



Guidance on How to Obtain Testing for Influenza A(H5N1) Virus in Florida as of August 29, 2006

For consultation on testing or management of possible human cases of avian influenza contact your County Health Department or the Bureau of Epidemiology (850) 245-4401

Clinical laboratories should immediately upon receipt of a physician's orders for an influenza A H5 ("avian flu") test, contact the County Health Department or Bureau of Epidemiology (BOE: 850-245-4401). If the BOE deems this a credible potential case, the affected hospital shall immediately collect specimens for shipment to the Florida Department of Health Bureau of Laboratories (BOL). Guidance on Who Should be Tested for Influenza A (H5N1) Virus in Florida is available at http://www.doh.state.fl.us/disease_ctrl/epi/htopics/BirdFlu.htm

Duplicate specimens may be processed by those clinical laboratories desiring to perform in-house testing under BSL2 conditions **for RT-PCR or antigen testing only.**

Do not wait for results of these tests to report the case for evaluation by the BOE.

Viral culture should NOT be attempted on specimens from patients suspected to have influenza H5N1, unless conducted under Biosafety Level 3 conditions with enhancements.

Specimen Collection, Packing, and Shipping Instructions

These recommendations are based on the particular Asian strain of H5N1 that is currently causing widespread disease in birds with occasional human cases, and are subject to change.

Most human cases of Asian avian influenza A/H5 have been lower respiratory tract infections, thus collecting only a nasopharyngeal or nasal swab is not recommended. In a clinical setting, nasopharyngeal swabs have been a traditional specimen for detection of seasonal influenza along with the use of rapid test kits. Because A H5 infections are deep in the lung, such samples will produce false negative influenza results. This may lead to non-reporting of the case and a lack of further testing performed. As such, important identification of early cases will be missed unless additional specimens are collected.

If a suspect case is evaluated by the BOE as being acceptable for testing, *multiple sample types should be collected over several days*. Specimens for virus detection must be collected prior to treating the patient with antivirals. Beyond the first three to five days post onset of disease, the probability of recovering virus is low. Sputum, tracheal aspirates or bronchiolar lavage specimens are recommended.

The recommendation to try to get sputum or lower airway material for culture is based on the known cellular affinities of this virus -- which also explain why it is hard for people to acquire and to transmit this agent. If the cellular tropism changes from alveolar cells to upper airway cells, both the epidemiology and the preferred laboratory specimens will change.

- Oropharyngeal swab specimens and lower respiratory tract specimens (e.g., bronchoalveolar lavage or tracheal aspirates) are preferred because they appear to contain the highest quantity of virus for influenza H5N1 detection, as determined on the basis of available data. Nasal or

nasopharyngeal swab specimens are acceptable, but may contain less virus and therefore not be optimal specimens for virus detection.

- Detection of influenza H5N1 is more likely from specimens collected within the first 3 days of illness onset. If possible, serial specimens should be obtained over several days from the same patient.
- Bronchoalveolar lavage is considered to be a high-risk aerosol-generating procedure. Therefore, infection control precautions should include the use of gloves, gown, goggles or face shield, and a fit-tested respirator with an N-95 or higher rated filter. A loose-fitting powered air-purifying respirator (PAPR) may be used if fit-testing is not possible (for example, if the person has a beard). Detailed guidance on infection control precautions for health care workers caring for suspected influenza H5N1 patients is available.||
- Swabs used for specimen collection should have a Dacron tip and an aluminum or plastic shaft (e.g., viral culturettes). Swabs with calcium alginate or cotton tips and wooden shafts dry swabs or culturettes designed for bacterial samples are not acceptable.
- Maintain specimens at 4°C after collection and ship as soon as possible at 4°C.
- Influenza H5N1-specific RT-PCR testing conducted under Biosafety Level 2 conditions is the preferred method for diagnosis. The Florida Department of Health Bureau of Laboratories is able to perform influenza H5N1 RT-PCR testing, and is the recommended sites for initial diagnosis.
- Viral culture should NOT be attempted on specimens from patients suspected to have influenza H5N1, unless conducted under Biosafety Level 3 conditions with enhancements.¶
- Commercial rapid influenza antigen testing in the evaluation of suspected influenza H5N1 cases should be interpreted with caution. Clinicians should be aware that these tests have relatively low sensitivities, and a negative result would not exclude a diagnosis of influenza H5N1. In addition, a positive result does not distinguish between seasonal and avian influenza A viruses.
- Serologic testing for influenza H5N1-specific antibody, using appropriately timed specimens, can be considered if other influenza H5N1 diagnostic testing methods are unsuccessful (for example, due to delays in respiratory specimen collection). Paired serum specimens from the same patient are required for influenza H5N1 diagnosis: one sample should be tested within the first week of illness, and a second sample should be tested 2-4 weeks later. A demonstrated rise in the H5N1-specific antibody level is required for a diagnosis of H5N1 infection.

Specimen collection:

- Oropharyngeal swab
 - Have patient give a deep cough.
 - Swab the oropharynx, or far back in the throat, using a Dacron tipped plastic shaft swab.
 - Reinsert the swab back into the culturette and crush the ampoule at the base of the culturette. OR: Insert the swab into a tube of transport media; remove excess shaft from the swab and close the tube tightly.
- Sputum, tracheal or nasopharyngeal washes/aspirates or bronchial lavage: follow facility protocols for collection of these specimen types.
- Label each specimen with the patient's name and specimen type.

- Place each specimen in a separate ziplock bag with absorbent material to trap any spills.
- **Do not freeze.** Hold the specimen in the back of a refrigerator until shipped. Ship promptly!

Shipping

- Wrap the culturette or the tube of viral transport medium containing the swab in a paper towel and place in a zip-lock plastic bag.
- Place the swabs in a small styrofoam cooler with a frozen blue-ice or gel cold-pack.
- Add sufficient “stuffing” to prevent the items from shifting during transport.
- Put the lid on the cooler and place the paper-work/submittal sheets on top of the lid.
 - Please completely fill out the requested patient information on the submission sheets. The laboratory specimen submission sheet may be downloaded from http://www.doh.state.fl.us/lab/PDF_Files/doh_form.pdf
 - Be sure that the name, address and phone number of the person to whom the report is to be sent is clearly written.
- Tape the outer cardboard box shut.
- The box should have a “Biological Substance, Category B” label on it (not a biohazard label).
- Ship overnight to the Bureau of Laboratories.
- If you are shipping on a Friday, be sure the mailing label has “Saturday Delivery” indicated.
- Specimens should be sent without delay to the nearest Bureau of Laboratories location, e.g., Jacksonville, Tampa, Miami or Pensacola with a note indicating that this is a suspect avian flu. The submitter should also call the laboratory before sending the sample. Between 8 AM and 5 PM Monday thru Friday, you may contact the laboratory directly as follows.

 Jacksonville: Valerie Mock, 904-791-1539

 Tampa: Lillian Stark, 813-974-5990

 Miami: Peter Shih, 305-324-2432

 Pensacola: Leah Gillis, 850-595-8895

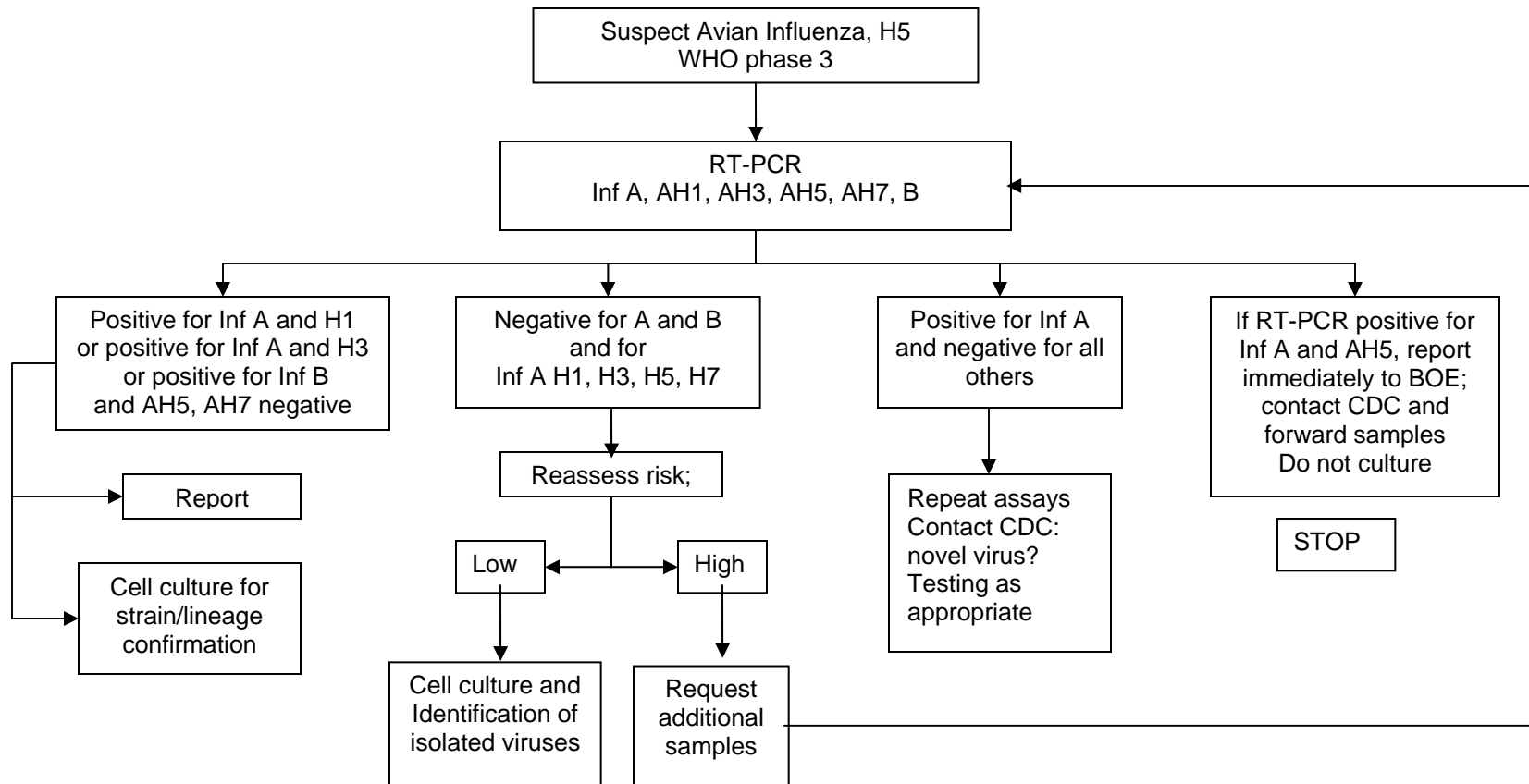
 The after hours number is 1-866-FLA-LABS. (1-866-352-5227)

Specimens will be screened at the BOL with a real-time RT-PCR molecular assay for Influenza A, B and A types H1, H3, H5. Specimens positive for Influenza A H5 will be sent to the CDC. Specimens negative for Influenza A H5 may then be inoculated into a variety of cell cultures for confirmation and detection of additional viral agents.

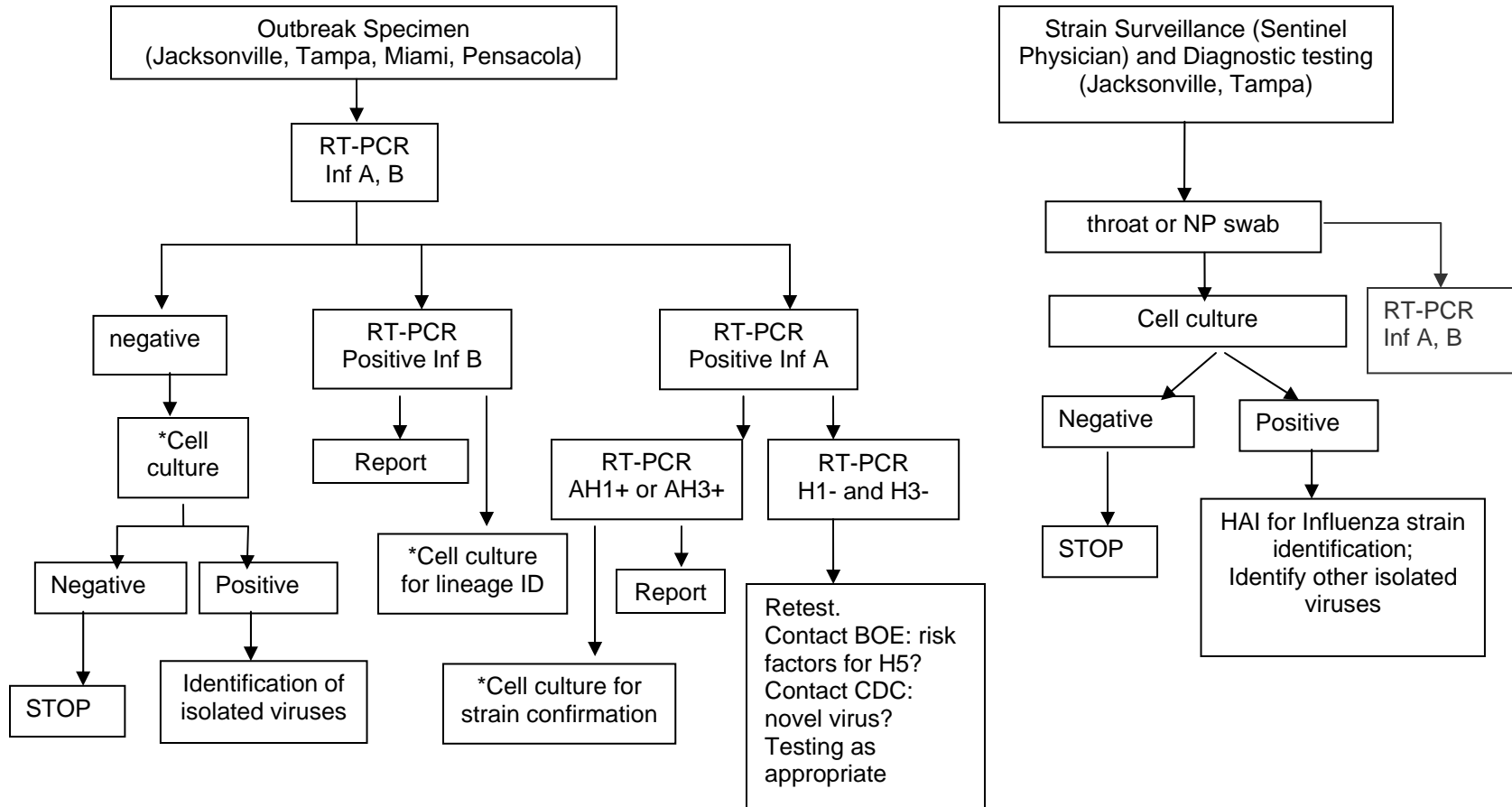
|| Interim recommendations for infection control in health-care facilities caring for patients with known or suspected avian influenza are available at <http://www.cdc.gov/flu/avian/professional/infect-control.htm>.

¶ Information regarding Laboratory Biosafety Level Criteria can be found at <http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4s3.htm>.

Attachment 1: BOL Influenza Test Algorithm



Routine Testing Algorithms for Influenza specimens



* Miami/Pensacola BOL will send specimens to the Tampa or Jacksonville Virology Laboratory for cell culture after PCR assays.