Louisiana Office of Public Health Laboratories	
Test Name	Influenza Real Time RT-PCR Detection and Characterization
PHL Location	Office of Public Health Laboratory Baton Rouge
CPT Code	**87502 -** Infectious agent detection by nucleic acid (DNA or RNA); influenza virus, for multiple types or sub-types, multiplex reverse transcription and amplified probe technique, first 2 types or sub-types. **87503 -** Infectious agent detection by nucleic acid (DNA or RNA); influenza virus, for multiple types or sub-types, multiplex reverse transcription and amplified probe technique, each additional influenza virus type or subtype beyond two.
Synonyms	Flu, H1N1, Influenza
Brief Description of Test	Multiple target Real Time RT-PCR for Influenza Detection and Further Characterization InfA and InfB are designed for universal detection of type A and type B influenza viruses YAM and VIC are designed to genotype Influenza B H3 and H5 are designed to specifically detect contemporary human A/H3, and A/H5 (Asian lineage) influenza viruses pdmInfA and pdmH1 are designed to detect the nucleoprotein gene and hemagglutinin gene RNA from 2009 H1N1 influenza virus RP is designed to detect the human RNase P gene RNA and is used as an internal specimen control as well as an extraction control
Possible Results	This laboratory will determine the best testing algorithm for your sample based on current reagent availability, epidemiologic data and specimen volume. Available options include: Influenza A/B Typing – (infA, infB and RP) • If influenza A is detected, the sample will automatically be reflex tested for subtypes • If influenza B is detected, the sample will automatically be reflex tested for YAM and VIC genotyping (currently reported to Epidemiology only) Influenza A/B plus Subtyping – (infA, infB, H3, pdmInfA, pdmH1 and RP)

	Influenza A/H5 Subtyping Assay – (InfA, H5a, H5b and RP) • Testing for avian influenza A/H5N1 is considered on a case-by-case basis in consultation with the Infectious Disease Epidemiology department for hospitalized or ambulatory patients with: o Documented temperature of > 38°C **AND** o One or more of the following: cough, sore throat, shortness of breath, **AND** o History of contact with poultry or a known or suspected case of influenza A (A/H5N1) in an A/H5N1-affected country within 10 days of symptom onset. Influenza euH7 (emergency use authorization for H7 testing) is considered on a case-by-case basis in consultation with the Infectious Disease Epidemiology department. All H3N2v presumptive positive clinical samples must be sent to CDC for confirmation. Influenza A unsubtypable with infA Ct values <35 must be sent to CDC for further testing. Influenza A unsubtypable with infA Ct values >35 will be reported as with a comment to indicate that the sample was unsubtypable due to low viral titer. Each Influenza target tested will be listed on the report with a result of Positive or Negative. After each target is listed, a report conclusion will follow. RP will not be printed on the report. Non-Standard result combinations such as being positive for Influenza A and Influenza B will have INCONCLUSIVE listed as the report conclusion. Additional comments may be added as warranted by the specific result combination.
Reference Range	Not Detected
Specimen Type	Specimens are submitted in Viral Transport Media (VTM) Tubes • Upper respiratory tract clinical specimens o nasopharyngeal swabs [NPS] o nasal swabs [NS] o nasal aspirates [NA] o nasal washes [NW] o dual nasopharyngeal/throat swabs [NPS/TS] • lower respiratory tract specimens o bronchoalvolar lavage [BAL] o bronchial wash [BW] o tracheal aspirate [TA] o sputum o lung tissue

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- viral culture

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<tr><td>Specimen Container(s):</td><td>VTM</td></tr>
<tr><td>Minimum volume accepted:</td><td>Request 1 mL
Testing may be performed on as little as 100µL</td></tr>
<tr><td>Collection Instructions</td><td>
<table>
<tr><th>If</th><th>Then</th></tr>
<tr><td>Nasal swabs (NS), nasopharyngeal swabs (NPS), dual nasopharyngeal/throat swabs (NPS/TS)</td><td>Swab should be placed and mixed well in a VTM tube immediately after collection. Swab specimens should be collected only on swabs with a synthetic tip (such as polyester or Dacron) and an aluminum or plastic shaft. Swabs with cotton tips and wooden shafts are NOT recommended. Specimens collected with swabs made of calcium alginate are NOT acceptable.</td></tr>
<tr><td>Nasal Aspirates (NA), Nasal Washes (NW), Broncheoalveolar lavage (BAL), Tracheal Aspirate (TA) and Bronchial Wash (BW), sputum</td><td>Aseptically dilute liquid sample with an equal volume of VTM. Portion of transport media may be removed so that the volume of the sample equals the volume of the transport media.</td></tr>
<tr><td>Lung tissue or culture</td><td>Add available portion to transport media</td></tr>
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Label specimen with Patient Name and a 2nd Unique Identifier such as a chart number or medical record number. DOB is not considered unique.

Complete a LAB Form 96 to accompany the sample. Lab submission form must be thoroughly completed with patient’s first and last name, 2nd patient identifier, gender, date of birth, date and time of collection, specimen source, test requested, submitter’s name, address, fax and contact number. Additional information regarding patients’ address is requested.

Transport specimen to laboratory as soon as possible after collection/incubation. Keep submission forms insulated from specimens.
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<tr><td>Storage and Transport Instructions</td><td>Transport specimen to laboratory as soon as possible after collection. If sample will be delivered to the laboratory within 72 hours after collection, hold the specimen at 2-8°C and ship specimen at 2-8°C.</td></tr>
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	To minimize the effects of multiple freezing and thawing every attempt should be made to deliver the specimen to the laboratory within 72 hours from collection. If delivery to the laboratory within 72 hours from collection is not possible (ie. Sample collected on a Friday), freeze the specimen upon collection and ship to the laboratory on dry ice for delivery within 5 days. If the sample is frozen at any point, it must remain and be shipped frozen. Document the date/time of freezing on the specimen submission form. Follow shipping company guidelines for Category B transport.
Causes for Rejection	• Received outside acceptable transport conditions • Incorrect source • Incorrect labeling • Expired collection tubes
Limitations of the Procedure	Negative results do not preclude influenza virus infection and should not be used as the sole basis for treatment or other patient management decisions. A false negative result may occur if a specimen is improperly collected, transported or handled. False negative results may also occur if amplification inhibitors are present in the specimen or if inadequate numbers of organisms are present in the specimen. Children tend to shed virus more abundantly and for longer periods of time than adults. Therefore, testing specimens form adults will have lower sensitivity than testing specimens from children. Positive and negative predictive values are highly dependent on prevalence. False negative test results are more likely during peak activity when prevalence of disease is high. False positive test results are more likely during periods of low influenza activity when prevalence is moderate to low. The performance of the assay has not been established in individuals who received nasally administered influenza vaccine. Individuals who received nasally administered influenza A vaccine may have positive test results for up to three days after vaccination. Optimum specimen types and timing for peak viral levels during infections caused by a novel influenza A virus have not been determined. Collection of multiple specimens from the same patient may be necessary to detect the virus. If the virus mutates in the rRT-PCR target region, a specific novel influenza A virus may not be detected or may be detected less predictably.

	Inhibitors or other types of interference may produce false negative results. An interference study evaluating the effect of common cold medications was not performed. Test performance can be affected because the epidemiology and pathology of disease caused by a specific novel influenza A virus is no fully known. For example, clinicians and laboratories may not know the optimum types of specimens to collect, and when during the course of infection these specimens are most likely to contain levels of virus that can be readily detected. Detection of viral RNA may not indicate the presence of infectious virus or that influenza is the causative agent for clinical symptoms. The performance of this test has not been established for monitoring treatment of influenza A or 2009 H1N1 influenza infection. The performance of this test has not been established for screening of blood or blood product for the presence of influenza A or 2009 H1N1 influenza infection. This test cannot rule out disease caused by other bacterial or viral pathogens.
Interfering Substances	Nasally administered influenza A vaccine may produce positive test results for up to 3 days or longer in some instances. Specimens collected with calcium alginate or cotton swabs
References	CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel
Additional Information	Currently accepting specimens from Sentinel Sites and from hospital submitter's with hospitalized patients presenting with influenza like illness.
Release Date	05/16/2018
Warning: If you have printed a copy of this information please be advised that the Louisiana Office of Public Health Laboratories website and methods are updated on a regular basis. Please check the on-line version of this document to ensure you are relying on the most recent release.	