LIGHT DIAGNOSTICS™

SimulFluor® RSV/FLU A DFA KIT

A Direct Immunofluorescence Assay for the Differentiation of RSV and Flu A in Cell Culture

3129

125

CE

EMD Millipore Corporation
28820 Single Oak Drive Temecula, CA 92590 • United States
Tel. : +1 (951) 676-8080 • Fax : +1 (951) 676-9209
www.millipore.com

Millipore (UK) Ltd.
Fleming Road, Kirkton Campus • Livingston EH54 7BN
United Kingdom
Tel. +44 1506 404000 • Fax +44 1506 404001

This page intentionally left blank

Intended Use

The **Light Diagnostics™ SimulFluor® RSV/Flu A DFA Kit** is intended for use in respiratory specimens such as throat, nasal and nasopharyngeal swabs, throat washes, aspirates, broncho-alveolar lavages from patients with febrile respiratory illness following amplification of virus in cell culture. Negative results do not preclude influenza virus infection and should not be used as the sole basis for treatment or other management decision.

Performance characteristic 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 department for testing. Viral culture should not be attempted in these cases unless a Biosafety Level 3 (BSL 3+) facility is available to receive and culture specimens.

IVD

Summary and Explanation

Respiratory syncytial virus (RSV) occupies the genus *Pneumovirus* in the family *Paramyxoviridae*. It is a pleomorphic virus ranging from approximately 100 to 300 nm in diameter containing a linear, non-segmented, single-stranded, negative sense RNA. The envelope contains projections on the outer surface composed of two highly antigentic glycoproteins, F and G, and a major structural protein, N, in the nucleocapsid. The F, G, and N structural proteins divide RSV into two major antigenic groups; the “prototype” strains are Long (group A) and CH-18537 (group B) (1-4). RSV causes major epidemics of severe respiratory illness during the winter and spring seasons throughout the world. Because natural immunity to RSV is weak, these epidemics tend to occur every or every other year.

RSV can be isolated from nasopharyngeal aspirates, nose or throat swabs, or lung specimens taken within the first few days of illness. The virus grows in human epithelial cells such as HEp2, primary rhesus monkey kidney (RMK),

A549, MRC-5, and NCI-H292. Rapid and direct tests on suitable clinical specimens are diagnostic of RSV infections provided that standardized reagent and adequate controls are used. Confirmation by virus isolation is desired wherever possible.

Hospitalized RSV-infected infants have been successfully treated with ribavirin, administered as an aerosol. However, this requires specialized care, and is not given routinely (4). Immune globulin is also available for treatment. RSV vaccines have a long history of failure but are still under intense development.

Influenza A virus is a member of the family of *Orthomyxoviridae*. It is a large, enveloped virus, 110 nm in diameter, and containing a segmented, single-stranded RNA, with hemagglutinin (HA) and neuraminidase (NA) projections protruding through the glycoprotein membrane (5-7). Influenza A has a high frequency of mutation and cause periodic epidemics of influenza worldwide. Epidemics are particularly severe when the mutations have resulted in dramatic shifts in the HA or NA structure such that circulating antibodies in a community of people who do not recognize the virus strains (6-8). Specificity in influenza viruses is conferred by antigenic differences in two of the major structural proteins, the internal nucleoprotein (NP) and the matrix protein (M) (5-8).

Influenza infections are characterized by tracheobronchitis, pharyngitis, myalgia, fever, headache, and malaise (5,6,9). Minimal coryza often distinguishes influenza from other viral respiratory illnesses. It is highly contagious among adults, being spread by aerosolized droplets and fomites. As with RSV, an incubation period of 1 to 4 days helps the virus spread rapidly within communities and within hospital wards. The most significant complication of influenza is pneumonia, which occurs by itself or may be mixed with bacterial pneumonia. Pneumonia, occurs most frequently in the elderly, in patients with weakened immune systems, or in patients with underlying diabetes, heart or lung disease, or chronic kidney disease. Children are more likely to experience additional symptoms of gastrointestinal pain, vomiting, myositis, otitis media, conjunctivitis, and croup.

The increase in deaths from pneumonia during “flu season” is assumed to be due to influenza virus and is used to track influenza epidemics and to check the efficacy of influenza vaccines. Less common complications in adults include Reye’s syndrome or other CNS involvement, cardiac symptoms, sinusitis, and otitis media.

Amantadine and its analog, rimantadine, are effective in preventing up to 90% of influenza A infections and 100% of illnesses if taken prophylactically (5,10). They are also effective therapeutically by reducing the duration of illness if taken

within the first 2 days of illness. Ribavirin may be effective in treating influenza A when administered by an aerosol route. Vaccines against influenza A constitute a major public health effort as new strains are identified. Current influenza vaccines generally have an efficacy rate of 70-90% (5,7).

Influenza viruses can be grown in RMK, MDCK (Madin-Darby canine kidney), and occasionally other cell lines, depending on the particular strain of virus (11-13). Trypsin added to the culture fluid at ~2 µg/mL concentration greatly aids in influenza virus isolation in MDCK cells.

Both standard culture and shell vials may be used. Direct tests, including immunofluorescence, (FA) and enzyme immunoassays (EIA) (11,14-17).

Adequate specimens include nasal washes, throat swabs, and broncholaveolar lavages. Any direct test with standardized reagents is diagnostic of influenza infection, especially if confirmed by virus isolation.

Test Principle

Light Diagnostics™ SimulFluor® RSV/Flu A DFA Kit utilizes a single reagent for the simultaneous detection and identification of RSV and influenza A. The primary component, specific for RSV, will bind to RSV F and G antigens in RSV-infected cells. The secondary component, specific for influenza A, will bind to influenza A nucleoprotein in influenza A-infected cells. Unbound reagent is removed by rinsing with phosphate buffered saline (PBS). The antigen-antibody complex can be visualized by fluorescence microscopy. The RSV antigen-antibody complex will exhibit an apple-green fluorescence and the influenza A antigen-antibody complex will appear yellow-gold. Uninfected cells stain a dull red due to the presence of Evans blue in the reagent.

Kit Components

1. SimulFluor® RSV/Flu A Reagent - REF 5245: One (1) 5 mL dropper vial containing a primary component specific for RSV and a secondary component specific for influenza A, protein stabilizer, Evans blue and 0.1% sodium azide (preservative).

   Amount provided is sufficient for 125 tests. Estimate is based on test drop of 40µL; actual number of tests may vary.

2. RSV Control Slides - REF 5012: Two (2) slides containing one well of RSV-infected cells and one well of uninfected cells.

3. Influenza A & B Control Slides - REF 5010: Two (2) slides containing one well of influenza A-infected cells, one well of influenza B-infected cells and two wells of uninfected cells.

4. Phosphate-Buffered Saline (PBS) - REF 5087: One (1) packet of phosphate buffered saline (PBS) salts to yield 1 liter upon reconstitution with distilled water. Store in a clean, closed container at room temperature.

5. Tween® 20/Sodium Azide Solution (100X) - REF 5037: One (1) 10 mL vial containing Polyoxyethylene Sorbitan Monolaurate (Tween 20) and Sodium Azide (NaN_3) concentrate to be diluted 1:100 in PBS.

6. Mounting Fluid - REF 5013: One (1) 10 mL dropper vial containing Tris-buffered glycerin, a fluorescence enhancer and 0.1% sodium azide (preservative). Store at 2°C - 25°C.

Materials Required But Not Provided

- Acetone, reagent grade or better; stored in glass
- Deionized or distilled water
- Virus culture controls (reference RSV and influenza A strains available from American Type Tissue Culture Collection (ATCC®), Manassas, VA.
- Sodium hypochlorite solution, 0.05% (1:100 dilution of household bleach)
- RSV and influenza susceptible cell lines such as HEp-2, MRC-5, primary monkey kidney (PMK), MDCK cell lines (14,15,16), or other influenza susceptible cell lines (e.g. LLC-MK2, etc.) Appropriate cell lines can be obtained from the American Type Tissue Culture Collection (ATCC®), Manassas, VA. Tissue culture media such as RPMI or Eagle's Minimum Essential Medium (EMEM) with fetal bovine serum (FBS) and antibiotics, or equivalent
- Viral transport medium, which is non-inhibitory to RSV or influenza A (Hanks balanced salt solution (HBSS) with antibiotics, or equivalent)
- Acetone cleaned glass slides, non-fluorescing

- No. 1 coverslips
- Aspirator device with disposable sterile Pasteur pipettes
- Centrifuge capable of 700 to 950 x g with biohazard buckets and adapters for shell vials
- Fluorescence microscope with 100 watt mercury or halogen lamp, appropriate filter combination for FITC (excitation peak = 490 nm, emission peak = 520 nm), 160 to 200x and 400x magnification (dry objective)
- Optional: filter combination for TRITC (excitation peak = 550 nm, emission peak = 570 nm)
- Forceps
- Humid chamber
- Incubator, 37 ± 1°C
- Syringe and needle or other implement to remove coverslip from shell vial
- Ultrasonic water bath
- Vortex mixer or sonicator

Warnings and Precautions

- The sodium azide used as a preservative in the **SimulFluor®** reagent, PBS/Tween 20 solution and mounting fluid is toxic if ingested. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. Upon disposal, flush with large volumes of water to prevent build-up in plumbing.
- Pooling or alteration of any reagent may cause erroneous results.
- Do not substitute reagents from other manufacturers.
- Incubation times or temperatures other than those specified may give erroneous results. Any such change must be validated by the user.
- Do not allow shell vials or slides to dry at any time during the staining procedure.
- Handle all specimens and materials coming in contact with them as potentially infectious and dispose of with proper precautions. Decontaminate with 0.05% sodium hypochlorite.

- Acetone is extremely flammable and harmful if swallowed or inhaled. Keep away from heat, sparks or flame. Avoid breathing vapor. Use adequate ventilation.
- Do not mouth pipette reagents.
- Avoid contact with Evans blue (present in **SimulFluor®** RSV/Flu A Reagent REF 5245) as it is a potential carcinogen. If skin contact occurs, flush with large volumes of water.
- Mounting Fluid REF 5013 contains a fluorescence enhancer that may be destructive to mucous membranes. Avoid direct skin or mucous membrane contact. If contact occurs, flush with large volumes of water.
- 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 department for testing. Viral culture should not be attempted in these cases unless a Biosafety Level 3 (BSL 3+) facility is available to receive and culture specimens.

Stability and Storage

When stored at 2° to 8°C, the **SimulFluor® RSV/Flu A DFA Kit** is stable up to the expiration date printed on the kit label. Do not freeze or expose to elevated temperatures. Discard any remaining reagents after the kit expiration date.

Specimen Collection

Appropriate specimen collection, transport, processing, and storage is of fundamental importance in isolation and confirmation of RSV and influenza A infection. Aspirations of respiratory secretions are the specimens of choice for culture although nasopharyngeal or throat swabs may also be used.

Nasal washes and nasopharyngeal or tracheal aspirates are ideal for specimen collection, as ample epithelial cells may be obtained. Nasopharyngeal wash specimens are collected by placing 3-5 mL of saline into the patient's nostril while the patient is in a supine position. The saline is gently suctioned out using a syringe, rubber bulb or catheter attached to a mucous trap.

Nasopharyngeal swabs, throat swabs, or nasal swabs often do not contain adequate numbers of ciliated and columnar epithelial cells.

Transport specimen on wet ice or cold pack to the laboratory immediately after collection. Specimen for RSV should be processed and inoculated in cell culture as soon as possible upon receipt. Specimens to be used for culture isolation should be frozen at -70°C if processing will not be performed within 72 hours of collection. Avoid repeated freeze-thaw cycles.

Training in specimen collection is highly recommended because of the importance of specimen quality. Additional information on specimen collection techniques can be found in Manual of Clinical Microbiology, Balows, A. *et al*., eds. 5th ed. (1991). *American Society for Microbiology, Washington, D.C.,* Influenza Viruses, Ch. 81; Respiratory Syncytial Virus, Ch. 83; Animal and Animal Cell Culture Systems, Ch. 19 and Clinical Microbiology Procedures Handbook, Volume 2, Isenberg, H.D. ed. (1992). *American Society for Microbiology, Washington, D.C*., Selection, Collection and Transport of Specimens for Viral and Rickettsial Cultures, Section 8.2.

For appropriate specimen transport refer to 42 CFR (Code of Federal Regulations) part 72.

Specimen Processing

Before processing specimens, ascertain that they have been properly collected and transported. Improper handling of specimens may cause erroneous results.

Processing Specimens for Culture/Isolation:

<u>Nasopharyngeal aspirate, tracheal aspirate, nasal wash, bronchial wash:</u>

1. Remove specimen from original container and place in 10 to 15 mL sterile centrifuge tube.
2. Add sufficient cold PBS to bring the volume to 2.0 to 2.5 mL.
3. Vortex to mix. If specimen is to be used for viral isolation only, proceed to step 4.
4. Sonicate specimen at 8 to 12 Kc/sec for 30 to 60 seconds to disrupt cells and release viral particles.
5. Centrifuge specimen at 300 to 500 x g at 2° to 8°C for 10 minutes to sediment cellular debris.
6. Remove supernatant from cellular debris.

7. The supernatant can be mixed with 10X antibiotic solution and allowed to stand at 2° to 8°C for 30 to 60 minutes before inoculation to avoid bacterial overgrowth.

Nasopharyngeal swab, throat swab, nasal swab:

1. Vortex or agitate specimen vigorously to dislodge cells from the swab.
2. For increased viral recovery, add a few sterile glass beads to the specimen and vortex for one minute or sonicate at 8 to 12 Kc/sec for 30 to 60 seconds.
3. Discard the swab into sodium hypochlorite solution.
4. Centrifuge the specimen at 300 to 500 x g at 2° to 8°C for 10 minutes.
5. Use the supernatant as inoculum material.

Culture/Isolation and Preparation for Staining

Inoculation of Standard Tubes or Shell Vials:

1. Immediately prior to inoculation with specimens, examine cell cultures for proper morphology.
2. Aspirate growth medium from standard tubes or shell vials to be inoculated.
3. Add 0.2 to 0.5 mL of the inoculum to each tube or shell vial.
4. Inoculate specimens into appropriate cell lines.
5. Centrifuge shell vials at room temperature for 30 minutes at 500 to 700 x g.
6. Adsorb inoculum onto standard tube monolayers by incubation on a slant rack at 35-37°C for 1 hour.
7. After adsorption or centrifugation, aspirate inoculum and add sufficient maintenance medium to completely cover the cell monolayer.
8. Incubate at 35-37°C in roller drums or stationary racks.
9. The monolayer can be gently rinsed 2 to 3 times with pre-warmed maintenance media if it appears that the specimen may be toxic to the cell monolayer.

10. Renew the cell culture medium every 3 to 5 days.

    ***Note**: A sample of each lot of the cell lines used for cell culture should be inoculated with representative RSV and influenza A strains to establish susceptibility to infection and subsequent development of CPE. Uninoculated cell cultures should also be grown and examined daily for contaminating viruses or mycoplasma. This will act as a control for normal cell morphology and could be useful in detecting early CPE. Unless these control cell cultures show appropriate growth, the results of the cell culture isolation should be considered invalid.*

Preparation of Tissue Culture for Staining:

1. Examine tissue culture tubes and/or shell vials daily for cytopathic effect. Shell vials can be stained when CPE is present or at optimal time points determined by the individual laboratory. If CPE is observed, the cells can be scraped or trypsinized from the tube (or shell vials) and a multi-welled slide prepared for staining.

2. To prepare multi-welled slides, aspirate the medium from the tube (or shell vial). Store unused medium at 2° to 8°C until testing has been completed. If a repeat is required, viral isolation can be attempted from this medium.

3. Rinse the cell culture gently three times with 1 to 2 mL of HBSS. Discard all rinses into sodium hypochlorite solution.

4. Add one tenth of the original culture volume of trypsin and let stand for 30 seconds. Gently tap the culture vessel to loosen the cells. Resuspend cells with 2 mL HBSS. Centrifuge the cell suspension at 300-500 x g for 10 minutes. Resuspend the cell pellet in 0.3 mL of sterile PBS to give a slightly suspension. The slides must exhibit at least two cells per field at 250x magnification to be considered adequate for detection.

   ***Note:** Alternately, the cell monolayer may simply be scraped from the tube using a glass rod or sterile pipette. Resuspend and centrifuge cells as described above.*

5. Spot the cell suspension onto an acetone cleaned slide and allow to dry. Fix the slide in chilled acetone (2° to 8°C) for 10 minutes and air dry completely. Store unused slides with desiccant at -20°C.

Test Procedure

Reagent Preparation:

PBS/Tween 20 Solution - Dissolve the contents of the PBS packet in 950 mL of deionized or distilled water. Add the contents of the Tween 20/Sodium Azide vial to the PBS. Mix thoroughly; Q.S. to one liter with deionized or distilled water. Transfer to a clean labeled storage container and cap tightly; store at room temperature. Discard if PBS/Tween becomes turbid or a precipitate develops.

All other reagents are provided ready to use.

Suggested Direct Immunofluorescence (Staining) Procedure:

1. Allow the acetone fixed control slide and/or test slide and reagents to equilibrate to room temperature.

   Note: *Do not allow slides to dry at any time during the staining procedure.*

2. Add sufficient **SimulFluor® RSV/Flu A Reagent** REF 5245 to cover the cells; 1 drop for cell spots and 4-6 drops for shell vials.
3. Incubate the slide at 37°C for 15 minutes in a humid chamber.
4. Rinse the slide gently with a squirt bottle of PBS/Tween 20 solution for 10-15 seconds to remove excess monoclonal antibody solution, taking care to direct the stream away from the well. For shell vials: aspirate reagent from vial and gently wash each shell vial 3 times with 1mL PBS/Tween 20 solution.
5. Shake off excess reagent from the slide.
6. Mount under a coverslip using an aqueous Mounting Fluid pH 8.5,

   REF 5013 or equivalent. For shell vials: Aspirate PBS/Tween from shell vials. Raise each coverslip using a bent needle affixed to a small syringe and carefully remove with forceps. Mount each coverslip CELL SIDE DOWN on a glass slide with Mounting Fluid.
7. Wipe excess fluid from the edges of the slide.

   Note: *For best results, read slides immediately after preparation. If slides are to be stored after staining, store at 2° to 8°C, in a secure container in the dark.*

8. Examine slides, using a fluorescence microscope at 100-200x for cells exhibiting fluorescence. Detailed examination may be carried out at 400x magnification.

   Note: *Performance of the fluorescence microscope is of critical importance in achieving satisfactory test results. While objectives, bulb intensity and wattage, and filters may affect results, use of a positive control will verify functioning of reagents, culture methodology and microscope.*

Interpretation of Results

Quality Control

Control slides should be tested with each batch of samples. The control slides supplied in the kit are intended to demonstrate the proper function of kit components and immunofluorescence staining procedure.

Control slides prepared from RSV-infected, influenza A-infected, and uninfected cells may also be tested to ensure proper staining procedures during direct specimen analysis. In addition, standard tubes or shell vials inoculated with reference RSV and influenza A strains and uninoculated cultures should be maintained and tested to ensure proper culture isolation and staining procedures.

Note: *Scan the entire slide well for the presence of cell-associated fluorescence.* Shell vials and/or control slides prepared from RSV-infected, influenza A-infected and uninfected cells should be tested to ensure proper staining procedures during specimen analysis.

When viewed in a fluorescence microscope an RSV- positive reaction is indicated by a bright apple-green fluorescence in the nucleus and/or cytoplasm of the infected cells. An influenza A-positive reaction is indicated by a yellow-gold fluorescence in the nucleus and/or cytoplasm of the infected cells.

Positive staining for either RSV or influenza A is indicated by the presence of 2 or more intact cells exhibiting specific fluorescence. A presumptive negative result is indicated by the absence of fluorescence in a minimum sampling of 20 epithelial cells. A sample containing fewer than 20 epithelial cells is considered inadequate, and the test is thus invalid.

Caution: Fluorescent staining of cell fragments, due to trapping of reagent in such debris, should be ignored. If the positive and negative controls cannot be clearly distinguished, the test should be considered invalid.

If desired, a TRITC filter set may be used to confirm the staining. The RSV-infected cells will no longer be visible while the influenza A- infected cells will exhibit bright pink fluorescence staining.

The positive result does not rule out co-infections with other pathogens or identify any specific virus subtype.

Cell Culture Isolation/Confirmation:

Standard tubes or shell vials inoculated with reference RSV and influenza A strains and uninoculated cultures should be maintained and tested to ensure proper culture isolation and staining procedures.

When viewed under a fluorescence microscope using a FITC filter set, an RSV-positive reaction is indicated by a bright apple-green fluorescence in the nucleus and/or cytoplasm of the infected cells. An influenza A-positive reaction is indicated by a yellow-gold fluorescence in the nucleus and/or cytoplasm of the infected cells.

Positive staining for either RSV or influenza A is indicated by the presence of 2 or more intact cells exhibiting specific fluorescence. A presumptive negative reaction is indicated by the absence of fluorescence and presence of a dull red color due to the Evans blue counterstain.

A negative result does not rule out RSV and/or influenza A infection. A negative result may be due to a variety of factors such as: inadequate sample, improper specimen collection and handling, improper culture technique, use of an inappropriate cell line or temperature during isolation, or other factors mentioned in the "Troubleshooting" section. All presumptive negative results should be reported as "No virus observed".

Limitations

- A negative result in cell culture does not rule out RSV and/or influenza A infection. A negative result may be due to a variety of factors such as: time of collection during infection, inadequate sample, improper specimen collection and handling, improper culture technique, use of an inappropriate cell line or temperature during isolation, or other factors mentioned in the "Troubleshooting" section.
- The use of a 10x objection (100x magnification) may not provide sufficient magnification to see cell morphology and staining pattern, particularly for cells infected in influenza A.

- The monoclonal antibodies used in this kit were prepared using a prototype strain and may not detect all antigenic variants or new strains of RSV or influenza A.
- Monoclonal antibodies may fail to detect strains of RSV and influenza A that have undergone minor amino acid changes in the target epitope region.
- Protein A, produced by certain bacteria, will bind the Fc portion of the monoclonal antibodies used in the **Light Diagnostics™ SimulFluor® RSV/Flu A DFA Kit.** Bacterial contamination would be identifiable in a culture isolate and such samples should be eliminated from analysis. Staining however, could be differentiated by size and morphology. The presence of *Staphylococcus aureus* (producing protein A) will result in fluorescence of the cell wall, which is bright, small (0.8 to µm) and spherical.
- Performance of the fluorescence microscope is of critical importance in achieving satisfactory test results. While objectives, bulb intensity and wattage and filters may affect results, use of appropriate controls will verify functioning of reagents, culture methodology and microscope.
- The performance characteristics for this test have not been established for monitoring therapy.
- The performance characteristics for this test have not been fully established for sample types such as nasophyaryngeal aspirates or biopsy specimens from the respiratory tract.
- The monoclonal antibodies may fail to detect, or detect with less sensitivity, influenza A viruses that have undergone minor amino acid changes in the target epitope region.
- Additional testing is required to differentiate any specific influenza A subtypes or strains, in consultation with state or local public health departments.
- Children tend to shed virus more abundantly and for longer periods of time than adults. Therefore, testing specimens from adults will have lower sensitivity than testing specimens from children.
- Positive and negative predictive values are highly dependent on prevalence. False negative test results are more likely during peak activity when prevalence of disease is high. False positive test results are more likely during periods of low influenza activity when prevalence is moderate to low.

- Individuals who received nasally administered influenza A vaccine may have positive test results for up to three days after vaccination.
- Possibility of false negative or false positive results may occurs due to interference such as commonly used collection materials or substances that may be endogenously or exogenously introduced into a specimen prior to testing.

Specificity and Cross-Reactivity

The monoclonal antibodies used in the **SimulFluor® RSV/Flu A** reagent were tested against a variety of viruses and bacteria found in the respiratory tract, and cell lines commonly used to isolate RSV and influenza A viruses. The results are indicated in the table below.

Table 1a. Cross-reactivity against common viruses

Microorganisms	**SimulFluor® RSV/Flu A**
Viruses	
Adenovirus; AD-75 CDC V5-002	-
Coxsackievirus A9; ATCC VR-186	-
Coxsackievirus B1; NIH	-
Cytomegalovirus; clinical isolate	-
Enterovirus 70; ATCC VR-836	-
Enterovirus 71; ATCC VR-784	-
Echovirus 4; ATCC VR-34	-
Echovirus 6; ATCC VR-36	-
Echovirus, 9; ATCC VR-39	-
Echovirus 11; ATCC VR-41	-
Echovirus 30; ATCC VR-322	-
Herpes simplex virus type 1; clinical isolate	-
Herpes simplex virus type 2; clinical isolate	
Influenza A: H1N1: 6 strains H2N2: 1 strain H3N2: 8 strains	+
Influenza B; 6 strains	-
Mumps;	-
Measles	-
Parainfluenza 1; CDC V6-004	-
Parainfluenza 2; CDC V7-003	-

Microorganisms	SimulFluor® RSV/Flu A
Parainfluenza 3; CDC V5-003	-
Parainfluenza 4A; VR1378 Strain M-25	-
Respiratory syncytial virus; clinical isolates - 6 Strain Long Strain 'B'	+
Pneumocystis carinii, rat lung	-
Varicella zoster virus; clinical isolate	-

Table 1b. Cross-reactivity against common bacteria and cell lines

Microorganisms	SimulFluor® RSV/Flu A
Bacteria	
Bordetella bronchiseptica; ATCC 10580	-
Bordetella pertussis; ATCC 9340	-
Branhamella catarrhalis; ATCC 25238	-
Chlamydia trachomatis; ATCC	-
Chlamydia pneumoniae	-
Corynebacterium diptheriae; ATCC 13812	-
Legionella micdadei; ATCC 33204	-
Legionella pneumophila; ATCC 33156	-
Mycobacterium tuberculosis; ATCC 25177	-
Mycoplasma hominis; ATCC 23114	-
Mycoplasma pneumoniae; ATCC 15531	-
Neisseria meningitidis; ATCC 13077	-
Cell Lines	
HEp-2	
RMK	-
MRC5	-
A549	-
Vero	-
LLC-MK2	-

Expected Values

Five hundred and twenty-seven specimens were received at 2 sites between September, 1997 and March, 1998, 253 from the southeast portion of the United States and 274 from the northeast.

In the southeast, RSV was identified by culture in 52 specimens for a prevalence of 20.6%, while influenza A was isolated from 31 specimens (prevalence 12.3%). All of the patients from whom RSV was identified were under the age of 3 years. In contrast, 19 of 31 (61.3%) patients positive for influenza A were older than 10 years of age.

In the northeast, RSV was identified in 111 specimens (prevalence 40.5%) and influenza A in 23 specimens (8.4% prevalence). With the exception of 11 throat swabs taken from adults in a nursing home, all specimens were nasopharyngeal aspirates from children. A similar age distribution was seen for RSV and influenza A infections as was found at the other study site. All patients from whom RSV was identified were under the age of 3 years, while 3 of the 23 influenza A isolates were from elderly patients. Differences in performance are expected when this test is used on specimens from adult versus from children, but specific differences are not known.

Table 2: Prevalence of RSV and Influenza A in the Eastern United States in Children Under 3 Years of Age

	Southeastern U.S.			**Northeastern U.S.**		
	Total	**Positive**	**% < 3yrs**	**Total**	**Positive**	**% < 3yrs**
RSV	52/253	20.6%	100%	111/274	40.5%	100%
Flu A	31/253	12.3%	38.7%	23/274	8.4%	13.0%

Five different specimen types were received for testing at the two study sites. The numbers of specimens and positivity rate for either RSV or influenza A are summarized below. Nasal swabs and nasopharyngeal aspirates were found to be the best specimen types for virus isolation.

Table 3: Specimens Received for RSV and Influenza A Testing

	TS	NS	TW	NPA	Sput	Bronch	Other
# Specimens	63	137	17	263	37	2	3
# Positive	15	70	4	134	13	0	0
% Positive	23.8	51.1	23.5	50.9	35.1	0	0

TS=throat swab; NS=nasal swab; TW=throat wash; NPA=nasopharyngeal; Sput = Sputum; Bronch= bronchal brushings; O=Other

Performance Characteristics

SITE 1

Two hundred and fifty-three specimens were received for RSV and influenza A testing at a reference laboratory in southeastern United States. Specimens, including nasal swabs, throat swabs, nasal washings, etc., were from both children and adults. Slides were made from each specimen and stained with the **SimulFluor® RSV/Flu A** reagent and the results compared to those from an fluorescence assay Predicate Device. Specimens were also inoculated into culture for virus isolation. Slides were made from inoculated cultures and were stained with the **SimulFluor® RSV/Flu A** reagent and an fluorescence assay Predicate Device.

Twenty-six viruses other than RSV and influenza A were isolated including rhinovirus (9), CMV (6), adenovirus (4), and enterovirus (2) and one parainfluenza 2. Four dual infections were also identified including one each of CMV/adeno, influenza A/adeno, CMV/RSV and CMV/parainfluenza 3.

Detection of RSV

RSV was detected in 52 patient specimens by culture isolation. The **SimulFluor® RSV/Flu A** reagent had complete agreement with the Predicate Device for culture confirmation.

The relative sensitivity and specificity of the **SimulFluor® RSV/Flu A** reagent was 100% respectively for culture confirmation, while the relative sensitivity.

Table 4: Detection of RSV in Culture

SimulFluor® RSV/Flu A/ Predicate Device	Culture Positive	Culture Negative
SimulFluor® RSV/Flu A Positive	13	0
SimulFluor® RSV/Flu A Negative	0	240
Total	13	240

Relative Sensitivity Culture Confirmation = 100% (13/13)
(95% Confidence Interval 27.6 – 86.2%)
Relative Specificity Culture Confirmation = 100% (240/240)
(95% Confidence Interval 91.4 – 97.2%)

Detection of Influenza A

Influenza A was isolated in culture from 31 of the 253 specimens. The **SimulFluor® RSV/Flu A** and Predicate Device had complete correlation for culture confirmation.

Table 5 Detection of Influenza A in Culture

SimulFluor® RSV/Flu A/ Predicate Device	Culture Positive	Culture Negative
SimulFluor® RSV/Flu A Positive	31	0
SimulFluor® RSV/Flu A Negative	0	222
Total	31	222

Relative Sensitivity Culture Confirmation = 100% (31/31)
(95% Confidence Interval 82.1 – 163%)
Relative Specificity Culture Confirmation = 100% (222/222)
(95% Confidence Interval 83.7 – 91.8%)

Performance characteristics for detecting influenza A virus from human specimens when these or other influenza A virus subtypes are emerging as human pathogens have not been established.

SITE 2

Two hundred and seventy-four specimens were received at a hospital laboratory during from November, 1997 to March 1998. With the exception of 11 throat swabs taken from adults in a nursing home, all specimens were nasopharyngeal aspirates from children. Each specimen was also inoculated into cell culture for the isolation and detection of influenza A and RSV using a commercially available immunofluorescence reagent, and the SimulFluor® RSV/ Flu A reagent.

Twenty-two viruses other than RSV and influenza A were isolated from the specimens. These included 9 CMV (including 1 co-infection with influenza A and 4 with RSV), 8 adenovirus (including 3 co-infections with RSV), 3 enterovirus (2 co-infections with RSV) and 1 HSV.

Detection of RSV

Table 6: Comparison of **SimulFluor® RSV/Flu A** *vs.* Predicate Device

SimulFluor® RSV/Flu A/ Predicate Device	Culture Positive	Culture Negative	Total
SimulFluor® RSV/Flu A Positive	88	2*	90
SimulFluor® RSV/Flu A Negative	0	183	184
Total	88	185	274

*1 specimen was also positive by Membrane EIA
Relative Sensitivity: (88/90) 97.8% (95% Confidence Interval: 92.2 – 99.7%)
Relative Specificity: (183/185) 98.9% (95% Confidence Interval: 96.1-99.9%)

Four specimens were positive for both RSV and influenza A and all four RSV were identified by both reagents in culture.

Detection of Influenza A

Twenty-three specimens were positive for influenza A by the Predicate Device in culture; one sample was positive by **SimulFluor® RSV/Flu A** reagent on culture, but negative in culture by the Predicate Device. Three specimens were positive for both RSV and influenza A in culture, and 1 dual infection with CMV was identified. Of the 32 specimens, which were inadequate for testing with **SimulFluor® RSV/Flu A**, only 1 was positive for influenza A in culture.

Table 7: Detection of Influenza A in Culture

SimulFluor® RSV/Flu A/ Predicate Device	Culture Positive	Culture Negative
SimulFluor® RSV/Flu A Positive	23	1
SimulFluor® RSV/Flu A Negative	0	250
Total	23	251

Relative Sensitivity Culture Confirmation = 95.8% (23/24)
(95% Confidence Interval: 78.9 – 99.9%)
Relative Specificity Culture Confirmation = 99.6% (250/251)
(95% Confidence Interval: 97.8 - 100 %)

Performance characteristics for detecting influenza A virus from human specimens when these or other influenza A virus subtypes are emerging as human pathogens have not been established.

Troubleshooting

Specimen preparation is technique dependent and may affect the results obtained. In order to resolve any performance questions, all steps in the process must be analyzed.

A marked decrease in fluorescence may indicate:

1) Reagent deterioration,
2) Microscopy problems or
3) Other equipment or technique effects.

- Verify expiry date for all reagents used.
- If reagents are in-dating, verify microscope performance; re-read positive control.
- If problem is still not determined, verify all equipment operation as per package insert and repeat test.

Contact **EMD Millipore Corporation** - Technical Service at (800) 437-7500 for United States and Canada or (951) 676-8080. For additional assistance, visit www.millipore.com/offices.

References

1. Hierholzer, J C. 1993. Viral cause of respiratory infections. Immunol. Allergy Clin. North Amer. 13: 27-42
2. Hierholzer, J C., et. al. 1994. Subgrouping of respiratory syncytial virus strains from Australia and Papua New Guinea by biological and antigenic characteristics. Arch. Virol. 136:133-147
3. Mufson, M A. and R B. Belshe. 1997. Respiratory syncytial virus and the parainfluenza viruses. *In*: Manual of Clinical Laboratory Immunology, 5th Edition. N.R. Rose, E.C. deMacario, J.D. Folds, H.C. Lane, and R.M. Nakamura, eds.; American Society for Microbiology Press, Washington, D.C. pp. 679-687.
4. Tristram, D.A. and R.C. Welliver. 1995. Respiratory syncytial virus. *In:* Diagnostic Procedures for Viral, Rickettsial, and Chlamydial Infections, 7th Edition. E.H. Lennette, D.A. Lennette, and E.T. Lennette, eds.; American Public Health Assn., Washington , D.C. pp. 539-552
5. Couch, R.B. and J.A. Kasel. 1995. Influenza. *In:* Diagnostic Procedures for Viral, Rickettsial, and Chlamydial Infections, 7th Edition. E.H. Lennette, D.A. Lennette, and E.T. Lennette, (eds.). American Public Health Assn., Washington, D.C. pp. 431-446.
6. Krug, R.M. (ed.) 1989. The Influenza Viruses. Plenum Press, New York.
7. Ziegler, T., J.M. Katz, N.J. Cox, and H.L. Regnery. 1997. Influenza viruses. *In:* Manual of Clinical Laboratory Immunology, 5th Edition. N.R. Rose, E.C. deMacario, J.D. Folds, H.C. Lane, and R.M. Nakamura, eds.; American Society for Microbiology Press, Washington, D.C. pp. 673-678.
8. Xu, X., et. al. 1993. Genetic and antigenic analyses of influenza A (H1N1) viruses, 1986-1991. Virus Res. 28:37-55.
9. Kilbourne, E.D. 1987. Influenza. Plenum Publ. Co., New York.
10. Mostow, S.R. 1987. Prevention, management, control of influenza: Role of amantadine. Amer. J. Med. 82 (suppl. A):35-41.
11. Hierholzer, J.C. and R.A. Killington. 1996. Virus isolation and quantitation. *In:* Virology Methods Manual; B.W.J. Mathy and H.O. Kangro, (eds.). Academic Press, London. pp. 25-46
12. Espy, M.J., et. al. 1986. Rapid detection of influenza virus by shell vial assay with monoclonal antibodies. J. Clin. Microbiol. 24:677-679.
13. Spada, B., K. Biehler, P. Chegas, J. Kaye, and M. Riepenhoff-Talty. 1991. Comparison of rapid immunofluorescence assay to cell culture isolation for the detection of influenza A and B viruses in nasopharyngeal secretions from infants and children. J. Virol. Meth. 33:305-310.

14. Ziegler, T., et. al. 1995. Type- and subtype-specific detection of influenza viruses in clinical specimens by rapid culture assay. J. Clin. Microbiol. 33:318-321.

15. Ziegler, T. and N.J. Cox. 1995. Influenza viruses. *In:* Manual of Clinical Microbiology, 6th Edition. P.R. Murray et. al. (eds.) American Society for Microbiology Press, Washington, D.C. pp. 918-925.

16. Claas, E.C., et. al. 1983. Prospective application of reverse transcriptase polymerase chain reaction for diagnosing influenza infections in respiratory samples from a children's hospital. J. Clin. Microbiol. 31:2218-2221.

17. Harmon, M.W. and A.P. Kendal. 1991. Influenza viruses. Ch. 81. *In:* Manual of Clinical Microbiology, Fifth Edition, A. Balows, et. al. (eds.), American Society for Microbiology, Washington, D.C. p. 870.

Glossary of Symbols

Symbol	Used for	Symbol	Used for
REF	Catalog number		Use by YYYY-MM-DD or YYYY-MM
	Manufacturer	EC REP	Authorized representative in the European Community
	Caution, consult accompanying documents	Σ	Contains sufficient for <n> tests
IVD	*In vitro* diagnostic medical device		Temperature limitation
i	Consult instructions for use		Biological risks
CONTROL	Control	CONTROL –	Negative control
CONTROL +	Positive control		

Warranty:

EMD Millipore warrants that its products will meet their applicable published specifications when used in accordance with their applicable instructions for the expressly stated duration. This warranty does not extend to any product which has been subjected to misuse, neglect, or to use in violation of instructions furnished by EMD Millipore. For products EXPRESSLY SOLD FOR USE IN FOOD, DRUG OR COSMETIC APPLICATIONS, EMD Millipore guarantees that no such product is adulterated or misbranded within the meaning of the Federal Food, Drug and Cosmetic Act (the “Act”) or within the meaning of any other applicable law in which the definitions of adulteration or misbranding are substantially the same as those contained in the Act, as such laws are constituted and effective at the time of shipment, or is an article which may not, under Sections 404 or 505 of the Act, be introduced into interstate commerce.

EMD MILLIPORE MAKES NO OTHER WARRANTY, EXPRESS OR IMPLIED, AND EXPRESSLY DISCLAIMS WARRANTY OF MERCHANTABILITY OR WARRANTY OF FITNESS FOR A PARTICULAR PURPOSE. The warranty provided herein and the data, specifications and descriptions of EMD Millipore products appearing in EMD Millipore’s published catalogues and product literature may not be altered except by express written agreement signed by an officer of EMD Millipore. Representations, oral or written, which are inconsistent with this warranty or such publications are not authorized and if given, should not be relied upon.

In the event of a breach of the foregoing product warranty, EMD Millipore’s sole obligation shall be to repair or replace, at its option, the applicable product or part thereof, provided the customer notifies EMD Millipore promptly of any such breach. If after exercising reasonable efforts, EMD Millipore is unable to repair or replace the product or part, then EMD Millipore shall refund to the customer all monies paid for such applicable product or part.

EMD Millipore warrants that it shall provide services in a professional and workmanlike manner and that it will attempt in good faith to perform the services to Customer’s reasonable satisfaction. **EMD MILLIPORE MAKES NO OTHER WARRANTY EXPRESS OR IMPLIED.** If EMD Millipore breaches this warranty, then Customer shall be entitled to a refund of up to the amount paid for such services. The above represents Customer’s sole remedy for breach of this services warranty.

Buyer assumes all risk and liability for loss, damage or injury to persons or to property of Buyer or others arising out of the presence or use of the products or EMD Millipore’s provision of services. Except as expressly provided otherwise herein, EMD Millipore shall not indemnify nor be liable to Buyer, Buyer’s customers, successors, or to any person or entity for any claims, damages or losses arising out of the sale or use of products or the provision of services, where liability is premised upon any theory including, but not limited to, warranty, negligence or strict liability. **EMD MILLIPORE SHALL NOT IN ANY EVENT BE LIABLE FOR INCIDENTAL, CONSEQUENTIAL, INDIRECT, EXEMPLARY OR SPECIAL DAMAGES OF ANY KIND RESULTING FROM ANY USE OR FAILURE OF THE PRODUCTS OR SERVICES INCLUDING, WITHOUT LIMITATION, LIABILITY FOR LOSS OF USE, LOSS OF WORK IN PROGRESS, LOSS OF REVENUE OR PROFITS, OR ANY LIABILITY OF BUYER TO A THIRD PARTY. THE TOTAL LIABILITY OF EMD MILLIPORE UNDER THESE TERMS AND CONDITIONS OF SALE SHALL NOT EXCEED THE PURCHASE PRICE OF THE PRODUCTS OR SERVICES INVOLVED.** All claims must be brought within one (1) year of delivery, regardless of their nature.

Tween® is a registered trademark of ICI Americas Inc.
ATCC® is a registered trademark of the American Type Culture Collection Corporation.

©2012 EMD Millipore Corporation a division of Merck KGaA, Darmstadt, Germany. All rights reserved. EMD, EMD Millipore, Millipore, the M mark, Light Diagnostics, SimulFluor and all other trademarks, unless specifically identified above in the text as belonging to a third party, are owned by Merck KGaA, Darmstadt, Germany.

3129

August 2012
Revision A; 3129MAN