

BD Directigen™ EZ Flu A+B
For the Differentiated, Direct Detection of Influenzae A and B Viral Antigens

I. INTENDED USE

The **Directigen™** EZ Flu A+B test is a rapid chromatographic immunoassay for the direct and qualitative detection of influenza A and B viral antigens from nasopharyngeal washes/aspirates, nasopharyngeal swabs and throat swabs of symptomatic patients. The **Directigen** EZ Flu A+B test is a differentiated test, such that influenza A viral antigens can be distinguished from influenza B viral antigens from a single processed sample using a single device. The test is to be used as an aid in the diagnosis of influenza A and B viral infections. All negative test results should be confirmed by cell culture because negative results do not preclude influenza virus infection and should not be used as the sole basis for treatment or other management decisions.

Performance characteristics for Influenza B using nasopharyngeal swabs (NPS) were established primarily with retrospective, frozen specimens. Users may wish to establish the sensitivity of this test for Influenza B using fresh nasopharyngeal swab specimens.

II. SUMMARY AND EXPLANATION

Influenza illness classically presents with sudden onset of fever, chills, headache, myalgias, and a non-productive cough. Epidemics of influenza typically occur during winter months with estimated 114,000 hospitalizations¹ and 36,000 deaths² per year in the U.S. Influenza viruses can also cause pandemics, during which rates of illness and death from influenza-related complications can increase dramatically.

Patients who present with suspected influenza may benefit from treatment with an antiviral agent especially if given within the first 48 h of onset of illness. It is important to rapidly distinguish influenza A from influenza B in order to allow physicians a choice in selective antiviral intervention. Moreover, it is important to determine if influenza A or B 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.

Diagnostic tests available for influenza include rapid immunoassay, immunofluorescence assay, polymerase chain reaction (PCR), serology, and viral culture.³⁻¹⁰

Immunofluorescence assays entail staining of specimens immobilized on microscope slides using fluorescent-labeled antibodies for observation by fluorescence microscopy.^{5,11,12}

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.¹²⁻¹⁴

The **Directigen** EZ Flu A+B test is a chromatographic immunoassay to detect influenza A or B antigens from respiratory specimens of symptomatic patients with time to results of 15 min.

The speed and simplified workflow of the **Directigen** EZ Flu A+B test make it applicable as a "STAT" influenza A and B antigen detection test providing relevant information to assist with the diagnosis of influenza. The use of the **Directigen** EZ Flu A+B test 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** EZ Flu A+B test is a chromatographic assay to qualitatively detect influenza A and B viral antigens in samples processed from respiratory specimens. When specimens are processed and added to the test device, influenza A or B viral antigens bind to anti-influenza antibodies conjugated to visualizing particles in the corresponding A and B test strips. The antigen-conjugate complex migrates across the test strip to the reaction area and is captured by the line of antibody on the membrane. A positive result for influenza A is visualized as a reddish purple line at the Test "T" position and the Control "C" position in the **Directigen** EZ Flu A read window. A positive result for influenza B is visualized as a reddish purple line at the Test "T" position and the Control "C" position in the **Directigen** EZ Flu B read window.

IV. REAGENTS

The following components are included in the **Directigen** EZ Flu A+B kit:

BD™ Flu A+B Devices	30 Devices	Foil pouched device containing two reactive strips. Each strip that has a test line of monoclonal antibody specific to either Flu A or Flu B influenza viral antigen and a control line of anti-species antibody.
Reagent E	4.7 mL	Detergent, with 0.2% sodium azide (preservative).
Flu A+/B- Control Swab	1 each	Flu A Positive and Flu B Negative Control, influenza A antigen (inactive recombinant nucleoprotein) with < 0.1% sodium azide (preservative).
Flu B+/A- Control Swab	1 each	Flu B Positive and Flu A Negative Control, influenza B antigen (inactive recombinant nucleoprotein) with < 0.1% sodium azide (preservative).
DispensTube™ Tubes	30	Tubes for specimen processing and sample delivery into devices.

Materials Required But Not Provided: Pipette (capable of delivering 300 µL), timer, vortex mixer, transport media (see Specimen Collection and Handling), normal saline, and swabs.

Warnings and Precautions:

For *in vitro* Diagnostic Use.

1. If infection with a novel influenza A virus is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions for novel virulent influenza viruses and sent to state or local health departments for testing. Viral culture should not be attempted in these cases unless a BSL 3+ facility is available to receive and culture specimens.
2. Pathogenic microorganisms, including hepatitis viruses, Human Immunodeficiency Virus and novel influenza viruses, may be present in clinical specimens. "Standard Precautions"¹⁵⁻¹⁸ and institutional guidelines should be followed in handling, storing and disposing of all specimens and all items contaminated with blood and other body fluids.
3. Reagents contain sodium azide, which is harmful if inhaled, swallowed or in contact with skin. 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.
4. Do not use kit components beyond the expiration date.
5. Do not mix reagents from different kit lot numbers.
6. Do not reuse the device.
7. Do not use the kit if the Controls do not yield appropriate results.

Storage and Handling: Kits may be stored at 2–25°C. DO NOT FREEZE. Reagents and devices must be at room temperature (15–25°C) when used for testing.

V. SPECIMEN COLLECTION AND HANDLING

Specimen Transport and Storage:

Freshly collected specimens should be processed immediately. If necessary, specimens (other than throat swabs) may be stored in suitable transport medium and maintained at 2–8°C for up to 72 h or at –20°C for up to seven days.

It is essential that correct specimen collection and preparation methods be followed. Do not centrifuge specimens prior to use, as the removal of cellular material may adversely affect test sensitivity. Specimens obtained early in the course of the illness will contain the highest viral titers.

Transport Media: The following transport media have been tested and found to be compatible with the **Directigen EZ Flu A+B Test**:

Amies Medium (liquid)	PBS plus 0.5% BSA
Bartel ViraTrans™ Medium	PBS plus 0.5% gelatin
Earle's Minimal Essential Medium (EMEM)	Phosphate Buffered Saline (PBS)
EMEM plus 0.5% BSA	Starplex Multitrans™
EMEM plus 1% BSA	Sucrose Phosphate (2-SP)
Hanks Basal Salt Solution	Trypticase™ Soy Broth
M4 Medium	Trypticase Soy Broth + 0.5% gelatin
M4 RT Medium	Trypticase Soy Broth + 0.5% BSA
M5 Medium	BD™ Universal Viral Transport Medium
Modified Stuart's Medium (liquid)	Veal Infusion Broth (VIB)
Normal Saline*	VIB plus 0.5% BSA

* Frozen storage of specimens collected in normal saline is not recommended for testing with the **Directigen EZ Flu A+B test**.

Other transport media may be utilized if an appropriate qualification exercise is performed.

NOTE: EMEM media containing lactalbumin (i.e., 0.5% or 1.0%) or any other transport media containing lactalbumin are not compatible with the **Directigen EZ Flu A+B test**.

Specimen Collection and Preparation: Acceptable specimens for testing with the **Directigen EZ Flu A+B test** include nasopharyngeal washes/aspirates, nasopharyngeal swabs and throat swabs. Inadequate specimen collection, improper specimen handling and/or transport may yield a false negative result; therefore, training in specimen collection is highly recommended due to the importance of specimen quality to accurate test results.

Procedure for Nasopharyngeal Wash/Aspirate and Nasopharyngeal Swab Specimens:

1. For NP washes/aspirates, sample volumes of 1 to 3 mL are recommended. If transport medium is used, minimal dilution of specimen is preferred.
2. Excessive wash volumes should be avoided as they may result in decreased test sensitivity.
3. For NP swabs, place swabs in 1 to 3 mL transport medium after collection.
4. Process specimen as described in "Test Procedure."

Procedure for Throat Swab Specimens (polyurethane [foam] swabs are recommended – See "Availability" Section):

1. Throat swab specimens must be processed without transport medium as described in "Test Procedure."

VI. PROCEDURE

Test Procedure

NOTES:

- Reagents, specimens and devices must be at room temperature (15–25°C) for testing.
 - Thoroughly mix all specimens prior to removal of an aliquot for processing. Do not centrifuge specimens.
 - **DispensTube** tubes and reagent bottles must be held vertically (approximately one inch above the **Directigen** EZ Flu A+B device sample well or **DispensTube** tube), while gently dispensing one drop at a time.
1. Remove the **Directigen** EZ Flu A+B device from its foil pouch immediately before testing.
 2. Label one device and one **DispensTube** tube for each control and specimen to be tested.
 3. Place the labeled **DispensTube** tube(s) in the designated area of workstation or rack.
 4. Gently mix Reagent E. Dispense 4 drops into the **DispensTube** tube. Hold reagent bottle vertically (approximately one inch above the **DispensTube** tube) while dispensing drops.
 5. Process specimens or controls as directed below:
 - a. **For nasopharyngeal wash/aspirate specimens and nasopharyngeal swab specimens in transport media:**
 1. Vortex or thoroughly mix specimen. Do not centrifuge.
 2. Pipette 300 µL of specimen into **DispensTube** tube (containing Reagent E).
 - b. **For throat swab specimens:**
 1. Add 300 µL of normal saline, to the **DispensTube** tube (containing Reagent E).
 2. Insert swab and express by rotating swab 6 – 8 times while pinching tube.
 3. Remove swab (pinch tube to remove excess fluid from the swab tip).
 - c. **For Kit Controls:**
 1. Add 300 µL of normal saline, to the Dispense tube (containing Reagent E)
 2. Insert control swab and express by rotating swab 6-8 times while pinching tube.
 3. Remove control swab while pinching tube to remove excess fluid from the swab tip.

6. Insert a **DispensTube** Tip into the **DispensTube** tube containing the processed specimen or control.
Note: Do not use tips from other Directigen products.
7. Vortex or mix thoroughly.
8. Invert the **DispensTube** tube and, holding the tube on the upper half away from the tip, gently squeeze three (3) drops of the processed specimen into the Flu A sample well and three (3) drops into the Flu B sample well of the appropriately labeled **Directigen** EZ Flu A+B device. To assure proper delivery, **DispensTube** tubes must be held vertically (approximately one inch from the **BD** Flu A+B device sample well), while gently dispensing one drop at a time, in quick succession.

Note: Squeezing the tube close to the tip may result in ejection of the tip and leakage of the contents from the tube.

9. After sample addition, read result at 15 min and record test result.

VII. QUALITY CONTROL:

Quality control requirements must be performed in accordance with local, state and/or federal regulations or accreditation requirements and your laboratory's standard Quality Control procedures.

Each **Directigen** EZ Flu A+B device contains both positive and negative internal/procedural controls:

- The appearance of a reddish purple control line in the Flu A and/or Flu B read windows at the Control "C" position provides an internal positive control that validates the proper reagent function and assures that the correct test procedure was followed.
- The membrane area surrounding the Flu A and/or Flu B test and control lines is the internal negative control for the device. A background area that is white to light pink indicates that the test is performing correctly.

Each **Directigen** EZ Flu A+B kit contains swab Control A+/B- and B+/A-:

These controls are tested in the same manner as patient specimens and provide a means of external quality control. At a minimum, these controls should be run as a quality control procedure for each new kit lot or shipment received. If desired, appropriate reagent performance and proper testing technique may also be determined by using specimens qualified as positive or negative for the influenza A or B virus.

The formation of a reddish purple line on the membrane in the Flu A read window at the Test (T) position and Control (C) position when the A+/B- Control is tested, and in the Flu B read window at the Test (T) position and Control (C) position when the B+/A- Control is tested, indicates that the influenza antigen binding property of the test strip is functional.

The formation of a reddish purple control line at the Control (C) position in the Flu B read window and the absence of a reddish purple test line at the Test (T) position when the A+/B- Control is employed is an appropriate Flu B negative control result that indicates proper reagent function and that the proper test procedure was followed. Similarly, the formation of a reddish purple control line at the Control (C) position on the Flu A read

window and the absence of a reddish purple test line at the Test (T) position when the B+/A- Control is tested indicates an appropriate Flu A negative control result.

If the kit controls do not perform as expected, do not report patient results. Contact your local BD representative or Technical Services for assistance.

VIII. INTERPRETATION OF RESULTS

NOTE: The control line intensity may vary between the Flu A and Flu B read windows. Variability in control line intensity is acceptable. The background area should be white to light pink and may vary in intensity between the Flu A and Flu B read windows.

Positive Test for Flu A (influenza A antigen present) - A reddish purple line appears at the Test "T" position and the Control "C" position in the **Directigen** EZ Flu A read window. This result does not identify any specific influenza A virus subtype. A reddish purple line should also appear at the Control "C" position in the **Directigen** EZ Flu B read window. This indicates influenza A antigen was detected in the specimen. The background area should be white to light pink.

Positive Test for Flu B (influenza B antigen present) - A reddish purple line appears at the Test "T" position and the Control "C" position in the **Directigen** EZ Flu B read window. A reddish purple line should also appear at the Control "C" position in the **Directigen** EZ Flu A read window. This indicates influenza B antigen was detected in the specimen. The background area should be white to light pink.

Negative Test for Flu A or Flu B (no antigen detected) - No reddish purple line visible at the Test "T" position in either the Flu A or the Flu B read window indicates that influenza A antigen, or influenza B antigen, or both, were not detected in the specimen. These results do not exclude influenza viral infection. A reddish purple line at the Control "C" position in the Flu A read window, the Flu B read window, or in both read windows indicates proper performance of test procedure and reagents. The background area should be white to light pink.

Invalid Test - The test is invalid either for Flu A, or Flu B, or for both Flu A and Flu B, if no reddish purple line is visible next to the Control "C" position in the respective read window(s). The test is also invalid if a reddish purple line appears at the Test "T" position in both the Flu A and Flu B read windows for the same specimen. If invalid, the test must be repeated.

IX. REPORTING OF RESULTS

Positive Test Positive for the presence of influenza A or influenza B antigen. A positive result may occur in the absence of viable virus.

Negative Test	Negative for the presence of influenza A or influenza B antigen. Infection due to influenza cannot be ruled-out because the antigen present in the sample may be below the detection limit of the test. Culture confirmation of negative samples is recommended.
Invalid Test	Test result is inconclusive. Do not report results.

X. LIMITATIONS OF THE PROCEDURE

- The etiology of respiratory infection caused by microorganisms other than influenza A or B virus will not be established with this test. **Directigen** EZ Flu A+B is capable of detecting both viable and non-viable influenza particles. The **Directigen** EZ Flu A+B test performance depends on antigen load and may not correlate with cell culture performed on the same specimen.
- Low levels of virus shedding may yield a false-negative result; therefore, a negative test result does not eliminate the possibility of an influenza A or influenza B, or both influenza A and B infection.
- The validity of **Directigen** EZ Flu A+B has not been proven for identification or confirmation of cell culture isolates.
- Performance characteristics for influenza A were established when influenza A/H3 and A/H1 were the predominant influenza A viruses in circulation. When other influenza A viruses are emerging, performance characteristics may vary.
- Children tend to shed virus for longer periods of time than adults, which may result in differences in sensitivity between adults and children.
- Positive and negative predictive values are highly dependent on prevalence. False positive test results are more likely during periods of low influenza activity when prevalence is moderate to low.

XI. EXPECTED VALUES

The rate of positivity observed in respiratory 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. The overall prevalence observed with culture during the 2003-2004 clinical study was 24.3% for influenza A and 3.8% for influenza B. During the 2004-2005 clinical study, the overall prevalence observed with culture was 26.0% for influenza A and 31.2% for influenza B. During the 2006 clinical study, the overall prevalence observed with culture was 33.9% for influenza A and 0.2% for influenza B.

XII. PERFORMANCE CHARACTERISTICS

Performance characteristics for the **Directigen** EZ Flu A+B test were established in multi-center studies conducted at five trial sites during the 2003-2004 respiratory season, 13 trial sites during the 2004-2005 respiratory season and 2 trial sites during the 2006 respiratory season. The clinical

centers were located in geographically diverse areas within the United States, Japan, Hong Kong and New Zealand.

A total of 1191 prospective specimens and 59 retrospective specimens were evaluated using the **Directigen EZ Flu A+B** test and cell culture. These specimens consisted of nasopharyngeal washes, nasopharyngeal aspirates, nasopharyngeal swabs and throat swabs from patients suspected of having influenza.

Clinical Performance:

The performance characteristics for **Directigen EZ Flu A+B** test as compared to cell culture for each specimen type are presented in Table 1 through Table 6.

Table 1: Summary of the Performance of the Directigen EZ Flu A+B Test Compared to Culture for all Specimen Types - Influenza A Combined 2003-2004, 2004-2005 and 2006 Respiratory Seasons

Specimen Type	Directigen™ EZ Flu A+B Test	Cell Culture	
		P	N
Nasopharyngeal washes/aspirates	P	115	2
	N	19	330
Sensitivity: 86% (95% CI: 79% - 91%) Specificity: 99% (95% CI: 98% - 100%)			
Throat swabs	P	43	29
	N	13	184
Sensitivity: 77% (95% CI: 64% - 87%) Specificity: 86% (95% CI: 81% - 91%)			
Nasopharyngeal swabs	P	146	20
	N	15	275
Sensitivity: 91% (95% CI: 85% - 95%) Specificity: 93% (95% CI: 90% - 96%)			

Table 2: Summary of the Performance of the Directigen EZ Flu A+B Test Compared to Culture for all Specimen Types - Influenza B Combined 2003-2004, 2004-2005 and 2006 Respiratory Seasons

Specimen Type	Directigen™ EZ Flu A+B Test	Cell Culture	
		P	N
Nasopharyngeal washes/aspirates	P	57	1
	N	14	394
Sensitivity: 80% (95% CI: 69% - 89%) Specificity: 100% (95% CI: 99% - 100%)			
Throat swabs	P	38	3
	N	17	211

Sensitivity: 69% (95% CI: 55% - 81%)			
Specificity: 99% (95% CI: 96% - 100%)			
Nasopharyngeal swabs	P	1	0
	N	0	455
Sensitivity: 100% (95% CI: 3% - 100%)			
Specificity: 100% (95% CI: 99% - 100%)			

Table 3: Summary of the Performance of the Directigen EZ Flu A+B Test Compared to Culture for Retrospective Nasopharyngeal Swabs - Influenza B from 2005 Respiratory Season

Specimen Type	Directigen™ EZ Flu A+B Test	Cell Culture	
		P	N
Nasopharyngeal swabs	P	32	0
	N	11	16
Positive agreement: 74% (95% CI: 59% - 87%)			
Negative agreement: 100% (95% CI: 79% - 100%)			

Table 4: Summary of the Performance of the Directigen EZ Flu A+B Test Compared to Culture for all Specimen Types by Population - Influenza A Combined 2003-2004, 2004-2005 and 2006 Respiratory Seasons

Specimen Type	Directigen™ EZ Flu A+B Test	Cell Culture			
		Pediatric (≤ 21 years)		Adult (> 21 years)	
		P	N	P	N
Nasopharyngeal washes/aspirates	P	94	2	21	0
	N	11	294	8	36
Sensitivity: Pediatric: 90% (95% CI: 82% - 95%); Adult: 72% (95% CI: 53% - 87%)					
Specificity: Pediatric: 99% (95% CI: 98% - 100%); Adult: 100% (95% CI: 90% - 100%)					
Throat swabs	Directigen™ EZ Flu A+B Test	Pediatric (≤ 21 years)		Adult (> 21 years)	
		P	N	P	N
		P	38	23	5
	N	10	151	3	33
Sensitivity: Pediatric: 79% (95% CI: 65% - 90%) Adult: 63% (95% CI: 24% - 91%)					
Specificity: Pediatric: 87% (95% CI: 81% - 91%) Adult: 85% (95% CI: 69% - 94%)					
Nasopharyngeal swabs	Directigen™ EZ Flu A+B Test	Pediatric (≤21 years)		Adult (> 21 years)	
		P	N	P	N
		P	62	11	84
	N	6	84	9	191

Sensitivity: Pediatric: 91% (95% CI: 82% - 97%); Adult: 90% (95% CI: 82% - 96%) Specificity: Pediatric: 88% (95% CI: 80% - 94%); Adult: 96% (95% CI: 92% - 98%)
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Table 5: Summary of the Performance of the Directigen EZ Flu A+B Test Compared to Culture for all Specimen Types by Population - Influenza B Combined 2003-2004, 2004-2005 and 2006 Respiratory Seasons

Specimen Type	Directigen™ EZ Flu A+B Test	Cell Culture			
		Pediatric (≤ 21 years)		Adult (> 21 years)	
		P	N	P	N
Nasopharyngeal washes/aspirates	P	49	1	8	0
	N	13	338	1	56
Sensitivity: Pediatric: 79% (95% CI: 67% - 88%); Adult: 89% (95% CI: 52% - 100%) Specificity: Pediatric: 100% (95% CI: 98% - 100%); Adult: 100% (95% CI: 94% - 100%)					
Throat swabs	Directigen™ EZ Flu A+B Test	Pediatric (≤ 21 years)		Adult (> 21 years)	
		P		N	
		P	19	1	19
	N	12	190	5	21
Sensitivity: Pediatric: 61% (95% CI: 42% - 78%); Adult: 79% (95% CI: 58% - 93%) Specificity: Pediatric: 99% (95% CI: 97% - 100%); Adult: 91% (95% CI: 72% - 99%)					
Nasopharyngeal swabs	Directigen™ EZ Flu A+B Test	Pediatric (≤ 21 years)		Adult (> 21 years)	
		P	N	P	N
		P	0	0	1
	N	0	163	0	292
Sensitivity: Pediatric: No Sensitivity Calculation; Adult: 100% (95% CI: 3% - 100%) Specificity: Pediatric: 100% (95% CI: 98% - 100%); Adult: 100% (95% CI: 99% - 100%)					

Table 6: Summary of the Performance of the Directigen EZ Flu A+B Test Compared to Culture for Retrospective Nasopharyngeal Swabs by Population - Influenza B from 2005 Respiratory Season

Specimen Type	Directigen™ EZ Flu A+B Test	Cell Culture			
		Pediatric (≤ 21 years)		Adult (> 21 years)	
		P	N	P	N
Nasopharyngeal	P	20	0	12	0

swabs	N	6	10	5	6
Positive agreement: Pediatric: 77% (95% CI: 56% - 91%); Adult: 71% (95% CI: 44% - 90%)					
Negative agreement: Pediatric: 100% (95% CI: 69% - 100%); Adult: 100% (95% CI: 54%-100%)					

Reproducibility: The reproducibility of the **Directigen** EZ Flu A+B test was evaluated at four sites. The reproducibility panel was composed of 20 simulated influenza samples. These samples included low Flu A or Flu B positive samples (near the assay limit of detection), moderate Flu A or Flu B positive samples and negative samples. The overall reproducibility for the **Directigen** EZ Flu A+B test was 99.6%.

Analytical Studies

Analytical Sensitivity (Limit of Detection)

The limit of detection (LOD) for the Directigen EZ Flu A+B test was established for a total of 17 influenza strains; 11 influenza A and six influenza B.

Type	Influenza Viral Strain	LOD (CEID ₅₀ /mL*)	LOD (TCID ₅₀ /mL**)
A	A/PR/8/34 (H1N1)	1.75 X 10 ⁴	-
A	A/FM/1/47 (H1N1)	1.98 X 10 ³	-
A	A/NWS/33 (H1N1)	1.00 X 10 ⁴	-
A	A1/Denver/1/57 (H1N1)	5.56 X 10 ³	-
A	A/New Jersey/8/76 (H1N1)	4.45 X 10 ³	-
A	A/Port Chalmers/1/73 (H3N2)	1.00 X 10 ³	-
A	A/Hong Kong/8/68 (H3N2)	2.78 X 10 ²	-
A	A2/Aichi2/68 (H3N2)	3.50 X 10 ³	-
A	A/Victoria/3/75 (H3N2)	2.78 X 10 ⁴	-
A	A/California/04/09 (H1N1)	-	5.37 X 10 ²
A	A/California/07/09 (H1N1)	-	1.86 X 10 ³
B	B/Lee/40	6.95 X 10 ⁵	-
B	B/Allen/45	2.00 X 10 ³	-
B	B/GL/1739/54	5.56 X 10 ³	-
B	B/Taiwan/2/62	3.50 X 10 ²	-
B	B/Maryland/1/59	2.23 X 10 ⁴	-
B	B/Hong Kong/5/72	2.23 X 10 ⁴	-

* CEID₅₀/mL = Chick Embryo Infectious Dose at which 50% of the embryos perish

** TCID₅₀/mL = 50% Tissue Culture Infectious Dose

Although this test has been shown to detect the 2009 H1N1 virus cultured from positive human respiratory specimen, the performance characteristics of this device with clinical specimens that are positive for the 2009 H1N1 influenza virus have not been established. **BD Directigen** EZ Flu A+B can distinguish between Influenza A and B viruses, but it can not differentiate influenza subtypes.

Analytical Specificity (Cross Reactivity)

The **Directigen** EZ Flu A+B test was evaluated with a total of 98 microorganisms. The 51 bacteria and two yeasts were tested at a concentration of $\sim 10^8$ CFU/mL (CFU - Colony Forming Units). *Mycoplasma pneumoniae* and *Mycoplasma orale* were tested at concentrations of $> 10^6$ and $> 10^7$ CCU/mL (CCU - Color Changing Units), respectively. The three mycobacteria strains were inoculated at 10^7 CFU/mL and *Chlamydia trachomatis* LGVII was tested at 2.5×10^8 EB/mL (EB = elementary bodies). The 39 viruses tested were evaluated at concentrations of 10^4 to 10^9 TCID₅₀/mL (TCID₅₀ - Tissue Culture Infectious Dose at which 50% of the cells are lysed) or 10^4 to 10^8 CEID₅₀/mL (CEID₅₀ - Chick Embryo Infectious Dose at which 50% of the chick embryos are infected). All microorganisms (other than influenza viruses) resulted in negative Flu A and Flu B test results. The influenza A viruses tested (n = 2) resulted in Flu A positive and Flu B negative test results. The influenza B viruses tested (n = 6) resulted in Flu B positive and Flu A negative test results.

<i>Acinetobacter baumannii</i> (<i>calcoaceticus</i>)	<i>Neisseria meningitidis</i>	Coxsackievirus Type A9 (Griggs)
<i>Actinobacillus suis</i>	<i>Neisseria mucosa</i>	Coxsackievirus Type A9 (P.B. Bozek)
<i>Bacteroides fragilis</i>	<i>Neisseria sicca</i>	Coxsackievirus Type B5 (Faulkner)
<i>Bordetella pertussis</i>	<i>Neisseria subflava</i>	Coxsackievirus Type B6 (Schmitt)
<i>Candida albicans</i>	<i>Peptostreptococcus anaerobius</i>	Coxsackievirus Type A21 (Kuykendall)
<i>Candida glabrata</i>	<i>Porphyromonas</i> <i>asaccharolyticus</i>	Cytomegalovirus (AD-169)
<i>Cardiobacterium hominis</i>	<i>Prevotella oralis</i>	Echovirus Type 2 (Cornelis)
<i>Chlamydia trachomatis</i> LGVII	<i>Proteus mirabilis</i>	Echovirus Type 3 (Morrisey)
<i>Corynebacterium</i> <i>diphtheriae</i>	<i>Proteus vulgaris</i>	Echovirus Type 6 (D'Amori)
<i>Eikenella corrodens</i>	<i>Pseudomonas aeruginosa</i>	HSV Type 1 (HF)
<i>Enterococcus faecalis</i>	<i>Salmonella choleraesuis</i> sub <i>minnesota</i>	HSV Type 2 (MS)
<i>Enterococcus gallinarum</i>	<i>Serratia marcescens</i>	Influenza A/PR/8/34 (H1N1)
<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>	Influenza A/Victoria/3/75 (H3N2)
<i>Fusobacterium nucleatum</i>	<i>Staphylococcus aureus</i> -Cowan 1	Influenza B/Hong Kong/5/72
<i>Gardnerella vaginalis</i>	<i>Streptococcus bovis</i> II group D	Influenza B /Lee/40
<i>Haemophilus aphrophilus</i>	<i>Staphylococcus epidermidis</i>	Influenza B/Allen/45
<i>Haemophilus influenzae</i>	<i>Streptococcus mutans</i>	Influenza B/GL/1739/54
<i>Haemophilus</i> <i>parainfluenzae</i>		Influenza B/Maryland/1/59
<i>Haemophilus</i> <i>paraphrophilus</i>	<i>Streptococcus oralis</i>	Influenza B/Taiwan/2/62
<i>Kingella kingae</i>	<i>Streptococcus pneumoniae</i>	Measles virus (Edmonston)
<i>Klebsiella pneumoniae</i>	<i>Streptococcus pyogenes</i> group A	Mumps virus (Enders)
<i>Lactobacillus casei</i>	<i>Streptococcus sanguis</i>	Parainfluenza Type 1 (Sendia/52)
<i>Lactobacillus fermentum</i>	<i>Streptococcus</i> sp. group B	Parainfluenza Type 2 (Greer)

<i>Lactobacillus plantarum</i>	<i>Streptococcus</i> sp. group C	Parainfluenza Type 3 (C243)
<i>Legionella pneumophila</i>	<i>Streptococcus</i> sp. group F	Rhinovirus Type 1A (2060)
<i>Listeria monocytogenes</i>	<i>Streptococcus</i> sp. group G	Rhinovirus Type 2 (HGP)
<i>Moraxella catarrhalis</i>	<i>Veillonella parvula</i>	Rhinovirus Type 13 (353)
<i>Mycobacterium avium</i>	Adenovirus, Type 3 (GB)	Rhinovirus Type 15 (1734)
<i>Mycobacterium intracellulare</i>	Adenovirus, Type 5 (Adenoid 75)	Rhinovirus Type 16 (11757)
<i>Mycobacterium tuberculosis</i>	Adenovirus, Type 7 (Gomen)	Rhinovirus Type 37 (151-1)
<i>Mycoplasma orale</i>	Adenovirus, Type 10 (J.J.)	RSV Type A (Long)
<i>Mycoplasma pneumoniae</i>	Adenovirus, Type 18 (D.C.)	RSV Type B (Wash/18537/62)
<i>Neisseria gonorrhoeae</i>	Coronavirus (229E)	VZV (Ellen)

Strain Reactivity with Influenza A and B viruses

The **Directigen** EZ Flu A+B test was evaluated using a panel of 39 influenza strains. All known hemagglutinin (15) and neuraminidase (9) subtypes of influenza A were represented in this panel. All of the human and animal influenza A strains showed positive Flu A test results and negative Flu B test results. Conversely, all of the influenza B strains showed positive Flu B test results and negative Flu A test results.

Influenza Virus (Human Strain)	Viral Type	Influenza Virus (Animal Strain)	Viral Type
A/NWS/33	A (H1N1)	A/Turkey/Kansas/4880/80	A (H1N1)
A/PR/8/34	A (H1N1)	A/Mallard/New York/6750/78	A (H2N2)
A1/FM/1/47	A (H1N1)	A/Turkey/England/69	A (H3N2)
A1/Denver/1/57	A (H1N1)	A/Chicken/Alabama/75	A (H4N8)
A/New Jersey/8/76 (Hsw N1)	A (H1N1)	A/Turkey/Wisconsin/68	A (H5N9)
A/Port Chalmers/1/73	A (H3N2)	A/Turkey/Canada/63	A (H6N8)
A/Victoria/3/75	A (H3N2)	A/Turkey/Oregon/71	A (H7N3)
A/Vietnam/3028/04	A (H5N1)	A/Turkey/Ontario/6118/67	A (H8N4)
A/Thailand/MK2/04	A (H5N1)	A/Turkey/Wisconsin/66	A (H9N2)
A/Hong Kong/486/97	A (H5N1)	A/Chicken/Germany/N/49	A (H10N7)
A/California/07/2004	A (H3N2)	A/Duck/Memphis/546/74	A (H11N9)
A/California/04/2009	A (H1N1)	A/Duck/Alberta/60/76	A (H12N5)
A/California/07/2009	A (H1N1)	A/Gull/MD/704/77	A (H13N6)
B/Lee/40	B	A/Mallard/Gurjev/263/82	A (H14N5)
B/Allen/45	B	A/Shearwater/WA/2576/79	A (H15N6)
B/GL/1739/54	B		
B/Maryland/1/59	B		
B/Taiwan/2/62	B		
B/Mass/3/66	B		
B/Hong Kong/5/72	B		
B/Victoria/504/00	B		
B/Tokyo/53/99	B		
B/Quingdao/102/91	B		
B/Leningrad/86/93	B		

NOTE: Performance characteristics for detecting influenza A virus from human specimens when non-A/H3 and non-A/H1 influenza A virus subtypes are emerging as human pathogens have not been established.

Although this test has been shown to detect the 2009 H1N1 virus cultured from positive human respiratory specimen, the performance characteristics of this device with clinical specimens that are positive for the 2009 H1N1 influenza virus have not been established. **BD Directigen EZ Flu A+B** can distinguish between Influenza A and B viruses, but it can not differentiate influenza subtypes.

Interfering Substances

Various substances were evaluated with the **Directigen EZ Flu A+B** test. These substances included whole blood (2%) and various medications. No interference was noted with this assay for any of the substances tested.

Medications Evaluated

Three over the counter mouthwashes at 25%	Pseudoephedrine HCl (20 mg/mL)
Three OTC throat drops at 25%	Guaiacol Glyceryl Ether (20 mg/mL)
Three OTC nasal sprays at 10%	Ibuprofen (10 mg/mL)
4-Acetamidophenol (10 mg/mL)	Oxymetazoline (0.05 mg/mL)
Acetylsalicylic acid (20 mg/mL)	Phenylephrine (1 mg/mL)
Chlorpheniramine maleate (5 mg/mL)	Loratidine (100 ng/mL)
Dextromethorphan (10 mg/mL)	Diphenhydramine HCl (5 mg/mL)
Fexofenadine (500 ng/mL)	Zanamivir (1 mg/mL)
Amantadine (500 ng/mL)	Rimantadine (500 ng/mL)
Albuterol (0.083 mg/mL)	Oseltamivir (500 ng/mL)
Ribavirin (500 ng/mL)	Synagis™ (0.1 mg/mL)

XIII. AVAILABILITY

Cat. No.	Description
256050	Directigen™ EZ Flu A+B 30 Test Kit
220144	BBL™ CultureSwab™ EZ 100 (single swab) for throat swab specimens
220115	BBL™ CultureSwab™ Sterile Single Swab, Pkg. of 100
220131	BBL™ CultureSwab™ Liquid Amies, Flexible Aluminum Wire, Pkg. of 50
220134	BBL™ CultureSwab™ Liquid Stuart, Flexible Aluminum Wire, Pkg. of 50
221819	Normal, saline, 5 mL, Ctn. of 100
220220	BD™ Universal Viral Transport 3 mL Vial, Ctn. of 50
220250	Regular Flocked Swab, Sterile Single Wrapped, Ctn. of 100
220251	Minitip Flocked Swab, Sterile Single Wrapped, Ctn. of 100
220252	Flexible Minitip Flocked Swab, Sterile Single Wrapped, Ctn. of 100
256033	Directigen™ EZ Flu A+B Control Set , Ctn. of 10

XIV. REFERENCES

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