventilated area and keep away from flames and other sources of ignition.
- Evans Blue counter-stain is a potential carcinogen. If skin contact occurs, flush with water immediately.

- The Metapneumovirus DFA Reagent is supplied at working strength; any dilution will decrease sensitivity.
- Reagents should be used prior to their expiration date.
- Each hMPV Antigen Control Slide should be used only once. Do not re-use a Control Slide.
- Microbial contamination of the Metapneumovirus DFA Reagent may cause a decrease in sensitivity. Check for turbidity that may indicate contamination.
- Store 1X PBS in a clean container at 20°C to 25°C to prevent contamination. Check for contamination and discard solution if cloudy.
- Reusable glassware must be washed and thoroughly rinsed free of all detergents.
- Do not expose Metapneumovirus DFA Reagent to bright light during staining or storage.
- 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.
  - 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.
  - Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. Avoid disposal of these solutions down sanitary or industrial plumbing systems.
  - Avoid release to the environment.
- Use of other reagents 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 Solution 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 ambient temperature.

80% Acetone Solution

- Add 20 mL of distilled or de-mineralized water to a 100 mL container.
- Add 80 mL of acetone to the container and mix by inversion.
- Label the container as to contents, the date diluted, and technologist's initials.
- Store the acetone solution at ambient temperature.

Storage

Table 1. Reagent Storage Conditions

Metapneumovirus DFA Reagent	Store at 2°C to 8°C in the dark.
Mounting Fluid	
hMPV Antigen Control Slides	Store at 2°C to 8°C.
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.	Store liquid at 2°C to 8°C prior to dilution.
1X PBS	Store at ambient temperature (20°C to 25°C).

Stability

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

Discard 1X PBS if it becomes cloudy or after sixty (60) days.

SPECIMEN COLLECTION AND PREPARATION

Proper collection and handling of the patient specimen are the most important factors in successful hMPV 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.[16]

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.

Freezing and thawing specimens should be avoided since this will result in a loss of viability of viruses leading to decreased sensitivity for cell culture isolation.

PROCEDURE

Preliminary Comments and Precautions

- Adhere to the recommended volumes and times in the procedure to ensure accurate results.
- When staining with fluorescent reagents and examining cells microscopically for specific viral fluorescence, include both positive and negative controls to monitor the procedure and performance of the reagents. Run controls with each batch of patient specimens.
- Place the closed, humidified chamber for holding slides during staining into the incubator for equilibration to 35°C to 37°C prior to staining. By doing this, the test slides will come to temperature quickly, yielding more rapid, intense staining.
- Bring the Metapneumovirus DFA Reagent to ambient temperature (20°C to 25°C) prior to use, and immediately return to refrigerator after use for storage at 2°C to 8°C.

Regarding Cell Culture Testing

- Good Laboratory Practice dictates that positive and negative virus controls be run with each new batch of cells to confirm their performance in culturing specific viruses.
- It is good practice to retain the medium removed from the monolayers until after staining results have been obtained. If there is any question concerning the specimen results, the medium can be passed to another monolayer for repeat testing.
- When staining cell monolayers in polystyrene multi-well plates, fix the cells with acetone that has been diluted to 80% by adding 20 mL of demineralized water to 80 mL of acetone. (See *MATERIAL REQUIRED BUT NOT PROVIDED*.)

- Do not allow the monolayers to dry before fixing; this can lead to high background, non-specific staining, and decreased sensitivity.
- Do not allow the DFA Reagent to dry on the monolayers; this can lead to high background, non-specific staining, and decreased sensitivity.

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). Do not report results for patient samples until 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, the emission output decreases, resulting in lower fluorescence intensity of the stained material. Change the fluorescent bulb according to manufacturer's recommendations.
  - The light source is focused by a number of lenses and mirror(s). For maximum intensity, ensure that the lenses and mirrors are 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 a part of the monolayer. Cell debris, lint, etc. can non-specifically adsorb fluorescent stain resulting in highly intense fluorescence.
  - Intense fluorescence around the periphery of slide wells indicates drying of the DFA Reagent, suggesting that incubation was too long or the humidity was not properly controlled.
  - Inadequate removal of mucus from direct specimen material can lead to non-specific staining when conducting the test on direct specimens.
  - On direct specimens, leukocytes and monocytes may trap fluorescence or RBC may leave a green haze on the sample.
  - Generalized, low-grade, yellow-green fluorescence may be seen particularly in areas that have clumped cells or near holes in the monolayer in cell culture. Diffusion of the trapped fluorescent stain is impeded during the washing steps resulting in non-specific fluorescence.
- 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 of time.

Specimen Preparation

For specimen processing recommendations, refer to the CLSI standard, "Approved Viral Culture Guidelines."[16]

Direct Specimen Testing

1. Spot 25 µL of a prepared cell suspension onto a labeled, acetone-cleaned slide. Repeat this step for each specimen.
2. Air dry the slides completely.
3. Fix the cells to the slides using fresh, chilled 100% acetone for 5 to 10 minutes at 20°C to 25°C.
4. **Caution: Acetone is volatile and flammable; keep away from open flames.**
5. Remove the slides from the fixative and allow to air dry.
6. Add one drop of the Metapneumovirus DFA Reagent to completely cover the dried, fixed cells on the prepared slide.
7. Add one drop of the Metapneumovirus DFA Reagent to the positive and negative wells of a fresh hMPV Antigen Control Slide.

8. **Note:** Use antigen control slides only one time; do not re-stain.
9. Place the slides in a pre-warmed, covered chamber at 35°C to 37°C for 15 to 30 minutes.
10. Rinse the stained cells using the 1X PBS. For only a few slides, use a wash bottle, beaker or Coplin jar of 1X PBS. For many slides, place a slide carrier that holds 10 to 20 slides in a container of 1X PBS. For effective rinsing, dip the slide(s) up and down a minimum of four times.
11. Discard the used 1X PBS and repeat the washing step using fresh 1X PBS.
12. Perform a final rinse of the stained slides using de-mineralized water. For only a few slides, use a wash bottle, beaker or Coplin jar of de-mineralized water. For many slides, place a slide carrier that holds 10 to 20 slides in a container of de-mineralized water. For effective rinsing, dip the slide(s) up and down a minimum of four times.
13. Gently blot the excess de-mineralized water from the slides.
14. Add a small drop of Mounting Fluid to each cell-containing well and cover the wells with a coverslip.
15. Examine the stained, mounted slides using a fluorescence microscope with 200X to 400X magnification. (See *Regarding Immunofluorescence Microscopy*.)
16. Refer to *INTERPRETATION OF RESULTS*.

Cell Culture Testing – Shell-Vial

1. Examine the monolayers for proper cell morphology and confluency prior to inoculation.
2. Aspirate maintenance medium from the monolayers and add 1 mL of appropriate refeed medium to each shell-vial.
3. Add 0.2 to 0.4 mL of prepared specimen or control virus to each shell-vial.
4. Centrifuge the shell-vials at 700xg for 1 hour at 20°C to 25°C.
5. Place stoppered shell-vials in an incubator at 35°C to 37°C for up to two days.
6. When a monolayer is ready for staining, remove the medium by aspiration and add 1 mL of 1X PBS to wash.
7. Swirl to mix and then aspirate.
8. Repeat the wash step with 1 mL 1X PBS, and aspirate the liquid from the monolayer.
9. Add 1 mL of fresh, chilled, 100% acetone and allow to fix for 5 to 10 minutes at 20°C to 25°C.
10. **Caution: Acetone is volatile and flammable; keep away from open flames.**
11. Remove the acetone-fixative by aspiration.
12. Add 0.5 mL of 1 X PBS to wet the monolayer.
13. Swirl and then aspirate completely.
14. Add 4 drops of the Metapneumovirus DFA Reagent to the fixed monolayers of patient and control samples. Rock **to ensure complete coverage** of the monolayer by the DFA Reagent.
15. Place stoppered shell-vials in an incubator at 35°C to 37°C for 15 to 30 minutes.
16. Aspirate the Metapneumovirus DFA Reagent from the monolayers.
17. Add 1 mL of 1X PBS to wash.
18. Remove the 1X PBS by aspiration; repeat the wash step, and again remove by aspiration.
19. Add 1 mL of de-mineralized water to each shell-vial.
20. Remove the de-mineralized water by aspiration.
21. Lift the coverslip from the bottom of the shell-vial using a bent-tip needle on a syringe barrel. Grasp the cover slip with the fine-tipped forceps, transfer it, monolayer-side down, to a small drop of Mounting Fluid on a standard microscope slide.
22. Examine the stained monolayers using a fluorescence microscope with 200X to 400X magnification. (See *Regarding Immunofluorescence Microscopy*.)
23. Refer to *INTERPRETATION OF RESULTS*.

Cell Culture Testing – Multi-Well Plate

1. Examine the monolayers for proper cell morphology and confluency prior to inoculation.
2. Aspirate maintenance medium from the monolayers and add 1 mL of appropriate refeed medium to each well of a 24-well plate; add 0.8 mL to each well of a 48-well plate.
3. Add 0.2 to 0.4 mL of prepared specimen or control virus to the appropriate wells of a multi-well plate.
4. Centrifuge the multi-well plates at 700xg for 1 hour at 20°C to 25°C.
5. Place the covered multi-well plates in an incubator at 35°C to 37°C in a humidified atmosphere of 5% CO_2 for up to two days post-inoculation.
6. When a monolayer is ready for staining, remove the medium by aspiration and add 1 mL of 1X PBS to wash.
7. Swirl and then aspirate.
8. Repeat the wash step with 1 mL 1X PBS and aspirate.
9. Add 1 mL of 80% aqueous acetone and allow to fix for 5 to 10 minutes at 20°C to 25°C.
10. **NOTE:** Do not allow the 80% acetone fixative to remain in the polystyrene wells longer than 10 minutes since it may craze and cloud the plastic making it difficult to examine the monolayers.
11. **Caution: Acetone is volatile and flammable; keep away from open flames.**
12. Remove the acetone-fixative by aspiration.
13. Add 0.5 mL of 1X PBS to wet the monolayer.
14. Swirl and aspirate completely.
15. Add 4 drops of the Metapneumovirus DFA Reagent to the fixed monolayers of patient and control samples in each well of a 24-well plate; add 3 drops of the DFA Reagent to the fixed monolayers of patient and control samples in each well of a 48-well plate. Rock to **ensure complete coverage** of the monolayer by the DFA Reagent.
16. Place the covered multi-well plate in a humidified incubator at 35°C to 37°C for 15 to 30 minutes.
17. Aspirate the Metapneumovirus DFA Reagent from the monolayers.
18. Add 1 mL of 1X PBS and mix to wash.
19. Remove 1X PBS by aspiration; repeat the wash step, and aspirate.
20. Add 1 mL of de-mineralized water to each well.
21. Remove the de-mineralized water by aspiration.
22. Add 2 to 3 drops of Mounting Fluid to each well, and cover the plate.
23. Examine the stained monolayers using a fluorescence microscope with 200X to 400X magnification. (See *Regarding Immunofluorescence Microscopy*.)
24. Refer to *INTERPRETATION OF RESULTS*.

QUALITY CONTROL

Reagents

- A fresh hMPV Antigen Control Slide should be stained each time the staining procedure is performed to ensure proper test performance.
- The positive well will show multiple infected cells with bright, apple-green fluorescence.
- The negative well will show only negative cells staining a dull red due to the Evans Blue that is included as a counter-stain.
- 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.

Cell Culture

- Positive and negative hMPV controls should be run with each new batch of cells to confirm their performance in culturing hMPV.
- To ensure viral sensitivity, an hMPV-inoculated control monolayer should be included each time a new lot of cell culture is used.

- A non-inoculated monolayer from each lot should be stained to serve as a negative control. Adverse storage conditions or handling procedures will also be reflected in the negative control.
- If control cultures fail to perform correctly, results are considered invalid.

INTERPRETATION OF RESULTS

Examination of Samples and Controls

- Examine controls first to ensure proper test performance before examining patient specimens.
- A positive reaction for hMPV is one in which apple-green fluorescence is observed in the cytoplasm.
- Non-infected cells will fluoresce dull red due to the Evans Blue counter-stain included in the DFA Reagent.
- Examine the entire cell spot or monolayer of cells before reporting final negative results. Do not report results for patient samples unless controls perform as expected.

Artifacts of Staining

- Dried edges of the monolayer or cell clumps may non-specifically fluoresce due to antibody trapping.
- Dead, rounded cells may non-specifically fluoresce dull olive-green due to specimen toxicity or improper cell storage.
- Properly controlled humidity during staining and adequate washing between steps helps to eliminate non-specific staining.

Results From Direct Specimen Testing

- The quality of the specimen with respect to the number of columnar epithelial cells in the sample is assessed by examining different fields at a magnification of 200X.
- If fluorescent cells are observed in a sample, report: “Metapneumovirus viral antigens detected by direct specimen testing.”
- To be reported as negative, a sample must contain 20 or greater columnar epithelial cells. Specimens containing fewer than 20 epithelial cells are invalid. A new specimen should be obtained and tested. A satisfactory specimen with no virus-specific fluorescent cells should be reported as “No metapneumovirus antigens detected by direct specimen testing”. Such negative results, however, should be confirmed using an FDA-cleared hMPV molecular assay.

Results From Culture Isolation/Confirmation

- Examine the entire cell spot or monolayer of cells for hMPV-specific fluorescent cells. If no fluorescent cells are found, report: “No metapneumovirus isolated by cell culture.”
- If hMPV-specific fluorescence is observed, report as “Metapneumovirus isolated by cell culture.”

LIMITATIONS OF THE PROCEDURE

- The detection of hMPV-specific viral antigens is dependent upon proper specimen collection, handling, transportation, storage, and preparation. Failure to observe proper procedures in any one of these steps can lead to incorrect results.[17]
- Assay performance characteristics have not been established for direct specimen staining on respiratory specimens other than nasal/nasopharyngeal swabs and aspirates/washes. The use of the D^3 DFA Metapneumovirus 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 hMPV will vary greatly depending upon the specimen quality at the time of collection and subsequent handling. A negative result does not exclude the possibility of hMPV infection and should not be used as the sole basis for diagnosis, treatment or other patient management decisions. Results of the test should be interpreted by trained health care professionals in conjunction with information available

from epidemiological studies, clinical signs and symptoms, patient's medical history, and the results of other diagnostic procedures.

- The monoclonal antibodies used in this kit are from hybridomas created using virus-infected cells as the immunogen. The specific viral antigens detected by the antibodies are undetermined.
- Since the monoclonal antibodies have been prepared using defined hMPV strains, they may not detect all antigenic variants or new strains of the virus, should they arise. Monoclonal antibodies may fail to detect virus strains which have undergone minor amino acid changes in the target epitope region.
- The Metapneumovirus DFA Reagent detects but does not differentiate between the four recognized subtypes of hMPV (subtypes A1, A2, B1 and B2).
- The effects of antiviral therapy on the performance of this kit have not been established.
- A negative result on a direct or cultured specimen does not rule out the presence of hMPV. Such a negative result should be confirmed using an FDA-cleared hMPV molecular assay.
- The hMPV viral antigens detected in some direct specimens may be from non-viable virus and cannot be isolated by culture.
- The performance of the D^3 DFA Metapneumovirus Identification Kit has not been established in immunocompromised patients.
- Performance of the kit can be assured only when components used in the assay are those supplied by Quidel.
- Prolonged storage of the Metapneumovirus DFA Reagent under bright light will decrease the staining intensity.
- Light background staining may occur with specimens contaminated with Staphylococcus aureus strains containing large amounts of protein A. Protein A binds to the Fc portions of the conjugated antibodies. Such binding can be distinguished from viral antigen binding on the basis of morphology, e.g., S. aureus-bound fluorescence appears as small (~1 micron diameter), bright dots. Results from cell cultures with bacterial contamination must, therefore, be interpreted with caution.

EXPECTED VALUES

Many respiratory virus infections are seasonal. This seasonality is still being established for hMPV. In the D^3 DFA Metapneumovirus Identification Kit (D^3 DFA MPV Kit) multicenter prospective clinical study testing direct respiratory specimens, a total of 2109 respiratory specimens were tested from three U.S. clinical laboratories across the United States during the 2005 and 2006 respiratory virus seasons (December 2005-April 2006 and

December 2006-March 2007). hMPV prevalence as determined by the D^3 DFA MPV Kit direct specimen testing varied from 8.1% to 11.3% by site and averaged 9.3% overall. The number and percentage of hMPV positive cases by the D^3 DFA MPV Kit direct specimen testing, calculated by age group, are presented in Table 2 below.

Table 2. Prevalence of hMPV in Clinical Studies

Age Group	Total Specimens Evaluated	hMPV Positive by the D^3 DFA MPV Kit	
		Number Positive	Observed Prevalence
< 1 month	104	1	1.0%
≥ 1 month to < 2 years	1249	124	9.9%
≥ 2 years to < 5 years	293	30	10.2%
≥ 5 years to < 12 years	151	11	7.3%
≥ 12 years to < 18 years	65	1	1.5%
≥ 18 years to < 21 years	10	1	10.0%

Age Group	Total Specimens Evaluated	hMPV Positive by the D³ DFA MPV Kit	
		Number Positive	Observed Prevalence
≥ 21 years to < 60 years	90	3	3.3%
≥ 60 years	123	13	10.6%
Age Not Reported	24	0	0%
Totals	2109	184	8.7%

SPECIFIC PERFORMANCE CHARACTERISTICS

Clinical Performance Studies

Direct Specimen Testing

Performance characteristics of the D³ DFA MPV Kit testing direct respiratory specimens were established during prospective studies at 3 geographically diverse U.S. clinical laboratories during the 2005 and 2006 respiratory virus seasons (December 2005-April 2006 and December 2006-March 2007). 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 DFA MPV Kit was assessed and compared to a predetermined algorithm that used composite comparator methods at clinical Study Sites 1 and 2. The composite comparator methods consisted of viral culture and a validated hMPV RT-PCR comparator assay targeting the hMPV nucleocapsid gene followed by bi-directional genetic sequencing analysis.[1] The Quidel hMPV RT-PCR comparator assay targets the hMPV Nucleocapsid gene. "True" hMPV positive was defined as any sample that either tested positive by 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" hMPV negative was defined as any sample that tested negative by both viral culture and the hMPV real-time RT-PCR comparator assay.

Performance of the D³ DFA MPV Kit at clinical Study Site 3 was evaluated and compared only to the validated hMPV real-time RT-PCR followed by bi-directional sequencing analysis comparator assay described above. Any sample that 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, was considered as hMPV-positive, and the real-time hMPV RT-PCR comparator assay negatives were considered as hMPV-negatives at this site.

Study Site 1

Study Site 1 evaluated a total of 1564 fresh respiratory specimens submitted, December 2006 through March 2007, to the laboratory for respiratory virus testing. Slides were prepared from Phosphate Buffered Saline

[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 to 50 $TCID_{50}$/mL.

[2]The E-values generated from the clinical trials range from a low of 2e-77 to a high of 2e-67. 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). Therefore an E-Value ranging from 2e-67 to 2e-77 has a very low probability of occurring purely by chance.

(PBS)-washed cells from the fresh specimens and fixed according to the prescribed protocol. The slides were stained in accordance with the procedure in the product insert.

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

Table 3. Study Site 1 – Age and Gender Distribution

Sex	**Female**	**Male**
Total	**687**	**877**
Age		
< 1m	42	50
≥ 1m to < 2y	444	617
≥ 2y to < 12y	164	185
≥ 12y to < 18y	30	20
≥ 18y to < 21y	4	3
≥ 21y	3	2
Age Not Reported	0	0

Of the 1564 fresh respiratory specimens tested, 1509 were nasal wash/nasopharyngeal aspirate specimens. Due to insufficient sample numbers to establish performance of the D^3 DFA MPV Kit, 55 other types of respiratory specimens were removed from performance analysis. Of the 1509 fresh nasal wash/nasopharyngeal aspirate specimens tested, 27 were further excluded from the performance analysis due to insufficient volume for the comparator methods, resulting in a total of 1482 fresh nasal wash/nasopharyngeal aspirate specimens for analysis. Table 4 below shows the study results of the claimed specimen type at Study Site 1.

Table 4. Study Site 1 – Comparison of Results Using D^3 DFA MPV Kit, with Results Using the Composite Comparator Methods

Fresh Nasal Wash/ Nasopharyngeal Aspirate	**Composite Comparator Methods**		
DS-FA	Positive	Negative	Total
Positive	122	3	125
Negative	108	1249	1357
Total	230	1252	1482
			95% CI
Sensitivity	122/230	53.0%	46.6% to 59.5%
Specificity	1249/1252	99.8%	99.3% to 99.9%

Study Site 2

Study Site 2 evaluated a total of 371 fresh respiratory specimens submitted, December 2005 through January 2006, to the laboratory for respiratory virus testing. Slides were prepared from Phosphate Buffered Saline (PBS)-washed cells from the fresh specimens and fixed according to the prescribed protocol. The slides were stained in accordance with the procedure in the product insert.

Table 5 below shows the age and gender distribution for individuals studied at Study Site 2.

Table 5. Study Site 2 – Age and Gender Distribution

Sex	Female	Male
Total	**155**	**216**
Age		
< 1m	2	5
≥ 1m to < 2y	50	83
≥ 2y to < 12y	26	37
≥ 12y to < 18y	2	5
≥ 18y to < 21y	1	0
≥ 21y	74	86
Age Not Reported	0	0

Of the 371 fresh respiratory specimens tested, all were nasal/nasopharyngeal swab specimens. Three (3) were excluded from the performance analysis due to insufficient volume for the comparator methods, resulting in a total of 368 fresh nasal/nasopharyngeal swab specimens for analysis. Table 6 below shows the study results of the claimed specimen type at Study Site 2.

Table 6. Study Site 2 – Comparison of Results Using D^3 DFA MPV Kit, with Results Using the Composite Comparator Methods

Fresh Nasal/ Nasopharyngeal Swab	**Composite Comparator Methods**		
DS-FA	Positive	Negative	Total
Positive	41	1	42
Negative	17	309	326
Total	58	310	368
			95% CI
Sensitivity	41/58	70.7%	57.3% to 81.9%
Specificity	309/310	99.7%	98.2% to 100%

Study Site 3

Study Site 3 evaluated a total of 174 fresh respiratory specimens submitted, March 2006 through April 2006, to the laboratory for respiratory virus testing. Slides were prepared from Phosphate Buffered Saline (PBS)-washed cells from the fresh specimens and fixed according to the prescribed protocol. The slides were stained in accordance with the procedure in the product insert.

Table 7 below shows the age and gender distribution for individuals studied at Study Site 3.

Table 7. Study Site 3 – Age and Distribution

Sex	Female	Male	Sex Not Reported
Total	**78**	**95**	**1**
Age			
< 1m	1	1	0
≥ 1m to < 2y	19	37	0
≥ 2y to < 12y	16	17	0
≥ 12y to < 18y	3	6	0
≥ 18y to < 21y	2	0	0
≥ 21y	26	22	0
Age Not Reported	11	12	1

Of the 174 fresh respiratory specimens tested, 62 were nasal wash/nasopharyngeal aspirate specimens, and 110 were nasal/nasopharyngeal swab specimens. Of the 62 nasal wash/nasopharyngeal aspirate specimens, 30 were excluded from the performance analysis due to insufficient volume for the comparator method, resulting in a total of 32 fresh nasal wash/nasopharyngeal aspirate specimens for analysis. Of the 110 nasal/nasopharyngeal swab specimens, 44 were excluded from the performance analysis due to insufficient volume for the comparator method, resulting in a total of 66 fresh nasal/nasopharyngeal swab specimens for analysis. Tables 8 and 9 below show the study results of the claimed specimen types at Study Site 3.

Table 8. Study Site 3 - Comparison of Results Using D³ DFA MPV Kit, with Results Using the hMPV Real-time RT-PCR Followed by Bi-directional Sequencing Analysis Comparator Assay

Fresh Nasal Wash/ Nasopharyngeal Aspirate	**hMPV RT-PCR Followed by Sequencing Comparator Assay**		
DS-FA	Positive	Negative	Total
Positive	9	0	9
Negative	0	23	23
Total	9	23	32
			95% CI
Positive Percent Agreement*	9/9	100.0%	66.4% to 100%
Negative Percent Agreement*	23/23	100.0%	85.2% to 100%

Table 9. Study Site 3 - Comparison of Results Using D³ DFA MPV Kit, with Results Using the hMPV Real-time RT-PCR Followed by Bi-directional Sequencing Analysis Comparator Assay

Fresh Nasal/ Nasopharyngeal Swab	**hMPV RT-PCR Followed by Sequencing Comparator Assay**		
DS-FA	Positive	Negative	Total
Positive	3	0	3
Negative	1	62	63
Total	4	62	66
			95% CI
Positive Percent Agreement*	3/4	75.0%	19.4% to 99.4%
Negative Percent Agreement*	62/62	100.0%	94.2% to 100%

*Since the performance of the D³ DFA MPV Kit at clinical Study Site 3 was not assessed against the predetermined composite comparator methods, positive and negative percent agreements, instead of sensitivity and specificity, are used in the performance presentation.

Cultured Cells Testing

Performance characteristics of the D³ DFA MPV Kit testing cultured cell specimens were established during a prospective study at Quidel during the 2007 respiratory virus seasons (January-April 2008). 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 collection site, and that otherwise would have been discarded. Individual specimens were delinked from all patient identifiers and given a study sample code.

Performance of the D³ DFA MPV Kit testing cultured cell specimens was evaluated and compared to the same validated hMPV real-time RT-PCR followed by bi-directional genetic sequencing analysis comparator assay as described earlier, at clinical Study Site 4. Any cultured cell specimens that 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, were considered as hMPV-positives, and the real-time hMPV RT-PCR comparator assay negative cultured cell specimens were considered as hMPV-negatives.

Study Site 4

Study Site 4 evaluated a total of 74 freeze-thawed nasopharyngeal swab specimens that were cultured and stained in accordance with the D^3 DFA MPV Kit procedure. Table 10 below shows the study results testing cultured cell specimens at Study Site 4.

Table 10. Study Site 4 – Comparison of Results Using D^3 DFA MPV Kit, with Results Using the hMPV Real-time RT-PCR Followed by Bi-directional Sequencing Analysis Comparator Assay

Freeze-thawed Nasopharyngeal Swab Amplified in Cell Culture	**hMPV RT-PCR Followed by Sequencing Comparator Assay**		
DFA	Positive	Negative	Total
Positive	5	0	5
Negative	1	68	69
Total	6	68	74
			95% CI
Positive Percent Agreement	5/6	83.3%	35.9% to 99.6%
Negative Percent Agreement	68/68	100.0%	99.7% to 100%

Analytical Studies

Analytical study to support the cultured cells testing claim for the D^3 DFA MPV Kit

An analytical study using well characterized hMPV isolates in cell cultures was carried out by operators in three laboratories to complement the clinical study data generated by testing cultured cells at Study Site 4, in supporting the cultured cells claim for the D^3 DFA MPV Kit.

Cultured LLC-MK_2 cells were inoculated with known (well-characterized) isolates of hMPV obtained from the University of Iowa Emerging Pathogens Laboratory located in Coralville, IA. The cell culture was incubated for 48 hours to amplify the virus. Cell spots were prepared on glass slides as described in the D^3 DFA MPV Kit, using cell suspensions from each of the cultures at three concentration levels of infected cells (less than 10%, between 20 and 30%, and between 40 and 50%). The slides were sent to three outside investigators where they were stained using the D^3 DFA MPV Kit and examined. Each investigator provided interpretations of test results, i.e., presence or absence of fluorescent cells. Replicate prepared slides were evaluated at Quidel by staining and examining with an alternative non-labeled murine monoclonal antibody (MAb-8) to hMPV strain MPV75-1998/CAN98-75, which was developed at the CDC by standard methods. [**Note:** The MAb-8 is a research use only device that has not been cleared by the FDA. However, there is data presented in the literature addressing its specificity and sensitivity to hMPV (Landry et al, 2005)**Error! Bookmark not defined.**.] Quidel also performed hMPV RT-PCR/Sequencing Assay on each of the virus isolates to verify their identities as hMPV.

The D^3 DFA MPV Kit detected 98.7% (74/75) of the suspensions expected to contain infected cells from cell culture. The CDC MAb-8 reagent detected 100% (75/75) of the suspensions expected to contain infected cells from cell culture. One suspension, which was hMPV strain B2, was missed by the D^3 DFA MPV Kit reagent. The estimated infected level, as seen on the CDC MAb-8 stained suspension, was less than 2% infected. It is probable that due to sampling issues, the D^3 DFA MPV Kit reagent-stained slide did not contain any infected cells.

Table 11 below summarizes the data from the three external sites using the D^3 DFA MPV Kit, compared to the MAb-8 results generated at the Quidel facility.

Table 11. Comparison of Results Using D³ DFA MPV Kit, with Results Using the CDC MAb-8 Results (data from all 3 sites combined)

Culture Slides Made From Culture Suspensions	**CDC MAb-8**		
DFA	Positive	Negative	Total
Positive	74	0	74
Negative	1	51	52
Total	75	51	126
			95% CI
Positive Percent Agreement	74/75	98.7%	92.9% to 99.8%
Negative Percent Agreement	51/51	100.0%	93.0% to 100%

Table 12 below summarizes the data from the three external sites using the D³ DFA MPV Kit, compared to the hMPV RT-PCR/Sequencing Assay results generated at the Quidel facility.

Table 12. Comparison of Results using D³ DFA MPV Kit, with Results using the hMPV real-time RT-PCR followed by Bi-directional Sequencing Analysis Comparator Assay (data from all 3 sites combined)

Culture Slides Made From Culture Suspensions	**hMPV RT-PCR Followed by Sequencing**		
DFA	Positive	Negative	Total
Positive	74	0	74
Negative	1	51	52
Total	75	51	126*
			95% CI
Positive Percent Agreement	74/75	98.7%	92.9% to 99.8%
Negative Percent Agreement	51/51	100.0%	93.0% to 100%

*hMPV RT-PCR followed by sequencing data extrapolated from testing the lowest positive cell suspension.

Reproducibility

Assay reproducibility was assessed at 3 laboratory sites with a panel of proficiency-level antigen control slides. The reproducibility panel consisted of 5 panel members. Each panel member was a 2-well slide spotted with the same cell preparation in each well. The cell preparations used to construct the slides are the following:

- Non-infected LLC-MK_2 cells
- Low level hMPV (A1 strain) grown in LLC-MK_2 cells (manufactured to contain between 4-10% infected cells)
- Mid-level hMPV (A1 strain) grown in LLC-MK_2 cells (manufactured to contain between 20-30% infected cells)
- High level hMPV (A1 strain) grown in LLC-MK_2 cells (manufactured to contain between 50-75% infected cells)

Each panel was tested daily in two separate runs for 5 days by three different laboratories (30 total runs). The panel members were randomized with different slide identification numbers to act as a "blinded" panel. An hMPV Antigen Control Slide (two-well slide, one well contains cell culture-derived hMPV-positive cells and one well contains cell culture-derived negative cells) was stained according to the D³ DFA MPV Kit instructions for use with each run. The following results were recorded for both the control slides and the panel slides:

- Presence or absence of apple-green fluorescence
- Percent of cells exhibiting apple-green fluorescence

A single lot of D^3 DFA MPV Kit reagent was used. A total of 210 data points were included in the reproducibility study data analysis (7 samples and controls/run X 2 runs/day X 5 days X 3 sites = 210). The combined data from the three sites demonstrated that the detection of hMPV occurs in a reproducible manner. The presence of hMPV-infected cells was reported in 100% (120/120) of the wells in which infected cells were present. The combined data from the three sites also demonstrated that no hMPV was detected in non-infected cells. The absence of hMPV was reported in 100% (90/90) of the wells in which infected cells were not present. The total percent agreement for the D^3 DFA MPV Kit was 100% (210/210). Table 13 below summarizes the reproducibility study results.

Table 13. Reproducibility Study Results

	Panel Member	hMPV A1 Low Level	hMPV A1 Mid-Level	hMPV A1 High Level	hMPV A1 Negative	Positive Control	Negative Control	Total % Agreement
	Concentration	4 to 10% infected cells	20 to 30% infected cells	50 to 75% infected cells	Non-infected cells	50 to 75% infected cells	Non-infected cells	
Site 1	Agreement with Expected result	10/10 (100%)	10/10 (100%)	10/10 (100%)	20/20 (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%)	20/20 (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%)	20/20 (100%)	10/10 (100%)	10/10 (100%)	**70/70 (100%)**
	Total Agreement with Expected result	30/30 (100%)	30/30 (100%)	30/30 (100%)	60/60 (100%)	30/30 (100%)	30/30 (100%)	**210/210 (100%)**
	95% CI	88.4% to 100%	88.4% to 100%	88.4% to 100%	94.0% to 100%	88.4% to 100%	88.4% to 100%	**98.3% to 100%**

Analytical Sensitivity and Reactivity

Analytical limits of detection were addressed for each of 4 recognized genetic sublineages of hMPV (A1, A2, B1, and B2) for direct specimen testing and for testing of cultured specimens. Results of these studies also provide evidence of analytical reactivity with representatives of each of the four recognized genetic sub-lineages of hMPV.

Limit of Detection for Direct Specimens

Analytical detection limits on direct specimens for the D^3 DFA MPV Kit were addressed using quantified cultures of characterized isolates of each of the four recognized genetic sublineages of hMPV (A1, A2, B1, and B2). The infected culture cells from a 1,000 infected cells/mL culture were serially diluted with a suspension of non-infected LLC-MK$_2$ cells. Twenty-five microliter (25 µL) aliquots from each dilution level were spotted onto 10 replicate microscope slides, then fixed and 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 four hMPV genetic sublineages were defined as the lowest dilutions at which at least 9 out of 10 replicates were detected. Results are summarized in Table 14 below.

Table 14. Limit of Detection of the D³ DFA MPV Kit for Direct Specimens

Virus Strain	Infected Cells/mL	Number of replicates with positive cells	LOD determination
hMPV A1	1000	10/10	25 infected cells/mL
	200	10/10	
	100	10/10	
	50	9/10	
	25	9/10	
	12.5	2/10	
	6	0/10	
	3	2/10	
	1.5	0/10	
	0.8	0/10	
hMPV A2	1000	10/10	200 infected cells/mL
	200	10/10	
	100	8/10	
	50	6/10	
	25	6/10	
	12.5	0/10	
	6	1/10	
	3	1/10	
	1.5	0/10	
	0.8	0/10	
hMPV B1	250	10/10	50 infected cells/mL
	50	10/10	
	5	5/10	
	2.5	1/10	
	1.3	0/10	
	0.6	0/10	
	0.3	0/10	
	0.2	0/10	
	0.1	0/10	
	0.04	0/10	
hMPV B2	1000	10/10	100 infected cells/mL
	200	10/10	
	100	9/10	
	50	2/10	
	25	0/10	
	12.5	1/10	
	6	0/10	
	3	0/10	
	1.5	0/10	
	0.8	0/10	

Limit of Detection for Cell Culture Amplified Specimens

Detection limit on cell culture amplified specimens of the D³ DFA MPV Kit was addressed using a cell culture system. Analytical detection limits on cell culture amplified specimens for hMPV subtypes A1, A2, B1, and B2

were established with results reported in numbers of fluorescent cells per cell monolayer. Each master stock virus preparation was diluted in a ten-fold manner. Eight wells of a 48-well R-Mix cell culture plate were inoculated with 0.2 mL volumes of each dilution. The plates were centrifuged at 700 xg for 60 minutes, and then incubated at 35°C to 37°C for 48 hours. Each well was stained with the D^3 DFA MPV Kit then examined at 200x magnification and the number of fluorescent cells counted. In this study, the detection limit for the test on cell culture amplified specimens is defined as the lowest inoculum level at which positive wells (i.e., containing fluorescent cells) are observed, in terms of $TCID_{50}$. Table 15 below summarizes the results.

Table 15. Limit of Detection of the D^3 DFA MPV Kit for Cell Culture Amplified Specimens

Virus Strain	Concentration of Inoculum	Fluorescent staining cells/well
hMPV A1	50-$TCID_{50}$	47,39,41,31,26,30,21,29
	5-$TCID_{50}$	0,0,0,3,1,0,2,0
	0.5-$TCID_{50}$	0,0,0,0,0,0,0,0
hMPV A2	50-$TCID_{50}$	10,13,23,13,23,15,17,12
	5-$TCID_{50}$	3,1,1,4,2,2,0,0
	0.5-$TCID_{50}$	0,0,0,0,0,0,0,0
hMPV B1	50-$TCID_{50}$	36,56,23,41,28,29,34,28
	5-$TCID_{50}$	4,7,0,3,1,0,4,4
	0.5-$TCID_{50}$	0,0,0,0,0,0,0,0
hMPV B2	50-$TCID_{50}$	25,49,36,41,53,68,43,27
	5-$TCID_{50}$	0,3,1,1,5,6,3,5
	0.5-$TCID_{50}$	0,0,0,0,0,0,0,0

NOTE: Values are numbers of fluorescent staining cells per cell monolayer

Analytical Specificity

The D^3 DFA MPV Kit was tested for cross-reactivity against a variety of cells and microorganisms. Stringent conditions for cross-reactivity testing were achieved by using a high concentration of the D^3 DFA MPV Kit reagent and relatively high titers of microorganisms. The D^3 DFA MPV Kit reagent was prepared at 1.5X the concentration that is provided in the kit. No cross-reactivity was observed for 59 virus strains or for 16 host culture cell types. Twenty-five (25) bacterial strains, one yeast, three *Chlamydia* spp. and one protozoan were evaluated for cross-reactivity, including *Staphylococcus aureus*, a protein-A-producing bacterium. Except for *S. aureus*, which was cross-reactive with the D^3 DFA MPV Kit, all other microorganisms tested negative. Staining of *S. aureus* appeared as small points of fluorescence. The protein A produced by the bacterium, *S. aureus*, may bind the Fc portion of some fluorescein-labeled monoclonal 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 cell cultures with known or suspected bacterial contamination must, therefore, be interpreted with caution.

Fifty-nine (59) virus strains were tested for cross-reactivity. Depending on the particular virus, 71 to 714 $TCID_{50}$ viruses were inoculated into shell vial or multi-well plate cultures and incubated for 24 to 48 hours, to yield a 1+ to 4+ cytopathic effect, processed and stained with the 1.5X Metapneumovirus DFA Reagent according to the procedure as detailed in the product insert. Stained cells were examined at 200X magnification. No cross-reactivity was observed for the viruses listed below in Table 16 below.

Table 16. Virus Strains Tested for Cross-Reactivity with D³ DFA MPV Kit Reagent

Viruses	Strain or Type	D³ DFA hMPV Results	Inoculum ($TCID_{50}$)
Adenovirus	Type 1	–	714
	Type 3	–	714
	Type 5	–	714
	Type 6	–	714
	Type 7	–	714
	Type 10	–	714
	Type 13	–	714
	Type 14	–	714
	Type 18	–	714
	Type 31	–	714
	Type 40	–	714
	Type 41	–	714
Influenza A	Aichi (H3N2)	–	714
	Mal (H1N1)	–	714
	Hong Kong (H3N2)	–	714
	Denver (H1N1)	–	714
	Port Chalmers (H3N2)	–	714
	Victoria (H3N2)	–	714
	New Jersey (HSWN1)	–	714
	WS (H1N1)	–	714
	PR (H1N1)	–	714
Influenza B	Hong Kong	–	714
	Maryland	–	714
	Mass	–	714
	GL	–	714
	Taiwan	–	714
	JH-001 isolate	–	714
	Russia	–	714
RSV	Long	–	714
	Wash	–	714
	9320	–	714
Parainfluenza 1	C-35	–	714
Parainfluenza 2	Greer	–	714
Parainfluenza 3	C-243	–	714
Parainfluenza 4	M-25	–	714
Parainfluenza 4b	CH-19503	–	714
HSV-1	1(f)	–	71
	MacIntyre	–	71
HSV-2	MS	–	71
	Strain G	–	71
CMV	Towne	–	714
	Davis	–	714
	AD169	–	714
Varicella-zoster	Webster	–	71
	Ellen	–	71

Viruses	Strain or Type	D^3 DFA hMPV Results	Inoculum ($TCID_{50}$)
Echovirus	4	–	Control Slide
	6	–	Control Slide
	9	–	Control Slide
	11	–	Control Slide
	30	–	Control Slide
	34	–	Control Slide
Coxsackievirus	B1	–	Control Slide
	B2	–	Control Slide
	B3	–	Control Slide
	B4	–	Control Slide
	B5	–	Control Slide
	B6	–	Control Slide
Mumps	Bion (CDC V5-004)	–	Control Slide
Rubeola (Measles)	Bion	–	Control Slide

Seventeen (16) host culture cell types were tested for cross-reactivity. Cells were tested as intact monolayers or scraped and dotted cell spots and all were fixed in acetone. Confluent monolayers or cell spots were stained with the 1.5X preparation of the Metapneumovirus DFA Reagent according to the procedure as detailed in the product insert, and then examined for cross- reactivity. No cross-reactivity was observed for the following cell lines listed in Table 17 below.

Table 17. Cell lines Tested for Cross-Reactivity with D^3 DFA MPV Kit Reagent

Cell lines	Type	D^3 DFA hMPV Results	Monolayer/cell spot
A549	Human lung carcinoma	–	monolayer
Vero	African green monkey kidney	–	monolayer
HEp-2	Human epidermoid larynx carcinoma	–	monolayer
MRC-5	Human embryonic lung	–	monolayer
Mv1Lu	Mink lung	–	monolayer
MDCK	Canine kidney	–	monolayer
pRK	Rabbit kidney, primary	–	cell spot
pCMK	Cynomolgus monkey kidney, primary	–	cell spot
pRhMK	Rhesus monkey kidney, primary	–	monolayer
R-Mix	Mixed A-549 + Mv1Lu	–	monolayer
LLC-MK_2	Rhesus monkey kidney	–	monolayer
BGMK	African green monkey kidney	–	monolayer
MRHF	Human foreskin fibroblast	–	monolayer
WI-38	Human embryonic lung	–	cell spot
NCI-H292	Human pulmonary mucoepidermoid carcinoma	–	monolayer
RD	Human rhabdomyosarcoma	–	monolayer

Thirty (30) microorganisms, including 25 bacterial and one yeast cultures, three *Chlamydia* spp. and one protozoan commercially available control slides were tested for cross reactivity. Bacteria were cultured, processed as suspensions, then spotted on microscope slides at CFUs (colony forming units) ranging from 6.4 x 10^4 to 2.93 x 10^7/well in a 10 µL dot, depending on the bacterium, then stained with the 1.5X Metapneumovirus DFA Reagent according to the procedure as detailed in the product insert. Stained cells

were examined at 400X for cross-reactivity. Concentrations for each bacterial organism cultured by Quidel for cross reactivity testing were determined from suspensions of the bacteria in PBS by spectrophotometer according to McFarland standards of levels 1.0 and 2.0 (equaling approximately 3.0×10^6 and 6.0×10^6 CFU per mL). Slides were prepared with spots of 10 µL of the suspensions to give either 3.0×10^4 or 6.0×10^4 per spot. At the same time, 1 mL of each suspension was plated on an appropriate agar dish for colony confirmation. According to the confirmation agar cultures, initial concentrations of the bacterial organisms in the study ranged from 6.4×10^4 to 2.93×10^7 CFU/well. Except for *Staphylococcus aureus*, which was cross-reactive with the Metapneumovirus DFA Reagent, all other microorganisms tested negative. Reactivity with *S. aureus* is more than likely due to binding the protein A produced by *S. aureus*. Microorganisms tested are listed in Table 18 below.

Table 18. Microorganisms Tested for Cross-Reactivity with D³ DFA MPV Kit Reagent

Organism	D³ DFA hMPV Results	CFU tested
Bacteria		
Acholeplasma laidlawii	–	~1.0×10^7
Acinetobacter calcoaceticus	–	9.7×10^5
Bordetella bronchiseptica	–	1.8×10^5
Bordetella pertussis	–	4.7×10^6
Chlamydophila pneumoniae	–	Control Slides
Chlamydophila psittaci	–	Control Slides
Chlamydia trachomatis	–	Control Slides
Corynebacterium diphtheriae	–	2.5×10^6
Escherichia coli	–	2.6×10^5
Gardnerella vaginalis	–	5.0×10^5
Haemophilis influenzae type A	–	9.3×10^5
Klebsiella pneumoniae	–	6.4×10^6
Legionella pneumophila	–	6.5×10^4
Moraxella cartarrhalis	–	6.4×10^4
Mycoplasma hominis	–	~1.0×10^4
Mycoplasma orale	–	~1.0×10^4
Mycoplasma pneumoniae	–	~1.0×10^4
Mycoplasma salivarium	–	~1.0×10^7
Neisseria gonorrhoeae	–	1.3×10^6
Proteus mirabilis	–	2.1×10^6
Pseudomonas aeruginosa	–	1.0×10^7
Salmonella enteriditis	–	2.5×10^6
Salmonella typhimurium	–	1.8×10^6
Staphylococcus aureus	+	1.0×10^7
Streptococcus agalactiae	–	9.6×10^6
Streptococcus pneumoniae	–	8.0×10^5
Streptococcus pyogenes	–	2.9×10^7
Ureaplasma uralyticum	–	~1.0×10^4
Protozoan		
Trichomonas vaginalis	–	Control Slides
Yeast		
Candida glabrata	–	8.7×10^6

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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

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