



MLABS – DEPARTMENT OF PATHOLOGY
ATTENTION: IMPORTANT TEST INFORMATION

NOTICE DATE: September 30, 2009

EFFECTIVE DATE: September 30, 2009

NEW PCR ASSAY FOR RESPIRATORY VIRUSES NOW AVAILABLE FROM MLABS

Respiratory Viruses by PCR

Order Code: PCRIR

Fee Code: 40087

Effective September 30, 2009, the MLabs Virology Laboratory will begin offering PCR testing for Influenza A, Influenza B and Respiratory Syncytial Virus (RSV). The new assay, **Respiratory Viruses by PCR** (order code PCRIR) replaces both Influenza Virus Types A and B by Rapid PCR (order code PFLU) and Respiratory Viruses Antigen Screen by IFA (order code RESPSN).

Collection Instructions: **SWAB:** Use flocked swab, dacron or rayon swab to obtain specimen and place into M4-RT transport medium. Refrigerate. **NASAL WASH:** Use a 1 oz rubber bulb containing 3 - 5 mL of sterile preservative-free saline and rapidly instill and aspirate the contents from a nostril in a single motion. Empty material into M4-RT transport medium (preferred) or sterile container and close cap tightly. Refrigerate. **NASAL ASPIRATE:** Gently insert a number 5 - 8 French pediatric-feeding tube attached to a suction strap through both nostrils into the nasopharynx. Aspirate contents into M4-RT transport medium and close cap tightly. Refrigerate. **BAL FLUID:** Place in sterile cup and refrigerate. M6-RT or UTM transport media is also acceptable. Deliver all specimen types to the laboratory as soon as possible. The possibility for virus isolation decreases as the length of storage time increases. Many viral agents will survive at room temperature for several hours; however, for optimum recovery of viruses, please refrigerate; do not freeze.

Additional Information: If the test is positive for Influenza A, a sub-typing test will be performed at an additional charge (cpt 87798 x3). The Influenza A Subtype assay includes typing for H1N1 seasonal, H1N1 2009 (swine), and H3 seasonal. If the test is negative, a culture will be performed at an additional charge (Viral Culture, Respiratory excluding CMV). The culture includes screening for respiratory viruses Influenza A and B, Parainfluenza 1, 2, and 3, Respiratory Syncytial Virus (RSV), Adenovirus, and human Metapneumovirus. By ordering this test the clinician acknowledges that additional reflex testing will be performed and billed at a separate additional charge if indicated.

MLabs

Spectrum Highlight

October 8, 2009

Laboratory Testing for Novel H1N1 Influenza Virus

A number of laboratory diagnostic tests can be used to detect the presence of influenza viruses in respiratory specimens, including direct antigen detection tests, virus isolation in cell culture, or detection of influenza-specific RNA by real-time reverse transcriptase-polymerase chain reaction (rRT-PCR). These tests differ in their sensitivity and specificity in detecting influenza viruses, their commercial availability, the time from specimen collection until results are available, and their ability to distinguish between different influenza virus types (A versus B) and influenza A subtypes (e.g., novel H1N1 versus seasonal H1N1 versus seasonal H3N2 viruses).

Rapid influenza diagnostic tests (RIDTs) detect influenza viral nucleoprotein antigen. Most of these provide results within 30 minutes, a relevant period to inform clinical decisions. These assays may be referred to as "point-of-care" tests since CLIA-waived RIDTs (not all RIDTs are CLIA-waived) may be used in facilities with a certificate of waiver or in locations outside a central laboratory. RIDTs can either: i) detect and distinguish between influenza A and B viruses; ii) detect both influenza A and B but not distinguish between influenza A or B viruses; or, iii) detect only influenza A viruses. None of the currently FDA approved RIDTs can distinguish between influenza A virus subtypes [e.g. seasonal influenza A (H3N2) versus seasonal influenza A (H1N1) viruses]. For detection of seasonal influenza A virus infection in respiratory specimens, RIDTs have low to moderate sensitivity compared to viral culture or RT-PCR. The sensitivities of RIDTs to detect influenza B viruses are lower than for detection of influenza A viruses. The sensitivities of RIDTs appear to be higher for specimens collected from children than specimens collected from adults as children appear to shed more virus during infection.

Although few assessments of RIDTs for the detection of novel influenza A (H1N1) virus compared to seasonal influenza viruses have been published, recent analytical studies indicate that commercially available RIDTs *are reactive* with the nucleoprotein of novel influenza A (H1N1) virus (1). However, only limited data have been published on the performance of RIDTs compared with RT-PCR for detecting the presence of novel influenza A (H1N1) virus in clinical specimens. While limited by small numbers, currently published, side-by-side comparisons of RIDTs suggest the sensitivity of RIDTs to detect novel influenza A (H1N1) virus is equal to or lower than the sensitivity to detect seasonal influenza viruses; compared to RT-PCR the sensitivity of RIDTs for detecting novel (H1N1) and seasonal (H1, H3) influenza A viruses infections ranged from 10-70% (2-5). **Therefore, a negative RIDT result does not rule out novel influenza virus infection.** Factors that might contribute to



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the lower sensitivity of influenza laboratory tests to detect novel influenza A (H1N1) virus infection include the type of respiratory specimen (i.e., nasal aspirate vs. nasopharyngeal swab), quality of the specimen (i.e., abundance of respiratory epithelial cells), time from illness onset to specimen collection, the age of the patient, time from specimen collection to testing, and the storage and processing of the specimen prior to testing.

Although cell culture is very sensitive and specific, it can take up to 5 days for results to be available. Since RT-PCR exhibits analytical performance equal to or better than cell culture, and because it can provide results more quickly, MLabs Clinical Microbiology and Virology Laboratories have implemented a new test for the detection of influenza and other respiratory viruses. "Respiratory Viruses by PCR" (order code PCRIR) will use RT-PCR to detect influenza A virus, influenza B virus, and/or respiratory syncytial virus (RSV) from respiratory specimens. Samples positive for influenza A virus will be reflexed to an assay to specifically identify novel H1, seasonal H1 or seasonal H3 subtypes. Specimens negative for influenza A virus, influenza B virus, and RSV will be reflexed to "Viral Culture, Respiratory excluding CMV" (order code RCULT) for the detection of parainfluenza viruses 1-3, adenovirus and human metapneumovirus. Reflex testing for influenza A subtype determination will continue during the season as long as this information remains clinically relevant. MLabs will provide notification if this changes in the future.

The University of Michigan Health System has chosen to limit influenza testing at this time to hospitalized patients with influenza-like illness, and patients presenting in an outpatient setting whose severity of illness warrants hospitalization as testing will have a significant impact on therapeutic management and implementation of strategies to prevent nosocomial transmission. In this context, clinicians in ambulatory care settings should consider the low utility for diagnostic testing in otherwise healthy individuals, with no co-morbidities or other risk factors for complications from influenza, prior to requesting testing.

1. Hurt AC et al. Performance of influenza rapid point-of-care tests in the detection of swine lineage A(H1N1) influenza viruses. *Influenza and Other Respiratory Viruses* 2009;3(4):171-76.
2. Faix DJ, Sherman SS, Waterman SH. Rapid-Test Sensitivity for Novel Swine-Origin Influenza A (H1N1) Virus in Humans. *N Engl J Med*. 2009 Jun 29 [Epub ahead of print].
3. Ginocchio CC et al. Evaluation of multiple test methods for the detection of the novel 2009 influenza A (H1N1) during the New York City outbreak. *J Clin Virol*. 2009 Jul;45(3):191-5.
4. Chan KH et al. Analytical sensitivity of rapid influenza antigen detection tests for swine-origin influenza virus (H1N1). *J Clin Virol*. 2009 Jul;45(3):205-7.
5. CDC. Evaluation of Rapid Influenza Diagnostic Tests for Detection of Novel Influenza A (H1N1) Virus --- United States, 2009. August 7, 2009 *MMWR*, 58(30):826-829.

This document was prepared in part using public information available from the following websites, which should be checked frequently for updates in content:

CDC H1N1 Flu page (<http://www.cdc.gov/h1n1flu/>)

MDCH H1N1 Flu page (<http://www.michigan.gov/mdch>)