LABORATORY PROCEDURE
DIRECTIGEN™ FLU A
FOR THE DIRECT DETECTION OF INFLUENZA A ANTIGEN

I. INTENDED USE

The Directigen™ Flu A Test is an in vitro enzyme immunoassay (EIA) membrane test for the direct rapid and qualitative detection of influenza A viral antigen from suitable specimens of symptomatic patients. Nasopharyngeal wash and aspirate specimens have been shown to be superior to nasopharyngeal and throat swab specimens and are the specimens of choice with **Directigen** Flu A test.

II. SUMMARY AND EXPLANATION

Procedures currently used to diagnose influenza Type A infection include serologic assays, direct specimen immunofluorescence (IF) and culture isolation with confirmation procedures.[1,2,3] The latter is considered the standard method and employs initial viral isolation in cell culture followed by hemadsorption inhibition, immunofluorescence, or neutralization assays to confirm the presence of the influenza virus.[4,5,6] The **Directigen** Flu A antigen detection test employs an enzyme immunomembrane filter assay to detect influenza A antigen extracted from suitable specimens from 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 Test because the target antigen is the nucleoprotein which is type specific and highly conserved.[8]

The speed and workflow of **Directigen** Flu A make it applicable as a "STAT" influenza A antigen detection test - providing rapid, relevant information to assist with specific antiviral intervention and other clinical or support decisions.

III. PRINCIPLES OF PROCEDURES

Extracted nasopharyngeal or pharyngeal specimens are added to a **ColorPAC**™ test device, and any influenza A antigen present is non-specifically bound to the membrane surface as the specimen passes through the membrane. Detector enzyme conjugated monoclonal antibodies (2) specific for the influenza A nucleoprotein antigen 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** Flu A Test kit includes:

Component	Quantity	Description
ColorPAC Devices	(20)	20 Test kit. Devices with flow controller units, containing a control dot of inactivated influenza A (H1N1) antigen in the center of the membrane.
DispensTube™ Tubes and Tips	(20)	20 Test kit.
Reagent A	(5.0mL)	Extraction, 1.6% mucolytic agent and 6.4 % detergent, with 0.2% sodium azide (preservative).
Reagent 1	(8.5mL)	Wash, 150mM citric acid.
Reagent 2	(4.7mL)	Wash, 50mM Tris and rabbit IgG with 0.2% sodium azide (preservative).
Reagent 3	(4.3mL)	Detection, anti influenza A monoclonal murine antibodies (2) - enzyme conjugated, with 0.2% sodium azide (preservative).
Reagent 4	(8.5mL)	Wash, 5% butanol, 2M urea and 100mM HEPES with 0.2% sodium azide (preservative).
Reagent 5	(7.3mL)	Wash, 50mM Tris and 150mM NaCl with 0.2% sodium azide (preservative).
Reagent 6	(4.5mL)	Substrate A, 0.4mM chromogen, with 0.02% sodium azide (preservative).
Reagent 7	(3.9mL)	Substrate B, 7.8mM chromogen with 0.2% sodium azide (preservative).
Reagent 8	(4.1mL)	Stop, 150mM citric acid.
Control +	(2.0mL)	Positive Control, detergent-treated influenza A antigen (H1N1) with 0.2% sodium azide (preservative).
Control -	(2.0mL)	Negative control, detergent-treated non-infected egg fluid, with 0.2% sodium azide (preservative).

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.

The Influenza A **Control** + has been prepared from influenza A (HlNl)-infected egg fluid which has 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 liberate 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 **Directigen** Flu A.

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

ColorPAC Device: Remove the device from the foil pouch just prior to use.

Storage: Store at room temperature (15 to 30^0C). DO NOT FREEZE.

V. SPECIMEN COLLECTION AND HANDLING

Refer to illustration on Page 48 of Package insert for Specimen Collection.

Transport fresh specimens to the laboratory as rapidly as possible in a suitable liquid transport system maintained at 2 to 8°C (handle according to 42 CFR [Code of Federal Regulations] Part 72 for interstate transport of etiological materials if applicable). Process specimens as soon as possible after collection. Do not centrifuge specimens prior to use with **Directigen** Flu A, as the removal of cellular material will adversely affect the sensitivity of the test. 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.

Fresh specimens are preferable to frozen, as decreased sensitivity may result. Avoid multiple freeze-thaw cycles. Do not store specimens in self-defrosting freezer.

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.

VI. SPECIMEN PREPARATION

Acceptable specimens include nasopharyngeal washes, swabs, aspirates, and pharyngeal swabs. Nasopharyngeal washes and aspirates, however, have been shown to be superior to nasopharyngeal and pharyngeal swabs and are the specimens of choice.

NOTE: Excessively mucoid specimens may occasionally 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 to a McFarland standard #1, mixed well, and a 125µl aliquot retested.

Procedure for use with Nasopharyngeal Washes:[2]

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

Procedure for use with Nasopharyngeal Aspirates:[2]

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

Procedure for use with Nasopharyngeal or Pharyngeal Swabs:[7]

1. Swab specimens should be added to 1-2 mL of transport medium or saline immediately after collection.
2. Mix the swab well in the transport medium or saline solution.
3. Remove as much liquid from the swab as possible.
4. Discard the swab into appropriate container.
5. Process specimen as described in "Test Procedure."

VII. PROCEDURES

Materials Provided: See "Reagents" for materials provided.

Materials 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** Flu A 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)	**Trypticase®** Soy Broth plus 0.5% gelatin
Veal Infusion Broth (VIB)	Minimal Essential Media (MEM) with 1% BSA
Viral **Culterette™** (see "Availablity")	M5 Media

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 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", and "Results". Reagents, specimens, and **ColorPAC** devices must be at room temperature (15 to 30°C) when used.

VIII. TEST PROCEDURE

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

A. **ColorPAC** Preparation

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

B. Specimen Extraction

Mix specimen well. Pipette 125µl of specimen into the **DispensTube** device or fill to line on calibrated transfer pipet. Transfer sample to **DispensTube** device.

Reagent A - gently mix. **Dispense 8 drops into DispensTube** device.

Vortex or mix thoroughly.

NOTE: Quality Control

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

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

Insert a tip into the **DispensTube** device. **NOTE: Do not use tips from other Directigen products.**

Invert and carefully squeeze.

Dispense all of the extracted specimen dropwise (avoiding excess bubble addition) into the ColorPAC test well.

Allow 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

Reagent 1 - gently mix.
Rapidly add drops until well is filled. (Approximately 10 drops).
Allow to absorb completely.

Remove the Flow Controller. Discard as biohazard.
Reagent 2 - gently mix.
Add 4 drops onto the ColorPAC membrane.
Allow to absorb completely.

Reagent 3 - gently mix.
Add 4 drops onto the ColorPAC membrane.
Allow to absorb completely.
Allow to stand 2 minutes.

Reagent 4 - gently mix.
Rapidly add drops until ColorPAC well is filled. (Approximately 12 drops.)
Allow to absorb completely.

Reagent 5 - gently mix.
Add 4 drops onto the ColorPAC membrane.
Allow to absorb completely.

Reagent 6 - gently mix.
Add 4 drops onto the ColorPAC membrane.
Allow to absorb completely
NOTE: Membrane will turn yellow.

Reagent 7 - gently mix.
Add 4 drops onto the ColorPAC membrane.
Allow to absorb completely.
Allow to stand 5 min.

IX. RESULTS

Read the results in a well-lighted area within 25 min and record the test results.

OPTIONAL -To further extend the readout time period to a maximum of 12 hours, add 4 drops of **Reagent 8** (Stop Solution) onto the **ColorPAC** membrane.

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

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

Uninterpretable Test - The test is uninterpretable if neither a purple dot nor a purple triangle is 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. To correct this problem, dilute the sample 1:4 in 0.9% saline, or transport media and repeat the test .

Excessively mucoid samples 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 assistancce, telephone Technical Services in the United States at 1-800-638-8663.

X. QUALITY CONTROL

Each **Directigen** Flu A **ColorPAC** device contains both internal positive and negative procedural controls (i.e., two levels). The appearance of a purple control dot provides an internal positive reactivity 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 control for the device. The lack of any significant 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 influenza A 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.

Patient results should not be reported if positive and negative controls do not yield appropriate results.

XI. LIMITATIONS OF PROCEDURE

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

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 totally eliminate the possibility of an influenza A infection. Patient diagnosis should always include laboratory test results in concert with all other clinical information available.

The validity of **Directigen** Flu A has not been proven for identification/confirmation of cell culture isolates and should not be utilized in this capacity.

XII. 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.

XIII. PERFORMANCE CHARACTERISTICS

Clinical Accuracy: The clinical performance of the **Directigen** Flu A test was determined by prospective evaluations conducted by five independent investigators from three geographic regions of the U.S. during the 1989-1990 flu season.

A total of 1100 fresh specimens consisting of nasopharyngeal wash (NPW), swab (NPS), aspirate (NPA), and pharyngeal swab (PS) specimens from influenza symptomatic patients were evaluated with **Directigen** Flu A. In addition 29 frozen NPS specimens were also retrospectively evaluated in-house. Cell culture (CC) was the primary reference method, with blocking assays (BA) used to resolve discrepancies, when applicable and when sufficient specimen volume permitted. Results are summarized in Table 1 for each specimen type.

TABLE 1

Agreement between **Directigen**™ Flu A and Reference Results					
Specimen Type	Total Number	Sensitivity		Specificity	
		CC* only	CC + BA**	CC only	CC + BA
NPA	717	92% (68/74)	94% (90/96)	88% (568/643)	95% (568/597)
NPW	175	96% (23/24)	96% (24/25)	90% (136/151)	91% (136/150)
PS	183	67% (10/15)	80% (20/25)	92% (156/168)	99% (156/158)
NPS	54	88% (21/24)	88% (22/25)	97% (29/30)	100% (29/29)

*Cell Culture reference method: Two tubes of Primary Rhesus Monkey Kidney cells were inoculated and held 10-21 days for cultivation. Hemadsorption with guinea pig erythrocytes was performed on all CPE negative cultures at least once after 3-5 days, and again on the last day of culture. Influenza A virus was definitively identified in hemadsorption positive cultures using influenza A specific fluorescent conjugated antisera.

24/75 culture negative, **Directigen positive specimens were QNS for blocking assay resolution and were deleted from the database.

Overall, the sensitivity of the **Directigen** Flu A test for all of the specimen types, using cell culture and blocking results as the reference, is 91% (156/171) and the specificity is 95% (889/934). There were a total of 28 specimens (2.6%) in this study that originally yielded uninterpretable results that were resolved following dilution and retesting.

Physician Office Evaluation and Reproducibility: An evalutation of this test in the physician's office setting was determined at four office sites using a total of 160 in vitro prepared nasal wash specimens. Two small (1 physician), one medium (6 physicians) and one large (9 physicians) office settings were involved in this evaluation and the actual testing was conducted by a physician, a receptionist, a nurse, and a laboratory certified technician. The samples tested consisted of 13 negative, 15 low level positive and 12 mid to high positive specimens being run at each site in a blind fashion over a 3 day testing period. There was 100% correlation between the results obtained at these four sites and the expected results.

Cross Reactivity/Interference Studies: The bacterial and viral microorganisms listed in Tables 2 and 3 were used to assess both cross reactivity and interference in the **Directigen** Flu A Test. No cross reactivity or interference was noted in the assay for any of the microorganisms listed.

Table 2

Bacterial Cross-Reactivity /Interference Panel			
Streptococcus pyogenes	*Lactobacillus casei*	*Streptococcus agalactiae*	*Streptococcus sanguis*
Staphylococcus aureus	*Gardnerella vaginalis*	*Streptococcus* sp Group C	*Chlamydia trachomatis*
Pseudomonas aeruginosa	*Streptococcus pneumoniae*	*Streptococcus* sp Group F	*Chlamydia psittaci*
Serratia marcescens	*Neisseria meningitidis*	*Streptococcus* sp Group G	*Mycoplamsa pneumoniae*
Staphylococcus epidermidis	*Haemophilus infuenzae*	*Lactobacillis plantarum*	*Mycobacterium avium*
Acinetobacter calcoaceticus	*Neisseria gonorrhoeae*	*Listeria monocytogenes*	*Mycobacterium intracellulare*
Klebsiella pneumoniae	*Corynebacterium diphtheriae*	*Mycobacterium tuberculosis*	*Legionella pneumophila*
Escherichia coli	*Bordetella pertussis*	*Proteus vulgaris*	*Mycoplasma orale*
Enterococcus faecalis	*Bacteroides fragilis*	*Moraxella catarrhalis*	
Candida albicans	*Streptococcus mutans*	*Neisseria sicca*	

Table 3

Viral Cross Reactivity/Interference Panel			
Influenza B Great Lakes	HSV Type 1	Cytomegalovirus Type A9	Rhinovirus Type 13
Influenza B Hong Kong	HSV Type 2	Coxsackievirus Type A9	Rhinovirus Type 15
Adenovirus Type 3	Parainfluenza Type 1	Coxsackievirus Type B5	Rhinovirus Type 37
Adenovirus Type 5	Parainfluenza Type 2	Coxsackievirus Type B6	VZV
Adenovirus Type 7	Parainfluenza Type 3	Echovirus Type II	RSV
Adenovirus Type 10	Rhinovirus Type 1A	Echovirus Type 3	
Adenovirus Type 18	Rhinovirus Type 2	Echovirus Type 6	

Reactivity

The reactivity of **Directigen** Flu A was tested with the following strains of influenza A virus and found to be reactive.

A/Puerto Rico/8/34(H1N1)
A/Taiwan/1/86(H1N1)
A/New Jersey/8/76(HSW1N1)
A2/Taiwan/1/64(H2N2)
A/Japan/170/62(H2N2)
A/Japan/305/57(H2N2)
A/England/648/89(H3N2)
A/Victoria/5/89(H3N2)
A/Czechoslovakia1/6/89(H3N2)
A/Shanghai/11/87(H3N2)
A/NWS/33(H1N1)
A/Port Chalmers/1/73(H3N2)
A/Victoria/3/75(H3N2)
A/Beijing/352/89(H3N2)
A/Fukushima/2/88(H1N1)
A/Trinidad/2/B6(H1N1)
A/Victoria/43/88(H1N1)
A/Sichuan/4/88(H1N1)

Reproducibitity

The reproducibility of a test result with the **Directigen** Flu A Test was evaluated in a multiple specimen study. A panel consisting of five different levels of influenza A antigen (from weak to strong) plus a negative control were repeated five times with a single lot of the **Directigen** Flu A product. The testing was performed on one day with results interpreted by multiple readers. 100% accuracy was obtained for all the positive specimens and negative controls.

XIV. AVAILABILITY

Catalog #	Description
256020	**Directigen**™ Flu A (Influenza A Virus) Kit, 20 Determinations.
261514	Viral **Culturette**™, Single Swab, carton of 100.

XV. REFERENCES

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7. Dowdle, W.R., Kendal, A.P., and Noble, G.R. 1980 "Influenza Virus, Chapter 82", In: Manual of Clinical Microbiology, 3rd edition, Lennette, et. al., American Society for Microbiology, 836-884.
8. Kendal, A.P., and Dowdle, W.R. 1986 "Influenza Virus, Chapter 79th , In: Manual of Clinical Laboratory Immunology, 3rd edition, Lennette et. al., American Society for Microbiology, 515-520.

TECHNICAL INFORMATION: In the United States, telephone BD Diagnostic Systems Technical Services, toll free (800) 638-8663, Prompt 2.

Approved by:

Date Effective: ___________________

Supervisor: ___________________________ Date: __________

Director: ___________________________ Date:__________

Reviewed:

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