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 instructions for use (IFU) be maintained as a supplement.

PROCEDURE

Title: Sekisui Diagnostics OSOM® Ultra Plus Flu A & B Test (P/N 1032)

Procedure #:

Institution: _____

Prepared by: _____ Date: _____

Title: _____

Accepted by: _____ Date adopted: _____

Title: _____

Reviewed by: _____ Date: _____

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Discontinued by: _____ Date:

I. TEST NAME

OSOM® Ultra Plus Flu A & B Test

CLIA Complexity: Waived

II. INTENDED USE

The OSOM ULTRA PLUS FLU A&B Test is an in vitro rapid diagnostic immunochromatographic assay intended for the qualitative detection of influenza type A and type B nucleoprotein antigens directly from nasal and nasopharyngeal swab specimens from patients with signs and symptoms of respiratory infection.

It is intended to aid in the rapid differential diagnosis of influenza A and B viral infections. This test is not intended for the detection of influenza C viruses. A negative test result is presumptive, and it is recommended these results be confirmed by viral culture or an FDA-cleared influenza A and B molecular assay. Negative test results do not preclude influenza virus infection and should not be used as the sole basis for treatment or other patient management decisions.

Performance characteristics for influenza A were established during the US 2018-2019 influenza season when A/H1N1pdm09 and influenza A/H3N2 were the predominant influenza A viruses in circulation, and the influenza B Yamagata and Victoria lineages were in co-circulation. When other influenza A or B viruses are emerging, performance characteristics may vary.

If infection with a novel influenza 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 your state or local health department for testing. Viral culture should not be attempted in these cases unless a BSL 3+ facility is available to receive and culture specimens.

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 5% – 20% of the United States population annually, resulting in more than 200,000 hospitalizations and 36,000 deaths.[1] The influenza A virus is typically more prevalent and is associated with the most serious influenza epidemics, while influenza B infections usually present with milder 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 antiviral therapy. Initiation of antiviral therapy should begin as soon as possible after onset, ideally within 48 hours of the appearance of symptoms, as treatment may reduce the duration of symptoms.[2] The OSOM ULTRA PLUS FLU A&B Test can provide rapid detection of influenza A and/or B viral antigens from symptomatic patients.

IV. PRINCIPLE OF TEST

The OSOM ULTRA PLUS FLU 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 sample by mixing the swab in an Extraction Buffer vial. 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 rat anti-influenza A and/or mouse anti-influenza B antibody coated on the nitrocellulose membrane. A pink to purple control line must appear in the control region of the Test Stick for results to be valid. The appearance of a second and possibly a third light pink to purple line in the test line region indicates an A, B or A and B positive result. A visible control line with no test line is a negative result.

V. REAGENTS AND MATERIALS PROVIDED

25 – Test Sticks
25 – Sterile Nasal Swabs
25 – Extraction Buffer vials each containing: 0.25 mL phosphate buffered salt solution (with 0.09% sodium azide as preservative)
1 – Influenza A+ Control Swab (packaged with a desiccant tablet): coated with non-infectious recombinant influenza A containing 0.05% sodium azide
1 – Influenza B+ Control Swab (packaged with a desiccant tablet): coated with non-infectious recombinant influenza B containing 0.05% sodium azide
1 – Instructions for Use (IFU)
1 – Quick Reference Guide (QRG)
1 – Workstation

NOTE: Two extra Test Sticks and Extraction Buffer vials have been included in the kit for External Quality Control (QC) testing.

STORAGE AND STABILITY

Store the OSOM ULTRA PLUS FLU A&B Test at room temperature (15-30ºC/59-86ºF) in the original packaging, away from direct sunlight. Kit contents are stable until the expiration date printed on the kit box.

- Do not freeze any of the test kit components.
- Do not use Test Sticks or Extraction Buffer after expiration date.
- Recap the desiccated Test Stick canister immediately after removing a Test Stick.
- Test sticks that have been outside of the desiccated container for more than 30 minutes should be discarded.

At this facility, kits are stored:

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VI. MATERIALS REQUIRED BUT NOT PROVIDED

- Timer or watch
- If needed, sterile nasopharyngeal swabs (Puritan Catalog #25-1406 1PF)

VII. PRECAUTIONS/WARNINGS

- For in vitro diagnostic use only.
- Caution: Federal Law restricts this device to sale by or on the order of a licensed practitioner.
- Do not use the kit contents beyond the expiration date printed on the outside of the box.
- To obtain accurate results, the Instructions for Use (IFU) must be followed.
- Swabs, Extraction Buffer vials, and Test Sticks are for single use only (do not reuse).
- The Extraction Buffer vial only contains enough liquid for one test. Do not add a second Test Stick to the same Extraction Buffer vial as invalid or incorrect results may occur.
- Do not interchange or mix components from different kit lots.
- Follow your clinical and/or laboratory safety guidelines and use appropriate precautions in the collection, handling, storage, and disposal of patient samples and all used kit contents.[3]
- Use of nitrile or latex (or other equivalent) gloves is recommended when handling patient samples.[3]
- Inadequate or inappropriate sample collection, storage, and transport may yield false test results.
- For optimal results, use the nasal swabs provided in the kit.
- Dispose of unused contents and containers in accordance with federal, state, and local regulations.

VIII. SPECIMEN COLLECTION, PREPARATION AND STORAGE

This facility's procedure for patient preparation is:

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This facility's procedure for sample labeling is:

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Specimen Collection and Preparation:

Only nasal/nasopharyngeal swabs can be used with this test. Use of nasal washes or aspirates has not been validated.

NOTE: Freshly collected patient samples should be processed in the Extraction Buffer vial as soon as possible after collection. If the sample cannot be processed immediately, the patient swab may be stored at room temperature (15-30ºC/59-86ºF) for up to 8 hours or refrigerated (2-8ºC/36-46ºF) for up to 24 hours prior to testing. Refrigerated samples should come to room temperature before testing.

NOTE: For optimal results, use only the nasal swabs supplied in the OSOM ULTRA PLUS FLU A&B Test kit (or the nasopharyngeal swab - Puritan® Catalog #25-1406 1PF). Do not use swabs that have cotton, rayon, or polyester tips or wooden shafts.

Nasal Swab Sample (Provided in the kit)

1. Gently insert the sterile swab into the nostril that appears to have the most secretion. Insert until resistance is met at the level of the turbinates (less than 1 inch into the nostril).
2. Rotate the swab several times against the nasal wall and remove from the nostril.
3. Sample should be processed in the Extraction Buffer vial within 8 hours after collection.

Nasopharyngeal Swab Sample (Use a foam tip nasopharyngeal swab, not provided)

1. Gently insert the sterile swab into the nostril that appears to have the most secretion.
2. Keep the swab near the septum floor of the nose while gently pushing the swab into the posterior nasopharynx.
3. Rotate the swab several times and remove from nostril.
4. Sample should be processed in the Extraction Buffer vial within 8 hours after collection.

Sample Handling

- 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 sample collection and/or handling. Training in specimen collection is highly recommended because of the importance of specimen quality.
- To obtain accurate results, do not use visually bloody or overly viscous samples.
- If a culture result is desired, a separate swab must be collected for the culture.
- Once the swab has been mixed in the Extraction Buffer vial, the extracted sample must be used within 2 hours.

This facility's procedure for transporting specimens is:

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This facility's procedure for rejected specimen is**:**

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Specimen Transport and Storage

Patient swabs may be stored and transported in a clean, dry container such as a plastic or glass tube. If the use of media is required, the following transport media have been tested and shown not to interfere with the performance of the test. Please note, when the sample is diluted in

media the sensitivity of the test could be decreased. Storage in other transport media is not recommended.

Transport Media	Storage Conditions	
	15-30°C	2-8°
BD™ Universal Viral Transport Medium	Up to 24 hours	Up to 48 hours
Remel™ Micro Test M4, M4RT, M5, M6 Medium	Up to 24 hours	Up to 48 hours
Bartels Flex Trans™ Medium	Up to 24 hours	Up to 48 hours

NOTE: The performance of samples diluted in transport media was not evaluated in clinical studies.

IX. TEST PROCEDURE

1. Twist cap off an Extraction Buffer vial.
   NOTE: Specimen must be extracted in the Extraction Buffer vial within 8 hours of collection.
2. Insert the swab through the ridges into the liquid in the Extraction Buffer vial. Spin the swab in the liquid vigorously at least 10 times (while submerged).
   NOTE: Nasal swabs may not reach the bottom of the vial. Ensure that the swab is fully immersed in the liquid when mixing. Best results are obtained when the specimens are vigorously mixed in the solution.
3. Remove the swab and discard in biohazard waste. Remove a Test Stick from the canister. Recap the cannister immediately. Insert the Test Stick (arrows pointing downward) into the vial. Start timing.
4. Read test results at 10 minutes.
   NOTE: For help in reading the Test Stick or for correct line placement, refer to Interpretation of Results or the Test Stick diagram. You may need to remove the Test Stick from the vial to read the test results. Discard used vials and Test Sticks in biohazard waste.

For this facility, sample swabs, used extraction reagent capsules, and used test devices are disposed:

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X. INTERPRETATION OF TEST RESULTS

Positive Result

The Control Line must be present for the result to be valid

The appearance of **ANY** shade of a very light or faint pink to purple line at the **A Test Line and/or B Test Line** along with a **C Control Line** indicates a positive result for the presence of influenza A and/or B viral antigen. A positive result does not rule out coinfections with other pathogens or identify any specific influenza A or B virus subtypes.

NOTE: Positive test lines are usually very prominent but at times may vary in shade and intensity. A pink to purple line of any intensity or thickness in the A or B region is considered a positive result. The intensity of the Control Line should not be compared to that of the Test Line for the interpretation of the test result.

Take time to look at test lines very carefully, if you see a very light or faint pink to purple Test Line, this is considered a POSITIVE result.

NOTE: It is possible to have 3 lines, which would indicate a positive test for both influenza A and influenza B. Co-infection with influenza A and B is rare. OSOM ULTRA PLUS FLU A&B Test "dual positive" clinical specimens (influenza A and influenza B positive) should be retested with a new patient sample, Extraction Buffer vial, and Test Stick. Repeatable influenza A and B "dual positive" results should be confirmed by viral culture or an FDA-cleared influenza A and B molecular assay before reporting results.

Negative Result

At 10 minutes, the appearance of **ONLY** the pink to purple Control Line indicates that influenza A or B viral antigen has **NOT** been detected. A negative result should be reported as a presumptive negative for the presence of influenza antigen.

NOTE: A negative test result does not exclude infection with influenza A or B. 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 or an FDA-cleared molecular assay before reporting results.

Invalid Result

If the pink to purple Control Line does not appear, even if **ANY** shade of a very light or faint pink to purple line appears, the result is considered invalid. If at 10 minutes, the background color does not clear, and it interferes with the reading of the test, the result is considered invalid. If the test is invalid, a new test should be performed with a new patient sample, Extraction Buffer vial, and Test Stick.

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

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XI. RESULT REPORTING

This facility's procedure for patient result reporting is:

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XII. LIMITATIONS

- The contents of this kit are to be used for the qualitative detection of influenza type A and B antigens from direct nasal and nasopharyngeal swab samples.
- This test detects both viable (live) and non-viable influenza A and B. Test performance depends on the amount of virus (antigen) in the sample and may or may not correlate with viral culture or molecular results performed on the same sample.
- A negative test result may occur if the level of antigen in a sample is below the detection limit of the test or if the sample was collected or transported improperly.
- Failure to follow the TEST PROCEDURE may adversely affect test performance and/or invalidate the test result.
- Test results must be evaluated in conjunction with other clinical data available to the physician.
- Positive test results do not rule out co-infections with other pathogens.
- Positive test results do not identify specific influenza A subtypes or influenza B lineages.
- Negative test results cannot rule out diseases caused by other bacterial or viral pathogens.
- Children tend to shed virus more abundantly and for longer periods of time than adults. Therefore, testing samples from adults will often yield lower sensitivity than testing samples from children.
- Positive and negative predictive values are highly dependent on prevalence. False negative test results are more likely during peak activity when prevalence of disease is high. False positive test results are more likely during periods of low influenza activity when prevalence is moderate to low.
- Individuals who received nasally administered influenza vaccine may have positive test results for up to 3 days after vaccination.
- Monoclonal antibodies may fail to detect, or detect with less sensitivity, influenza viruses that have undergone minor amino acid changes in the target epitope region.
- If differentiation of specific influenza A or B subtypes and strains is needed, additional testing, in consultation with state or local public health departments, is required.
- The performance of this test has not been evaluated for use in patients without signs and symptoms of respiratory infection.
- The performance of this test has not been evaluated for monitoring antiviral treatment of influenza.

XIII. USER QUALITY CONTROL

Internal Quality Control

Several controls are incorporated into each Test Stick for routine quality checks for the test system and operator.

1. The appearance of the control line in the results window is an internal procedural control. It also verifies proper assembly of the Test Stick. 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 is another 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.

Contact Sekisui Diagnostics Technical Services at (800) 332-1042 or SDADiagnosticsTSDL@sekisuidiagnostics.com if you experience a problem.

External Quality Control

The OSOM ULTRA PLUS FLU A&B Test kit includes one Influenza A+ Control Swab and one Influenza B+ Control Swab, each of which contains recombinant antigen, 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 acts 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.

- When the Influenza A+ Control Swab is tested, the appearance of ANY shade of a very light or faint pink to purple line at the A Test Line along with a C Control Line, indicates that the influenza antigen binding property of the Test Stick is functional.
- When the Influenza B+ Control Swab is tested, the appearance of ANY shade of a very light or faint pink to purple line at the B Test Line along with a C Control Line, indicates that the influenza antigen binding property of the Test Stick is functional.

External controls are intended to monitor substantial reagent failure.

If External Quality Control testing fails, repeat the testing of the failed control or contact Sekisui Diagnostics Technical Services at (800) 332-1042 or SDADiagnosticsTSDL@sekisuidiagnostics.com before running patient samples.

External quality control requirements should be established in accordance with your local, state, and federal regulations or accreditation requirements. Minimally, Sekisui Diagnostics recommends that positive and negative external controls be run with each new lot, shipment received, and with each new untrained operator.

QC Testing Frequency and Documentation

For this facility, External QC is run:

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

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XIV. EXPECTED VALUES

The prevalence of influenza varies year to year typically peaking in the winter months. The rate of positivity in influenza testing is dependent on many factors including specimen collection and handling, test method used, patient age, time of year, geographic location, and local disease prevalence. The overall positivity rate as determined by the OSOM ULTRA PLUS FLU A&B Test during the 2018-2019 clinical study was 33.0% for influenza A and 1.7% for influenza B. The observed results by age are presented in the tables below.

Influenza A Positives by the OSOM ULTRA PLUS FLU A&B Test per Age Group

Age Group	Number of Specimens	Number of Influenza A Positives	Influenza A Positivity Rate
≤ 5 years of age	362	127	35.1%
6 to 21 years of age	479	211	44.1%
≥ 22 years of age	369	61	16.5%
Total	1210	399	33.0%

Influenza B Positives by the OSOM Ultra Plus Flu A&B Test per Age Group

Age Group	Number of Specimens	Number of Influenza B Positives	Influenza B Positivity Rate
≤ 5 years of age	362	5	1.4%
6 to 21 years of age	479	9	1.9%
≥ 22 years of age	369	6	1.6%
Total	1210	20	1.7%

XV. CROSS REACTIVITY

The OSOM ULTRA PLUS FLU A&B Test was evaluated with 41 organisms (bacterial, viral, fungal) and human DNA, listed below. Bacterial isolates were tested at concentrations of approximately 10^6 colony forming units per mL (CFU/mL). *Chlamydia pneumoniae* was tested at a concentration at least 2.0 x 10^2 CFU/mL. *Corynebacterium ulcerans* and *Streptococcus pyogenes* were tested at a concentration of at least 1.0 x 10^3 CFU/mL. Viral isolates were tested at approximately 10^5 copy number per mL (CP/mL) or 10^4 – 10^5 tissue culture infectious dose 50% per mL ($TCID_{50}$/mL). Human genomic DNA was diluted to a level greater than the minimum recommended concentration of 10^4 copies/mL in viral transport media (VTM). No cross-reactivity was observed at the concentrations tested, as all of the microorganisms and human genomic DNA produced negative results.

Bacterial / Fungal Panel

Bordetella pertussis
Candida albicans
Chlamydia pneumoniae
Corynebacterium ulcerans
Escherichia coli
Mycoplasma hominis
Mycoplasma pneumoniae
Neisseria meningitidis
Neisseria gonorrhoeae
Pseudomonas aeruginosa

Haemophilus influenzae
Klebsiella pneumoniae
Lactobacillus acidophilus Z048
Legionella pneumophila
Moraxella catarrhalis
avirulent *Mycobacterium tuberculosis*
Staphylococcus aureus MRSA
Staphylococcus aureus MSSA
Staphylococcus epidermidis MRSE
Streptococcus pneumoniae
Streptococcus pyogenes
Streptococcus salivarius

Virus / Viral Panel

Adenovirus type 1
Adenovirus type 7A
Coronavirus NL63
Coxsackievirus
Cytomegalovirus (CMV)
Epstein-Barr Virus (EBV)
Human Herpes Virus 6 (HHV6), Z29
Human Herpes Virus 7 (HHV7), SB Strain
Parainfluenza virus 1
Parainfluenza virus 2
Parainfluenza virus 3
Measles Virus
Mumps
Metapneumovirus 3 type B1
Metapneumovirus 9 type A1
Rhinovirus type 1A
Enterovirus 68
Respiratory Syncytial virus type A2 (RSV-A)
Respiratory Syncytial virus type B (RSV-B)

XVI. INTERFERING SUBSTANCES

The OSOM ULTRA PLUS FLU A&B Test was evaluated with potential interferents that may be encountered in respiratory specimens. The substances were tested at the concentrations listed in the table below. No interference was observed with the test for any of the substances at the concentrations listed.

Substance	Potential Interferent	Concentration Tested
Substance Control	Dry Swab	N/A
Study Control	Viral Transport media (VTM)	N/A
Mucus (Bovine)	Mucin Protein	19 mg/mL
Whole Blood	Whole Blood with EDTA	5% vol/vol
Analgesic	Acetaminophen	0.1 mg/mL
NSAIDs	Aspirin	16.2 mg/mL
	Ibuprofen	40 mg/mL
	Naproxen	55 mg/mL
Nasal Corticosteroids	Dexamethasone	0.5 mg/mL
	Fluticasone	50 mg/mL
	Mometasone furoate	2.5 µg/mL
	Budesonide	25 µg/mL
	Flunisolide	68.8 µg/mL
	Triamcinolone acetonide	5.5 µg/mL
	Beclomethasone	16 µg/mL
Nasal Sprays	Oxymetazoline	0.025% vol/vol
	Phenylephrine	0.5% vol/vol
	Sodium Chloride	0.325% vol/vol
Nasal Gel	Sabadilla	4x
	Galphimia glauca	4x, 12x, 30x
	Histaminum hydrochloricum	12x, 30x, 200x

	Luffa operculata	4x, 12x, 30x,
	Sulphur	12x, 30x, 200x
Antiviral	Oseltamivir	5 mg/mL
Antibacterial	Tobramycin	40.0 µg/mL
Throat Lozenge	Benzocaine	2.5% soln.
Antibiotic Nasal Ointment	Mupirocin	0.15mg/mL
Allergy medicine	Histamine hydrochloricum	1%

Competitive Interference

The performance of the OSOM ULTRA PLUS FLU A&B Test was evaluated in the presence of high levels of influenza A and influenza B. Contrived high and low titer influenza A (H1N1 and H3N2) and B positive samples were prepared and applied to swabs. The high titer for influenza A was at a concentration of $7.1x10^3$ $TCID_{50}$/mL for H1N1, and $2.2x10^7$ $CEID_{50}$/mL for H3N2; the high titer for influenza B was set at $1.6x10^4$ $TCID_{50}$/mL. The low titer for influenza A was at a concentration of $1.4x10^2$ $TCID_{50}$/mL for H1N1, and $4.4x10^5$ $CEID_{50}$/mL for H3N2; the low titer for influenza B was set at $3.2x10^2$ $TCID_{50}$/mL. High and low viral concentrations of influenza A and B were mixed and tested. No competitive interference on test performance was observed.

XVII. PERFORMANCE CHARACTERISTICS & POL STUDIES

Refer to Instructions for Use – OSOM® Ultra Plus Flu A&B

XVIII. REFERENCES

Refer to Instructions for Use – OSOM® Ultra Plus Flu A&B

XIX. ASSISTANCE

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

OSOM® is a registered trademark of Sekisui Diagnostics, LLC.