**PROCEDURE: GENMARK EPLEX RESPIRATORY PATHOGEN PANEL 2**

1. **PRINCIPLE**

The GenMark ePlex Respiratory Pathogen Panel 2 (RP2) is a qualitative nucleic acid multiplex *in vitro* diagnostic test intended for use on the ePlex System for the simultaneous detection and identification of multiple respiratory virlal and bacterial nucleic acids in nasopharyngeal swabs (NP swabs).

The ePlex system automates all aspects of nucleic acid testing including extraction, amplification, and detection, combining electrowetting and GenMark’s eSensor technology in a single use cartridge.

Electrowetting, or digital microfluidics, uses electrical fields to directly manipulate discrete droplets on the surface of a hydrophobically coated printed circuit board. Sample and reagents are moved in a programmable fashion in the ePlex cartridge to complete all portions of the sample processing from nucleic acid extraction to detection.

A sample is loaded onto the ePlex cartridge and nucleic acids are extracted and purified from the sample via magnetic solid phase extraction. For RNA targets, a reverse transcription step is performed to generate complimentary DNA from the viral RNA, followed by PCR to amplify the target. Exonuclease digestion creates single-stranded DNA in preparation for eSensor detection.

The target DNA is mixed with ferrocene-labeled signal probes that are complementary to the specific targets on the panel. Target DNA hybridizes to its complementary signal probe and capture probes, which are bound to gold-plated electrodes, as shown below in **Figure 1**. The presence of each target is determined by voltammetry which generates specific electrical signals from the ferrocene-labeled signal probe.



**Figure 1**: Hybridization complex. Target specific capture probes are bound to the gold electrodes in the eSensor microarray on the ePlex cartridge. The amplified target DNA hybridizes to the capture probe and to a complimentary ferrocene-labeled signal probe. Electrochemical analysis determines the presence or absence of targets using voltammetry.

Table 1. Targets Detected by the ePlex RP2 Panel

|  |  |  |  |
| --- | --- | --- | --- |
| **TARGET** | **CLASSIFICATION** | **SEASONAL PREVALENCE\*** | **MOST COMMONLY INFECTED DEMOGRAPHIC** |
| Adenovirus (A-F) | Adenovirus (DNA) | Late winter to early summer | All ages, immunocompromised |
| Coronavirus(229E, HKU1, NL63, OC43) | Coronavirus (RNA) | Winter, spring | All ages |
| SARS-CoV-2 | Coronavirus (RNA) | Unknown | Not established |
| Human Metapneumovirus | Paramyxovirus (RNA) | Winter, spring | Children, elderly, immunocompromised |
| Human Rhinovirus/Enterovirus | Picornavirus (RNA) | Fall, spring, summer | All ages, immunocompromised |
| Influenza A | Orthomyxovirus (RNA) | Winter | All ages |
| Influenza A H1 |
| Influenza A H1-2009 |
| Influenza A H3 |
| Influenza B |
| Parainfluenza Virus 1 | Paramyxovirus (RNA) | Fall | All ages |
| Parainfluenza Virus 2 | Fall, early winter |
| Parainfluenza Virus 3 | Spring, summer |
| Parainfluenza Virus 4 | Fall, early winter |
| Respiratory Syncytial Virus A | Paramyxovirus (RNA) | Winter | Infants, children, older adults |
| Respiratory Syncytial Virus B |
| *Chlamydia pneumoniae* | Bacterium (DNA) | No peak season |  |
| *Mycoplasma pneumoniae* | Bacterium (DNA) | Late summer, Fall | Children, young adults |
| \*Based on northern hemisphere seasons |  |  |

1. **AVAILABILITY**

Specimens will be run on all 3 shifts, 7 days a week

1. **TEST CODE**

RP2

1. **SPECIMEN**
	1. Nasopharyngeal swab collected and transported in viral/universal transport medium (UTM). Minimum volume of 200 µL required for testing.
2. **MATERIALS AND EQUIPMENT**
	1. Materials
		1. ePlex Respiratory Pathogen Panel 2 Test Kits (EA001222)
		2. Sterile, nuclease free disposable extended pipette tips with filters (Art XL P-200)
	2. Equipment
		1. GenMark ePlex System and Software
		2. 200uL Sartorius pipette
		3. Vortex mixer for specimen set up
		4. Freezer (manual defrost) at -20 to -80 oC
		5. Refrigerator at 2 to 8 oC
3. **STORAGE AND HANDLING**
	1. ePlex RP2 Panel reagents are shipped at room temperature. Upon receipt, they should be stored at 2-8 oC
		1. Cartridges can be used immediately from refrigerator storage
		2. Cartridges are single use and should be discarded in biohazard receptacles after use
		3. Once the foil packaging of the cartridge is opened, the cartridge must be used within 2 hours
	2. ePlex RP2 panel controls should be stored at 2-8 oC until needed
		1. Control tube must be flicked several times then vortexed for 3-5 seconds
		2. Tap on bench to force fluid from the cap
		3. Controls are one time use and any remaining fluid should be discarded in biohazard trash.
	3. Patient specimen should be run immediately after receipt in the lab. If the specimen cannot be run immediately, it should be stored in the refrigerator at 2 to 8 oC. Once run, the specimen should be stored in the refrigerator.
		1. Specimens can be held at Room Temperature (15 to 30 oC) for up to 12 hours or at 2 to 8 oC for 10 days after collection in UTM.
		2. Specimens can also be stored at -20 oC or -80 oC for 12 months with up to 2 freeze/thaw cycles.
4. **QUALITY CONTROL**
	1. External control
		1. Previously characterized positive samples or viral transport medium spiked with well characterized organism can be used as an external positive control.
		2. Sterile, viral transport media can be used as a negative control.
		3. Controls are run with every new lot, new shipment of ePlex RP2 Cartridges, or after a major system maintenance (software upgrade, annual PM, or replacement of multiple modules).
		4. No patient results will be released until required controls are resulted and confirmed correct. Bring any discrepant control results to the attention of the Senior Medical Technologist, Lead Medical Technologist, or Manager.
	2. Internal Control- Each Cartridge includes internal controls that monitor performance of each step of the testing process.
		1. A DNA control verifies extraction, amplification, and detection of DNA targets, and RNA controls verify amplification and detection of RNA targets.
		2. Either the internal control or the target must generate a signal above the threshold for a valid test result.
	3. Environmental wipe testing is performed monthly. All test areas are swabbed and run as test patients. Refer to ***Appendix AP25 – CORE Environmental QC*** in the STAT Binder for environmental testing instructions.
	4. Positivity rates are monitored monthly.
	5. All QC failures are documented in the QC Failures Binder.
5. **EQUIPMENT MAINTENANCE**
	1. On a monthly basis, the exterior (front, sides, and top) of the ePlex unit must be cleaned with 10% bleach- let sit for 5 minutes, water, then 70% alcohol using lint-free wipes.
		1. Avoid getting any liquid in the area of the bays.
	2. On a weekly basis, the exterior (front, sides, and top) of the ePlex unit must be cleaned with 70% alcohol using a lint-free wipe.
	3. The screen should be cleaned when needed with DI water and a WipeAll.
6. **TEST PROCEDURE**

**PATIENT SAMPLES SHOULD BE SET UP IN THE HOOD ONE AT A TIME.**

1. Thoroughly decontaminate the bench area, molecular hood, pipette, and corresponding tip box with bleach, followed by DI water, then 70% alcohol. **Change gloves**.
	* 1. The cartridge must be set up on a dry surface. A WipeAll on the working surface of the hood may be used.
2. Place one cartridge and one patient specimen in the hood.
3. Open the foil packaging of the cartridge and place label within the rectangular space on top of the cartridge.
4. Vortex the patient sample for 3-5 seconds.
5. Pipette **200uL** of patient sample and dispense into the sample loading port of the RP2 Test cartridge.
6. Slide the cap over the port and push cap into place. There should be a click.
	1. Note: Bubbles may be present when closing the cap.
7. **Change gloves**.
8. Bring the cartridge to the ePlex and place on a dry surface or WipeAll.
9. Log into ePlex if necessary.
10. Scan the patient barcode label and cartridge barcode using the scanner adhered to the ePlex.
11. The barcode reader will beep once to indicate it has read both barcodes
12. The patient identifier may be manually entered into the ePlex by selecting the keyboard at the bottom of the ePlex screen. Enter the identifier into the window and select enter.
13. Gently insert the cartridge into any available slot on the ePlex indicated by **white** flashing lights.
14. Once the pre-flight checks have been made, the white light will turn **blue.**
15. If pre-flight fails, remove the cartridge and place into another available slot. After three attempts GenMark Technical Services must be called
16. At this time, another patient can be set up. **Change gloves**, place a new WipeAll on to the hood, repeat starting with step B above
17. Alternatively, up to 3 specimens can be set up one-at-a-time in the hood before walking over to the e-Plex. Cartridges **cannot** be stacked, but must be placed on a sterile surface to be carried over to the equipment
18. Once the test is complete, the ePlex will eject the cartridge. At this time, it should be removed from the ePlex and placed in biohazard waste
19. A full report may be printed if needed when the test is complete
20. **INTERPRETATION**
	1. Internal Control (IC) – The internal control will result 1 of 4 ways
		1. **PASS** – Test is valid and all results will generate and can be reported
		2. **FAIL** – Test is not valid because neither the IC nor any target generated a signal above the threshold. **The specimen should be repeated.**
		3. **N/A** – Test is valid and results will generate and can be reported. An N/A result for IC indicates the internal control is not valid but detection of signal above threshold for a target in every amplification reaction indicates valid results were generated.
		4. **INVALID** – Test is not valid due to an error during processing on the instrument or a software error. **The specimen should be repeated.**
	2. Patient Specimen
		1. **DETECTED** – The detected target has generated signal above the defined threshold. Report target as “Detected”.
		2. **NOT DETECTED** – The test was completed successfully, and the target did not generate signal above the defined threshold. Report target as “Not Detected”.
		3. **INVALID** – The test did not complete successfully. Results are invalid and test **will be repeated one additional time**. Notify a nurse or clinician about the delay in resulting for any inpatient specimens. Record invalid in ePlex Invalid/Error log.
21. **RESULTING**
	1. Refer to ***Appendix AP80 - SARS-COV-2 SoftLabMic resulting: GenMark Dx ePlex*** for further instructions.
22. **LIMITATIONS OF TEST**
	1. This product can be used only with the GenMark ePlex instrument.
	2. Due to the genetic similarity between human rhinovirus/enterovirus and poliovirus, the ePlex RP2 Panel cannot reliably differentiate them. If a poliovirus infection is suspected, an ePlex RP2 human rhinovirus/enterovirus result of Detected should be confirmed using an alternate method (e.g. cell culture).
	3. At high titers, cross-reactivity with SARS-CoV-1 was observed with the ePlex RP2 Panel.
	4. Due to the genetic similarity between human rhinovirus and enterovirus, this test cannot reliably differentiate them. An ePlex RP2 Panel Rhinovirus/Enterovirus positive result should be followed up using an alternate method (e.g. cell culture or sequence analysis) if differentiation between the viruses is required.
	5. This test is a qualitative test and does not provide a quantitative value of detected organism present.
	6. The performance of the test has been evaluated for use with human sample material only.
	7. This test has not been validated for testing samples other than nasopharyngeal swab samples in viral transport media.
	8. The performance of this test has not been established for immunocompromised individuals.
	9. The performance of this test has not been established for patients without signs and symptoms of respiratory infection.
	10. Results from this test must be correlated with the clinical history, epidemiological data, and other data available to the clinician evaluating the patient.
	11. The effect of antibiotic treatment on test performance has not been evaluated.
	12. The performance of this test has not been established for screening of blood or blood products.
	13. Targets (viral and bacterial nucleic acids) may persist *in vivo*, independent of viral or bacterial viability. Detection of target(s) does not imply that the corresponding virus(es) or bacteria are infectious or are the causative agents for clinical symptoms.
	14. The detection of viral or 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 samples.
	15. There is a risk of false negative values due to the presence of sequence variants in the viral or bacterial targets of the test, the presence of inhibitors, technical error, sample mix-up, or an infection caused by an organism not detected by the panel. Test results may be affected by concurrent antibacterial or antiviral therapy or levels of bacteria or virus in the sample that are below the limit of detection for the test. A result of No Targets Detected on the ePlex RP2 Panel should not be used as the sole basis for diagnosis, treatment or other patient management decisions.
	16. A result of No Targets Detected on the ePlex RP2 Panel 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 sample.
	17. There is a risk of false positive results due to contamination of the sample with target organisms, their nucleic acids, or amplicons. Particular attention should be given to the Laboratory precautions noted under the *Warnings and Precautions* section.
	18. There is a risk of false positive results due to non-specific amplification and cross-reactivity with organisms found in the respiratory tract. Erroneous results due to cross-reactivity with organisms that were not specifically evaluated or new variant sequences that emerge are possible.
	19. If four or more organisms are detected in a sample, retesting is recommended to confirm polymicrobial result.
	20. The ePlex RP2 Panel influenza A subtyping reagents target the influenza A hemagglutinin gene only. The ePlex RP2 Panel does not detect or differentiate the influenza A neuraminidase gene.
	21. The performance of this test has not been established for monitoring treatment of infection with any of the panel organisms.
	22. 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.
	23. Clinical performance was established when influenza A H3 and influenza A H1-2009 were the predominant influenza A viruses in circulation. When other influenza A viruses emerge, performance may vary.
	24. Due to the small number of positive samples collected for *Chlamydia pneumoniae* during the prospective and retrospective clinical studies, performance characteristics for *Chlamydia pneumoniae* were established primarily with contrived clinical specimens. Performance characteristics for Influenza A H1 were established using contrived clinical specimens only.
	25. Clinical evaluation indicates a lower sensitivity for the detection of coronavirus OC43. If infection with coronavirus OC43 is suspected, negative samples should be confirmed using an alternative method.
	26. The effect of interfering substances has only been evaluated for those listed in this package insert. Interference due to substances other than those described in the “Interfering Substances” section can lead to erroneous results.
	27. At concentrations greater than 1% weight/volume in the sample, tobramycin was found to inhibit assay performance.
	28. The performance of this test has not been specifically evaluated for specimens collected from individuals who recently received influenza vaccine. Recent administration of a live intranasal influenza virus vaccine may cause false positive results for influenza A, H1, H3, H1-2009, and/or influenza B.
	29. The ePlex RP2 Panel cannot differentiate variant viruses, such as H3N2v, from seasonal influenza A viruses. 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.
23. **NOTES**
	1. Wet cartridges should never be used.
	2. Specimens must be processed in a biosafety hood. Technologist must be wearing protective gear such as sterile gloves and disposable lab coats
	3. A trained healthcare professional should interpret assay results in conjunction with the patient’s medical history, clinical signs and symptoms, and the results of other diagnostic tests.
	4. Reagents within the ePlex RP2 cartridge may cause irritation to skin, eyes, and respiratory tract and are harmful if swallowed. Do not pierce reagent blisters on the ePlex cartridge
	5. Once the sample is loaded into the ePlex RP2 test cartridge, the sample should be tested within 2 hours.
24. **TECHNICAL SUPPORT**

Phone: 1-800-373-6767, option 2

Email: technicalsupport@genmarkdx.com

1. **REFERENCES**
	1. ePlex Respiratory Pathogen Panel 2 Package Insert (EUA) PI1114 – B
2. **ATTACHMENTS**
	1. Appendix AP80 – SoftLabMic resulting: GenMark Dx ePlex