

Specimen Requirements for Respiratory Viruses Detected by Molecular Methods

- Methodology:**
1. Real time (rti) Reverse Transcriptase Polymerase Chain Reaction (RT-PCR)
 2. Respiratory Viral Assay
 3. Influenza Mutations Detection Sequencing
- Performed:**
1. rti RT-PCR is for qualitative detection of Influenza virus Type A or B.
 2. Influenza A subtyping and Influenza B genotyping.
 3. MultiPlex Respiratory Viral Assay is used to detect respiratory viral pathogens that may be associated with severe respiratory disease.
 4. Influenza Mutations Detection sequencing is performed in a subset of Influenza A 2009 H1N1, Influenza A (H3) or Influenza B.
- Turn-Around-Time:**
1. rti-RT-PCR results are reported 2-3 business days after approval and receipt of specimen(s).
 2. Multiplex Respiratory Viral Assay results are reported 2-3 business days after approval and receipt of specimens.
 3. Influenza Mutations Detection Sequencing results are reported 2-3 business days after sequencing approval.
- Specimen required:**
- The following respiratory specimens are acceptable:
Upper Respiratory Tract clinical specimens: nasopharyngeal (NP) or oropharyngeal (OP) aspirates or washes, NP or OP swabs, nasal swabs (NS), throat swabs (TS), or dual nasopharyngeal/throat swabs
Lower Respiratory Tract specimens: Bronchoalveolar lavage (BAL), bronchial wash (BW), tracheal aspirate (TA) or sputum
- Note: For the MultiPlex Respiratory Viral Assay, ONLY nasopharyngeal (NP) specimens are acceptable**
- Specimen Collection:**
- Follow specimen collection devices manufacturer instructions for proper collection methods.
Use only Dacron tip swabs with an aluminum or plastic shaft. Calcium alginate swabs or cotton swabs with wooden sticks are unacceptable because they may cause PCR inhibition and may contain substances that inactivate or may be toxic to some viruses.

<http://health.hawaii.gov/statelab/files/2013/05/sld-brl-vir-flu-sample-instruction.pdf>)

For NP swabs- Insert swab into the nostril parallel to the palate and leave in place for a few seconds to absorb secretions.

For OP swabs- swab both posterior pharynx and tonsillar areas, avoiding the tongue.

Place swabs immediately into sterile vials containing 1-3 ml of viral transport media. Break the shaft and tighten the cap of the vial. Label each specimen with a unique identifier, type of specimen and date of collection.

Note: Only swabs in viral transport media (VTM) will be accepted.

For NP wash/aspirate- Have the patient sit with the head tilted slightly backward. Instill 1-1.5 ml of non-bacteriostatic saline (pH 7.0) into one nostril. Flush a plastic catheter or tubing with 2-3 ml of saline. Insert the tubing into the nostril parallel to the palate. Aspirate NP secretions. Repeat this procedure with each nostril. Collect NP/OP wash or aspirate in sterile vials. Label each specimen with a unique identifier, type of specimen and date of collection. *NP aspirates are the specimen of choice for the detection of respiratory viruses.*

Note: Respiratory specimens should be collected as soon as possible in the course of illness. Recovery of viruses diminishes markedly >72 hours after onset of symptoms.

Specimen storage, packing and transport:

Respiratory specimens should be kept at 4°C for no longer than 3 days. Ship specimens with cold packs to keep the specimens at 4°C.

If storage/transport will exceed 72 hours, freeze specimens at $\leq -70^{\circ}\text{C}$ and ship on dry ice. Avoid freezing and thawing specimens. Viability of some pathogens from specimens that were frozen and thawed is greatly diminished.

Follow packing and shipping instructions of the U.S. Department of Transportation (U.S. DOT), and the International Air Transport Association (IATA) for Biological Substance, Category B.

Specimen submission:

Submitters: Clinical Laboratories and the Disease Investigation Branch (DIB)

Note: It is the responsibility of the submitter to track the arrival of the specimens along with Influenza Submission

Form at the State Laboratories Division to ensure that these specimens are received at the respective testing laboratories.

For MultiPlex Respiratory Viral Assay and Influenza Mutations Detection sequencing: Clinical Laboratories **must** consult with the Disease Investigation Branch. No testing will be performed if no investigation by the DIB was conducted.

Unacceptable conditions:

- Specimen is received in a container that is leaking;
- Specimen is not collected in a proper container or special handling instruction is not followed;
- Viral transport media (VTM) is expired;
- Specimens collected with swabs with cotton tips or calcium alginate, and swabs with wooden shafts;
- Specimen is not received at 4°C or packed in blue ice;
- Specimen quantity is insufficient to perform the tests;
- Unlabeled specimens;
- Incomplete specimen labeling and documentation;
- Specimen label does not match the requisition.

Stability:

All specimens must be refrigerated at 2-8°C immediately after collection. If storage/transport will exceed 72 hours, freeze specimens at $\leq -70^{\circ}\text{C}$ and ship on dry ice. Avoid freezing and thawing specimens. Viability of some pathogens from specimens that were frozen and thawed is greatly diminished.

Requisition Form:

State Laboratories Division Requisition **Flu-On-Line Form** (<http://health.hawaii.gov/statelab/files/2013/05/sld-flu-submission-form2.pdf>).

Each specimen submitted must have a completed Flu Form, with the patient's unique identifier, submitter, specimen site/specimen type, date of onset, travel history, date of collection, date shipped/sent to the SLD, test(s) requested and other pertinent information.

- Illegible Influenza Specimen Submission Form or forms that are not consistent with the specimen submitted will be rejected and requesting facility will be asked to re-submit.
- Requisition forms shall be placed in a separate bag and shall not be packed with the specimen(s).

Normal Value(s):

No Influenza A or B Nucleic Acid Detected
No Respiratory virus nucleic acid detected by SLD algorithm

No mutations found commonly associated with drug resistance for Influenza A 2009 H1N1 or Influenza A (H3) or Influenza B.

Result Notification: Laboratory reports are reported to the submitters by electronic reporting system (Dashboard, SharePoint).

Laboratory reports for the DIB other than the Flu Online will be posted to the DOH Disease Outbreak Control Division (DOCD) SharePoint and/or Dashboard.


Influenza Mutations Detection Sequencing results are reported to the DIB by electronic reporting system.


Test performed at: Virology Section, Medical Microbiology Branch (MMB) and
Biological Response Section, Laboratory Preparedness and Response Branch (BRS, LPRB)
State Laboratories Division
Department of Health
2725 Waimano Home Road
Pearl City, Hawaii 96782

Contact: MMB:
Roland Lee, Section Supervisor or Derek Harauchi, Unit Supervisor at 808-453-6705


For Influenza Mutations Detection Sequencing and USAPI Laboratories:
BRS-LPRB:
Remedios Gose, LPRB Chief at 808-453-5993 or Cheryl-Lynn Daquip at 808-453-5984


Reviewed By:


Remedios Gose, MSPH
Chief, Laboratory Preparedness and Response Branch


Date

Reviewed By:


Dayna Ornelas, M.S.
Chief, Medical Microbiology Branch


Date

Approved by:

Edward P. Desmond

Edward P. Desmond, Ph.D.
Administrator, State Laboratories Division

11/29/19

Date