

Directigen™ Flu A+B

For the Differentiated, Direct Detection of Influenza A and B Antigens

I. INTENDED USE

The **Directigen™** Flu A+B test is a rapid *in vitro* enzyme immunoassay (EIA) membrane test for the direct and qualitative detection of influenza A and B viral antigens from nasopharyngeal wash, nasopharyngeal aspirate, nasopharyngeal swab, lower nasal swab, throat swab and bronchoalveolar lavage specimens of symptomatic patients. The **Directigen** Flu A+B test is a differentiated test, and therefore influenza A viral antigens can be distinguished from influenza B viral antigens in a single test. The test is to be used as an aid in the diagnosis of influenza A and B viral infections. Negative test results should be confirmed by cell culture. The **Directigen** Flu A+B test is not intended for detection of influenza C.

II. SUMMARY AND EXPLANATION

Influenza is an acute viral disease that is seasonal in incidence. The illness classically presents with sudden onset of fever, chills, headache, myalgias, and a non-productive cough. Clinical manifestations usually resolve within one week unless complications develop. Influenza A or B virus cause the majority of clinically significant disease, with influenza C virus being responsible only for mild, predominately upper respiratory tract illness.

Patients who present with suspected influenza might benefit from treatment with antiviral agents. Amantadine¹ and rimantadine¹ are available for both the prevention and treatment of influenza A disease only. Zanamivir¹ and oseltamivir¹ are available for the treatment of both influenza A and B disease. In adults, therapy with these agents may reduce the severity and duration of illness if given within the first 48 hours of onset of illness. Since the therapeutic options have expanded to include options for the treatment of influenza B disease, it is important to rapidly distinguish influenza A from influenza B in order to allow physicians a choice in selective antiviral intervention. Moreover, since only amantadine and rimantadine have indications for influenza prophylaxis, it is important to determine if influenza A is causing symptomatic disease in a particular institution (e.g., nursing home) or community, so that appropriate preventative intervention can be taken for susceptible individuals. It is therefore important to not only rapidly determine whether influenza is present, but also which type of influenza virus is present.

Procedures used to diagnose influenza type A and B infections include rapid immunoassay, direct specimen immunofluorescence assay, reverse transcription-polymerase chain reaction (RT-PCR), serologic assay, and culture isolation with confirmation.²⁻⁸ Immunofluorescence assays entail staining of specimens immobilized on microscope slides using fluorescent-labeled antibodies for observation by fluorescence microscopy.^{4,9,10} Culture methods employ initial viral isolation in cell culture, followed by hemadsorption inhibition, immunofluorescence, or neutralization assays to confirm the presence of the influenza virus.¹⁰⁻¹² The **Directigen** Flu A+B antigen detection test is an enzyme immunomembrane filter assay to detect influenza A or B antigens extracted from suitable specimens of symptomatic patients. Total test time is less than 15 minutes with reactivity determined by visual color development. Antigenic drift is not an

issue with the **Directigen** Flu A+B test because the target antigens are the nucleoproteins, which are type-specific and are highly conserved.¹³⁻¹⁵

The speed and workflow of **Directigen** Flu A+B make it applicable as a "STAT" influenza A and B antigen detection test, providing rapid, relevant information to assist with the diagnosis of influenza. The use of **Directigen** Flu A+B to differentiate Flu A from Flu B infection can provide the opportunity for greater selectivity of antiviral intervention.

III. PRINCIPLES OF THE PROCEDURE

The **Directigen** Flu A+B test begins with the extraction of influenza A or B viral antigens from respiratory specimens. The extracted specimen is expelled through a filter assembly into each of two wells of a **ColorPAC**[™] test device. Influenza A or B antigens from viable or non-viable viral particles present in the specimen are non-specifically bound in a triangular shape to the membrane surface in the A and B wells as the specimen passes through the flow controller. Detection of antigen captured on the membrane is initiated after a membrane wash step. Detector enzyme-conjugated monoclonal antibodies (2) specific for influenza A nucleoprotein antigen are added to the upper A well of the test device. Detector enzyme-conjugated monoclonal antibody specific for influenza B nucleoprotein antigen is added to the lower B well of the test device. The enzyme-antibody conjugates are bound to trapped antigen following their addition to the **ColorPAC** membrane. The substrate is then added after washing the membrane and allowed to incubate for five minutes. Addition of a reagent to stop further color development provides time during which results may be interpreted. Development of a purple triangle on the membrane in either the A well or the B well of the test device indicates a positive test for Flu A or for Flu B, respectively. Formation of a purple control dot only with no visible triangle formation indicates a negative test.

IV. KIT COMPONENTS AND REAGENTS

The following are included in the **Directigen** Flu A+B test kit.

ColorPAC Devices	20	Each device with flow controller unit, containing control dots of recombinant influenza A (H1N1) and B (Lee 40) antigens in the center of the respective A and B well membrane contained in a foil pouch with desiccant.
Extraction Reagent E	9.9 mL	Extraction, 1.6% mucolytic agent and 7.4 % detergents, with 0.2% sodium azide (preservative).
Wash Reagent 1	5.1 mL	Wash, 50 mM Tris and rabbit IgG with 0.2% sodium azide (preservative).
Detection Reagent 2	2.3 mL	Detection, anti-influenza A monoclonal antibodies (mouse, clones 84 and 85)-enzyme conjugate, with 0.2% sodium azide (preservative).
Detection Reagent 3	2.3 mL	Detection, anti-influenza B monoclonal antibody (mouse, clone B.9)-enzyme conjugate, with 0.2% sodium azide (preservative).
Wash Reagent 4	6.5 mL	Wash, 5% butanol, 2 M urea and 100 mM HEPES with 0.2% sodium azide (preservative).
Wash Reagent 5	10.5 mL	Wash, 50 mM Tris and 150 mM NaCl with 0.2% sodium azide (preservative).

Substrate Reagent 6	5.8 mL	Substrate, 0.73 mM chromogen.
Stop Reagent 7	4.1 mL	Stop, 150 mM citric acid.
Control A+/B-	2.0 mL	Flu A Positive and Flu B Negative Control, influenza A antigen (recombinant nucleoprotein, H1N1) with 0.1% sodium azide (preservative).
Control B+/A- 2.0 mL		Flu B Positive and Flu A Negative Control, influenza B antigen (recombinant nucleoprotein, B Lee 40) with 0.1% sodium azide (preservative).
DispensTube™ Tubes	20	Tubes for specimen extraction and sample delivery into device.
DispensTube Tips	20	Tips to filter sample when delivered into device.

Warnings and Precautions: For *in vitro* Diagnostic Use.

Reagents: Do not use beyond the expiration date. Do NOT mix reagents from different kit lot numbers or mix reagent bottle caps. Do not reuse **ColorPAC** device. Incubation times and temperatures other than those specified may give erroneous results.

To ensure proper drop delivery, 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. 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: 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 for any potentially infectious samples. Prior to discarding, sterilize containers and other contaminated materials by autoclaving.

Warning: Reagents contain sodium azide, which is very toxic by inhalation, in contact with skin, and if swallowed. Contact with acids liberates very toxic gas. If there is 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. It has been determined that calcium alginate swabs can not be used with **Directigen Flu A+B**.

Sample Processing: Do not centrifuge specimens prior to use with **Directigen Flu A+B**, as the removal of cellular material will adversely affect the sensitivity of the test.

Controls: The Control A+/B- and Control B+/A- reagents have been prepared from recombinant cell cultures, not from viral cultures. Do not use the kit if the Control A+/B- and Control B+/A- reagents do not yield appropriate results.

ColorPAC Device: Remove the device from the foil pouch just prior to use. Do not use if desiccant is missing from pouch.

Storage: Store at ambient or refrigerated temperatures (2-25°C). **DO NOT FREEZE.**

V. SPECIMEN HANDLING

Transport fresh specimens to the laboratory as rapidly as possible in a suitable liquid transport system maintained on ice or refrigerated at 2 – 8°C. Process specimens as soon as possible after collection. If necessary, specimens may be stored at 2 - 8°C for up to 72 hours or at -20°C for up to 7 days from time of collection. Fresh specimens are preferable to frozen, as decreased sensitivity may result. Avoid multiple freeze-thaw cycles. Do not store specimens in self-defrosting freezer. It is essential that correct specimen collection and preparation methods be followed. Specimens obtained early in the course of the illness will contain the highest virus titers.

VI. SPECIMEN PREPARATION

Acceptable specimens include nasopharyngeal washes, nasopharyngeal aspirates, nasopharyngeal swabs, lower nasal (turbinate) swabs, throat swabs and bronchoalveolar lavages.

NOTE: Excessively mucoidal specimens may occasionally fail to be absorbed through the **ColorPAC** membrane or may yield uninterpretable results. These specimens may either be diluted 1:4 with 0.9% saline or viral transport medium, or adjusted to a McFarland standard 1.0, mixed well, and a 200 µL aliquot retested.

Procedure for use with Nasopharyngeal Washes and Bronchoalveolar Lavages

1. Wash or lavage volumes of 2-3 mL are recommended.
2. Excessive wash or lavage volumes may result in decreased test sensitivity.
3. Process specimen as described in "Test Procedure."

Procedure for use with Nasopharyngeal Aspirates

1. Nasopharyngeal aspirate specimens of less than 0.5 mL in volume must be dispersed in 2 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 than or equal to 4 mL.
3. Process specimen as described in "Test Procedure."

Procedure for use with Nasopharyngeal, Lower Nasal, or Throat Swabs

1. Throat swab specimens may be processed without dilution in transport medium by direct extraction as described in "Test Procedure." Throat swabs may also be added to 1 – 2 mL of transport medium media or saline immediately after collection.
2. Nasopharyngeal and lower nasal swab specimens should be placed into 1 – 2 mL of transport medium or saline immediately after collection.
3. Mix the swab well in the transport medium or saline solution.
4. Remove as much liquid from the swab as possible.

5. Discard the swab into appropriate container.
6. Process specimen as described in "Test Procedure."

VII. PROCEDURES

Materials Provided: See "Kit Components and Reagents" for materials provided.

Materials Required but Not Provided: The necessary equipment and labware for transport, storage, handling and allocation of specimens. Vortex mixer.

Transport Media: Use of the following transport media has been tested and found to be compatible with the **Directigen Flu A+B** test:

Saline, Normal	Trypticase™ Soy Broth plus 0.5% BSA
Phosphate Buffered Saline (PBS)	Trypticase Soy Broth plus 0.5% gelatin
PBS plus 0.5% gelatin	Earle's Minimum Essential Media (EMEM)
PBS plus 0.5% Bovine Serum Albumin (BSA)	EMEM with 0.5% BSA
Veal Infusion Broth (VIB)	EMEM with 1% BSA
VIB plus 0.5% BSA	EMEM with 0.5% Lactalbumin Hydrolysate
Viral Culturette™ (see "Availability")	EMEM with 1.0% Lactalbumin Hydrolysate
M4 Medium	Stuart's Medium CultureSwab™
M5 Medium	(see "Availability")
Bartels Medium	Liquid Amies Medium CultureSwab
Sucrose phosphate	(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 Flu A+B** prior to use. To qualify transport media, aliquots of media may be seeded with a known positive material (other than the kit Control A+/B- or Control B+/A-) and a known negative material, and tested with the assay. Appropriate results must be obtained.

Performance of Test: Review "WARNINGS AND PRECAUTIONS," "SPECIMEN HANDLING" and "INTERPRETATION OF RESULTS." Reagents, specimens and **ColorPAC** devices must be at room temperature (15 – 30°C) when used.

VIII. TEST PROCEDURE

Place a **DispensTube** in the designated area of the workstation.

A. **ColorPAC** Device Preparation

1. Remove a **ColorPAC** device from its foil pouch immediately before use.
2. Push down to ensure Flow Controller is seated snugly in both wells in the **ColorPAC** device.

B. Specimen Extraction

- 1a. **All samples, except throat swabs without transport media:** Gently mix Reagent E, and dispense 8 drops of **Reagent E** into a **DispensTube**. Mix specimen well. Pipet 200 µL of specimen into the **DispensTube**.
- 1b. **Throat swabs without transport media:** Gently mix Reagent E, and dispense 16 drops of **Reagent E** into the **DispensTube**. Insert swab into **DispensTube**. Swirl swab while intermittently squeezing swab through the walls of the **DispensTube** for 15-30 seconds. Remove swab while squeezing to displace excess liquid.

NOTE: Quality Control

The **Control A+/B-** or **Control B+/A-** may be used in place of patient samples for quality control purposes.

Dispense 8 drops of **Reagent E** into the **DispensTube**, followed by 4 drops of well-mixed **Control A+/B-** or **Control B+/A-**.

Mix well. Follow Test Procedure to dispense extracted **Control A+/B-** in an alternating dropwise manner into both wells of a single test device. The **Control A+/B-** serves as the positive control for Flu A as well as the negative control for Flu B. Repeat the procedure in a separate device with **Control B+/A-**. The **Control B+/A-** serves as the positive control for Flu B as well as the negative control for Flu A.

2. Insert a **DispensTube** tip into the **DispensTube**. Vortex or mix thoroughly. **NOTE: Do not use tips from other Directigen products.**
3. Invert the **DispensTube** and holding the tube on the upper half, away from the tip, gently squeeze. **NOTE: Squeezing the tube close to the tip may result in ejection of the tip and leakage of contents from the tube.**
4. **Dispense the extracted specimen dropwise (avoiding excess bubble addition), alternating single drops between the A and B test wells until 4 drops have been added to each well of the ColorPAC test device. Thus a total of 8 drops from each extracted specimen is added to a single test device.**
When testing extracted Controls, the extracted Control A+/B- must be added to both wells of a single ColorPAC test device, and similarly, the extracted Control B+/A- must be added to both wells of a single ColorPAC test device.
4. Allow specimen to absorb completely. If specimen fails to be absorbed into the device within five minutes, dilute as described in "Specimen Preparation" section and retest.

C. Color Development

Remove the Flow Controller. Discard as biohazard.

1. **Reagent 1** - gently mix.
Add 2 drops to each well.
Allow to absorb completely
2. **Reagent 2** - gently mix.
Add 2 drops to the A well only.
Proceed immediately to Reagent 3 addition
3. **Reagent 3** - gently mix.
Add 2 drops to the B well only.
Allow Reagents in A and B wells to absorb completely.
Allow to stand 2 minutes.
4. **Reagent 4** - gently mix.
Add 3 drops to each well.
Allow to absorb completely.
5. **Reagent 5** - gently mix.
Add 3 drops to each well.
Allow to absorb completely.
6. **Reagent 6** - gently mix.
Add 3 drops to each well.
Allow to absorb completely (NOTE: Membrane may turn yellow).
Allow to stand 5 minutes.
7. **Reagent 7** – gently mix.
Add 2 drops to each well.
Allow to absorb completely.

IX. INTERPRETATION OF RESULTS

Read the test in a well-lighted area and record the results. If necessary, the results may be read up to 9 hours after adding Reagent 7 (Stop Reagent).

A positive result should be reported as positive for the presence of influenza A and/or B antigen. A negative result should be reported as a presumptive negative for the presence of influenza A/B antigen.

Positive Test for Flu A (antigen present) - A purple triangle (of any intensity) appears in the A well on the **ColorPAC** membrane and indicates influenza A antigen was detectable in the specimen. The background area should be a light yellow to light purple color. A purple control dot should be evident in the center of the triangle unless obscured by an intense positive reaction.

Positive Test for Flu B (antigen present) - A purple triangle (of any intensity) appears in the B well on the **ColorPAC** membrane and indicates influenza B antigen was detectable in the

specimen. The background area should be a light yellow to light purple color. A purple control dot should be evident in the center of the triangle unless obscured by an intense positive reaction.

Negative Test for Flu A or Flu B (no antigen detected in the respective well or wells) - No purple triangle is visible in either the A well, or the B well, or both wells, indicating that influenza A antigen, or influenza B antigen, or both, were not detectable in the specimen. A purple control dot appears in either the A well, the B well, or in both wells on the **ColorPAC** membrane indicating proper performance of test procedures and reagents. The background area should be a light yellow to light purple color.

Uninterpretable Test - The test is uninterpretable either for Flu A, or for Flu B, or for both Flu A and Flu B, if neither a purple dot nor a purple triangle is visible in the respective well(s). 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 either for Flu A, or for Flu B, or for both Flu A and Flu B, 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. Additionally, the test result is uninterpretable if the entire membrane area is purple and no control dot is observed. To correct these problems, dilute the sample 1:4 either in 0.9% saline or in transport media and repeat the test.

Excessively mucoidal samples may fail to be absorbed through the **ColorPAC** membrane or may yield uninterpretable results. These specimens may be diluted 1:4 with saline, mixed well, and retested.

Quality Control: Each **Directigen Flu A+B ColorPAC** device contains both internal positive and negative procedural controls. The appearance of a purple control dot in the A and B wells provides an internal positive procedural control 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 procedural control for the device. The lack of significant color development in this background area to obscure the triangle or control dot indicates that the test has been performed correctly.

Liquid Positive Control A+/B- and Control B+/A- kit controls are also supplied with each kit. These controls are provided as a means of additional quality control to demonstrate a positive or negative reaction. 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 in the **ColorPAC** device A well when the Control A+/B- is tested, and in the device B well when the Control B+/A- is employed, further indicate that the influenza antigen binding properties of the membrane are functional. The formation of only a purple control dot in the **ColorPAC** device B well when the Control A+/B- is employed is an appropriate Flu B negative control result that indicates proper reagent function and that the correct test procedure was followed. Similarly, the formation of only a purple control dot in the A well when the Control B+/A- is employed indicates an appropriate Flu A negative control result. Do not use the kit if Control A+/B- and Control B+/A- do not give appropriate results.

The liquid controls may also be used to demonstrate a low positive reaction. A weaker positive reaction may be demonstrated by dilution of both Control A+/B- and Control B+/A- together into the same **DispensTube** (2 drops of Control A+/B- and 2 drops of Control B+/A- added to 10 drops of Extraction Reagent E). Performance of reagents and technique may also be evaluated by using specimens known to be positive or negative.

X. LIMITATIONS OF THE PROCEDURE

- The **Directigen** Flu A+B test is capable of detecting both viable and non-viable influenza A and B virus particles. The **Directigen** Flu A+B test performance depends on antigen load and may not correlate with cell culture performed on the same specimen. The etiology of respiratory infection caused by microorganisms other than influenza A or B viruses will not be established with this test.
- Inadequate specimen collection, improper sample handling/transport, or low levels of viral shedding may yield a false-negative result. Accordingly, a negative test result does not totally eliminate the possibility of an influenza A, influenza B, or both influenza A and B infection. As with all diagnostic procedures, the results obtained with the **Directigen** Flu A+B test should be used in conjunction with other clinical information available to the physician.
- The validity of **Directigen** Flu A+B has not been proven for identification/confirmation of cell culture isolates and should not be utilized in this capacity.
- Performance of the test has not been established for monitoring antiviral treatment of influenza.

XI. EXPECTED VALUES

The rate of positivity observed in influenza testing will vary depending on 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. The prevalence of influenza varies from year to year. The prevalence in the past 3 years in the United States has ranged between 28 and 34%.¹⁶ The incidence of influenza B infection is more sporadic than influenza A infection. In the 1999 through 2000 season in the U.S. the prevalence of influenza A in those patients diagnosed with the flu was 99.6% as compared to a prevalence of 0.4% for influenza B.¹⁶ Based on culture method, the prevalence observed in the U.S. during the clinical trial period for this product ranged between 0% to 33.9% for influenza A and 0% to 16.2% for influenza B. The average prevalence observed in the **Directigen** Flu A+B clinical trial study was 17.9% for influenza A and 2.6% for influenza B.

XII. PERFORMANCE CHARACTERISTICS

The performance of the **Directigen** Flu A+B test was determined in a multi-center study conducted at six clinical centers and two physician office laboratories during the 1999-2000 influenza season. The clinical centers were located in Canada, Hong Kong and geographically diverse areas in the United States.

A total of 1262 specimens, consisting of nasopharyngeal aspirate, nasopharyngeal wash, nasopharyngeal swab, lower nasal swab, nose/throat swab, throat swab, and bronchoalveolar lavage specimens from 1046 influenza symptomatic patients were evaluated with the **Directigen** Flu A+B test. At one clinical site, both nasopharyngeal swabs and lower nasal swabs were collected from each of 216 patients.

The specimen populations included 58 frozen archived specimens consisting of preselected influenza A positive, influenza B positive and influenza A/B negative samples. Distribution of these frozen samples is designated for each data table provided below.

The specimens evaluated in the study were collected and transported to the laboratory according to each laboratory's procedure. The following tests were performed on each specimen: **Directigen** Flu A+B, cell culture and direct specimen DFA. Any remaining specimen was archived at or below -20°C.

Methods

Cell Culture: For cell culture, a portion of the specimen was inoculated into Rhesus Monkey Kidney (RMK) or Madin-Darby Canine Kidney (MDCK) cells. Cells were examined for the appearance of cytopathic effects (CPE). Infected cells were confirmed for influenza A or B by direct fluorescent antibody (DFA) staining. Specimens negative for CPE at fourteen days were stained for negative confirmation by DFA.

Direct Specimen DFA: For direct specimen DFA testing, a portion of each specimen was used to prepare a smear for examination by direct fluorescent antibody to influenza A and B.

RT-PCR: RT-PCR was performed on all available specimens that were culture negative and direct specimen DFA positive. In addition, a subset of specimens with other combinations of results was evaluated by RT-PCR.

Clinical Accuracy

For all specimens evaluated, the overall sensitivity of the **Directigen** Flu A+B test for influenza A when compared to culture was 86.2% and for influenza B was 80.8%. The overall specificity for influenza A when compared to culture was 90.7% and for influenza B was 99.5%. For influenza A, there were 96 samples that were culture negative, **Directigen** positive. Direct specimen DFA was positive for 77 of the 96 samples. RT-PCR was performed on 86 of the 96 specimens; a total of 78 specimens were positive by RT-PCR. The uninterpretable rate for the **Directigen** Flu A+B test was 0.08% for both influenza A and influenza B results.

Nasopharyngeal Aspirates (NPA):

n=350 (one uninterpretable **Directigen** result is not included in the table nor in estimating performance characteristics)

		Cell Culture Results		
		A+/B-	A-/B+	A-/B-
Directigen Flu A+B	A+/B-	44	0	26*
	A-/B+	0	28	6**
	A-/B-	2	4	239

95% C.I.

Influenza A	Sensitivity	95.7%	44/46	85.2-99.5
	Specificity	91.4%	277/303	87.6-94.3
Influenza B	Sensitivity	87.5%	28/32	71.0-96.5
	Specificity	98.1%	311/317	95.9-99.3

*Of the 26 specimens, 20/26 were positive by DFA and 23/25 were positive by RT-PCR.

** Of the 6 specimens, 6/6 were negative by DFA and 5/5 were negative by RT-PCR.

Nasopharyngeal Specimen (NP) includes: Nasopharyngeal Wash and/or Nasopharyngeal Swab

n=512

		Cell Culture Results		
		A+/B-	A-/B+	A-/B-
Directigen Flu A+B	A+/B-	100	0	41*
	A-/B+	0	12**	0
	A-/B-	13	5**	341

95% C.I.

Influenza A	Sensitivity	88.5%	100/113	81.1-93.7
	Specificity	89.7%	358/399	86.3-92.5
Influenza B	Sensitivity	70.6%	12/17	44.0-89.7
	Specificity	100%	495/495	99.3-100

*Of the 41 specimens, 32/41 were positive by DFA and 30/35 were positive by RT-PCR.

** Frozen (archived) specimens for influenza B.

Note: Of the 50 frozen archived specimens tested, 10/13 culture positive specimens were positive and 37/37 culture negative specimens were negative for influenza A using the Directigen Flu A+B test. For influenza B, 12/17 culture positive specimens were positive and 33/33 culture negative specimens were negative when tested with the Directigen Flu A+B test.

Nose/Throat Specimens (NTS) include: Throat Swabs (TS) and /or Lower Nasal Swabs (LNS)
n=389

		Cell Culture Results		
		A+/B-	A-/B+	A-/B-
Directigen Flu A+B	A+/B-	56	0	29*
	A-/B+	0	0	0
	A-/B-	17	1**	286

95% C.I.

Influenza A	Sensitivity	76.7%	56/73	65.4-85.8
	Specificity	90.8%	287/316	87.1-93.8
Influenza B	Sensitivity	0.0%	0/1	0.0-97.5
	Specificity	100%	388/388	99.1/100

*Of the 29 specimens, 25/29 were positive by DFA and 25/26 were positive by RT-PCR.

**Frozen (archived) specimens for influenza B.

Note: Of the 5 frozen archived specimens tested, 3/3 culture positive specimens were positive and 2/2 culture negative specimens were negative for influenza A using the Directigen Flu A+B test. For influenza B, 0/1 culture positive specimens were positive and 4/4 culture negative specimens were negative when tested with the Directigen Flu A+B test.

Bronchoalveolar Lavage (BAL):

n=11

		Cell Culture Results		
		A+/B-	A-/B+	A-/B-
Directigen Flu A+B	A+/B-	0	0	0
	A-/B+	0	2*	0
	A-/B-	0	0	9

95% C.I.

Influenza A	Sensitivity	N/A**	N/A**	N/A**
	Specificity	100%	11/11	71.5-100
Influenza B	Sensitivity	100%	2/2	15.8-100
	Specificity	100%	9/9	66.4-100

*Frozen (archived) specimens for influenza B.

**N/A = Not applicable

Note: Of the 3 frozen archived specimens tested, 3/3 culture negative specimens were negative for influenza A using the Directigen Flu A+B test. For influenza B, 2/2 culture positive specimens were positive and 1/1 culture negative specimens were negative when tested with the Directigen Flu A+B test.

Paired Specimen Analyses:

At one clinical site, both nasopharyngeal swabs and lower nasal swabs were collected from each of 216 patients. All of the specimens from this site were negative for influenza B by both culture and the **Directigen Flu A+B** test. There were no statistically significant differences detected between specimen types for either the **Directigen Flu A+B** test or cell culture.

Directigen Flu A+B

		nasopharyngeal swab	
		+	-
lower nasal swab	+	53	5
	-	5	153

% Agreement = 206/216 = 95.4%

Cell Culture

		nasopharyngeal swab	
		+	-
lower nasal swab	+	25	9
	-	7	175

% Agreement = 200/216 = 92.6%

Analytical Specificity and Cross-Reactivity

The **Directigen** Flu A+B test was evaluated using a total of 90 microorganisms (58 bacteria, two yeasts and 30 viruses). Bacteria and yeast isolates were tested at concentrations of 10^7 to 10^8 CFU/mL. *M. pneumoniae* was tested at a concentration of $> 10^8$ CCU/mL. Viral isolates were tested at titers between 10^4 to 10^{10} TCID₅₀/mL. Influenza C was tested at a titer of 1.6×10^{10} CEID₅₀/mL. None of the microorganisms or viruses listed below gave a positive result in the **Directigen** Flu A+B test.

Microorganism Panel		
<i>Acinetobacter baumannii</i>	<i>Kingella kingae</i>	<i>Prevotella oralis</i>
<i>Actinobacillus suis</i>	<i>Klebsiella pneumoniae</i>	<i>Proteus mirabilis</i>
<i>Bacteroides fragilis</i>	<i>Lactobacillus casei</i>	<i>Proteus vulgaris</i>
<i>Bordetella pertussis</i>	<i>Lactobacillus fermentum</i>	<i>Pseudomonas aeruginosa</i>
<i>Candida albicans</i>	<i>Lactobacillus plantarum</i>	<i>Salmonella choleraesuis</i> subsp. <i>minnesota</i>
<i>Candida glabrata</i>	<i>Legionella pneumophila</i>	<i>Serratia marcescens</i>
<i>Cardiobacterium hominis</i>	<i>Listeria monocytogenes</i>	<i>Staphylococcus aureus</i>
<i>Chlamydia psittaci</i>	<i>Moraxella catarrhalis</i>	<i>Staphylococcus aureus</i> -(Cowan 1)
<i>Chlamydia trachomatis</i>	<i>Mycobacterium avium</i>	<i>Staphylococcus epidermidis</i>
<i>Corynebacterium diphtheriae</i>	<i>Mycobacterium intracellulare</i>	<i>Streptococcus bovis</i> II Group D
<i>Eikenella corrodens</i>	<i>Mycobacterium tuberculosis</i>	<i>Streptococcus mutans</i>
<i>Enterococcus faecalis</i>	<i>Mycoplasma orale</i>	<i>Streptococcus oralis</i>
<i>Enterococcus gallinarum</i>	<i>Mycoplasma pneumoniae</i>	<i>Streptococcus pneumoniae</i>
<i>Escherichia coli</i>	<i>Neisseria gonorrhoeae</i>	<i>Streptococcus pyogenes</i> Group A
<i>Fusobacterium nucleatum</i>	<i>Neisseria meningitidis</i>	<i>Streptococcus sanguis</i>
<i>Gardnerella vaginalis</i>	<i>Neisseria mucosa</i>	<i>Streptococcus</i> sp. Group B
<i>Haemophilus aphrophilus</i>	<i>Neisseria sicca</i>	<i>Streptococcus</i> sp. Group C
<i>Haemophilus influenzae</i>	<i>Neisseria subflava</i>	<i>Streptococcus</i> sp. Group F
<i>Haemophilus parainfluenzae</i>	<i>Peptostreptococcus anaerobius</i>	<i>Streptococcus</i> sp. Group G
<i>Haemophilus paraphrophilus</i>	<i>Porphyromonas asaccharolyticus</i>	<i>Veillonella parvula</i>

Viral Panel			
Adenovirus	Type 3	HSV	Type 2
Adenovirus	Type 5	Influenza	C/Taylor/1233/47
Adenovirus	Type 7	Measles virus	Edmonston
Adenovirus	Type 18	Mumps virus	
Coronavirus		Parainfluenza	Type 1
Coxsackievirus	Type A9 (Griggs)	Parainfluenza	Type 2
Coxsackievirus	Type B5 (Faulkner)	Parainfluenza	Type 3
Coxsackievirus	Type B6 (Schmitt)	Rhinovirus	Type 1A
Coxsackievirus	Type A21 (Kuykendall)	Rhinovirus	Type 2
Coxsackievirus	Type A9 P.B. (Bozek)	Rhinovirus	Type 13
Cytomegalovirus	AD-169	Rhinovirus	Type 16
Echovirus	Type 2	Rhinovirus	Type 37
Echovirus	Type 3	RSV	A
Echovirus	Type 6	RSV	B
HSV	Type 1	VZV	

Interfering Substances

The following substances were tested in the **Directigen** Flu A+B test and no interference was noted in the assay for any substance at the levels tested: whole blood (2%), 4 nasal sprays (25%), acetylsalicylic acid (20 mg/mL), 3 mouthwashes (25%), ibuprofen (10 mg/mL), oseltamivir (0.5 mg/mL), zanamivir (1 mg/mL), chlorpheniramine maleate (5 mg/mL), 4 throat drops (25%), guaifenesin (20 mg/mL), diphenhydramine hydrochloride (5 mg/mL), dextromethorphan hydrobromide (10 mg/mL), pseudoephedrine hydrochloride (20 mg/mL), acetaminophen (216 mg/mL), clemastine fumarate (0.35 mg/mL), phenylpropanolamine hydrochloride (20 mg/mL).

Analytical Sensitivity

The analytical sensitivity was established for a total of 13 influenza strains; 7 influenza A and 6 influenza B strains.

Influenza Viral Strain	Type	Detection Limit (CEID₅₀)
A/PR/8/34 (H1N1)	A	8.2 X 10 ³
A1/FM/1/47 (H1N1)	A	5.9 X 10 ²
A/NWS/33 (H1N1)	A	1.6 X 10 ²
A1/Denver/1/57 (H1N1)	A	6.5 X 10 ¹
A/Port Chalmers/1/73 (H3N2)	A	2.9 X 10 ²
A/Victoria/3/73 (H3N2)	A	3.3 X 10 ⁴
A/New Jersey/8/76 (H1N1)	A	2.1 X 10 ²
B/Lee/40	B	1.2 X 10 ⁶
B/Allen/45	B	1.8 X 10 ²
B/Maryland/1/59	B	4.6 X 10 ¹
B/GL/1739/54	B	2.5 X 10 ³
B/Taiwan/2/62	B	6.6 X 10 ²
B/Hong Kong/5/72	B	2.3 X 10 ³

Reactivity and Specificity of Influenza A and B Strains

The **Directigen** Flu A+B test was evaluated using a panel of 62 influenza strains. All of the human and animal influenza A strains gave positive test results for Flu A and negative test results for Flu B. All the human influenza B strains gave positive test results for Flu B and negative test results for Flu A. All known influenza A hemagglutinin and neuraminidase subtypes are represented below.

Influenza Virus Human Isolate	Viral Type	Influenza Virus Human Isolate	Viral Type
A/PR/8/34	A (H1N1)	A/Human/HongKong/481/97	A (H5N1)
A1/FM/1/47	A (H1N1)	A/Human/HongKong/482/97	A (H5N1)
A/NWS/33	A (H1N1)	A/Human/HongKong/228156/97	A (H5N1)
A1/Denver/1/57	A (H1N1)	A/Human/HongKong/229540/97	A (H5N1)
A/New Jersey/8/76 (Hsw N1)	A (H1N1)	A/Human/HongKong/242095/97	A (H5N1)
A/HongKong/9821/2000	A (H1N1)	A/Human/HongKong/1073/99	A (H9N2)
A/HongKong/2997/98	A (H1N1)	A/Human/HongKong/1074/97	A (H9N2)
A/HongKong/5405/2000	A (H1N1)	B/Lee/40	B
A/HongKong/6611/2000	A (H1N1)	B/Allen/45	B
A/HongKong/15946/2000	A (H1N1)	B/GL/1739/54	B
A/HongKong/16051/2000	A (H1N1)	B/Taiwan/2/62	B
A/Port Chalmers/1/73	A (H3N2)	B/Maryland/1/59	B
A/Victoria/3/73	A (H3N2)	B/HongKong/5/72	B
A/HongKong/114313/2000	A (H3N2)	B/Hong Kong/28637/2000	B
A/HongKong/117393/2000	A (H3N2)	B/Hong Kong/27254/2000	B
A/HongKong/114591/2000	A (H3N2)	B/Hong Kong/28636/2000	B
A/HongKong/119563/2000	A (H3N2)	B/Hong Kong/29130/2000	B
A/HongKong/120277/2000	A (H3N2)	B/Hong Kong/29276/2000	B
A/Hong Kong/68012/2000	A (H3N2)	B/Hong Kong/35952/2000	B

Influenza Virus Animal Isolate	Viral Type	Influenza Virus Animal Isolate	Viral Type
A/Turkey/Kansas/4880/80	A (H1N1)	A/Turkey/Ontario/6118/67	A (H8N4)
A/Asia/57	A (H2N2)	A/Chicken/HongKong/G9/97	A (H9N2)
A/Mallard/New York/6750/78	A (H2N2)	A/Swine/HongKong/9/98	A (H9N2)
A/swine/HongKong/5212/99	A (H3N2)	A/Turkey/Wisconsin/66	A (H9N2)
A/Turkey/England/69	A (H3N2)	A/Quail/HongKong/G1/97	A (H9N2)
A/Duck/HongKong/477/78	A (H4N6)	A/Duck/HongKong/865/80	A (H10N3)
A/Chicken/Alabama/75	A (H4N8)	A/Chicken/Germany/N/49	A (H10N7)
A/Turkey/Wisconsin/68	A (H5N9)	A/Duck/Memphis/546/74	A (H11N9)
A/Goose/HongKong/38/79	A (H6N1)	A/Duck/Alberta/60/76	A (H12N5)
A/Turkey/Canada/63	A (H6N8)	A/Gull/MD/704/77	A (H13N6)
A/Turkey/Oregon/71	A (H7N3)	A/Mallard/Gurjev/263/82	A (H14N5)
A/Duck/HongKong/47/76	A (H7N2)	A/Shearwater/WA/2576/79	A (H15N6)

Reproducibility

The reproducibility of the **Directigen** Flu A+B test was evaluated with a 20 sample panel that included two levels (low positive and high positive) of influenza A, two levels (low positive and high positive) of influenza B and negative controls. The low positive samples for both influenza A and influenza B were at or near the lowest visual detection limit for the **Directigen** Flu A+B test. The overall accuracy for the **Directigen** Flu A+B test was 95% for influenza A and 98% for influenza B.

Physician Office Laboratory (POL) Reproducibility

An evaluation of the **Directigen** Flu A+B test was conducted by five people at three POL sites with the same 20 sample panel described in previous reproducibility section. Two technologists, a phlebotomist, a receptionist, and a medical assistant conducted the actual testing. The panel was evaluated on three successive days. The overall accuracy was 93% for influenza A and 92% for influenza B.

XIII. AVAILABILITY

Cat. No.	Description
256010	Directigen TM Flu A+B (Influenza A and B virus) kit, 20 Determinations.
261514	Viral Culturette TM , Single Swab, carton of 100.
220099	BBL TM CultureSwab TM , Single Swab, Liquid Stuart, package of 50.
220093	BBL TM CultureSwab TM , Single Swab, Liquid Amies, package of 50.

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XV. TECHNICAL INFORMATION

In the United States, telephone Technical Services, toll free (800) 638-8663.

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PIRev 03/2001

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