



SAMPLE PROCEDURE

This "Sample Procedure" is not intended as a substitute for your facility's Procedure Manual or reagent labeling, but rather as a model for your use in customizing for your laboratory's needs.

Space has been provided within the document to allow you to update this template with information specific to your facility. It is suggested that a current version of the manufacturer's directional insert be maintained as a supplement.

I. TEST NAME

OSOM® Influenza A & B Test
CLIA Complexity: Moderate

II. INTENDED USE

The OSOM Influenza A&B Test is an in vitro diagnostic immunochromatographic assay intended for the qualitative detection of influenza A and influenza B viral nucleoprotein antigens from nasal swab specimens in symptomatic patients. It is intended to aid in the rapid differential diagnosis of influenza A and/or B viral infections. This test is not intended for the detection of influenza C viruses. A negative test is presumptive and it is recommended these results be confirmed by cell culture. Negative results do not preclude influenza virus infection and should not be used as the sole basis for treatment or other management decisions.¹

III. SUMMARY AND EXPLANATION OF TEST

Along with the common cold, influenza is one of the most common acute respiratory infections, producing symptoms such as headache, chills, dry cough, body aches and fever. It affects 10%-20% of the United States population annually, resulting in more than 110,000 hospitalizations and 10,000 to 40,000 deaths.²

The influenza A virus is typically more prevalent and is associated with the most serious influenza epidemics, while influenza B infections usually present more mild symptoms. Diagnosis is difficult because the initial symptoms can be similar to those caused by other infectious agents. Considering that the influenza virus is highly contagious, accurate diagnosis and prompt treatment of patients can have a positive effect on public health. Accurate diagnosis and the ability to distinguish between A or B antigens can also help reduce the inappropriate use of antibiotics and gives the physician the opportunity to prescribe an appropriate antiviral therapy. Initiation of antiviral therapy within 48 hours of symptom onset is recommended for more rapid reduction of symptoms and to reduce viral shedding.³ The OSOM influenza A&B Test can provide rapid detection of influenza A and/or B viral antigens from symptomatic patients.

IV. PRINCIPLE OF THE TEST

The OSOM Influenza A&B Test consists of a test stick that separately detects influenza A and B. The test procedure requires the solubilization of the nucleoproteins from a swab by mixing the swab in Extraction Buffer. The test stick is then placed in the sample mixture, which then migrates along the membrane surface. If influenza A and/or B viral antigens are present in the sample, it will form a complex with mouse monoclonal IgG antibodies to influenza A and/or B nucleoproteins conjugated to colloidal gold. The complex will then be bound by another mouse anti-influenza A and/or B antibody coated on the nitrocellulose membrane. A pink to purple control line must appear in the control region of the stick for results to be valid. The appearance of a second and possibly a third light pink to purple line will appear in the test line region indicating an A, B or A and B positive result.

V. KIT CONTENTS AND STORAGE

25 Test Sticks
25 Test tubes
25 Foam Swabs
1 Extraction Buffer vial

- 12 mL (20 mM phosphate buffered salt solution (pH 7.6) , 0.25 % protein stabilizer, 0.6% detergent and 0.09% sodium azide as a preservative)

- 1 Extraction Buffer dropper top
- 1 Influenza A Positive Control Swab (packaged with a desiccant tablet)
 - Formalin inactivated influenza A/Kitakushu/159/93 containing 0.05% sodium azide. Inactivity confirmed by inability of virus to infect cell culture.
 - Result is representative of a mid-level positive
- 1 Influenza B Positive Control Swab (packaged with a desiccant tablet)
 - Formalin inactivated influenza B/LEE/40 containing 0.05% sodium azide. Inactivity confirmed by inability of virus to infect cell culture.
 - Result is representative of a mid-level positive
- 1 Directional Insert
- 1 Procedure/Result Interpretation Guide
- 1 Workstation

Note: Two extra test sticks have been included in the kit for external QC testing. In addition, extra components (swabs, tubes) have been provided for your convenience.

STORAGE CONDITIONS

- Store test sticks and extraction buffer tightly capped at room temperature (15°- 30°C/59°- 86°F).
- Do not freeze any of the test kit components.
- Do not use test sticks and reagents after expiration date.
- Recap the desiccated container immediately after removing a test stick.
- Test sticks that have been outside of the desiccated container for more than 1 hour should be discarded.

At this facility, kits are stored: _____.

VI. MATERIALS REQUIRED BUT NOT PROVIDED
--

A timer or a watch.

VII. WARNINGS AND PRECAUTIONS

- For in vitro diagnostic use only.
- Follow your clinical and/or laboratory safety guidelines in the collection, handling, storage, and disposal of patient specimens, and all items exposed to patient specimens.⁴
- Swabs, test tubes, and test sticks are for single use only.
- The Extraction Buffer contains a solution with a preservative (0.09 % sodium azide). If solution comes in contact with the skin or eyes, flush with ample volumes of water.
- Solutions that contain sodium azide may react explosively with lead or copper plumbing. Use large quantities of water to flush discarded solutions down a sink.
- Do not interchange or mix components from different kit lots.
- 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.¹

VIII. PATIENT PREPARATION & SPECIMEN COLLECTION

This facility's procedure for patient preparation is: _____

This facility's procedure for sample labeling is: _____

Specimen Collection and Handling:

- Only nasal swabs can be used with this test. Use of nasal washes or aspirates has not been validated.
- Insert the swab into the nostril that appears to have the most secretion. Using a gentle rotation, push the swab until resistance is met at the level of the turbinates (at least one inch into the nostril). Rotate the swab a few times against the nasal wall.
- Use only the swabs supplied in the OSOM influenza A&B Test kit. Swabs from other suppliers have not been validated. Do not use swabs that have cotton, rayon or polyester tips or wooden shafts.
Test the swab as soon as possible after collecting the specimen. If swabs cannot be processed immediately, specimens may be held at room temperature for no longer than 8 hours. Swabs may also be stored at 2°- 8°C (36°- 46°F) for up to 24 hours. Extracted samples may be held at room temperature or refrigerated (2°- 8°C/36°- 46°F) for up to 24 hours.
- To transport patient samples place swab in a clean, dry container such as a plastic or glass tube.
- **If a culture result is desired, a separate swab must be collected for the culture.**
- The test performance depends on the quality of the sample obtained as well as the handling and transport of the sample. Negative results can occur from inadequate specimen collection and/or handling. Training in specimen collection is recommended because of the importance of specimen quality.

This facility's procedure for transporting specimens is: _____

This facility's procedure for rejected specimens is: _____

IX. QUALITY CONTROL & ASSURANCE

The OSOM Influenza A&B Test provides two types of controls: procedural internal controls to aid in determining test validity, and two external positive and negative controls for influenza A and influenza B. The influenza A control swab acts as a negative control for the influenza B antigen and conversely, the influenza B control swab serves as a negative control for influenza A antigen.

Internal Procedural Controls

Several controls are incorporated into each Test Stick for routine quality checks.

1. The appearance of the control line in the results window is an internal positive procedural control:

Test System: The appearance of the control line assures that adequate Extraction Buffer volume was present and that adequate capillary migration of the extracted sample has occurred. It also verifies proper assembly of the Test Stick.

Operator: The appearance of the control line indicates that an adequate Extraction Buffer volume was present for capillary migration to occur. If the control line does not appear at the read time, the test is invalid.

2. The clearing of the background in the results area may be documented as an internal procedural control. It also serves as an additional capillary flow control. At the read time, the background should appear white to light pink and not interfere with the reading of the test. If the background color does not clear and interferes with the test result, the test is invalid. Call Genzyme Diagnostics Technical Service at (800) 332-1042 if you experience a problem.

External Quality Control Testing

The OSOM Influenza A&B Test kit includes one Influenza A Positive Control Swab and one Influenza B Positive Control Swab, each of which contains inactivated virus, for external quality control testing. The Influenza A control swab acts as a negative control for the influenza B antigen and conversely, the Influenza B control swab serves as a negative control for influenza A antigen.

Use the Controls to help ensure that the test sticks are functioning properly and to demonstrate proper performance by the test operator

- The presence of a light pink to purple line at the “A” test line position and at the “Control” line position when the Influenza A positive control swab is tested, indicates that the influenza antigen binding property of the test stick is functional.
- The presence of a light pink to purple line at the “B” test line position and at the “Control” line position when the Influenza B positive control swab is tested, indicates that the influenza antigen binding property of the test stick is functional.

External controls are intended to monitor substantial reagent failure. The positive controls will not challenge the assay at the cutoff.

Quality Control requirements should be established in accordance with local, state and federal regulators or accreditation requirements. Minimally, Genzyme Diagnostics recommends that positive and negative external controls be run with each new lot, shipment received and with each new operator. Additional controls may be purchased separately (OSOM Influenza A&B Control Kit #191).

QC Testing Procedures

The Positive Control Swabs are impregnated with sufficient influenza A or B antigen to produce a visible positive test result. To perform a positive or negative control test, complete the steps in the Test Procedure section treating the control swab in the same manner as a specimen swab. The influenza A control swab acts as a negative control for the influenza B antigen and conversely, the influenza B control swab serves as a negative control for influenza A antigen.

QC Testing Frequency and Documentation

For this facility, External QC is run: _____

Results of External QC and action(s) taken when control results are unacceptable are documented:

X. TEST PROCEDURE

When opening kit for the first time, unscrew the cap from the Extraction Buffer bottle and replace it with the dropper top included in the kit. Discard the original Extraction Buffer cap.

STEP 1: ADD EXTRACTION BUFFER

Using the supplied dropper top, add 0.3 mL of Extraction Buffer to each test tube. Fill the dropper to the line indicated on the barrel of the dropper top and expel entire contents into tube. **Note: Add Extraction Buffer to the tube before putting in the specimen swab to prevent contaminating the Extraction Buffer vial.**

STEP 2: MIX SWAB IN BUFFER

Put the specimen swab into the tube. Vigorously mix the solution by rotating the swab forcefully against the side of the tube at least ten times (while submerged). Best results are obtained when the specimen is vigorously mixed in the solution.

STEP 3: SQUEEZE LIQUID FROM SWAB

Squeeze out as much liquid as possible from the swab by pinching the side of the flexible test tube as the swab is removed. Discard the swab in a suitable biohazardous waste container.

STEP 4: ADD TEST STICK

Remove a Test Stick from the canister. Recap the canister immediately. Place the test stick (arrows pointing downward) into the tube with the extraction buffer solution.

Set a timer for 10 minutes.

STEP 5: READ RESULTS

At 10 minutes remove the test stick from the tube and read the results (some positive results may be seen earlier).

For help in reading the test stick or for correct line placement refer to the Result Interpretation Guide in the directional insert.

Discard used test tubes and Test Sticks in suitable biohazardous waste container.

For this facility, sample swabs, used test tubes and Test Sticks are disposed: _____

XI. INTERPRETATION OF RESULTS

The appearance of a Control Line, with or without a Test Line, indicates a valid result. A pink-to-purple line that appears uneven in color shading is still considered a valid line. In cases of moderate or high positive specimens, some color behind the Test Line may be seen. As long as the Test Line and the Control Line are visible, the results are valid. For help in reading the test stick or for correct line placement refer to the Results Interpretation Guide in the directional insert.

Influenza A Positive

A pink-to-purple Test Line at the “A” test line position and a pink-to-purple Control Line is a positive result for the detection of influenza A antigen. Note that the pink-to-purple lines can be any shade of that color and can be lighter or darker than the line in the package insert picture.

Influenza B Positive

A pink-to-purple Test Line at the “B” test line position and a pink-to-purple Control Line is a positive result for the detection of influenza B antigen. Note that the pink-to-purple lines can be any shade of that color and can be lighter or darker than the line in the package insert picture.

Negative

A pink-to-purple Control Line but no Test Line is a presumptive negative result. A negative result means that no influenza antigen was detected, or that the level of the antigen in the sample was below the detection limit of the assay.

Invalid

If no pink-to-purple Control Line appears or background color makes reading the pink-to-purple Control Line impossible, the result is invalid. If this occurs, repeat the test on a new Test Stick or contact Genzyme Diagnostics’ Technical Service.

In the event this test becomes inoperable, this facility’s course of action for patient samples is: _____

XII. RESULT REPORTING

- Report negative test results as influenza A (or B) virus antigen not detected. Infection due to influenza cannot be ruled out since the antigen may be present in the specimen below the detection limit of the test. Negative tests are presumptive and should be confirmed by culture.
- Report positive test results as positive for influenza A (or B) virus antigen. This result does not rule out co-infections with other pathogens or identify any specific influenza A virus subtype.
- If result is considered invalid, repeat the test using a new sample and a new test dipstick.

This facility’s procedure for patient result reporting is: _____

XIII. LIMITATIONS

- The OSOM Influenza A&B Test is for the qualitative detection of influenza A and B viral antigens. The test performance depends on antigen load and may not correlate with cell culture performed on the same specimen. Negative test results are not intended to rule out other non-influenza viral infections.
- Sensitivity can differ with various strains of influenza due to difference in antigen expression. Specimens might contain new, non-identified strains of influenza that express varying amounts of antigen.
- This test detects both viable and non-viable influenza A and B, and may yield a positive result in the absence of living organisms.
- The test performance depends on the quality of the sample obtained as well as the handling and transport of the sample. Negative results can occur from inadequate specimen collection and/or handling.
- As with all diagnostic assays, the results obtained with this test kit yield data that must be used only as an adjunct to other information available to the physician.
- Use of nasal wash or aspirate has not been validated.
- *Staphylococcus aureus* in specimens at concentrations greater than 9×10^8 cfu/mL may interfere with the test results. Bacterial levels in sinonasal infections have been reported at levels that are much less than those that affect the assay; typically ranging between 10^5 and 10^7 cfu/mL.⁵
- High levels of blood on specimen swabs might cause an intense red background on the test strip that could interfere with the test interpretation. Avoid samples that have been heavily contaminated with whole blood.
- It is well-recognized that testing done with children will appear more sensitive because children shed virus more abundantly and longer than adults.⁶
- Positive and negative predictive values of these diagnostic assays are highly dependent on prevalence or current level of influenza activity.⁶ During peak influenza activity in a season, positive predictive values are higher, with false positives less likely; and negative predictive values are lower, with false negatives more likely. Conversely, during low influenza activity (e.g., off-season or beginning of a season), negative predictive values are higher and positive predictive values lower, with false positive test results more likely.
- Additional testing is required to differentiate any specific influenza A subtypes or strains, in consultation with state or local public health departments.¹
- Individuals who received nasally administered influenza vaccine may have positive test results for up to three days after vaccination.¹
- Monoclonal antibodies may fail to detect with less sensitivity, influenza A viruses that have undergone minor amino acid changes in the target epitope region.¹

XIV. EXPECTED RESULTS

Influenza viruses can cause epidemics which typically occur during the winter months and can also cause pandemics, during which rates of illness and death from influenza-related complications can increase dramatically worldwide. Influenza viruses cause disease among all age groups. Rates of infection are highest among children, but rates of serious illness and death are highest among persons aged > 65 years and persons of any age who have medical conditions that place them at increased risk for complications from influenza.

XV. CROSS REACTIVITY

The OSOM Influenza A&B Test was evaluated with 44 bacterial and viral isolates. Cross-reactivity testing was performed with materials obtained from ATCC. Bacterial isolates were tested at a concentration of approximately $>10^8$ cfu/mL. Very high levels of *Staphylococcus aureus* ($>9 \times 10^8$ cfu/mL) produced a positive result for influenza A. All other bacteria listed gave negative responses. Viral isolates were tested at approximately 1.4×10^5 - 2.3×10^8 TCID₅₀/test.

All viruses listed produced negative responses.

Bacterial Panel:

<i>Acinetobacter calcoaceticus</i>	<i>Legionella pneumophila</i>	<i>Staphylococcus aureus</i>
<i>Bordetella pertussis</i>	<i>Moraxella catarrhalis</i>	<i>Staphylococcus epidermidis</i>
<i>Candida albicans</i>	<i>Mycobacterium avium</i>	<i>Streptococcus Group A</i>
<i>Corynebacterium diphtheriae</i>	<i>Mycobacterium tuberculosis</i>	<i>Streptococcus Group B</i>
<i>Enterococcus faecalis</i>	<i>Neisseria meningitidis</i>	<i>Streptococcus mutans</i>
<i>Enterococcus gallinarum</i>	<i>Proteus mirabilis</i>	<i>Streptococcus pneumoniae</i>
<i>Escherichia coli</i>	<i>Proteus vulgaris</i>	<i>Torulopsis glabrata</i>
<i>Haemophilus influenza</i>	<i>Pseudomonas aeruginosa</i>	
<i>Klebsiella pneumoniae</i>	<i>Serratia marcescens</i>	

Viral Panel:

<i>Adenovirus Type 1</i>	<i>Coxsackievirus B5</i>	<i>Parainfluenza Type 2</i>
<i>Adenovirus Type 2</i>	<i>Echovirus 6</i>	<i>Parainfluenza Type 3</i>
<i>Adenovirus Type 3</i>	<i>Echovirus 11 (Gregory)</i>	<i>Parainfluenza Type 4B</i>
<i>Adenovirus Type 6</i>	<i>Echovirus 30</i>	<i>Rhinovirus 3</i>
<i>Coxsackievirus B2</i>	<i>Measles</i>	<i>Rhinovirus 4</i>
<i>Coxsackievirus B3</i>	<i>Mumps (Enders strain)</i>	<i>Rhinovirus 7</i>
<i>Coxsackievirus B4</i>	<i>Parainfluenza Type 1</i>	<i>RSV (Long strain)</i>

XVI. INTERFERING SUBSTANCES

The following potential interferents were tested and were found to have no effect on the performance of the OSOM Influenza A&B Test.

Potential Interferent	Concentration
Acetyl Salicylic Acid	20 mg/mL
Acetamidophenol	10 mg/mL
Chlorpheniramine maleate	5 mg/mL
Dextromethorphan HBr	20 mg/mL
Diphenhydramine HCl	5 mg/mL
Ephedrine HCl	20 mg/mL
Guiacol Glyceryl Ether	20 mg/mL
Oxymetazoline HCl	10 mg/mL
Phenylephrine HCl	100 mg/mL
Phenylpropanolamine	20 mg/mL
Whole Blood	2%
<i>OTC Throat Drops</i>	
Throat Drop (Halls)	25%
Throat Drop (Zinc)	25%
Throat Drop (Ricola)	25%

<i>OTC Nasal Sprays</i>	
Nasal Spray (Zicam)	10%
Nasal Spray (Afrin)	10%
Nasal Spray (Vicks Sinex)	10%

Note: A very high hemoglobin concentration could interfere with the interpretation of the test result.

XVII. PERFORMANCE CHARACTERISTICS & POL STUDIES
--

Refer to directional insert – OSOM[®] Influenza A&B Test

XVIII. REFERENCES

Refer to directional insert – OSOM[®] Influenza A&B Test

XIX. ASSISTANCE

For technical assistance contact Genzyme Diagnostics Technical Service at (800) 332-1042.