



D³ Double Duet™

DFA Respiratory Virus
SCREENING & ID KIT

For export only – Not for sale in the U.S.

For the qualitative identification of influenza A, respiratory syncytial virus (RSV), metapneumovirus (MPV) and to screen for the presence of the viruses influenza B, adenovirus, parainfluenza virus types 1,2 and 3, in respiratory tract cells (direct specimens) by immunofluorescence using monoclonal antibodies.

FOR *IN VITRO* DIAGNOSTIC USE



INTENDED USE

The Diagnostic Hybrids, Inc. D³ Double Duet DFA (Direct Fluorescent Antibody) Respiratory Virus Screening & ID Kit is intended for the qualitative identification of influenza A, respiratory syncytial virus (RSV), metapneumovirus (MPV) and to screen for the presence of the viruses influenza B, adenovirus, parainfluenza virus types 1, 2 and 3, in respiratory tract cells (direct specimens) by immunofluorescence using monoclonal antibodies. Negative results do not preclude influenza virus infection and should not be used as the sole basis for diagnosis, treatment or other management decisions.

- Performance characteristics for influenza A were established when influenza A/H3 and A/H1 were the predominant influenza A viruses in circulation. When other influenza A viruses are emerging, performance characteristics may vary.
- If infection with a novel influenza A virus is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions for novel virulent influenza viruses and sent to state or local health departments for testing. Viral culture should not be attempted in these cases unless a BSL3+ facility is available to receive and culture specimens.¹

SUMMARY AND EXPLANATION OF THE TEST

With the addition of new antiviral drugs for the treatment of influenza,² more rapid and sensitive tests for respiratory virus detection^{3,4} and the increasing need to be more discriminating in the use of antibiotics,⁵ early detection and identification of the infecting viral agent has grown substantially in importance. Virus detection is becoming increasingly important in ruling out bacteria as the cause of respiratory infections. Virus detection and identification using fluorescent monoclonal antibodies continues to be the standard method of identification in virology laboratories.

Influenza A and B

Influenza viruses (family *Orthomyxoviridae*) contain a single-stranded RNA genome which is present in 8 separate segments of ribonucleoprotein. This segmentation of the genome is rare among viruses and probably contributes to the rapid development of new influenza strains through interchange of gene segments if two different viruses infect the same cell. There are 3 types of influenza, A, B and C. Type A has counterparts in birds and pigs, as well as humans while types B and C are known only in man. Due to the possibility of another

pandemic caused by influenza A, as occurred in 1918 when 25-35 million people worldwide died, the Centers for Disease Control (CDC) and the World Health Organization (WHO) maintain surveillance of influenza strains and make predictions of suitable strains for vaccine production.

Influenza infects an estimated 120 million people in the U.S., Europe and Japan each year and it is estimated that in the U.S. there are 75,000 deaths annually from pneumonia caused by influenza. Primary viral pneumonia and pneumonia secondary to bacterial infections are the primary causes of morbidity of the viral infection. Pandemics of influenza A occur about every 10 to 30 years and epidemics of either influenza A or B occur annually. Infections are seasonal, typically extending from November to April in the northern hemisphere. Complications tend to occur in the young, elderly and persons with chronic cardio-pulmonary diseases.

Incubation time is only 1-3 days with rapid spread by inhalation via aerial droplets and fomites. It is characterized by fever, myalgia, headache and pharyngitis. Superinfections by other viruses, e.g., adenovirus may occur.⁶

Adenovirus

Adenoviruses (family *Adenoviridae*) are non-enveloped, double-stranded DNA viruses. There are 49 serotypes, further divided into 6 groups, A to F, with most associated with respiratory and ocular infections. Generally, adenovirus infections in adults have a low morbidity with the exceptions of immunocompromised patients and individuals living in cramped quarters where infections can cause atypical pneumonia. Virus spread is commonly via aerial droplets and fomites where they infect the mucous membranes of the eye, respiratory tract and gut.⁷

Parainfluenza Virus types 1, 2 and 3

Parainfluenza viruses (family *Paramyxoviridae*) are enveloped viruses with a single, negative strand RNA genome. The 4 different types, 1 to 4, cause croup and viral pneumonia in children under the age of 5 years and cause upper respiratory illness in adults. Parainfluenza is the number two leading cause of lower respiratory illness in children (after RSV). Formation of IgA antibodies are induced with infection in the upper respiratory tract and are more protective than serum IgG. Since IgA antibodies do not cross the placenta, babies receive no maternal antibody protection from this virus and are likely to become infected during the first or second year after birth. Outbreaks caused by parainfluenza viruses occur during alternate years in the fall (P1 and P2) or throughout the year, with increased activity in the spring (P3).⁸

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.⁹ RSV is usually a seasonal (winter and early spring) infection with epidemics lasting up to 5 months. Peak mortality due to RSV occurs in 3-4-month old infants. There are two major subtypes, A and B; Subtype B is characterized as the asymptomatic strain that the majority of the population experiences. The more severe clinical illnesses involve Subtype A strains which tend to predominate in most outbreaks.¹⁰ RSV is the primary viral cause of lower respiratory disease in infants and young children. Re-infections do occur but tend to be limited to minor upper respiratory infections.¹¹

Human Metapneumovirus (hMPV)

Human metapneumovirus (hMPV) is a respiratory viral pathogen that causes a spectrum of illnesses, ranging from asymptomatic infection to severe bronchiolitis. In 2001, van den Hoogen et al.¹² described the identification of this new human viral pathogen 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 25% of all children aged 6-12 months who were tested in the Netherlands had detectable antibodies to hMPV; by age 5 years, 100% of patients showed evidence of past infection. A separate report¹³ from Australia 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 is likely to emerge in the coming years.

Little is yet known about the pathophysiology of hMPV infection, but similar to the related pneumovirus, human respiratory syncytial virus, hMPV appears to have a tropism for the respiratory epithelium. The patient may be asymptomatic or symptoms may range from mild upper respiratory tract complaints to severe bronchiolitis and pneumonia. Experts in the field of pneumovirus infections agree that the pathophysiology of hMPV infection likely parallels that of RSV infection, including the absence of viremia.

PRINCIPLE OF THE PROCEDURE

Diagnostic Hybrids, Inc. D³ Double *Duet* DFA Respiratory Virus Screening & ID Kit uses viral antigen-specific murine monoclonal antibodies that are directly labeled with either R-Phycoerythrin (influenza A or RSV) or Fluorescein Isothiocyanate (influenza B, adenovirus, parainfluenza 1, parainfluenza 2, parainfluenza 3, and metapneumovirus) for the rapid identification of these respiratory viruses.

The Kit contains two staining reagents, each includes a mixture of viral antigen-specific individual monoclonal antibodies: Reagent 1 - R-Phycoerythrin (influenza A) and Fluorescein Isothiocyanate (influenza B, adenovirus, parainfluenza virus types 1, 2, and 3); Reagent 2 - R-Phycoerythrin (RSV) and Fluorescein Isothiocyanate (MPV).

The cells to be tested, derived from either a clinical specimen or cell culture, are fixed in acetone. Reagents 1 and 2 are added to separate cell preparations and after incubating for 15 to 30 minutes at 35°C to 37°C, the stained cells are washed with the diluted Wash Solution (1X). 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.

With Reagent 1, the influenza A infected cells fluoresce golden-yellow, and cells infected with any of the other five viruses fluoresce apple-green, while non-infected cells contain no fluorescence but will be stained red by the Evans Blue counter-stain. If only golden-yellow fluorescent cells are seen the specimen can be reported as positive for influenza A. If apple-green fluorescent cells are seen, the particular virus may be identified using individual DFA Reagents on new, separate cell preparations (individual DFA Reagents are available from Quidel. If both golden-yellow and apple-green fluorescent cells are seen, the additional virus may be identified using individual DFA Reagents on new, separate cell preparations.

With Reagent 2, the RSV infected cells fluoresce golden-yellow, and cells infected hMPV will fluoresce apple-green, while non-infected cells contain no fluorescence but will be stained red by the Evans Blue counter-stain. If only golden-yellow fluorescent cells are seen the specimen can be reported as positive for RSV. If only apple-green fluorescent cells are seen the specimen can be reported as positive for MPV. If both golden-yellow and apple-green fluorescent cells are seen, the specimen can be reported as positive for both RSV and MPV.

REAGENTS AND MATERIALS PROVIDED

The D³ Double Duet DFA Respiratory Virus Screening & ID Kit contains the following:

Reagent 1 **2 mL**

One dropper bottle containing a blend of fluorescently labeled murine monoclonal antibodies directed against respiratory viral antigens of influenza A, influenza B, adenovirus, parainfluenza virus types 1, 2 and 3. The buffered, stabilized, aqueous solution contains Evans Blue as a counter-stain and 0.1% sodium azide as preservative.

Reagent 2**2 mL**

One dropper bottle containing a mixture of fluorescently labeled murine monoclonal antibodies directed against respiratory viral antigens of respiratory syncytial virus (RSV) and metapneumovirus (MPV). The buffered, stabilized, aqueous solution contains Evans Blue as a counter-stain and 0.1% sodium azide as preservative.

Respiratory Virus Antigen Control Slides**2 slides**

Individually packaged control slides containing wells with positive and negative control cells. Each positive well is identified according to the virus infected cells present, i.e., influenza A, influenza B, adenovirus, respiratory syncytial virus (RSV), parainfluenza 1, parainfluenza 2 and parainfluenza 3. The Negative well contains non-infected cells. Each slide is intended to be stained only one time.

hMPV Antigen Control Slides**2 slides**

Each contains one well of non-infected cells and one well each of MPV infected cells. Each slide is intended to be stained only one time.

40X Wash Solution Concentrate**25 mL**

One bottle of 40X PBS concentrate consisting of Tween 20 and 4% sodium azide (0.1% sodium azide after dilution to 1X using de-mineralized water).

Mounting Fluid**15 mL**

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

MATERIALS REQUIRED BUT NOT PROVIDED

- Fluorescence microscope with the correct filter combination for FITC (excitation peak = 490-nm, emission peak = 520-nm), which also can be used effectively for R-phycoerythrin (excitation peak = 490 nm, emission peak = 575 nm).
NOTE: The R-phycoerythrin fluorescence can also be observed using a rhodamine filter set (excitation peak = 566 nm, emission peak = 575 nm).
- **Influenza B DFA Reagent**, 2 mL. One dropper bottle containing fluorescein labeled murine monoclonal antibodies directed against antigens produced by influenza B virus infected cells. The buffered, stabilized, aqueous solution contains Evans Blue as a counter-stain and 0.1% sodium azide as preservative. (Available from Quidel)
- **Adenovirus DFA Reagent**, 2 mL. One dropper bottle containing fluorescein labeled murine monoclonal antibodies directed against antigens produced by adenovirus infected cells. The buffered, stabilized, aqueous solution contains Evans Blue as a counter-stain and 0.1% sodium azide as preservative. (Available from Quidel)
- **Parainfluenza 1 DFA Reagent**, 2 mL. One dropper bottle containing fluorescein labeled murine monoclonal antibodies directed against antigens produced by parainfluenza 1 infected cells. The buffered, stabilized, aqueous solution contains Evans Blue as a counter-stain and 0.1% sodium azide as preservative. (Available from Quidel)
- **Parainfluenza 2 DFA Reagent**, 2 mL. One dropper bottle containing fluorescein labeled murine monoclonal antibodies directed against antigens produced by parainfluenza 2 infected cells. The buffered, stabilized, aqueous solution contains Evans Blue as a counter-stain and 0.1% sodium azide as preservative. (Available from Quidel)
- **Parainfluenza 3 DFA Reagent**, 2 mL. One dropper bottle containing fluorescein labeled murine monoclonal antibodies directed against antigens produced by parainfluenza 3 infected cells. The buffered, stabilized, aqueous solution contains Evans Blue as a counter-stain and 0.1% sodium azide as preservative. (Available from Quidel)
- Coverslips (22 x 50 mm) for antigen control slides and for specimen slides.
- **Universal Transport Medium**. (Available from Quidel)
- Reagent grade acetone (>99% pure) chilled at 2°C to 8°C for fixation of prepared specimen slides.

NOTE: Keep the reagent grade acetone container tightly sealed to avoid hygroscopic absorption of water, which may cause a hazy, non-specific, background fluorescence. Glass pipettes should be used to pipette pure acetone since polystyrene disposable pipettes will be crazed and solubilized by the pure reagent.

- Sterile graduated pipettes: 10 mL, 5 mL, 1 mL.
- Sterile Pasteur pipettes or other “transfer”-type pipettes.
- Sodium hypochlorite solution (1:10 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 multi-well glass microscope slides (2-well and 8-well masked slides, available from Quidel).
- Blotters for multi-well glass microscope slides: Two- and 8-well absorbent blotters, used to blot excess liquid from the mask to prevent spread of liquid or stained cells from one well to the other.
- Sterile nylon flocked swab or polyester swab, non-inhibitory to respiratory viruses and tissue culture.
- Incubator, 35° to 37°C.
- Centrifuge with free-swinging bucket rotor.
- De-mineralized water for dilution of 40X Wash Concentrate Solution.
- PBS (Phosphate Buffered Saline), sterile, for use in rinsing and suspending cells.
- Wash Container: Beaker, wash bottle or Coplin jar for washing slides.
- Fixing Container: Coplin jar, slide dish or polyethylene holder for slides for use in fixing the cells on the slides.

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use.
- Manipulations which present potential hazards should be conducted in a Class II biosafety cabinet, with gloves worn at all times. Specimens should be autoclaved or disinfected prior to disposal.
- 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.
- Sodium azide is included in the 40X Wash Solution 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.
 - ▶ Avoid disposal of this material 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 skin contact occurs, flush with water immediately.
- Reagent 1 and Reagent 2 are supplied at working strength. Any dilution of these reagents will decrease sensitivity.
- Reagents should be used prior to their expiration date.
- Microbial contamination of reagents may cause a decrease in sensitivity.
- Store 1X Wash Solution in clean containers to prevent contamination.
- All specimens and materials used to process them should be considered potentially infectious and handled in a manner which prevents infection of laboratory personnel. Decontamination is most effectively accomplished using a solution of sodium hypochlorite (1:10 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.
- 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 cell culture procedures.
- Reusable glassware must be washed and thoroughly rinsed free of all detergents.
- Do not expose Reagent 1 or Reagent 2 to bright light during staining or storage.

- 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 WASH SOLUTION

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

STORAGE INSTRUCTIONS

TABLE 1. Reagent Kit Component Storage Conditions

Reagent 1	Store at 2°C to 8°C in the dark.
Reagent 2	
Mounting Fluid	
Respiratory Virus Antigen Control Slides	Store at 2°C to 8°C.
hMPV Antigen Control Slides	
40X Wash Solution Concentrate <u>NOTE:</u> The Concentrate may crystallize when stored at 2° to 8°C. The crystals will dissolve when the Concentrate is warmed to ambient temperature.	Store liquid at 2°C to 8°C prior to dilution.
1X Wash Solution	Store at ambient temperature (20°C to 25°C).

STABILITY

Reagent components will retain their full potency through the expiration date shown on the label of each bottle when stored at recommended temperatures. Light exposure of Reagent 1 and Reagent 2 should be kept to a minimum.

Discard 1X Wash Solution if it becomes cloudy.

SPECIMEN COLLECTION 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 staining of the respiratory tract cells should be attempted only by personnel that have been trained in such procedures. Care should be taken during all specimen collection and handling to avoid generation of aerosols.

Specimen Collection¹⁴

Aspirates and washes containing secretions from the nasopharyngeal epithelium contain large numbers of epithelial cells. Swabs from nasal, throat and nasopharyngeal areas also are appropriate specimen types for

cell culture testing method, although they may not contain as many columnar epithelial cells as do aspirates and washes.

Aspirates can be collected using a sterile, soft polyethylene #8 infant feeding tube attached to a disposable aspiration trap connected to a suction device.

Washes can be collected by instilling and aspirating 1 to 2 mL of saline in the patient's nostril while the patient is in a supine position.

Aspirates and washes should be diluted with equal volumes of transport medium contained in a centrifuge tube with several sterile glass beads.

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 on wet ice to the laboratory and processed and tested as soon as possible and then stored at 2°C to 8°C.

Specimens should be stored at 2°C to 8°C for no longer than 48 hours before being tested. If longer storage is required, the specimens should be frozen at –70°C or lower.

Freezing and thawing of specimens should be avoided since this will result in a loss of viability of viruses, leading to decreased sensitivity of the test.

Specimen Preparation

Vortex the specimen vigorously for 10 to 15 seconds prior to culture inoculation. Optional: Add a few sterile glass beads to the tube before vortexing, to break up the cells and release any virus. Repeat this step for each specimen.

PROCEDURE

Preliminary Comments, Precautions

- Adhere to the recommended volumes and times in the following procedure to ensure that accurate results are obtained.
- For specimen swabs received in transport medium with glass beads, vortex vigorously for about 15 seconds to dissociate adhered cells. For swabs not received in transport medium, transfer them to a tube of transfer medium containing glass beads and vortex vigorously for about 15 seconds to dissociate adhered cells.
- When staining with fluorescent reagents and examining cells microscopically for fluorescence, it is very important to include controls, both positive and negative, to monitor the procedure and performance of the reagents. It is recommended that such controls be run with each batch of patient specimens.
- The closed, humidified container for holding the slides during incubation should be kept in the incubator so it is at incubator temperature when the slides are placed in it. By doing this, the cells and antibody solution will come up to temperature more rapidly, yielding more intense stains in the specified periods of time.

Immunofluorescence Microscopy

- It is good practice to examine the positive and negative controls before examining the test specimens. If one of these fails to perform as expected, review the steps and conditions under which the test was performed to determine the cause(s). Do not report results until controls perform properly.
- There are three aspects of the fluorescence microscope that must be functioning properly and optimally in order to achieve maximum brightness of fluorescence:
 - ▶ The activation light source has a finite life and as it ages, its output decreases, resulting in lower fluorescence intensity from the stained cells.
 - ▶ 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 the particular fluor, in this case, fluorescein and R-phycoerythrin. Both fluorescein and R-phycoerythrin absorb strongly at 490 nm but fluorescein emits at 520 nm while R-phycoerythrin emits at 575 nm, allowing the visualization of both fluors as different colors, apple-green and golden-yellow, respectively, using the fluorescein filter set.
- There are several fluorescent artifacts that may be observed in the cell monolayers being examined:
 - ▶ Cell debris, lint, etc. can non-specifically adsorb antibodies in Reagent 1 and Reagent 2, resulting in highly intense fluorescence. These can be identified by their morphology, i.e., they don't have the appearance of a complete cell and typically do not appear to be a part of the monolayer like the other cells.
 - ▶ A low-grade hazy fluorescence may sometimes be seen, particularly in areas that have piled cells or are near holes in the cell monolayer. In both cases, the diffusion of entrapped antibodies is retarded during the wash step, resulting in the non-specific fluorescence.
 - ▶ Intense fluorescence around the periphery of slide wells is indicative of drying of Reagent 1 and Reagent 2 during incubation, suggesting that it was incubated too long or the humidity was not controlled.
 - ▶ Inadequate washing can lead to general low-grade fluorescence due to residual antibodies remaining on the monolayer of cells.
- Quenching or fading of the fluorescence of the stained cells may occur on exposure to light, particularly light of high intensity. Slides should be protected as much as possible during the assay.

DIRECT SPECIMEN TESTING

1. Vortex the specimen vigorously for 10 to 15 seconds.
2. Centrifuge at 400 to 600xg for 5 to 10 minutes.
3. Add 5 mL of PBS and vortex vigorously for 10 to 15 seconds.
4. Centrifuge at 400-600xg for 5 to 10 minutes.
5. Remove the supernatant and the mucus layer above the cell pellet taking care not to disturb the cell pellet.
6. Repeat steps 3 to 6 until the mucus layer has been completely removed.
NOTE: It is important to remove all the mucus since it can cause non-specific fluorescence.
7. Add 0.5 to 1-mL of PBS.
8. Mix the suspension by pipetting up and down to re-suspend the cell pellet, forming a slightly cloudy suspension.
NOTE: The quality of the slide preparation is dependent on the concentration of cells in the suspension; too many cells make it difficult to read the result and too few decrease the sensitivity of the procedure.
9. Spot 25 µL of the suspension onto two separate wells of a labeled, acetone-cleaned two well slide.
10. Repeat this step for each specimen.
11. Air dry the wells completely.
12. Fix the cells using fresh, chilled 100% acetone for 5 to 10 minutes at 20 °C to 25°C.
Caution: Acetone is volatile and flammable: keep away from open flames.
13. Remove the slides from the fixative and allow to air dry.

14. Add one drop of the Reagent 1 to completely cover the dried, fixed cells on the first well of each slide and to the appropriate wells of the Antigen Control Slide.
15. Add one drop of the Reagent 2 to completely cover the dried, fixed cells on the second well of each slide and to the appropriate wells of the Antigen Control Slide.
16. Place the slides in a covered, humidified chamber at 35° to 37°C for 15 to 30 minutes.
17. Rinse the stained cells using the 1X Wash Solution. For only a few slides, this can be done using a beaker of the 1X Wash Solution. For many slides, a slide carrier that holds 10-20 slides can be placed in its container of 1X Wash Solution and dipped up and down a minimum of four times for effective rinsing.
18. Discard the used Wash solution and repeat the washing step using new 1X Wash Solution.
19. Blot the excess 1X Wash Solution, add a small drop of Mounting Fluid to each cell-containing well and cover the wells with a coverslip.
20. Examine the stained, mounted cells using a fluorescence microscope with magnifications of 200X to 400X.

Quality Control

A fresh Respiratory Virus and hMPV Antigen Control Slide is stained each time the staining procedure is performed to ensure proper test performance. The positive wells will show multiple infected cells of either golden-yellow fluorescence (for influenza A or RSV) or apple-green fluorescence (for influenza B, adenovirus, parainfluenza 1, 2, or 3 or MPV), with negative cells staining a dull red due to the included Evans Blue counter-stain. The negative well will show only negative cells staining a dull red. Positive and negative controls must demonstrate appropriate fluorescence for specimen results to have validity. Controls may also aid in the interpretation of patient specimens.

INTERPRETATION OF RESULTS

It is recommended that controls be examined first to ensure proper test performance before examination of specimen results. A positive reaction is one in which golden-yellow or apple-green fluorescence is observed in the infected cells. Non-infected cells will stain dull red due to the Evans Blue counter-stain included in the Reagents. Technologists should not confuse cell clumps which may fluoresce due to entrapment of antibody with virus-specific staining. Occasionally, dead, rounded cells due to specimen toxicity or improper cell storage may non-specifically stain as a dull hazy fluorescence. Adequate washing between steps will help to eliminate this type of non-specific staining.

Fluorescent Staining Patterns of Respiratory Virus Infected Cells:

- The “typical” golden-yellow fluorescence staining pattern for each virus is as follows:
 - ▶ **Influenza A:** The fluorescence is cytoplasmic, nuclear or both. Cytoplasmic staining is often punctate with large inclusions.
 - ▶ **Respiratory Syncytial Virus:** The fluorescence is cytoplasmic and punctate with small inclusions in the syncytia.
- The “typical” apple-green fluorescence staining pattern for each virus is as follows:
 - ▶ **Influenza B:** The fluorescence is cytoplasmic, nuclear or both. Cytoplasmic staining is often punctate with large inclusions while nuclear staining is uniformly bright.
 - ▶ **Adenovirus:** The fluorescence is cytoplasmic and punctate or bright nuclear or both.
 - ▶ **Parainfluenza 1, 2, 3:** The fluorescence is cytoplasmic and punctate with irregular inclusions. Types 2 and 3 cause the formation of syncytia.
 - ▶ **Metapneumovirus:** The fluorescence is cytoplasmic and punctate with small inclusions in the syncytia.

The entire cell spot or monolayer of cells must be examined for virus-infected, fluorescent cells. If no fluorescent cells are found in either Reagent 1 or 2, the results of testing of the specimen should be reported as, “No influenza A or B, adenovirus, respiratory syncytial virus, parainfluenza 1, 2, 3, or metapneumovirus detected.”

If golden-yellow fluorescent cells are found in Reagent 1, the specimen should be reported as: “influenza A detected.”

If apple-green fluorescent cells are found in Reagent 1, the specimen may be reported as: “Presumptive detection of influenza B, adenovirus, parainfluenza 1, parainfluenza 2, or parainfluenza 3; identification of a specific virus not determined.”

In order to identify which of the other five respiratory viruses may be detected, one may continue with the Testing procedures, staining with individual virus DFA Reagents. The particular virus DFA Reagent that yields fluorescent cells represents the identification of the respiratory virus. In such a case, it should be reported as: “...detected by direct specimen testing”, where ‘...’ is the appropriate virus, e.g., influenza B, adenovirus, parainfluenza 1, parainfluenza 2, or parainfluenza 3.

If golden-yellow fluorescent cells are found in Reagent 2, the specimen should be reported as: “RSV Detected.”
If apple-green fluorescent cells are found in Reagent 2, the specimen should be reported as: “MPV Detected.”

Co-infection with more than one infecting virus present in the specimen has been reported in a number of studies. The presence of multiple viruses may be indicated when both golden-yellow and apple-green fluorescent cells are noted in a single result; also, a result of only apple-green fluorescent cells may be shown to be due to co-infection (which would be indicated when more than one well of the 8-well slide shows fluorescent cells during the individual virus DFA Reagent staining). In the case that multiple viruses are identified, the result should be reported as “... and ... detected by direct specimen testing.”

LIMITATIONS OF PROCEDURE

- Inappropriate specimen collection, storage, and transport may lead to false negative results.¹⁵
- Incubation times or temperatures other than those cited in the test instructions may give erroneous results.
- Detection of viruses 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 antigens detected by the antibodies are undetermined.
- Since the monoclonal antibodies have been prepared using defined 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.
- Performance of the kit can only be assured when components used in the assay are those supplied by Quidel.
- Prolonged storage of Reagent 1 and Reagent 2 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 will specifically bind 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.

EXPECTED VALUES

Respiratory virus infections are often seasonal, with influenza typically extending from November to April in the northern hemisphere, and adenovirus infections occurring more often during late winter to early summer. RSV is usually a seasonal (winter and early spring) infection as well, with epidemics lasting up to 5 months,

while outbreaks caused by parainfluenza viruses may occur throughout the year. Prevalence data for hMPV is still being generated.

The clinical studies described in Section X ('Specific Performance Characteristics') were comprised of respiratory specimens collected during the winter to early spring months of 2005/2006. Prevalence of the respiratory viruses within the population of specimens that was prospectively collected and tested fresh from one clinical site is noted in TABLE 2 below:

TABLE 2. Prevalence of Respiratory Viruses in Study Population

Expected Values	Adenovirus	Influenza A	Influenza B	Parainfluenza 1	Parainfluenza 2	Parainfluenza 3	Respiratory Syncytial Virus
Fresh Specimens (n = 326)	18	32	19	2	0	5	18
Prevalence	5.5%	9.8%	5.8%	0.6%	0%	1.5%	5.5%

SPECIFIC PERFORMANCE CHARACTERISTICS

Clinical Performance Characteristics

The monoclonal antibodies used in Reagent 1 (influenza A, influenza B, adenovirus, parainfluenza 1, parainfluenza 2 and parainfluenza 3) and the RSV and MPV monoclonal antibodies of Reagent 2 were evaluated at three sites against a currently-marketed DFA Screening & Identification Kit for the detection of these viruses in respiratory tract cells. This study included eight hundred and nineteen (819) respiratory specimens. Of these, 18 specimens had insufficient cell numbers to be evaluated, and one (1) which could not be evaluated because it exhibited non-specific staining from the Normal Mouse Gamma Globulin DFA Reagent, leaving 800 for analysis. The summary of these evaluations is given in Table 3 below. There were ten (10) specimens identified with co-infections as follows: three (3) Flu A+Para 3, one (1) Flu B+Para 2, one (1) Flu B+Para 3, one (1) RSV+Para 1, three (3) RSV+Para 3 and one (1) Adeno+Para 3. Because of these ten (10) co-infections, the ID screening results add up to 10 more than the Screen+ results.

Table 3. Direct Specimen Results for D³ DFA Reagents (Subject) Compared to Predicate Devices

	Negative	Screen +	Adenovirus	Influenza A	Influenza B	Parainfluenza 1	Parainfluenza 2	Parainfluenza 3	Respiratory Syncytial Virus
Predicate Results:	542	258	26	117	37	5	3	11	69
Subject Results:	538	262	26	117	38	5	3	14	69
Positive Percent Agreement ^a (PPA)		100%	100%	100%	100%	100%	100%	100%	100%

	Negative	Screen +	Adenovirus	Influenza A	Influenza B	Parainfluenza 1	Parainfluenza 2	Parainfluenza 3	Respiratory Syncytial Virus
95% CI ^b - PPA		98.5%-100%	87.1%-100%	96.8%-100%	90.6%-100%	56.5%-100%	43.9%-100%	74.1%-100%	94.7%-100%
Negative Percent Agreement ^c (NPA)	99.3%		100%	100%	99.9%	100%	100.0%	99.6%	100%
95% CI – NPA	98.1-99.7%		99.5-100%	99.4-100%	99.3-99.9%	99.5-100%	99.5-100%	98.9-99.9%	99.5-100%

^a "Positive Percent Agreement", or "PPA", values were calculated according to $\frac{\text{Total Number of Positive Results in Agreement by both Subject and Predicate Tests}}{\text{Total Number of Positive Results in Agreement by both Subject and Predicate Tests} + \text{Number of Results Positive by Predicate but Negative by Subject}}$ multiplied by 100%.

^b "95% CI" refers to 95% Confidence Intervals, which were calculated according to Exact method (Clopper, C. and S. Pearson, *Biometrika* 26:404-413, 1934).

^c "Negative Percent Agreement", or "NPA", values were calculated according to $\frac{\text{Total Number of Negative Results in Agreement by both Subject and Predicate Tests}}{\text{Total Number of Negative Results in Agreement by both Subject and Predicate Tests} + \text{Number of Results Negative by Predicate but Positive by Subject}}$ multiplied by 100%.

The MPV antibodies (D³ DFA MPV) used in Reagent 2 were evaluated by Direct Specimen (DS) DFA and rapid culture (RC) at two sites with a total of 681 specimens. Of these, 1-DS and 1-RC were indeterminate and 18 specimens had insufficient cell numbers to be evaluated, leaving 661 for analysis. The summary of these evaluations is given in Table 4, below:

Table 4. D³ DFA MPV Results for Direct Specimen Compared to Rapid Culture

	Site 1		Site 2		Combined				
	Rapid Culture		Rapid Culture		Rapid Culture				
	+	-	+	-	+	-			
Direct Specimen	+	14	6	+	24	7	+	38	13
	-	1	358	-	1	250	-	2	608
	PPA =	93.3%		96.0%		95.0%			
	95% CI PPA =	70.2 - 98.8%		80.5 - 99.3%		83.5 - 98.6%			
	NPA =	98.3%		97.3%		97.9%			
	95% CI PPA =	96.4 - 99.2%		94.5 - 98.7%		96.4 - 98.8%			

Analytical Performance Characteristics – Cross-reactivity Testing

Reagent 1 and 2 of the D³ Double Duet DFA Respiratory Virus Screening & ID Kit were tested for cross-reactivity against a wide variety of cells and microorganisms. No cross-reactivity was observed for sixty-seven (67) virus strains (cultured and processed for staining) or for eighteen (18) host culture cell types. Thirty (30) bacterial cultures were stained and examined for cross-reactivity, including *Staphylococcus aureus*, a protein-A-producing bacterium. Staining of *S. aureus* appeared as small points of fluorescence (see 'Limitations of Procedure', Section VIII.9) while all other bacterial cultures were negative.

Stringent conditions for cross-reactivity testing were achieved by using high concentration DFA Reagents and high titers of microorganisms. The DFA Reagents (i.e., directly fluoresceinated monoclonal antibodies) were prepared at 1.5X the concentration that is provided in the kit. Each of the tested viruses was prepared as infected cell monolayers (250 infected cells inoculated into a shell-vial culture and incubated for 24 to 48 hours, to yield a 3+ to 4+ infection), and processed and stained with the 1.5X DFA Reagents according to the procedure detailed in this product insert. Bacterial strains were cultured, processed as suspensions, then spotted on microscope slides (yielding > 150 bacteria per 400X microscope field), then stained with the 1.5X DFA Reagents according to the procedure in this product insert. Cell cultures were stained as confluent monolayers.

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., please contact your local distributor. Additional information about Quidel, our products, and our distributors can be found on our website quidel.com.

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I-01-320000 – D³ Double Duet DFA Respiratory Virus Screening & ID Kit

IVD



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GLOSSARY

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 NaN₃

Contents/Contains

NaN₃ 4%

Contents/Contains
