MAYO CLINIC LABORATORIES

Test Definition: RP

Respiratory Panel, PCR, Nasopharyngeal

Overview

Useful For

Rapid detection of respiratory infections caused by the following:
-Adenovirus
-Coronavirus serotypes HKU1, NL63, 229E, OC43
-SARS-CoV-2, the causative agent of COVID-19
-Human metapneumovirus
-Human rhinovirus/enterovirus
-Influenza A subtypes H1, H1-2009, H3, influenza A undifferentiated subtypes (including subtype H5N1)
-Influenza B
-Parainfluenza virus serotypes 1-4
-Respiratory syncytial virus (RSV)
-*Bordetella pertussis*
-*Bordetella parapertussis*
-*Chlamydia pneumoniae*
-*Mycoplasma pneumoniae*

This test is **not recommended** as a test of cure.

Reflex Tests

Test Id	Reporting Name	Available Separately	Always Performed
RPMPM	M. pneumoniae Macrolide Resist PCR	Yes	No

Testing Algorithm

If positive for *Mycoplasma pneumoniae*, *M pneumoniae* macrolide resistance will be performed at an additional charge.

For more information see Coronavirus Disease 2019 (COVID-19), Influenza, and Respiratory Syncytial Virus Testing Algorithm.

Special Instructions

- Coronavirus Disease 2019 (COVID-19), Influenza, and Respiratory Syncytial Virus Testing Algorithm

Highlights

This test is a multiplex polymerase chain reaction (PCR) test capable of qualitatively detecting DNA or RNA of 22 pathogens (bacteria and viruses) in approximately 1 hour using nasopharyngeal swab specimens.

This test may diagnose infections caused by adenovirus, coronavirus (HKU1, NL63, 229E, OC43), SARS-CoV-2, human metapneumovirus, human rhinovirus/enterovirus, influenza A subtypes H1, H1-2009, and H3, influenza A undifferentiated subtypes (including subtype H5N1),influenza B, parainfluenza (1, 2, 3, 4), respiratory syncytial virus, *Bordetella pertussis, Bordetella parapertussis, Chlamydia pneumoniae,* and *Mycoplasma pneumoniae*.

Method Name
Multiplex Polymerase Chain Reaction (PCR)

NY State Available
Yes

Specimen

Specimen Type
Varies

Ordering Guidance
This assay is not predicted to detect severe acute respiratory syndrome (SARS)-associated coronavirus or Middle East respiratory syndrome (MERS)-coronavirus.

This test is **not** intended for otherwise healthy, immunocompetent patients who are likely to have a mild, self-limited respiratory infection. If testing is desired, these patients should be tested using the more targeted diagnostic assays based on their exposure history and clinical presentation.
-FLUNP / Influenza Virus Type A and Type B, and Respiratory Syncytial Virus (RSV), Molecular Detection, PCR, Nasopharyngeal Swab
-BPRPV / *Bordetella pertussis* and *Bordetella parapertussis*, Molecular Detection, PCR, Varies
-MPRP / *Mycoplasma (Mycoplasmoides) pneumoniae* with Macrolide Resistance Reflex, Molecular Detection, PCR, Varies
-HPCOV /Severe Acute Respiratory Syndrome Coronavirus 2 (SARS CoV-2), Molecular Detection, Varies

It is **not** recommended that the following tests be concomitantly ordered when this test is ordered:
-FLUNP / Influenza Virus Type A and Type B, and Respiratory Syncytial Virus (RSV), Molecular Detection, PCR, Nasopharyngeal Swab
-LADV / Adenovirus, Molecular Detection, PCR, Varies
-LENT / Enterovirus, Molecular Detection, PCR, Varies
-BPRPV / *Bordetella pertussis* and *Bordetella parapertussis*, Molecular Detection, PCR, Varies
-MPRP / *Mycoplasma (Mycoplasmoides) pneumoniae* with Macrolide Resistance Reflex, Molecular Detection, PCR, Varies
-HPCOV /Severe Acute Respiratory Syndrome Coronavirus 2 (SARS CoV-2), Molecular Detection, Varies

This test is appropriate for nasopharyngeal swabs only. For bronchoalveolar lavage or bronchial washings specimens, order RPB / Respiratory Panel, PCR, Varies.

Shipping Instructions
Specimens that cannot be shipped refrigerated to Mayo Clinic Laboratories within 3 days (72 hours) should be frozen prior to shipment. Specimens received older than 72 hours (refrigerated) or older than 30 days (frozen) will be canceled.

Specimen Required

Specimen Type: Nasopharyngeal swab
Supplies:
-Culture Swab - Liquid Stuarts/Single Swab (NP Swab) (T515)
-M4-RT (T605)
Collection Container/Tube: Swab. See Additional Information for acceptable swab.
Submission Container/Tube: Transport medium. See Additional Information for acceptable media.
Specimen Volume: Nasopharyngeal swab in minimum volume of 1 mL of transport media
Collection Instructions: Nasopharyngeal swab specimens should be collected according to standard technique and immediately placed into transport media and submitted for testing.
Additional Information:
If any nasopharyngeal swab or transport media not listed below is utilized, testing may be canceled.
-Acceptable nasopharyngeal (NP) swabs are Copan Rayon Swabs, Copan Nylon Flocked Swabs, Copan Polyester Swabs, Puritan Calcium Alginate Swabs, SteriFlock NP Swab
-Acceptable transport media are Remel M4, Remel M4-RT, Remel M5, Remel M6, BD Universal Viral Transport Media (VTM), Copan Universal Transport Media (UTM), PrimeStore Molecular Transport Medium (MTM)
-Acceptable collection and transport system is Sigma-Virocult Viral Collection and Transport System (Swab and transport medium)

Forms

If not ordering electronically, complete, print, and send 1 of the following forms with the specimen:
-Kidney Transplant Test Request
-Microbiology Test Request (T244)

Specimen Minimum Volume

See Specimen Required

Reject Due To

Swabs other than NP swab (eg, any thick shafted swab such as an oropharyngeal swab or nasal swab)	Reject
Any transport media or NP swab type not listed in **Specimen Required**	Reject

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container

Varies	Refrigerated (preferred)	72 hours	
	Ambient	4 hours	
	Frozen	30 days	

Clinical & Interpretive

Clinical Information

Respiratory infections are common and generally cause self-limited illnesses in healthy, immunocompetent hosts. Viruses account for a significant percentage of respiratory diseases, but bacteria can be associated with respiratory infections. Although respiratory illnesses are frequently mild, viruses may cause significant morbidity and mortality in immunocompromised hosts (eg, transplant recipients, patients with underlying malignancies).

Influenza viruses (types A and B) and respiratory syncytial virus (RSV) are 2 common causes of viral respiratory illness, with peak incidence in the winter and spring months in the Northern hemisphere. Both viruses can cause a clinically indistinguishable syndrome characterized by fever, cough, headache, and general malaise. RSV is a leading cause of respiratory illness in young children. Early diagnosis of influenza and RSV is important so necessary infection control precautions can be taken if the patient is hospitalized, and antiviral therapy can be considered if the patient is hospitalized or considered at high-risk for severe disease.(1) Human metapneumovirus is also a cause of respiratory illness in both children and adults.

Human rhinovirus and coronavirus serotypes HKU1, NL63, 229E, and OC43 are the causative agents of the common cold, with symptoms including runny nose, sore throat, and malaise. Infections with rhinovirus and coronaviruses are extremely common, due to the large number of serotypes of these viruses. Most infections are mild and self-limiting; however, immunocompromised individuals may suffer more severe illnesses, including lower respiratory tract disease.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is an RNA virus that causes COVID-19. Like other coronaviruses that infect humans, SARS-CoV-2 can cause both upper and lower respiratory tract illness. Symptoms can range from mild (eg, the common cold) to severe (eg, pneumonia) in both healthy and immunocompromised patients. SARS-CoV-2 transmission occurs primarily via respiratory droplets. During the early stages of COVID-19 disease, the symptoms may be nonspecific and resemble other common respiratory infections, such as influenza.

Parainfluenza viruses and adenovirus are also common causes of viral infection, especially in young children. Parainfluenza viruses are most common during the spring, summer, and fall months, with symptoms including fever, runny nose, and cough. However, parainfluenza viruses may also cause more severe lower respiratory disease, such as croup or pneumonia. Adenoviruses may infect a range of organ systems, with sequelae ranging from cold-like symptoms (sore throat) to pneumonia, conjunctivitis (pink eye), or diarrhea. Similar to the viruses described above, parainfluenza viruses and adenoviruses generally cause mild, self-limited infections but may cause severe disease in immunosuppressed patients.

Respiratory infections may also be caused by bacterial pathogens, including *Bordetella pertussis*, *Bordetella parapertussis*, *Chlamydia pneumoniae*, and *Mycoplasma pneumoniae*. *B pertussis* is the causative agent of pertussis, or whooping cough, a disease characterized by a prolonged cough that may be associated with an inspiratory whoop and post-tussive vomiting. *B parapertussis* causes a similar, but generally less severe, illness. *M pneumoniae* is a cause of upper respiratory infection, pharyngitis, tracheobronchitis, and pneumonia. *C pneumoniae* is a rare cause of pneumonia.

Reference Values

Undetected (for all targets)

Interpretation

Results are intended to aid in the diagnosis of illness and are meant to be used in conjunction with other clinical and epidemiological findings.

A negative result should not rule out infection in patients with a high pretest probability for a respiratory infection. The assay does not test for all potentially infectious agents of respiratory disease. Specimens collected too early or too late in the clinical course may not yield the organism causing disease. Negative results should be considered in the context of a patient's clinical course and treatment history, if applicable.

For patients who are immunocompromised and have a negative FilmArray respiratory panel test from a nasopharyngeal sample but a high suspicion for infection, there may be additional value in testing a bronchoalveolar lavage specimen (RPB / Respiratory Panel, PCR, Varies).

Positive results do not distinguish between a viable or replicating organism and the presence of a nonviable organism or nucleic acid, nor do they exclude the potential for coinfection by organisms not included in the panel. Nucleic acid may persist in some patients for days to weeks, even following appropriate therapy. Detection of 1 or more organisms included in this test suggests that the virus or bacteria is present in the clinical sample; however, the test does not distinguish between organisms that are causing disease and those that are present but not associated with a clinical illness. Coinfections (eg, detection of multiple viruses or bacteria or viruses and bacteria) may be observed with this test. In these situations, the clinical history and presentation should be reviewed thoroughly to determine the clinical significance of multiple pathogens in the same specimen.

Cautions

Test results should be used as an aid in diagnosis. The single assay should not be used as the only criteria to form a clinical conclusion, but results should be correlated with patient symptoms and clinical presentation. A negative result does not negate the presence of the organism or active disease.

The detection of microbial DNA or RNA is dependent upon proper sample collection, handling, transportation, storage, and preparation. There is a risk of false-negative results due to the presence of strains with sequence variability or genetic rearrangements in the target regions of the assays or levels of the organism at or below the limit of detection of the test.

Positive results do not rule out coinfection with other pathogens.

Negative results combined with respiratory illness may be due to pathogens not detected by this panel.
Repeat testing should not be performed on samples collected less than 7 days apart.

For severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) results from this assay, if repeat testing is considered within a 7-day period after an initial negative SARS-CoV-2 result, consider ordering a targeted SARS-CoV-2 assay. If initial SARS-CoV-2 results from this assay were positive, it is recommended to wait 14 days until a subsequent test is performed, if desired.

Adenovirus
Assay may show variable detection with nonrespiratory serotypes within species A, D, F, and G.

Influenza A
Performance characteristics were established when influenza A H1-2009, A H1, and A H3 were the predominant influenza A viruses in circulation. Performance of detecting influenza A may vary if other influenza A strains are circulating or a novel influenza A virus emerges. The performance of the FilmArray respiratory panel has not been established in individuals who received the influenza vaccine. Recent administration of a nasal influenza vaccine may cause false-positive results for influenza A or influenza B. Some strains of human, swine, or avian origin are predicted to react with influenza A assays leading to an Influenza A (no subtype detected) result.

Assay detects and differentiates commonly occurring influenza A hemagglutinin subtypes based on only the hemagglutinin gene, through the use of 2 influenza A assays and 3 subtyping assays for the hemagglutinin gene. Results are reported as "detected" when at least one of the influenza A assays and one of the subtyping assays are both positive. If both influenza A assays are positive without a hemagglutinin subtype, results are reported as influenza A (no subtype detected). Equivocal results are reported following repeat testing in 2 scenarios:
-Neither of the influenza A assays are positive, but a hemagglutinin gene is positive.
-One of the influenza A assays is positive, and hemagglutinin genes are negative.
The assay does not detect or differentiate the influenza A neuraminidase gene.

Rhinovirus/Enterovirus Group
Due to the genetic similarity of these viruses, the assay is unable to reliably differentiate them.

Bordetella pertussis/Bordetella parapertussis
Some acellular vaccines contain polymerase chain reaction (PCR)-detectable DNA. Contamination of specimens with the vaccine can cause false-positive *Bordetella pertussis* PCR results. Specimens should not be collected or processed in areas that are exposed to *B pertussis* vaccine material. Assay targets the single-copy promoter region of the pertussis toxin gene. Results of this assay may not be concordant with commonly used *Bordetella* PCR assays, which target the multicopy insertions sequences (IS481). Cross reactivity could occur with high levels or rare sequence variants of other species such as *Bordetella bronchiseptica* and *Bordetella parapertussis*.

Coronavirus
Coronavirus OC43 assay may cross-react with coronavirus HKU1. As a result, when both HKU1 and OC43 are detected in the same patient specimen, the result may be due to assay cross-reactivity. A coinfection with these 2 viruses is also possible.

SARS-CoV-2
The following animal coronavirus strains, unlikely to be found in humans, may cross react with the SARS-CoV-2 target: Bat coronavirus RaTG13 (accession: MN996532), Pangolin coronavirus (accession: MT084071), and bat SARS-like coronavirus sequences (accession MG772933 and MG772934).

Supportive Data

This test is approved for testing nasopharyngeal (NP) swabs; the manufacturer has evaluated the clinical performance data of this sample type. The Clinical Bacteriology Laboratory at Mayo Clinic conducted a verification of the FilmArray Respiratory Panel 2.1 (RP2.1) assay for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) using 2 pools of

commercially available positive and negative control material. The assay demonstrated 100% overall agreement with expected results. The laboratory also conducted a separate verification of the FilmArray Respiratory Panel 2 (RP2) assay using 4 pools of known target analytes from a commercially available verification panel. The assay demonstrated 100% overall agreement with expected results. Additionally, the Clinical Bacteriology Laboratory tested 35 clinical NP samples side by side on the RP2 and compared the results to those of prior testing on the FilmArray Respiratory Panel (RP). The percent positive agreement was above 95% for all targets tested, with the exception of human rhinovirus/enterovirus for which it was 75%, as a result of one missed detection compared to the four detected with RP assay. Some targets were not represented in the clinical NP sample set, including coronavirus 229E, coronavirus NL63, human metapneumovirus, influenza B, and parainfluenza virus 1-4.

Clinical Reference

1. Lee N, Lui GC, Wong KT, et al. High morbidity and mortality in adults hospitalized for respiratory syncytial virus infections. Clin Infect Dis. 2013:57(8):1069-1077. doi:10.1093/cid/cit471
2. Miliander C, Espy M, Binnicker MJ. Evaluation of the BioFire FilmArray for the detection of respiratory viruses in clinical samples. Clinical Virology Symposium Annual Meeting. Daytona, Florida; April 2013
3. Ramanan P, Bryson AL, Binnicker MJ, Pritt BS, Patel R. Syndromic panel-based testing in clinical microbiology. Clin Microbiol Rev. 2017;31(1):e00024-17. doi:10.1128/CMR.00024-17

Performance

Method Description

The FilmArray Respiratory Panel is a closed system that performs all the chemistry required to isolate, amplify, and detect nucleic acid from multiple viral and bacterial respiratory pathogens within a single nasopharyngeal swab specimen. The panel contains reagents in freeze-dried form and is divided into discrete segments where the required chemical processes are carried out. Patient sample and hydration fluid are drawn by vacuum into the panel and then placed into the FilmArray instrument. The detection process operations are automated (nucleic acid purification, first stage polymerase chain reaction [PCR], second stage PCR, and melt analysis) and complete in about 45 minutes in this closed system.

Nucleic Acid Purification:
The sample is lysed by a combination of chemical and mechanical mechanisms and the liberated nucleic acid is captured, washed, and eluted using magnetic bead technology.

First-Stage PCR:
A reverse transcription step is performed to convert viral RNA into complementary DNA prior to amplification. The purified nucleic acid solution is combined with a preheated master mix to initiate the reverse transcription step and subsequent thermocycling for multiplex PCR.

Second-Stage PCR:
Products of first-stage PCR are diluted and mixed with fresh PCR reagents, which is distributed over the second stage PCR array. The individual wells of the array contain primers for different assays (in triplicate) that target specific nucleic acid sequences from each of the pathogens detected, as well as control template material.

DNA Melting Analysis:
Temperature is slowly increased and fluorescence in each well of the array is monitored and analyzed to generate a melt curve.

Analysis of Melt Curves:
The software evaluates the DNA melt curve for each well to determine if a PCR product was present in that well. If the melt profile indicates the presence of a PCR product, then the analysis software calculates the melting temperature of the curve, which is then compared against the expected range for the assay. When the software determines that the melt curve falls inside the assay-specific melt temp range, it is called positive. When it determines that the melt curve is not in the appropriate range, it is called negative.

Analysis of Replicates:
Melt curves of each of the 3 replicates for each assay are evaluated to determine the assay result. For an assay to be called positive, at least 2 of the 3 associated melt curves must be called positive, and the melting temperature (Tm) for at least 2 of the 3 positive melt curves must be similar (within 1 degree C). Assays that do not meet these criteria are called negative.(Instruction manual: FilmArray Respiratory Panel 2.1 (RP2.1). BioFire Diagnostics, LLC; VFR0000-8303 05/2020)

PDF Report
No

Day(s) Performed
Monday through Sunday

Report Available
1 to 2 days

Specimen Retention Time
7 days

Performing Laboratory Location
Mayo Clinic Laboratories - Rochester Main Campus

Fees & Codes

Fees
- Authorized users can sign in to Test Prices for detailed fee information.
- Clients without access to Test Prices can contact Customer Service 24 hours a day, seven days a week.
- Prospective clients should contact their account representative. For assistance, contact Customer Service.

Test Classification
This test has been cleared, approved, or is exempt by the US Food and Drug Administration and is used per manufacturer's instructions. Performance characteristics were verified by Mayo Clinic in a manner consistent with CLIA requirements.

CPT Code Information

0202U

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
RP	Respiratory Panel, PCR, NP	82159-5

Result ID	Test Result Name	Result LOINC® Value
SS021	Specimen Source	31208-2
609644	Adenovirus	82160-3
609645	Coronavirus 229E	82163-7
609646	Coronavirus HKU1	82161-1
609647	Coronavirus NL63	82162-9
609648	Coronavirus OC43	82164-5
609650	Human Rhinovirus/ Enterovirus	82175-1
609651	Human Metapneumovirus	82165-2
609652	Influenza A	82166-0
609653	Influenza B	82170-2
609654	Parainfluenza Virus 1	82171-0
609655	Parainfluenza Virus 2	82172-8
609656	Parainfluenza Virus 3	82173-6
609657	Parainfluenza Virus 4	82174-4
609658	Respiratory Syncytial Virus	82176-9
609660	Bordetella pertussis	82179-3
609659	Bordetella parapertussis	87621-9
609661	Chlamydia pneumoniae	82178-5
609662	Mycoplasma pneumoniae	82177-7
609663	Interpretation	59464-8
609649	SARS Coronavirus-2	94565-9