KAISER PERMANENTE®
COLORADO LABORATORY

FLU A AND FLU B TEST PROCEDURE

PURPOSE

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 an dB 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.

SCOPE

All Medical Technologists and Medical Laboratory Technicians working at the Medical Office Laboratory

SPECIMEN

1. Either a nasal wash or a nasopharyngeal swab is acceptable for this test. All other specimen types must be rejected; including calcium alginate swabs.
    a. Nasal washes are collected in the patient care areas and not by the laboratory. A nasal wash is suitable for testing if the specimen is maintained at 2-8° C for up to 72 hours or at -20 °C for up to seven days.
    b. Nasopharyngeal swabs must be submitted within 1 hour of collection.

Note: If the specimen is submitted by an external facility (i.e. nursing home), place swab in 1-3 ml of normal saline and transport on ice.

2. Allow the sample to warm to room temperature before testing with the Directigen kit.
3. Do not centrifuge specimens prior to use, as the removal of cellular material may adversely affect test sensitivity.

POLICY

1. Do not use kit components beyond the expiration date
2. Do not mix reagents from different kit lot numbers
3. Do not reuse the devise
4. Do not use the kit if the Control A+ / B- and Control B+ / A- do not yield appropriate results.

REAGENTS AND SUPPLIES

1. BD Directigen EZ Flu A+B Kit. (BD #256050 30 devices / kit)
   Each Kit includes the following items:
   - BD Flu A+B devices (30 devices) – Foil pouched device containing two reactive strips. Each strip 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 Positive and Flu B Negative control swab, influenza A antigen (inactive recombinant nucleoprotein) with < 0.1% Sodium azide (preservative)
   - Flu B Positive and Flu A Negative Control swab, influenza B antigen (inactive recombinant nucleoprotein) with < 0.1% sodium azide (preservative)
   - DispensTube Tubes (30) – Tubes for specimen processing and sample delivery into devices.
   - DispensTube Tips (30) – Tips to filter sample when delivered into devices

2. Adjustable pipette (capable of delivering 300 *ul*.
3. Timer
4. Vortex mixer
5. Normal Saline. Order through standard stock

TEST CODES

Cerner Test Code	Cerner Description	Health Connect Code	Health Connect Description
FLUAB	Influenza A & B Antigen Rapid	208463	Influenza A/B, rapid

STORAGE REQUIREMENTS

- Store the kit at room temperature 15-30 ^{0}C
- The Directigen kit and reagents are stable until the expiration dates marked on their packaging and containers.

QUALITY CONTROL

Daily Quality Control:

- The Directigen Flu A/B test has internal (built-in) procedural controls. For daily quality control, record these controls for each test run on the FLU A and FLU B log.

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.

External Positive and Negative Controls:

- Good laboratory practice suggests the use of positive and negative controls to ensure that test reagents are working and the test is correctly performed
- Directigen test kits contain positive and negative control specimens for both Flu A and Flu B.
- These controls are tested in the same manner a patient specimens and provide a means of external quality control.
- Run the control material with each new kit lot or shipment and write the result on the Flu A / Flu B log. External controls must be run a minimum of once per month.

***** NOTE: If the correct control results are not obtained, do not report patient results – notify the laboratory manager *****

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 the 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. Thoroughly mix the specimen or control and process as directed below:

   a. **For nasopharyngeal wash/aspirate specimens:**

      1. Vortex or thoroughly mix specimen. Do not centrifuge.

2. Pipette 300 µL of specimen into **DispensTube** tube (containing Reagent E).

b. **For Kit Controls:**

1. Add 300 µL of normal saline to the DispensTube (containing Reagent E) One tube for each control

2. Insert control swab and express by rotating swab 6-8 times while pinching tube

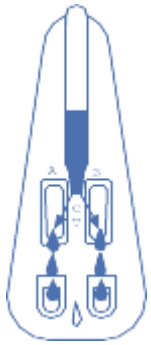
3. Remove control swab while pinching tube to remove excess fluid from swab tip

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.

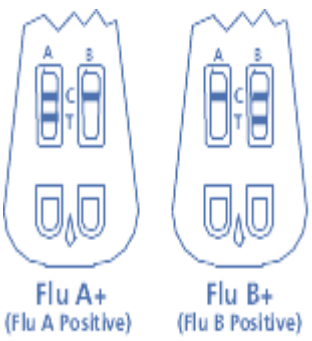


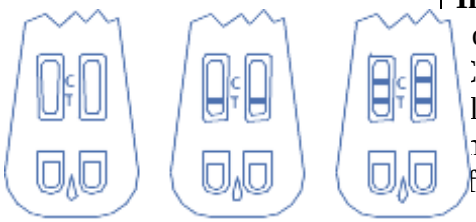
Note: Squeezing the tube close to the tip may result in ejection of the tip and leakage of the contents from the tube.

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

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) -** Any 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) - Any 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	**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 oth 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 lso invalid if a reddish purple line appears at the Test "T" position n both the Flu A and Flu B read windows for the same specimen. f invalid, the test must be repeated.

REPORTING OF RESULTS

1. Results are written on the BD FLU A and FLU B result log.
2. Put a check mark on the “control line” box to indicate that the internal control passed.
3. Put a check mark on the “background” box to indicate that the background passed (white to pink).
4. Write the result of the FLU A and FLU B (POS or NEG) in the appropriate box on the log.
5. Report as POSITIVE or NEGATIVE for both Influenza A AND Influenza B in the “ARE” application of the LIS.
6. Test must be repeated if invalid results are obtained.

PROCEDURAL NOTES

1. For in vitro diagnostic use only.
2. Leave test device sealed in its foil pouch until just before use.
3. DO NOT use kit past its expiration date.
4. For mucoid specimens: after thoroughly mixing the sample, allow sample to sit for a few (3) minutes and then pipette the liquid part of the sample. Or, the specimen can be mixed with an equal part of saline (i.e. 0.5 ml specimen with 0.5 ml saline).
5. For request from nursing homes and assisted living facilities, the lab will provide transport medium. Specimens will be retrieved by the TRiPS dept and transported on ice to the nearest MOL for testing.

LIMITATION 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.
- Calcium alginate swabs are unacceptable for this test.

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