DOH 301-018

Influenza Virus Testing at the Washington State Public Health Laboratories (WAPHL) Including Novel Influenza and Fatal Influenza September 15, 2016

The WAPHL performs influenza virus testing, subtyping, and antiviral resistance screening primarily for surveillance purposes. Results are used to monitor state influenza activity. In special situations, testing may be done to determine if novel influenza infection is occurring in humans. Testing and subtyping are performed using real-time reverse transcriptase polymerase chain reaction (RT-PCR) assays developed by the CDC. CDC-developed pyrosequencing protocols are also used to screen for some mutations associated with antiviral resistance in influenza A (H1N1) and (H3N2) viruses.

After approval from the local health jurisdiction, WAPHL will perform influenza testing and subtyping on specimens from:

1. Deceased patients suspected to have influenza. **NOTE:** Autopsy specimens are not a recommended specimen type for RT-PCR testing so viral isolation will be performed in conjunction with RT-PCR testing.
2. Patients with suspected novel influenza virus infection, such as infection with influenza A (H3N2v), (H5N1) or (H7N9) virus. **NOTE**: If novel influenza A virus infection is suspected, specimens should be collected using appropriate infection control precautions and sent IMMEDIATELY to WAPHL.
3. Patients associated with outbreaks.
4. Persons with exposure to avian influenza infected birds (including influenza testing of a symptomatic exposed person, and serology at CDC to determine asymptomatic infection as appropriate).

After approval from the local health jurisdiction, WAPHL or CDC will perform antiviral resistance testing for infection control purposes on specimens from:

1. Patients who develop laboratory-confirmed influenza while taking antiviral prophylaxis.
2. Severely immunocompromised patients with prolonged excretion of influenza virus despite antiviral treatment.
3. Patients in intensive care units with prolonged excretion of influenza virus despite antiviral treatment.

WAPHL additionally performs subtyping and antiviral resistance testing on a subset of specimens from the Influenza Sentinel Laboratory Network. Aggregate results are published weekly in the DOH Influenza Update: http://www.doh.wa.gov/Portals/1/Documents/5100/420-100-FluUpdate.pdf

Specimen Collection

The following specimen types are preferred* for seasonal influenza testing at WAPHL:

- Nasopharyngeal swab
- Nasal aspirate or wash
- Dual Nasopharyngeal / Throat swab

***The preferred specimen type** for detection of H5N1 or other novel influenza virus is a nasopharyngeal swab. For more about testing humans for avian flu, see CDC site: http://www.cdc.gov/flu/avianflu/severe-potential.htm.*

***For patients with severe lower respiratory tract disease, a lower respiratory tract specimen (e.g., BAL or tracheal aspirate) should be collected in addition to an upper respiratory tract specimen.*

The following specimen types are also acceptable for influenza testing at WAPHL:

- Nasal swab
- Throat swab
- Tracheal aspirate
- Bronchoalveolar lavage (BAL)
- Bronchial aspirate or wash
- Sputum
- Lung Tissue
- Viral culture
- For novel or avian influenza serology, 5 cc separated serum (not whole blood). Complete the WA PHL serology form if submitting serology, and get approval of WA DOH Communicable Disease Epidemiology 206-418-5500.

Key points for specimen collection:

- Collect specimens using appropriate infection control procedures. **At a minimum** use droplet precautions. For suspected novel influenza use airborne precautions (face shield and N95 mask in addition).
- Collect nasopharyngeal, nasal, and throat swabs using swabs with a synthetic tip, such as Dacron or nylon, and a plastic or wire shaft. Specimens collected with cotton or calcium alginate swabs with wooden shafts will not be tested.
- Immediately after collection, place the swab or aspirate material into a sterile vial with 2–3 ml of viral transport media; for swab specimens, aseptically break or cut off the end of the swab shaft. The shaft is most easily broken where it is scored.
- **Close vial tightly** to avoid leakage during transport.
- Do not let a swab come into contact with reagents used for other tests. If a swab contacts reagents for other tests, a new swab must be submitted.
- Label vial with patient's name AND a second identifier, specimen source, and date obtained.
- **Specimen Storage**: Optimal testing performance is obtained with freshly-collected specimens stored and shipped refrigerated (2–8°C) that arrive to the WAPHL for processing within 72 hours of collection. If you are unable to ship the specimen for testing within 72 hours of collection, any specimen except serum should be frozen at ≤ -70°C and shipped on dry ice. Serum should be refrigerated. All viral isolates should be frozen at ≤ -70°C prior to shipment.

Storage, packaging, and shipping of specimens in viral transport media

All persons shipping packages containing medical specimens must have documented shipping training (USDOT and USPS Regulations for Packaging and Labeling Infectious Substances). For more information, phone the Virology Lab (206-418-5458) or e-mail WAPHL Training Program (email: chuck.talburt@doh.wa.gov).

WAPHL is open to receive influenza specimens Mon – Fri 8am to 5pm. Special arrangements must be made with WAPHL in order for specimens to be received on weekends or holidays (Please contact WAPHL at 206-418-5409). Specimens that arrive at WAPHL on Saturdays or holidays will be received for processing the next business day. If this will delay specimen for processing > 72 hours from collection, freeze specimen and ship on dry ice. Ship specimens to:

Washington State Public Health Laboratories
Attn: Virology Laboratory
1610 NE 150th Street
Shoreline, WA 98155

It is your responsibility as shipper to correctly package and label specimens to meet shipping regulations.

When shipping influenza specimens please follow these steps:
You may use analogous supplies to what is recommended below.

- Check that the cap of the transport tube is securely closed; place tube in Biohazard Ziploc bag containing piece of super absorbent paper.
- Complete WAPHL Virology Specimen Submission Form. **Specimens will not be processed until ALL following information is known**:
    - Patient name, second identifier, and county of residence
    - Specimen type, date of collection and test requested
    - Submitter name, address, and telephone/FAX numbers
    - Reason for testing at WAPHL (i.e., which of the above criteria are met by the patient)
- Ensure patient's name and second identifier, are on specimen tube and **match** information on specimen submission form.
- Place completed WAPHL Virology Specimen Submission Form in OUTER pouch of Biohazard Ziploc bag (one specimen and one submission form per bag). Do not place any paperwork in the inner pouch along with the vial.
- Place up to three Biohazard Ziploc bags in a Saf-T-Pak plastic bag, then place Saf-T-Pak plastic bag into white Tyvek bag.
- Place white Tyvek bag into large plain plastic bag with frozen ice packs, then place all into the Saf-T-Pak fiberboard box. Add sufficient packing material (Styrofoam peanuts or other material) to prevent shifting of contents.
- Write shipper name/address in shipper label area; place WAPHL label as consignee.
- On side of box, check "Biological Substance, Category B".
- Choose shipping method for delivery ≤ 24 hours (e.g., FedEx, Greyhound, US Express Mail, private couriers). If using FedEx, shipper may use pre-paid FedEx air bill. Other shipping expenses paid by shipper. *FedEx Tip: Select FedEx Standard Overnight (will arrive by 10am next day like FedEx Priority Overnight but is less expensive).*

WAPHL testing procedures

Test results turnaround time: Projected turnaround time for influenza testing and subtyping using RT-PCR is up to 3 business days from specimen receipt. Results from additional work such as viral isolation, submission to CDC and pyrosequencing require additional time.

Reporting of test results: Test results are sent by fax to submitting facility. Test results will also be sent by fax and/or electronic reporting system to local health jurisdiction in which patient resides.