**1.0 PURPOSE**

To explain the testing procedure for the Veritor System Flu A+B test, a chromatographic immunoassay for the rapid detection of influenza A and B in nasopharyngeal washes/aspirates.

**2.0 SUMMARY AND EXPLANATION**

The Veritor System 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 Veritor System 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.

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

**3.0 PRINCIPLES OF THE PROCEDURE**

The **Veritor System** 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 determined by the BD Veritor System Reader when antigen-conjugate is deposited at the Test “A” position and the Control “C” position on the Veritor System Flu A+B assay device. A positive result for influenza B is determined by the BD Veritor System Reader when antigen-conjugate is deposited at the Test “B” position and the Control “C” position on the Veritor System Flu A+B assay device. The reader analyzes and corrects for non-specific binding and detects positives not recognized by the unaided eye to provide an objective digital result.

**4.0 REAGENTS**

 **1.** The following components are included in the **Veritor System** Flu A+B kit:

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| BD™ Veritor System Flu A+B Devices | 30 devices | Foil pouched device containing one reactive strips. Each strip has two test lines of monoclonal antibody specific to either Flu A or Flu B influenza viral antigen and a control line of anti-species antibody.  |
| RV Reagent C | 30 tubes with 100uL reagent | Detergent with <0.1% sodium azide (preservative) |
| Flu A+/B- cotton swab | 1 each | Flu A positive and Flu B negative control, influenza A antigen (inactive recombinant neucleoprotein) with < 1% sodium azide (preservative) |
| Flu B+/A- cotton swab | 1 each | Flu B positive and Flu A negative control, influenza B antigen (inactive recombinant neucleoprotein) with < 1% sodium azide (preservative) |
| 300uL Pipette | 30each | Transfer pipette |
| **Materials Required But Not Provided:** BD Veritor System Reader, timer, vortex mixer, transport media (see Specimen Collection and Handling), Distilled or deionized water, and tube rack. |

**2. 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.
8. Lot to Lot validation is recorded on IMV01-035 Form B.
9. Test results are not meant to be visually determined. **All results must be determined using the BD Veritor System Reader.**

**3. Storage and Handling**: Kits may be stored at 2–30°C. DO NOT FREEZE. Reagents and devices must be at room temperature (15–30°C) when used for testing.

**5.0 SPECIMEN COLLECTION AND HANDLING**

**1. Specimen Transport and Storage:**

Freshly collected specimens should be processed within 1 hour. 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.

 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.

**2. Transport Media**: The following transport media have been tested and found to be compatible with the **Veritor System** Flu A+B Test:

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| 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 |
| BD Universal Transport |  |

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

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

**Note** Media containing lactalbumin (i.e., 0.5% or 1.0%) or any other transport media containing lactalbumin may not be compatible with the **Veritor System** Flu A+B test.

**3. Specimen Collection and Preparation**: Acceptable specimens for testing with the **Veritor System** Flu A+B test include nasopharyngeal washes/aspirates and nasopharyngeal swabs in transport media. 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.

**4. Procedure for Nasopharyngeal Wash/Aspirate and Nasopharyngeal Swab Specimens:**

1. NP wash/aspirate is the preferred specimen. For NP washes/aspirates, sample volumes of 1 to 3 mL are recommended (we recommend 2-3 ml to our clinicians, see Guide to Laboratory Services (<http://e-net>). 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 in transport media, a minimal volume of transport media(1 mL) is recommended . **Swabs are not recommended due to difficulty in swabbing the nasopharyngeal space, requiring special swabs and technical expertise.**
4. Process specimen as described in "Test Procedure."

**6.0 PROCEDURE**

 **Notes:**

* + Reagents, specimens and devices must be at room temperature (15–30°C) for testing.
	+ Thoroughly mix all specimens prior to removal of an aliquot for processing. Do not centrifuge speci-mens.

1. Remove one **RV Reagent C** tube/tip and one **Veritor System Flu A+B** device from its foil pouch immediately before testing.

2. Label one **Veritor System** device and one **RV Reagent C** tube for each control and specimen to be tested.

3. Place the labeled **RV Reagent C** tube(s) in the designated area of workstation or rack.

4. 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. Remove and discard the cap from the RV Reagent C tube
3. Using the transfer pipette, transfer 300uL of specimen into the RV Reagent C tube. Discard pipette after use.

**c. For Kit Controls:**

* 1. Remove and discard the cap from the RV Reagent C tube
	2. Using the transfer pipette add 300uL of distilled or deionized water to the RV reagent C tube
	3. Insert the control swab into the tube and vigorously plunde the swab up and down in the liquid for a minimum of 15 seconds.
	4. Remove control swab while pinching tube to remove excess fluid from the swab tip.

5. Press the attached tip firmly onto the RV Reagent C tube containing the processed specimen or control

6. Vortex or mix thoroughly.

7. Invert the RV Reagent C tube and hold the tube vertically approximately one inch above the device sample well. Holding the tube at the ridged area, gently squeeze 3 drops of the processed sample into the sample well of the appropriately labeled BD Veritor System Flu A+B device.

**NOTE: When running the BD Veritor System RSV at the same time as the BD Veritor System Flu A+B, you may use the same RV Reagent C tube to dispense the processed sample into both of the appropriate testing devices.**

8. Allow the test to incubate at room temperature for 10 minutes before inserting into the reader.

9. Insert the BD Veritor System Flu A+B device into the BD Veritor System Reader. *The BD System Reader should be turned on prior to use and will indicate when it is ready for insertion of the* BD Veritor System Flu A+B device.

10. Follow the on-screen prompts to complete the procedure and obtain the test result.

11. You may insert another BD Veritor System Flu A+B (or RSV) device into the BD Veritor System Reader immediately after results are obtained.

**7.0 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 **Veritor System** Flu A+B device contains both positive and negative internal/procedural controls:

* The internal positive control validates the immunological integrity of the device, proper reagent function, and assures that the correct test procedure was followed.
* The membrane area surrounding test lines functions as a background check on the assay device.
* The positive and negative controls are evaluated by the BD Veritor System Reader. Failure of the internal/procedural controls will generate an invalid test result.

Each Veritor System Flu A+B kit contains swab Controls **A+/B-** and **B+/A-** to be used as external controls.

These controls provide additional quality control material to demonstrate positive or negative assay results using the BD Veritor System Reader and Veritor System test device. BD recommends that external positive and negative controls be run once for:

* Each new kit lot
* Each new shipment of test kits
* Each newly trained operator
* As required by internal control procedures and in accordance with local, state and federal regulations or accreditation requirements

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

 At **Elkins Park**, a positive and negative control is run each day a patient test is ordered prior to reporting

 patient results. QC Results are documented on Immunology QC form IMV01-003 Form E.

**8.0 INTERPRETATION OF RESULTS**

**Note**: The BD Veritor System Reader must be used for all interpretation of test results. Operators should not attempt to interpret assay results visually direct from the testing device..

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| **Reader Display** |  **Interpretation** |
| Flu A +Flu B - | Positive test for Flu A (Influenza A antigen present) |
| Flu A –Flu B + | Positive test for Flu B (Influenza B antigen present) |
| Flu A –Flu B - | Negative test for Flu A and Flu B (no antigen detected) |
| RESULT INVALID | Result invalid |
| CONTROL INVALID | Control line error |

**Invalid Test** – If the test is invalid, the BD Veritor System Reader will display “RESULT INVALID” or “CONTROL INVALID” and the test or control must then be repeated.

**9.0 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.

 When resulting at Elkins Park, document results on IMV01-035 Form A and IMV01-035 Form B.

**10.0 LIMITATIONS OF PROCEDURE**

1. The etiology of respiratory infection caused by microorganisms other than influenza A or B virus will not be established with this test. **Veritor System** Flu A+B is capable of detecting both viable and non-viable influenza particles. The **Veritor System** Flu A+B test performance depends on antigen load and may not correlate with other diagnostic methods performed on the same specimen.
2. A false-negative result may occur if the level of viral antigen is below the detection limit of the test or if the sample was collected or transported incorrectly; therefore, a negative test result does not eliminate the possibility of an influenza A or influenza B and should be confirmed by an alternate method.
3. Positive test results do not rule out co-infections with other pathogens and do not identify specific influenza A virus sub-types.
4. The BD Veritor System Reader reports dual positive influenza A and influenza B results as “RESULT INVALID”. Specimens generating a “RESULT INVALID” should be retested. Upon retesting, if the specimen produces a “RESULT INVALID” the user may want to consider other methods to determine whether the sample is positive or negative for influenza virus.
5. Children tend to shed virus for longer periods of time than adults, which may result in differences in sensitivity between adults and children.
6. 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.

**11.0 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 an FDA-cleared molecular influenza A and B assay in the US during the 2010-2011clinical study was 23.9% for influenza A and 7.5% for influenza B. The overall prevalence observed with an FDA-cleared molecular influenza A and B assay in the US during the 2011-2012 clinical study was 31.7% for influenza A and 4.5% for influenza B.

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**Signatures for Approval:**

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| --- | --- | --- |
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**History Review**

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| **Date Reviewed** | **Reviewed By** | **Revisions** |
| 4/15/16 | L. Provost | New Procedure |
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