

## **Annex G**

Procedures for Collecting, Storing, Testing by Rapid Diagnostic Test (RDT) and Shipping of Specimens for investigation on influenza.

# **G1 - Specimen collection**

## **General information**

Respiratory virus diagnosis depends on the collection of high-quality specimens, their rapid transport to the laboratory and appropriate storage before laboratory testing. Virus is best detected in specimens containing infected cells and secretions. Specimens for the direct detection of viral antigens or nucleic acids and virus isolation in cell cultures should be taken preferably during the first 3 days after onset of clinical symptoms.

## **Type of specimens**

A variety of specimens are suitable for the diagnosis of virus infections of the upper respiratory tract. For diagnosis of influenza the following are advisable and suitable in the Nauru conditions:

- nasal swab
- nasopharyngeal swab
- nasopharyngeal aspirate
- acute and convalescent serum specimens
- animal specimens - specimens need to be frozen and transported frozen in the reversed cold chain system.

## **Procedures for specimen collection**

Clinical specimens should be collected as described below and added to transport medium. Nasal or nasopharyngeal swabs can be combined in the same vial of virus transport medium. When possible, the following information should be recorded on the [Field Data Collection Form](#): general patient information, type of specimens, date of collection, and contact information of person completing the form, etc.

Standard precautions should always be followed, and barrier protections applied whenever samples are obtained from patients.

### *Nasal swab*

A dry polyester swab is inserted into the nostril, parallel to the palate, and left in place for a few seconds. It is then slowly withdrawn with a rotating motion. Specimens from both nostrils are obtained with the same swab. The tip of the swab is put into a plastic vial containing 2–3 ml of virus transport medium and the applicator stick is broken off.

### *Nasopharyngeal swab*

A flexible, fine-shafted polyester swab is inserted into the nostril and back to the nasopharynx and left in place for a few seconds. It is then slowly withdrawn with a rotating motion. A second swab should be used for the second nostril. The tip of the swab is put into a vial containing 2–3 ml of virus transport medium and the shaft cut.

### *Nasopharyngeal aspirate*

Nasopharyngeal secretions are aspirated through a catheter connected to a mucus trap and fitted to a vacuum source. The catheter is inserted into the nostril parallel to the palate. The vacuum is applied and the catheter is slowly withdrawn with a rotating motion. Mucus from the other nostril is collected with the same catheter in a similar manner. After mucus has been collected from both nostrils, the catheter is flushed with 3 ml of transport medium.

### *Acute and convalescent serum specimens*

## **Annex G**

Procedures for Collecting, Storing, Testing by Rapid Diagnostic Test (RDT) and Shipping of Specimens for investigation on influenza.

An acute-phase serum specimen (3–5 ml of whole blood) should be taken soon after onset of clinical symptoms and not later than 7 days after onset. A convalescent-phase serum specimen should be collected 14 days after the onset of symptoms. Where patients are near death, a second ante-mortem specimen should be collected.

Although single serum specimens may not provide conclusive evidence in support of an individual diagnosis, when taken more than 2 weeks after the onset of symptoms they can be useful for detecting antibodies against avian influenza viruses in a neutralization test.

A Rapid Diagnostic Test (RDT) is available at the Nauru Public Health Laboratory please refer to section G4 for the type of specimens required.

Specimens for referral to L2 and L3 laboratories:

Specimens for direct detection of viral antigens by immunofluorescence staining of infected cells should be refrigerated and processed within 1–2 hours. Specimens for virus isolation should be refrigerated immediately after collection and inoculated into susceptible cell cultures as soon as possible.

Respiratory specimens should be collected and transported in virus transport media. Commercial transport media are in store with the OIC, Laboratory, Nauru General Hospital.

### **G2 - Specimen storage**

Specimens in viral transport medium for viral isolation should be kept at 4 °C and transported to the laboratory promptly. Sera may be stored at 4 °C for approximately one week, but thereafter should be frozen at –20 °C.

Specimens should be collected and transported in a suitable transport medium on ice. Standard precautions should always be followed, and barrier protections applied whenever samples are obtained from patients.

The commercial transport media for referral of specimens are available at the Public Health Laboratory, Nauru General Hospital.

### **G3- Criteria for deployment and use of a Rapid Diagnostic Test (RDT)**

- ✓ The RDTs are very expensive (about USD 15 per test) and have to be used for Public Health purpose only.
- ✓ As such, the supply of the RDT will be under the direct control of the Director Public Health Services, or his/her proxy.
- ✓ In case of an occurrence/outbreak of ILI, specimens will be collected from only limited number of cases (5 – 10) for:
  - testing by the RDT in the Nauru Public Health laboratory, and
  - referral to L2 and/or L3 laboratories for further diagnostic procedures (immunofluorescence, virus isolation, nucleic acid technologies....)
- ✓ The cases selected for the specimen collection have to show signs and symptoms compatible with the influenza clinical case definition in Annex D of the National Influenza Pandemic Preparedness Plan (temperature > 38 C, cough or sore throat, myalgia).
- ✓ The results of the testing (both, local by RDT and overseas) have to be reported immediately to the Director Public Health, MOH, Nauru.

## **Annex G**

Procedures for Collecting, Storing, Testing by Rapid Diagnostic Test (RDT) and Shipping of Specimens for investigation on influenza.

### **G4 – Use of RDT - Binax Now Influenza A & B**

The BinaxNOW® Influenza A & B Test is an in vitro immunochromatographic assay for the qualitative detection of influenza A and B nucleoprotein antigens in nasopharyngeal (NP) swab and nasal wash/aspirate specimens. It is intended to aid in the rapid differential diagnosis of influenza A and B viral infections. Negative test results should be confirmed by cell culture.

**Principle of the test:** The BinaxNOW® Influenza A & B Test is an immunochromatographic membrane assay that uses highly sensitive monoclonal antibodies to detect influenza type A and B nucleoprotein antigens in NP specimens. These antibodies and a control antibody are immobilized onto a membrane support as three distinct lines and combined with other reagents/pads to construct a test strip. This test strip is mounted inside a cardboard, book-shaped hinged test device.

Swab specimens require a sample preparation step, in which the sample is eluted off the swab into elution solution, saline or transport media. Nasal wash/aspirate samples require no preparation.

#### **Composition of the Binax Now Kit:**

- ✓ **Test Devices:** A cardboard, book-shaped hinged test device containing the test strip.
- ✓ **Transfer Pipettes:** Fixed volume (100 µl) transfer pipettes used to transfer sample to the test devices. Use only pipettes provided by Binax or a calibrated pipette capable of delivering 100 µl sample volume.
- ✓ **Positive Control Swab:** Inactivated influenza A and B dried onto swab.
- ✓ **Negative Control Swab:** Inactivated Streptococcus Group A dried onto swab.
- ✓ **Elution Solution Vials for Control Swabs:** Vials containing a fixed volume (0.5 ml) of elution solution used to prepare the Control Swabs for testing.

Note: The materials provided in the test kit are sufficient for testing nasal wash/aspirate specimens only. If NP swab specimens will be tested, the Nasopharyngeal Swab Specimen Accessory Pack may be purchased.

#### **Composition of the Nasopharyngeal (NP) Swab Specimen Accessory Pack:**

- ✓ **NP Swabs:** Sterile foam swabs for use in the BinaxNOW® Influenza A & B Test. Other sterile flexible shaft NP swabs may be used in place of the Binax provided swabs.
- ✓ **Elution Solution Vials for Swab Specimens:** Vials containing a fixed volume (0.5 ml) of elution solution used to prepare the Control Swabs for testing. Transport media or saline may be used in place of Binax Elution Solution. Refer to Performance Characteristics – Transport Media section for details.

#### **Storage and stability:**

Store kit at room temperature (59-86°F, 15-30°C). The BinaxNOW® Influenza A & B Test kit and reagents are stable until the expiration dates marked on their outer packaging and containers.

#### **Specimen collection and handling**

## Annex G

Procedures for Collecting, Storing, Testing by Rapid Diagnostic Test (RDT) and Shipping of Specimens for investigation on influenza.

Use fresh NP swabs and nasal washes/aspirates for optimal test performance. Collect nasal washes in standard containers. Test as soon as possible. Washes can be held at 2-8°C for up to 24 hours prior to testing in the BinaxNOW® test. Use sterile cotton, rayon, foam or polyester flexible-shaft NP swabs to collect nasopharyngeal sample. Calcium alginate swabs are not recommended for use in this test. Elute sample within one hour of collection. Test as soon as possible.

Eluted swab samples can be held at 2-8°C for up to 24 hours prior to testing in the BinaxNOW® test. If needed, transport sample at 2-8°C in a leak proof container.

Allow samples to warm to room temperature before testing in the BinaxNOW® test. Swirl gently to mix before testing.

### Sample preparation procedure

#### Nasal Wash/Aspirate:

Nasal wash/aspirate samples do not need preparation. Go to Test Procedure.

#### Nasopharyngeal Swabs:

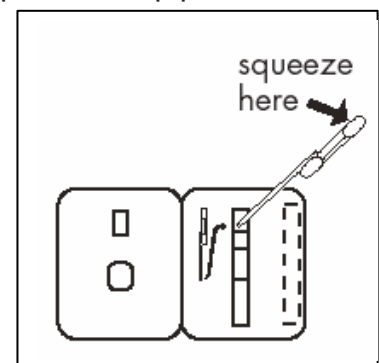
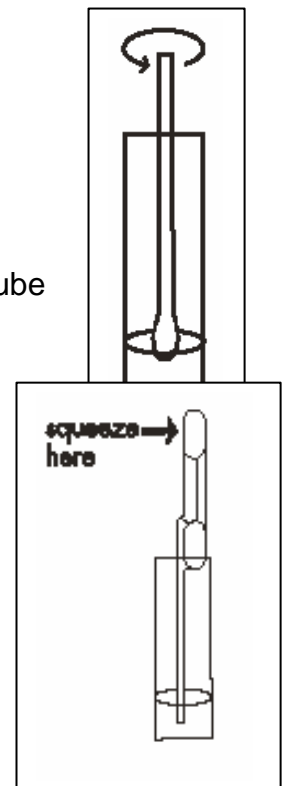
Elute swab in 0.5 ml of saline or Binax Elution Solution. The general procedure is as follows:

1. The Binax Specimen Accessory pack contains test vials pre-filled with elution solution. Twist off the test vial cap. Alternatively, use a microtube with 0.5 ml of sterile saline.
2. Put the swab to be tested into test vial/microtube. Rotate the swab vigorously three (3) times in the liquid.
3. Press the swab against the side of the vial and turn as you remove it from the vial. This removes sample from the swab.
4. Discard the swab.
5. Test the liquid sample (from the test vial) in the BinaxNOW® test as soon as possible.

#### Test Procedure.

1. Remove device from the pouch just prior to testing and lay flat on work bench.
2. Fill the Binax transfer pipette by firmly squeezing the top bulb and placing pipette tip into sample. Release bulb while tip is still in sample. This will pull liquid into the pipette. Make sure there are no air spaces in the lower part of the pipette.
3. See arrow on test device to find White Sample Pad. **SLOWLY** (drop by drop) add entire contents of pipette (100 µl) to the **MIDDLE** of this pad by squeezing the top bulb.
4. Immediately peel off brown adhesive liner from the test device. Close and securely seal the device. Read result in window 15 minutes after closing the device. Results read before or after 15 minutes may be inaccurate.

#### Result interpretation



## Annex G

Procedures for Collecting, Storing, Testing by Rapid Diagnostic Test (RDT) and Shipping of Specimens for investigation on influenza.

For a **NEGATIVE SAMPLE**, the BLUE Control Line in the **BOTTOM THIRD** of the window turns a pink-to-purple color. No other line appears.

For a **FLU A POSITIVE SAMPLE**, the BLUE Control Line turns a pink-to-purple color AND a second pink-to-purple Sample Line appears above it in the **MIDDLE THIRD** of the window. Any Sample Line, even when very faint, is positive.

For a **FLU B POSITIVE SAMPLE**, the BLUE Control Line turns a pink-to-purple color AND a second pink-to-purple Sample Line appears above it in the **TOP THIRD** of the window. Any Sample Line, even when very faint, is positive. A test is **INVALID** if the Control Line remains BLUE or is not present at all, whether a Sample Line(s) is present or not. Repeat Invalid tests with a new test device. Call Binax Technical Service if the problem persists. 800-323-3199 or 207-772-3988

### Result Suggested Report

**Positive for Flu A** – “Positive for Flu A protein antigen by Binax Now Influenza A & B test”.

**Positive for Flu B** – “Positive for Flu B protein antigen by Binax Now Influenza A & B test”.

**Negative** – “Negative for Flu A and Flu B protein antigens by Binax Now Influenza A & B test”. Infection due to Flu A and Flu B cannot be ruled out. Flu A and/or Flu B antigen in the sample may be below the detection limit of the test.

### Procedural Controls:

A. An untested device has a blue line at the "Control" position. If the test flows and the reagents work, this blue line will always turn pink in a tested device.

B. The background color in the result window should be light pink to white within 15 minutes. Background color should not hinder reading of the test.

### External Positive and Negative Controls:

BinaxNOW® test kits contain Positive and Negative Control Swabs (one each). These swabs will monitor the entire assay. Test these swabs once with each new test kit opened. This is to ensure that:

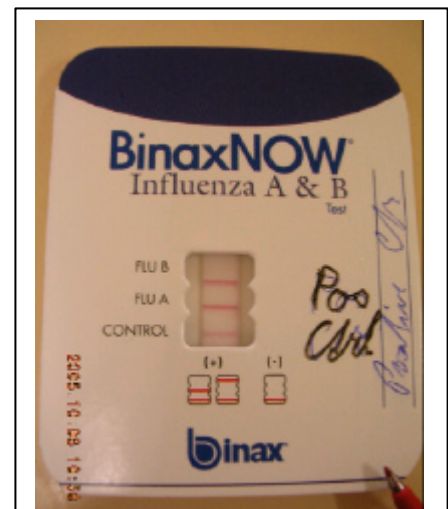
- ✓ test reagents are working; and
- ✓ the test is correctly performed.

Record the date and results of the positive and negative controls testing on the outer package of the kit to validate the use of the particular kit.

### Limitations:

A negative test result does not exclude infection with influenza A and B. Therefore, the results obtained with the BinaxNOW® Influenza A & B Test should be used in conjunction with clinical findings to make an accurate diagnosis.

The BinaxNOW® Influenza A & B Test detects both viable and non-viable influenza A and B. Test performance depends on antigen load in the specimen and may not correlate with cell culture performed on the same specimen.



Valid test run for Positive Control – both Influenza A & B positive, control line also showing.

**Annex G**

Procedures for Collecting, Storing, Testing by Rapid Diagnostic Test (RDT) and Shipping of Specimens for investigation on influenza.

Performance of the BinaxNOW® Influenza A & B Test has not been established for monitoring antiviral treatment of influenza.

Visibly bloody samples may not be appropriate for use in the BinaxNOW® Influenza A & B Test.

**Contact Information:****Binax, Inc.**

217 Read Street

Portland, Maine 04103 USA

Tel: 207-772-3988 or 800-323-3199

Fax: 207-761-2074

Internet: [www.binax.com](http://www.binax.com)

**Annex G**

Procedures for Collecting, Storing, Testing by Rapid Diagnostic Test (RDT) and Shipping of Specimens for investigation on influenza.

## G5 - Specimen referral to L2 and L3 levels laboratories

### General:

Transport of specimens should comply with [WHO Guidance on regulations for the Transport of Infectious Substances](#) (WHO/CDS/CSR/LYO/2005.22, revised September 2005).

The receiving laboratory should be notified before shipment of specimens.

Transport of specimens within national borders should comply with the procedures detailed within each country's regulations.

International air transport of human specimens known or suspected to contain the influenza must follow the current edition of the International Air Transport Association (IATA)

Dangerous Goods Regulations.

[IATA Dangerous Goods Regulations](#)

[Consignment of Diagnostic Specimens, 2003](#)

The IATA Regulations, Consignment of Diagnostic Specimens, 2003 allow specimens known or suspected to contain the influenza virus to be transported as UN 3373 "diagnostic specimens" when they are transported for diagnostic or investigational purposes.

Specimens transported for any other purposes, and cultures (as defined in the IATA Regulations) prepared for the deliberate generation of pathogens, must be transported as UN 2814 or UN 2900, as appropriate.

All specimens to be transported (UN 3373, UN 2900, or UN 2814) must be packaged in triple packaging consisting of three packaging layers as indicated in the [IATA Dangerous Goods Regulations](#)

UN 3373, Diagnostic Specimens, shall be packed in good quality packaging, which shall be strong enough to withstand the shocks and loads normally encountered during transport. Packaging shall be constructed and closed so as to prevent any loss of contents that might be caused under normal conditions of transport, by vibration or by changes in temperature, humidity or pressure.

Primary receptacles shall be packed in secondary packaging in such a way that, under normal conditions of transport, they cannot break, be punctured or leak their contents into the secondary packaging. Secondary packaging shall be placed in a final outer package with suitable cushioning material. Any leakage of the contents shall not compromise the protective properties of the cushioning material or of the outer packaging.

For air transport, the smallest overall external dimension of a completed package must be at least 10 cm.

### Specific:

Nauru Health Department has a current (issued 10 May 2005, expiry 10 May 2007) AQIS Permit organized by WHO for Victorian Infectious Disease Laboratory (VIDRL, L3).

Dangerous Goods Officer (Approval Current)

Mr. Rhondo Dowabobo is currently the approved Dangerous Goods Officer. Air Nauru also has two other approved Officers who can carry out the process in absence of the RON Dangerous Goods Hospital Officer. The Dangerous Goods Officer is responsible of packing and signing the necessary documents for the consignments.

Steps to be taken for referring specimens for the laboratory influenza examination from Nauru to L2/L3 laboratory:

Collect appropriate specimen as per section G1 above;

Put in the viral transport medium (follow the specific instructions by the L2/L3 lab for the respective type of examinations);

**Annex G**

Procedures for Collecting, Storing, Testing by Rapid Diagnostic Test (RDT) and Shipping of Specimens for investigation on influenza.

Store appropriately as per section G2 above;

Advise in writing Mr. Rhondo Dowabobo, or his proxy (airport operators), Ph. 4443883 at the RON Hospital stores the exact details of the consignment;

Mr. Rhondo Dowabobo carries out the packing accordingly (as per the section above);

Advise the recipient laboratory by phone/fax/email

The following documentation needs to be prepared and accompany the shipment, so that the consignment is clearly identified and the content linked to the relevant item(s) on the Import Permit:

Valid Import Permit,

Shipper's Declaration of Infectious Substances under the 2814 packing rules

Accompanying letter from the Department of Health, describing the content,

Accompanying invoice or airway bill

Labeling - Mr. Rhondo Dowabobo

As of October 2005, Nauru "Cargo cut-off times" are:

Monday 9:30 - MAJ/TAR/NAD

Tuesday 11:00 a.m., HON/BRI

Thursday 9:30 a.m. – TAR/NAD

Friday 13:00 – HON/BRI/MEL

Until Mataika House laboratory fully assumes its regional function, specimens for examination on influenza need to be sent to:

WHO CCRRINF – Melbourne, Address, Contact: Dr Ian Barr,

Victorian Infectious Diseases Reference Laboratory

10 Wreckyn St, Nth Melbourne

Victoria, Australia, 3051

Telephone: +61 3 9342 2600

Fax: +61 3 9342 2660

The copies of the Current Import Permit and samples of the Shipper's Declaration and DOH Accompanying letter are attached for easy reference.