Lab Dept: Microbiology

Test Name: RESPIRATORY PATHOGEN PANEL PCR, NP SWABS

General Information

Lab Order Codes: RESP2

Synonyms: Respiratory pathogen panel PCR; PCR respiratory pathogen panel

CPT Codes:
87633 – Infectious agent detection by nucleic acid (DNA or RNA); respiratory virus (eg, adenovirus, influenza virus, coronavirus, metapneumovirus, parainfluenza virus, respiratory syncytial virus, rhinovirus), includes multiplex reverse transcription, when performed, and multiplex amplified probe technique, multiple types or subtypes, 12-25 targets
87798 x2 – Infectious agent detection by nucleic acid (DNA or RNA), not otherwise specified; amplified probe technique, each organism (*B. pertussis* and *B. parapertussis*)
87486 – Infectious agent detection by nucleic acid (DNA or RNA); *Chlamydia pneumoniae*, amplified probe technique
87581 - Infectious agent detection by nucleic acid (DNA or RNA); *Mycoplasma pneumoniae*, amplified probe technique

Test Includes:
Detection of:
Viruses:
Adenoviruses (AdV)
Coronaviruses (CoV) - 229E, OC43, HKU1, NL63
Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)
Human Metapneumovirus (hMPV)
Influenza A – H1, H1-2009, H3
Influenza B|
Parainfluenza Viruses (PIVs) – PIV 1-4
Respiratory Syncytial Virus (RSV)
Rhinoviruses (HRV) and Enteroviruses (EV)
Bacteria:
Bordetella pertussis
Bordetella parapertussis
Chlamydia pneumoniae
Mycoplasma pneumoniae

NOTE: This test is not recommended for ED and ambulatory patients. In these areas, consider ordering rapid PCR for Influenza A/B, RSV, or RSV and Influenza A/B.

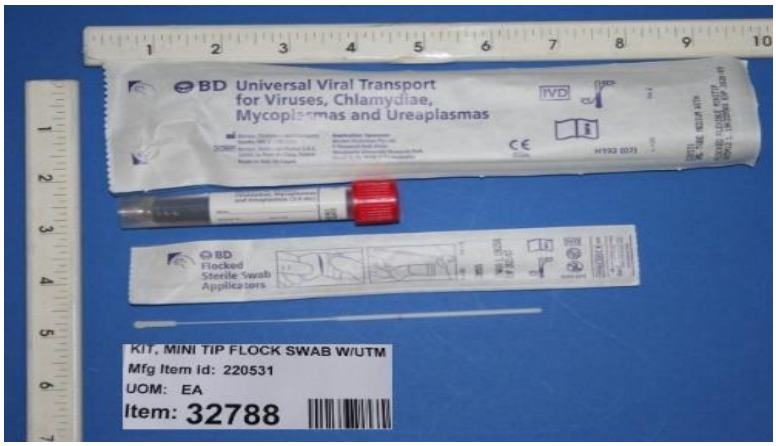
NOTE: The BioFire Respiratory Panel 2.1 was issued an Emergency Use Authorization (EUA) by the FDA on May 1, 2020.

Logistics

Lab Testing Sections:	Microbiology
Phone Numbers:	MIN Lab: 612-813-6280 STP Lab: 651-220-6550
Test Availability:	Specimens accepted daily, 24 hours
Turnaround Time:	3 - 24 hours from receipt in Minneapolis Lab Testing performed: Mon – Fri: 6:00 AM – 9:00 PM Sat – Sun: 6:00 AM – 2:00 PM
Special Instructions:	Requisition must state specific type of specimen and date/time of collection.

Specimen

Specimen Type:	Minitip Flocked Nasopharyngeal (NP) swab
Container:	**Flocked Flexible Minitip NP Swab:** mini tip flocked swab in Universal Transport Media (UTM) CHC # 32788: Kit, Mini Tip Flock Swab w/UTM



Draw Volume:	1 flocked NP swab in 3 mL UTM
Collection:	**Flocked NP swab (1)** 1. Carefully insert a flexible-shaft flocked mini-tip swab containing a dry tip into the nasopharyngeal cavity until resistance is encountered.| 2. Rotate the swab slowly on the nasopharyngeal membrane for 5-10 seconds to absorb secretions. 3. Remove the swab, break off into transport media at the score line, and send to the lab immediately.

Transport/Storage:	Transport to the Microbiology Lab immediately to maintain specimen integrity. Specimens are stable up to 4 hours at room temperature (15 - 25°C) and 3 days refrigerated (2 – 8 °C) in viral transport Media (VTM).
Patient Preparation:	N/A
Sample Rejection:	Calcium alginate swabs (inhibitory to PCR), sputum, transit time exceeding 1 hour after collection without refrigeration; dry swabs; improperly labeled specimen; insufficient volume; leaking or non-sterile containers. If an unacceptable specimen is received, the physician or nursing station will be notified and another specimen will be requested before the specimen is discarded.

Interpretive

Reference Range:	Negative (for all targets)
Alert Value:	Positive *Bordetella pertussis* and SARS-CoV-2 results will be phoned to the patient's caregiver.
Limitations:	● The use of this assay an in vitro diagnostics under US FDA Emergency Use Authorization (EUA) is limited to laboratories that are certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), to perform high and moderate complexity tests. ● FilmArray Respiratory Panel 2.1 (RP2) performance has only been established on the FilmArray 2.0 and FilmArray Torch systems. ● The BioFire RP2.1 is a qualitative test and does not provide a quantitative value for the organism(s) in the specimen. ● Results from this test must be correlated with the clinical history, epidemiological data, and other data available to the clinician evaluating the patient. ● The performance of the BioFire RP2.1 has been evaluated for use with human specimen material only. ● The BioFire RP2.1 has not been validated for testing of specimens other than nasopharyngeal swab (NP/NPS) specimens in transport medium. ● The performance of BioFire RP2.1 has not been established for specimens collected from individuals without signs or symptoms of respiratory infection. ● The performance of the BioFire RP2.1 has not been specifically evaluated for NPS specimens from immunocompromised individuals. ● The effect of antibiotic treatment on test performance has not been evaluated. ● The performance of the BioFire RP2.1 has not been established with potentially interfering medications for the treatment of influenza or cold viruses. The effect of interfering substances has only been evaluated for those listed in the Interference section. Interference from substances that were not evaluated could lead to erroneous results. ● The performance of the BioFire RP2.1 has not been established for monitoring treatment of infection with any of the panel organisms. ● The performance of BioFire RP2.1 has not been established for screening of blood or blood products. ● The detection of viral and bacterial nucleic acid is dependent upon proper specimen collection, handling, transportation, storage and preparation.

Failure to observe proper procedures in any one of these steps can lead to incorrect results. There is a risk of false positive or false negative values resulting from improperly collected, transported or handled specimens.

● A negative BioFire RP2.1 result does not exclude the possibility of viral or bacterial infection. Negative test results may occur from the presence of sequence variants in the region targeted by the assay, the presence of inhibitors, technical error, sample mix-up or an infection caused by an organism not detected by the panel. Test results may also be affected by concurrent antiviral/antibacterial therapy or levels of organism in the specimen that are below the limit of detection for the test. Negative results should not be used as the sole basis for diagnosis, treatment, or other patient management decisions. Negative results in the setting of a respiratory illness may be due to infection with pathogens that are not detected by this test or lower respiratory tract infection that is not detected by a nasopharyngeal swab specimen.

● There is a risk of false positive results due to cross-contamination by target organisms, their nucleic acids or amplified product. Particular attention should be given to the Laboratory Precautions noted under the Warnings and Precautions section of the RP2 Package Insert.

● There is a risk of false positive results due to non-specific amplification and cross-reactivity with organisms found in the respiratory tract. Observed and predicted cross-reactivity for BioFire RP2.1 is described in the Analytical Specificity section of the RP2 Package Insert. Erroneous results due to cross-reactivity with organisms that were not evaluated or new variant sequences that emerge is also possible.

● If four or more organisms are detected in a specimen, retesting is recommended to confirm the polymicrobial result.

● Viral and bacterial nucleic acids may persist in vivo independent of organism viability. Detection of organism target(s) does not imply that the corresponding organisms are infectious or are the causative agents for clinical symptoms.

● 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 when prevalence is moderate to low.

● Clinical performance was established when Influenza A H1-2009 (H1N1pdm09) was the predominant Influenza A virus in circulation. When other Influenza A viruses are emerging, performance may vary.

● Due to the small number of positive specimens collected for certain organisms during the prospective clinical study, performance characteristics for *Bordetella parapertussis*, *Bordetella pertussis*, *Chlamydia pneumoniae*, Coronavirus 229E, Influenza A H1, Influenza A H3, Influenza B, Parainfluenza Virus 1, and Parainfluenza Virus 4 were established primarily with retrospective clinical specimens. Performance characteristics for Influenza A H1 was established primarily using contrived clinical specimens.

● The BioFire RP2.1 influenza A subtyping assays target the influenza A hemagglutinin (H) gene only. The BioFire RP2.1 does not detect or differentiate the influenza A neuraminidase (N) subtypes.

● The BioFire RP2.1 may not be able to distinguish between existing viral strains and new variants as they emerge. For example, the BioFire RP2.1 can detect Influenza A H3N2v (first recognized in August, 2011), but will not be able to distinguish this variant from Influenza A H3N2 seasonal. If variant virus infection is suspected, clinicians should contact their state or local health department to arrange specimen transport and request a timely diagnosis at a state public health laboratory.

● Recent administration of nasal influenza vaccines (FluMist) prior to NPS

specimen collection could lead to accurate virus detection by the BioFire RP2.1 of the viruses contained in the vaccine, but would not represent infection by those agents.
● Due to the genetic similarity between Human Rhinovirus and Enterovirus, the BioFire RP2.1 cannot reliably differentiate them. A BioFire RP2.1 Rhinovirus/Enterovirus Detected result should be followed-up using an alternate method (e.g. cell culture or sequence analysis) if differentiation between the viruses is required.
● BioFire RP2.1 detects a single-copy Pertussis Toxin promoter target (ptxP, present at one copy per cell) in *B. pertussis*. Other PCR tests for *B. pertussis* target the multi-copy IS481 insertion sequence (present in both *B. pertussis* and *B. holmesii*) and are therefore capable of detecting lower levels of *B. pertussis* (i.e. more sensitive).
● BioFire RP2.1 should not be used if B. pertussis infection is specifically suspected; a B. pertussis molecular test that is FDA-cleared for use on patients suspected of having a respiratory tract infection attributable to B. pertussis only should be used instead.
● Due to lower sensitivity, the BioFire RP2.1 *B. pertussis* assay is less susceptible than IS481 assays to the detection of very low levels of contaminating *B. pertussis* vaccine material. However, care must always be taken to avoid contamination of specimens with vaccine material as higher levels may still lead to false positive results with the BioFire RP2.1 test.
● The IS481 sequence is also present in *B. holmesii* and to a lesser extent in *B. bronchiseptica*, whereas the BioFire RP2.1 assay (ptxP) was designed to be specific for *B. pertussis*. However, the BioFire RP2.1 *Bordetella pertussis* (ptxP) assay can also amplify pertussis toxin pseudogene sequences when present in *B. bronchiseptica* and *B. parapertussis*. Cross-reactivity was observed at high concentration (e.g., ≥1.2E+09 CFU/mL).
● Some strains of *B. bronchiseptica* (rarely isolated from humans) do carry IS1001 insertion sequences identical to those carried by most strains of *B. parapertussis*. These sequences will be amplified by the IS1001 assay and reported by BioFire RP2.1 as *Bordetella parapertussis* (IS1001).
● There is a risk of false positive results for *Bordetella species* and Human Rhinovirus/Enterovirus due to non-specific amplification and cross-reactivity for BioFire RP2.1. Erroneous results due to cross-reactivity with organisms that were not evaluated or new variant sequences that emerge is also possible.
● There is a risk of false positive results due to cross-contamination with organisms, nucleic acids or amplified products.
● Primers for both BioFire RP2.1 SARS-CoV-2 assays share substantial sequence homology with the Bat coronavirus RaTG12 (Accession: MN996532) and cross-reactivity with this closely-related viral sequence is predicted. In addition, the SARS-CoV-2 assay may cross-react with Pangolin coronavirus (accession: MT084071) and two other bat SARS-like coronavirus sequences (accession MG772933 and MG772934). It is unlikely that these viruses would be found in a human clinical nasopharyngeal swab; but if present, the cross-reactive product(s) produced by the BioFire RP 2.1 will be detected as Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2).

Methodology: Multiplex Polymerase Chain Reaction (PCR)

References:

FilmArray Respiratory Panel (RP2.1) Instructions for Use, REF 423738, May 2020, BioFire Diagnostics

Ramanan P, Bryson AL, Binnicker MJ, Pritt BS, et al: Syndromic panel-based testing in clinical microbiology. Clin Microbiol 2018 Rev 31:e00024-17. Available at: https://cmr.asm.org/content/31/1/e00024-17

Updates:

11/11/2020: Updated swab info.