

LABORATORY PROCEDURE
DIRECTIGEN™ RSV
For the direct detection of Respiratory Syncytial Virus (RSV)

I. INTENDED USE

The **Directigen™** Respiratory Syncytial Virus (RSV) Test is an *in vitro* enzyme immunoassay (EIA) membrane test for the rapid and qualitative detection of RSV antigen directly from nasopharyngeal specimens.

II. SUMMARY AND EXPLANATION

Procedures currently used to diagnose respiratory syncytial virus (RSV) infection include amplification/isolation in tissue culture followed by immunofluorescence (IF) confirmation of cell culture cytopathic effects (CPE), or demonstration of a four-fold rise in antibody titer in paired acute and convalescent sera. More recently, direct or indirect IF and micro well/tube enzyme immunoassays have been employed with or without culture confirmation as a “rapid” RSV detection method.¹⁻⁴ The **Directigen RSV** antigen detection test employs an enzyme immunomembrane filter assay to detect RSV antigen extracted from suitable specimens from symptomatic patients. Total test time is less than 15 minutes with reactivity determined by visual color development.

The speed and workflow of the **Directigen RSV** Test make it applicable as a “STAT” RSV test - providing rapid, relevant information to assist with specific antiviral intervention and support decisions.

III. PRINCIPLES OF THE PROCEDURE

Extracted nasopharyngeal specimens are added to a **ColorPAC™** test device, and RSV antigen is bound to the membrane surface as the specimen passes through the membrane. Detector enzyme conjugated monoclonal antibodies specific for RSV nucleoprotein and fusion protein are bound to the trapped antigen following addition to the **ColorPAC** membrane. Two substrates are then added sequentially and allowed to incubate for five minutes, resulting in a purple triangle developing on the membrane indicating a positive test.

IV. REAGENTS

The **Directigen RSV** Test includes:

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|-------------------------|------|--|
| ColorPAC Devices | (40) | 40 Test kit. |
| | (20) | 20 Test kit. Devices with flow controller units containing a control dot of inactivated RSV antigen in the center of the membrane. |

DispensTube™	(40)	40 Test kit.
Tubes and Tips	(20)	20 Test kit.
Reagent A	(4.5 ml)	Extraction, 4% mucolytic agent and 16% detergent, with 0.2% sodium azide (preservative).
Reagent 1	(7.0 ml)	Wash, 50 mM Tris-Saline, with 0.2% sodium azide (preservative).
Reagent 2	40 Tests (7.0 ml) 20 Test (3.5 ml)	Detector, anti-RSV (Mouse Monoclonal Antibodies) - Enzyme conjugated, with 0.2% sodium azide (preservative).
Reagent 3	(13.5 ml)	Wash, 50 mM Tris-Saline, with 0.2% sodium azide (preservative).
Reagent 4	(7.0 ml)	Color Development Substrate A, 0.4 mM chromogen, with 0.02% sodium azide (preservative).
Reagent 5	(7.0 ml)	Color Development Substrate B, 7.8 mM chromogen, with 0.2% sodium azide (preservative).
Reagent 6	(7.0 ml)	Stop, 150 mM citric acid.
Control +	(1.0 ml)	Positive Control, detergent-treated RSV antigen with 0.2% sodium azide (preservative).
Control -	(1.0 ml)	Negative Control, detergent-treated non-infected HEp-2 cells, with 0.2% sodium azide (preservative).

Precautions: *in vitro* Diagnostic

Reagents: Do not use beyond the expiration date. Do NOT mix reagents from different kit lot numbers or mix reagent bottle caps.

To assure proper delivery, **DispensTube** devices and reagent bottles must be held vertically (approximately one inch from the **ColorPAC** membrane surface or tube), while gently dispensing one drop at a time, in quick succession.

The RSV **Control +** and control spot on the **ColorPAC** membrane have been prepared from RSV-infected tissue culture cells which have been inactivated and subsequently tested by bio-assay procedures.

Avoid contact of reagents with skin and mucous membranes. If reagents come into contact with these areas, flush with water and contact your physician.

Warning: Reagents contain sodium azide. Very toxic by inhalation, in contact with skin, and if swallowed. Contact with acids liberates very toxic gas. After contact with skin, wash immediately with plenty of water. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up.

Swabs: For nasopharyngeal swabs (NPS), Dacron™ polyester or rayon-tipped swabs with an aluminum wire are recommended. Calcium alginate swabs are not recommended for use with the **Directigen** RSV Test.

Controls: Do not use the kit if the **Control +** and **Control -** do not yield appropriate results.

ColorPAC Device: Remove the device (with the inserted flow controller) from the foil pouch just prior to use.

Storage: Store at room temperature (15 - 30°C) or refrigerate. DO NOT FREEZE.

V. SPECIMEN COLLECTION

Specimen Handling

Transport fresh specimens to the laboratory (as rapidly as possible) in a suitable liquid transport system. Specimens may be stored at 2 - 8°C for up to 48 h or -20°C for up to one week prior to processing. Do not centrifuge specimens prior to use with the **Directigen** RSV Test, as the removal of cellular material will adversely affect the sensitivity of the test.

Excessively bloody specimens should not be tested with the **Directigen** RSV Test. Specimens containing visible blood have been found to yield either uninterpretable or false-positive results when processed with this test.

Observe established precautions against microbiological hazards throughout all procedures. All specimens should be handled according to CDC/NIH (Centers for Disease Control and Prevention/ National Institutes of Health) recommendations, NCCLS guidelines or local institution guidelines for any potentially infectious samples. Prior to discarding, sterilize containers and other contaminated materials by autoclaving.

Specimen Preparation

Acceptable specimens include fresh or frozen nasopharyngeal washes, aspirates, and swabs as well as tracheal aspirates. Nasopharyngeal washes and aspirates, however, have been shown to be superior to swabs and are the specimens of choice.⁵⁻⁷

NOTE: Excessively mucoid specimens may fail to be absorbed into the **ColorPAC** membrane or may yield uninterpretable results. These specimens may either be diluted 1:4 with 0.9% saline or adjusted with 0.9% saline to a McFarland standard #1, mixed well, and a 250 µl aliquot tested. As an alternate to performing a dilution, excessively mucoid specimens treated with extraction reagent may be subjected to a brief sonication procedure prior to addition to the **ColorPAC** device.

Procedure For Use with Nasopharyngeal Washes:

1. Nasopharyngeal wash volumes of 3 to 4 ml are recommended.
2. Excessive wash volumes may result in decreased test performance.
3. Process specimen as described in "Specimen Extraction."

Procedure For Use with Nasopharyngeal Aspirates:

1. Nasopharyngeal aspirate specimens of less than 0.5 ml in volume must be dispersed in at least 3 ml of transport medium or saline prior to processing.
2. Aspirate specimens of greater than 0.5 ml require a transport medium or saline volume addition of greater or equal to 4 ml.
3. Process specimen as described in "Specimen Extraction."

Procedure For Use with Nasopharyngeal Swabs:

1. Nasopharyngeal swab specimens should be added to 0.75 - 3 ml of transport medium or saline prior to processing.
2. Vortex the swab and transport media or saline solution.
3. Remove as much liquid from the swab as possible.
4. Dispose the swab into appropriate container.
5. Process specimen as described in "Test Procedure."

VI. PROCEDURES

Materials Provided: See "Reagents" for materials provided.

Materials Require But Not Provided: Required are the necessary equipment and labware used for transport, storage, handling and allocation of specimens.

Transport Media: The following transport media have been tested and found to be compatible with the **Directigen** RSV Test:

Saline, Normal	VIB plus 0.5% BSA
Phosphate Buffered Saline (PBS)	Earle's Minimum Essential Media (EMEM)
PBS plus 0.5% gelatin	EMEM with Lactalbumin Hydrolysate
PBS plus 0.5% Bovine Serum Albumin (BSA)	M5 media
Veal Infusion Broth (VIB)	
PBS plus 0.5% Bovine Serum Albumin (BSA)	
Trypticase TM Soy Broth plus 0.5% gelatin	
Viral CULTURETTE TM (See "Availability")	

Other transport media may be utilized if an appropriate qualification exercise is performed. As a precaution, all transport media should be qualified with **Directigen** RSV prior to use. To qualify transport media, aliquots of media may be seeded with a known positive material and a known negative material and tested with the assay. Appropriate results should be obtained.

Performance of Test: Review "Precautions," "Specimen Handling," "Specimen Preparation" and "Results." Reagents, specimens, and **ColorPAC** devices must be at room temperature (15 - 30°C) when used.

TEST PROCEDURE

Place a **DispensTube** device in the designated area of the work station.

ColorPAC Preparation

Ensure Flow Controller is seated snugly in the **ColorPAC** device.

Specimen Extraction

1. Vortex specimen. Pipette 250 µl of specimen into the **DispensTube** device.
2. **Reagent A - Dispense 3 drops into the DispensTube device.**
3. Vortex or mix thoroughly.

NOTE: Quality Control

- The **Control +** and **Control -** may be used in place of patient samples for quality control purposes.
- Dispense 5 drops of well mixed **Control +** or **Control -** into the **DispensTube** device, followed by 3 drops of **Reagent A**.
- Mix well.

4. Insert a tip into the **DispensTube** device. **NOTE: Do not use tips from other Directigen products.**
5. Invert and carefully squeeze.
6. **Dispense all of the extracted specimen dropwise in quick succession (avoiding excess bubble addition) into the ColorPAC test well.**
7. Allow to absorb completely.
8. If specimen fails to be absorbed into the device within 5 min, dilute as described in "Specimen Preparation" and retest.

Color Development

1. **Remove the flow controller from the ColorPAC device. Discard as biohazard.**
Reagent 1 - gently mix.
Add 4 drops onto the ColorPAC membrane.
Allow to absorb completely.
2. **Reagent 2** - gently mix.
Add 4 drops onto the ColorPAC membrane.
Allow to absorb completely.
Allow to stand for 2 min.
3. **Reagent 3** - gently mix.
Add 4 drops onto the ColorPAC membrane.
Allow to absorb completely.
4. **Reagent 4** - gently mix.
Add 4 drops onto the ColorPAC membrane.
Allow to absorb completely.

5. **Reagent 5** - gently mix.
Add 4 drops onto the ColorPAC membrane.
Allow to absorb completely.
Allow to stand 5 min.
Read in a well-lighted area and record the test results.

VII. RESULTS

NOTE: Results may be read for up to an additional 10 min prior to adding **Reagent 6**.

OPTIONAL - To further extend the readout time period to a maximum of 12 h, add 4 drops of **Reagent 6** (Stop solution) onto the **ColorPAC** membrane.

Positive Test (antigen present) - A purple triangle (of any intensity) appears on the **ColorPAC** membrane and indicates RSV antigen was detectable in the specimen. The background area should be white-to-light purple. A purple dot may be evident in the center of the triangle unless obscured by an intense positive triangle.

Negative Test (no antigen detected) - No purple triangle is visible indicating RSV antigen was not detectable in the specimen. A purple dot appears on the **ColorPAC** membrane indicating proper performance of test procedure and reagents. The background area should be white-to-light purple.

Uninterpretable Test - The test is uninterpretable if a purple dot is not visible. Any incomplete triangle is also to be regarded as an uninterpretable test. If uninterpretable, the test should be repeated. The test result is also uninterpretable if a white triangle appears on the **ColorPAC** membrane and the entire surrounding background membrane is purple in color. A muted control dot may be evident in the center of the white triangle. A minimum specimen dilution of 1:4 with 0.9% saline or an adjustment to a McFarland standard #1 will typically correct this problem and, when retested, yield an interpretable result.

Excessively mucoid specimens may fail to be absorbed into the **ColorPAC** membrane or may yield uninterpretable results. These specimens may be diluted 1:4 with saline, mixed well, and retested. If you need additional assistance, telephone Technical Services in the United States at 1-800-638-8663.

VIII. QUALITY CONTROL

Each **Directigen RSV ColorPAC** device contains both internal positive and negative controls (i.e., two levels). The appearance of a purple control dot provides an internal positive reactivity control (level 1) that validates the immunological integrity of the device, proper reagent function, and assures that the correct test procedure was followed. The membrane area surrounding the triangle is the internal negative control (level 2) for the device. The lack of any color development in this background area indicates that the test has been performed correctly.

Liquid Positive (**Control +**) and Negative (**Control -**) controls are also supplied with each kit. These controls are provided as a means of additional quality control. At a minimum, the liquid controls should be run as a quality control procedure for each lot of each shipment received. The formation of a purple triangle on the membrane when the **Control +** is employed further indicates that the RSV antigen binding property of the membrane is functional. Do not use if the **Control +** and **Control -** do not give appropriate results.

The liquid controls may also be used to demonstrate a positive or negative reaction. As described under "Test Procedure" the **Control +** will demonstrate a strong positive reaction. Dilution (1:2 maximum) of the **Control +** with saline may be performed and tested to demonstrate a weaker positive reaction. Performance of reagents and technique may also be evaluated by using specimens known to be positive or negative.

IX. LIMITATIONS OF THE PROCEDURE

The etiology of respiratory infection caused by microorganisms other than respiratory syncytial virus will not be established with this test. **Directigen** RSV is capable of detecting both viable and non-viable RSV particles. The **Directigen** RSV Test performance depends on antigen load and may not correlate with tissue culture performed on the same specimen.

It has been previously established that fresh specimens are preferable to frozen for RSV testing. Sub-optimal test performance may result with the latter. Inadequate specimen collection, improper sample handling/transport or low levels of virus shedding may yield a false-negative result. Accordingly, a negative test result does not eliminate the possibility of an RSV infection. Patient diagnosis should always include laboratory test results in concert with all other clinical information available.

The validity of **Directigen** RSV has not been proven for identification/confirmation of tissue culture isolates.

X. EXPECTED VALUES

The rate of positivity observed in respiratory syncytial virus testing will vary, depending on the method of specimen collection, handling/transport system employed, detection method utilized, the time of year, age of the patient, geographic location, and most importantly, local disease prevalence.

XI. PERFORMANCE CHARACTERISTICS

Clinical Accuracy: The performance of the **Directigen** RSV Test was determined in retrospective and prospective evaluations conducted by independent investigators.

A total of 281 frozen specimens consisting of nasopharyngeal wash specimens from hospitalized, RSV-symptomatic patients were tested with the **Directigen RSV Test** by two independent investigators. A total of 170 specimens were negative and 106 specimens were positive for RSV, as determined by tissue culture.

Results are summarized in Table 1. Compared to tissue culture, the sensitivity of the **Directigen RSV Test** is 97% (103/106), and the specificity is 97% (166/170). Five uninterpretable results 2% (5/281) were also obtained.

Table 1
Agreement between Directigen™ RSV
and Tissue Culture Results

Tissue Culture	Number	Directigen RSV	
		Positive	Negative
Positive for RSV	106	103	3
Negative for RSV	170	4*	166

*Three of four were tissue culture negative and IFA positive.

A total of 528 nasopharyngeal wash specimens from hospitalized, RSV symptomatic patients were externally evaluated with **Directigen RSV** during the 1988-89 RSV season by two independent investigators. Tissue culture was the primary reference method; IFA and blocking assays resolved discrepancies when applicable. A total of 334 specimens were negative and 194 specimens were positive for RSV by the reference methods. **Directigen RSV** results are summarized in Table 2. In this prospective study, the sensitivity of the **Directigen RSV Test** is 93% (181/194) and the specificity is 90% (302/334).

Table2
Agreement between Directigen™ RSV
and Tissue Culture Results

Reference Results	Number	Directigen RSV	
		Positive	Negative
Positive for RSV	194	181	13
Negative for RSV	334	32	302

XII. AVAILABILITY

Description

Directigen™ RSV (Respiratory Syncytial Virus) Kit, 20 Determinations.

Directigen™ RSV (Respiratory Syncytial Virus) Kit, 40 Determinations.

Viral **CULTURETTE™**, Single Swab, carton of 100.

XIII. REFERENCES

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2. Swenson, P.D., and M.H. Kaplan. 1986. Rapid detection of respiratory syncytial virus in nasopharyngeal aspirates by a commercial enzyme immunoassay. *J. Clin. Microbiol.* 23:485-488.
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7. Greenberg, S.B. and L.R. Krilov. 1986. Cumitech 21, Laboratory diagnosis of viral respiratory disease. Coordinating ed., W.L. Drew and S.J. Rubin. American Society of Microbiology, Washington, D.C.

XIV. TECHNICAL INFORMATION

In the United States, telephone Becton Dickinson Microbiology Systems Technical Services, toll free (800) 638-8663, Prompt 2.

Approved by:_____

Date Effective:_____

Supervisor: _____ Date: _____

Director: _____ Date: _____

Reviewed:

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