LAB NEWS

From the Department of Laboratory Medicine - Yale-New Haven Hospital Medical Center

Clinical Virology Laboratory Newsletter

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Novel H1N1 (Swine-origin) Influenza Virus Testing at YNHH

In the coming 2009-10 winter respiratory season, increased demand for influenza testing is anticipated. The main influenza tests performed in the Virology Laboratory, direct immunofluorescence (DFA) and PCR, are highly specialized and labor intensive (1-3). Demand for flu tests may exceed the capacity of the lab to handle, and still maintain other test services.

Thus, an "Influenza Testing Advisory Group" made up of clinicians from inpatient and outpatient areas at YNHH was formed to aid in setting priorities. During the season, we will continually reassess laboratory capabilities and priorities. As test algorithms change, notifications will be sent by email and if possible, posted on the Clinical Workstations.

Once pandemic influenza virus is widely circulating, a clinical diagnosis should suffice for "low risk" outpatients, and laboratory testing will focus on inpatients, health care workers and high-risk outpatients. For updates on seasonal viruses, newsletters, and tests, see the Virology Laboratory website: http://labmed.yale.edu/virology/.

I. TEST ALGORITHMS

Three phases of influenza testing are expected (see below): 1) *Enhanced surveillance,* beginning in September;
2) *Standard respiratory season protocol*, when influenza circulates in seasonal numbers;
3) *Pandemic protocol*, when demand for testing exceeds laboratory resources.

Positive detection depends on the amount of virus in the sample. False negatives occur even with PCR. Optimizing sample collection to increase the amount of virus and reduce false negatives is essential (see page 2).

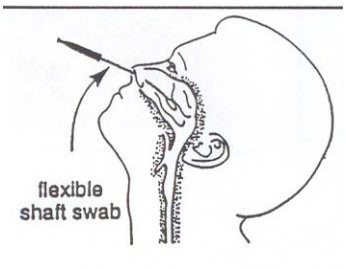
Tentative Plan for Use of Influenza Diagnostic Tests in the 2009-2010 Respiratory Virus Season

Test Information	Rapid Flu Test	Immunofluorescence (DFA)	Influenza PCR
Method	Simple, requires minimal training and no equipment Can be used at point of care	Multiplex Resp. Screen DFA detects Flu A & B, RSV, parainfluenza 1-3, adenovirus	CDC protocol for Flu A, Flu B, H1, H3, swine A and swine H1 real-time PCR
Sensitivity compared to PCR	30-35% for swine flu at Yale Rapid test detected only 9.7% of swine flu positives in NYC outbreak (4). False positives can occur.	DFA 83% for swine flu overall DFA 96% for children <5 yrs old who shed high virus titers	CDC PCR assay is "Gold standard" clinical test
Phase of testing			
Enhanced surveillance*	Not done	DFA main test, outpatient and inpatient	PCR when requested on outpatients or inpatients PCR to subtype all Flu A DFA positives
Standard respiratory season protocol	Done in Core Lab when Virology closed, only if needed for bed allocation. Call 688-2444.	DFA main test, outpatient and inpatient	PCR when requested on inpatients, health care workers, and outpatients identified as high risk[b]
Pandemic protocol	Done in Core Lab when Virology closed, only if needed for bed allocation. Call 688-2444.	Discontinue DFA for outpatients[a] except ED patients awaiting admission and some high risk patients	PCR is main test PCR for inpatients, health care workers, and high risk[b] outpatients

*Current phase. Transition to subsequent phases will be determined by test volume, number of flu positives, clinical priorities and ability of staff to maintain all virology services.
a, A clinical diagnosis should suffice for "low risk" outpatients
b, See Appendix for high risk patients

II. SAMPLE COLLECTION: *Proper sample collection is critical to optimize viral load in sample.*
FAILURE TO COLLECT OPTIMAL SAMPLES LEADS TO FALSELY NEGATIVE RESULTS.

INSTRUCTIONS: Insert swab deep into posterior nasopharynx (NP), past point of resistance and rotate to dislodge columnar epithelial cells. Alternatively, gently rub deep nasal turbinate. For the best results collect two swabs, one from each nostril, and combine into one vial (5). Place swabs in viral transport medium and transport promptly to the lab. NP washes and aspirates are excellent samples if available. Maximum titers are shed in the first 2-3 days of illness.



III. LABORATORY HOURS AND TEST AVAILABILITY
Standard hours are Monday-Friday 7 AM to 8:30 PM, Saturday-Sunday 8 AM to 4:30 PM. If staffing permits, hours may be extended during peak periods to facilitate respiratory virus testing.

Respiratory virus DFA is performed 7 days a week, usually with a 2-3 hr time to result once the sample is received in the lab. DFA cut–off for same day result is 90 minutes prior to closing time.

Influenza A & B PCR is performed once a day, Monday-Friday. If DFA testing is substantially reduced (see Pandemic protocol), staff will be available to perform PCR twice a day, 7 days a week.

Rapid Flu test is performed at Shoreline Medical Center, and in the YNHH Core Lab for bed allocation only, when Virology is closed. Call 688-2444 to request a rapid test only if needed for bed allocation. Note: Test is only 30-35% sensitive compared to PCR and false positives can occur.

Conventional Cell Culture will not be done for swine influenza unless isolates are needed for public health/vaccine purposes.

IV. TEST CHANGES DURING THE SEASON
Continual re-assessments will be made in consultation with the YNHH Influenza Testing Advisory Group, based on clinical priorities, staffing limitations, possible reagent shortages, CDC advisories, and public health needs. Test frequency and laboratory hours will be adjusted accordingly.

For questions or comments, contact Marie Landry, M.D., Laboratory Director, at 688-3475 or marie.landry@yale.edu, or David Ferguson, Laboratory Manager at 688-3524.

References
1. Landry ML and D Ferguson. 2000. SimulFluor Respiratory Screen for rapid detection of multiple respiratory viruses in clinical specimens by immunofluorescence staining. J Clin Microbiol 38:708-711.
2. Landry ML, S Cohen, and D Ferguson. 2008. Real-time PCR compared to Binax NOW and cytospin-immunofluorescence for detection of influenza in hospitalized patients. J Clin Virol. 43:148-151.
3. Landry ML and D. Ferguson. Cytospin-enhanced immunofluorescence and impact of sample quality on diagnosis of novel swine-origin (H1N1) influenza virus. Submitted.
4. Ginocchio CC, et al. 2009. Evaluation of multiple test methods for the detection of the novel 2009 influenza A (H1N1) during the New York City outbreak. J Clin Virol. 45:191-195.
5. Pollock NR, et al. 2009. Ruling Out Novel H1N1 Influenza Virus Infection with Direct Fluorescent Antigen Testing. Clin Infect Dis 49:e66–e68.

APPENDIX
Defined as high risk for influenza complications:
- Pregnant women
- Children or adolescents (<18) on long-term aspirin therapy
- Adults and children with chronic pulmonary, cardiovascular, hepatic, hematologic, neurologic, neuromuscular, metabolic disorders
- Adults and children who are immunosuppressed
- Residents of nursing homes and other chronic care facilities
- Persons 65 or older