



TECHNICAL NOTICE

SOUTH BEND MEDICAL FOUNDATION

September 2009

Testing for Seasonal and Novel Flu by EIA and RT-PCR (Influenza A and B and Respiratory Syncytial Virus Panel by RT-PCR and Influenza A Subtyping by RT-PCR)

Effective Date: October 12, 2009

Performing Department: Molecular Pathology

Overview:

Much media attention has been focused on Swine Flu which appeared last spring in Mexico and has spread to the United States and the rest of the World since. Although Influenza is rare during the summer months, cases of Swine Flu (now officially named 2009 [Novel] H1N1 Influenza A) have continued in the U.S. since the spring and now comprise 98-99% of all subtyped cases of Influenza A. South Bend Medical Foundation (SBMF) has performed Rapid Influenza Diagnostic Tests (RIDTs) to screen for cases of Influenza A and B since the spring. The Enzyme Immunoassay (EIA) screening test provides rapid turnaround time of 1-2 hours but its sensitivity for 2009 Novel H1N1 is only 50% at best. SBMF confirmatory DFA (Direct Immunofluorescent Assay) is more sensitive (70-75%), but is labor intensive and cannot readily be performed when test volume is high. Molecular tests to detect the virus have been performed at the Center for Disease Control (CDC) and some Public Health Laboratories since the spring, but these labs now perform the tests only for selected sentinel clients.

After recent FDA approval of a molecular screening test for Influenza A, SBMF has performed validation studies and two RT-PCR tests will be available in mid-October. The EIA screening test will continue to be available if the clinical situation requires a rapid test result. The DFA test for Influenza A will be replaced by the new RT-PCR tests. One RT-PCR test will distinguish Influenza A from Influenza B from Respiratory Syncytial Virus (RSV). This test is 95%-100% sensitive for 2009 Novel H1N1, but also detects other Influenza A subtypes. A second RT-PCR test (Influenza A subtyping) is available if the initial test indicates Influenza A and the physician and/or patient wants to know if the virus is 2009 Novel H1N1 or another Influenza A subtype. The turnaround time for these RT-PCR tests will be 24 hours. Once there is documented 2009 (Novel) H1N1 Influenza A outbreak in a community, routine testing for the virus is not necessary on most (non-hospitalized) patients.

Method: Real-Time Reverse Transcription Polymerase Chain Reaction (RT-PCR)

Use: Detection of Influenza Virus A & B and RSV and Influenza Virus A subtypes

Reference Range:

- No detectable Influenza Virus A nucleic acid (NA) by NA amplification
- No detectable Influenza Virus B nucleic acid (NA) by NA amplification
- No detectable Respiratory Syncytial Virus nucleic acid (NA) by NA amplification

Cautions:

- Results will vary depending on specimen quality/collection/handling, time of year, age of population, geographic location, etc.
- A negative test does not exclude infection, so that the results should be used in conjunction with clinical findings in order to make an accurate diagnosis.
- Both viable and nonviable viruses will be detected by this test.

SOUTH BEND MEDICAL FOUNDATION

530 N. Lafayette Boulevard • South Bend, IN 46601 • (574) 234-4176

Elkhart (574) 293-8441 • (800) 544-0925

Robert J. Tomec, M.D. • *Medical Director*

Testing for Seasonal and Novel Flu by EIA and RT-PCR (continued):

Specimen Requirements and Collection:

Note: • Attached are instruction sheets (SBMF P1035-1 and P1173) for collecting specimens in an office setting

Preferred Specimen: • Nasopharyngeal aspirate or washings, or swabs in viral transport media (M4 or UTM)

Requested Volume:

- Contents of syringe or bulb used for nasopharyngeal aspiration
- Contents of syringe or bulb used for nasopharyngeal saline washings
- One (1) flocked nasopharyngeal swab

Collection:

- Nasopharyngeal Aspirate Specimen Collection:
 - Insert tubing attached to syringe (or compressed bulb for infants) through nose and direct toward nasopharynx
 - Pull back on syringe (or decompress bulb for infants) to withdraw secretions
 - Expel secretions into viral transport media (M4, M6 or UTM) and securely tighten media vial cap
 - Immediately refrigerate and transport specimen
 - Must be received within 72 hours of collection time
- Nasopharyngeal Washings Specimen Collection:
 - Fill syringe with 5.0 mL sterile saline (or fill bulb for infants) and instruct patient not to swallow during procedure
 - Insert tubing attached to syringe (or bulb for infants) through nose and direct toward nasopharynx
 - Gently expel saline into the nasopharynx; wait 1-2 seconds and then pull back on syringe (or decompress bulb) to withdraw saline
 - Expel saline washings in syringe (or bulb) into viral transport media (M4, M6 or UTM)
 - Securely tighten media tube cap
 - Immediately refrigerate and transport specimen
 - Must be received within 72 hours of collection time
- Nasopharyngeal Flocked Swab Specimen Collection:
 - Insert flocked nasopharyngeal swab into patient's nasal passage until a slight resistance is met
 - Rotate swab 2-3 times and hold swab in place for 5 seconds to ensure maximum absorbency
 - Withdraw swab and repeat procedure in other nares with the same swab
 - Remove swab, place in vial of viral transport media (M4, M6 or UTM), and break off shaft at molded breakpoint
 - Securely tighten media tube cap
 - Immediately refrigerate and transport specimen
 - Must be received within 72 hours of collection time

Stability: • 3 days (72 hours) refrigerated (2-8°C) • If will not be received within 72 hours, deep-freeze specimen (-70°C)

Storage/Transport: • Refrigerated

Rejection Criteria: • Specimen not received within 72 hours of collection and not deep-frozen

Testing Schedule: • Daily • Result available within 24 hours

Order: • **Influenza A and B EIA and Influenza A&B/RSV RT-PCR Panel** Test #: **21992**..... CPT: • 87804 x 2 • 87801

• **Influenza A and B and RSV Panel, by RT-PCR**..... Test #: **36154**..... CPT: • 87801

• **Influenza A Subtyping, by RT-PCR** Test #: **36159**..... CPT: • 87801

• **Influenza A and B, by EIA only** Test #: **21132**..... CPT: • 87804 x 2

Please direct questions regarding this notice to C. Kurtis Kim, M.D., William J. Kaliney, M.D., Bobbie C. Sutton, M.D., Robert J. Tomec, M.D. or Sally Cornwall, South Bend Medical Foundation, (574) 234-4176 or (800) 544-0925.

SOUTH BEND MEDICAL FOUNDATION

530 N. Lafayette Boulevard • South Bend, IN 46601 • (574) 234-4176

Elkhart (574) 293-8441 • (800) 544-0925

Robert J. Tomec, M.D. • *Medical Director*