COVID-19 GenMark ePlex **Technical Procedure # 1247.T**

Purpose

The ePlex® SARS-CoV-2 cartridge is a multiplexed nucleic acid *in vitro* diagnostic test intended for the qualitative detection of SARS-CoV-2 nucleic acid in nasopharyngeal swab specimens (NPS) eluted in transport media (VTM/UTM) collected from individuals suspected of COVID-19 by their health care provider when used in conjunction with other clinical and epidemiological information.

Results are for the detection of SARS-CoV-2 RNA that are detectable in NPS specimens during infection. Positive results are indicative of active infection with SARS-CoV-2; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities. Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

Principle

The ePlex instrument automates all aspects of nucleic acid testing including extraction, amplification, and detection, combining electrowetting and GenMark's eSensor® technology in a single-use cartridge. eSensor technology is based on the principles of competitive DNA hybridization and electrochemical detection, which is highly specific and is not based on fluorescent or optical detection.
Electrowetting, or digital microfluidics, uses electrical fields to directly manipulate discrete droplets on the surface of a hydrophobically coated printed circuit board (PCB). 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 specimen via magnetic solid phase extraction. For RNA targets, a reverse transcription step is performed to generate complementary DNA from the RNA, followed by PCR to amplify the targets. 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 in Figure 1. The presence of each target is determined by voltammetry which generates specific electrical signals from the ferrocene-labeled signal probe.

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 complementary ferrocene-labeled signal probe. Electrochemical analysis determines the presence or absence of targets using voltammetry.

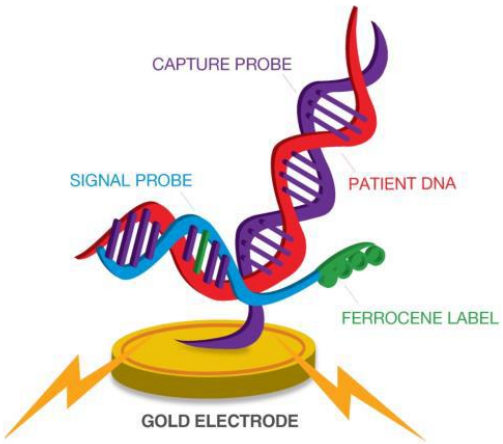


Figure 1 - Hybridization Complex

Clinical Significance

The novel coronavirus is a new coronavirus that has not been previously identified. The virus causing coronavirus disease 2019 (COVID-19), is not the same as the coronaviruses that commonly circulate among humans and cause mild illness, like the common cold. Reported illnesses have ranged from mild symptoms to severe illness and death for confirmed coronavirus disease 2019 (COVID-19) cases. The virus is thought to spread mainly from person-to-person: Between people who are in close contact with one another (within about 6 feet), through respiratory droplets produced when an infected person coughs, sneezes or talks via droplets that can land in the mouths or noses of people who are nearby or possibly be inhaled into the lungs. Some recent studies have suggested that COVID-19 may be spread by people who are not showing symptoms.

Specimen Collection

- Nasopharyngeal (NP) Swab in Universal Transport Media (UTM)
  Use the flocked swab packaged with UTM for collection.
- All human-sourced materials should be considered potentially infectious and should be handled with universal precautions and set up in the BSCII level hood.
- The detection of viral nucleic acid is dependent upon proper specimen collection, handling, transportation, storage and preparation.

Specimen Transport

- Specimens in UTM may be transported at ambient temperature or at 2-8°C.

Stability and Storage Requirements

- Specimens placed in UTM can be stored at room temperature for 12 hours.
- Specimens placed in UTM can be stored refrigerated at (2-8°C) for up to 10 days.
- Specimens placed in UTM should be frozen at -80°C if tested greater than 10 days post-collection

for 12 months with up to 2 freeze/thaw cycles.
  - All frozen samples should be thawed thoroughly at room temperature prior to testing

Unlabeled/Mislabeled Specimens
- Unlabeled or mislabeled specimens will be processed according to Departmental guidelines.

Unacceptable Specimens
- BAL or Bronchial Wash
- Dry NP swabs
- Swab samples collected using cotton, calcium alginate or wood-shafted swabs
- Specimens without a minimum sample volume of 200 µL
- Nonsterile containers
- Action: Specimens that are unacceptable will be cancelled according to Departmental guidelines and a "redraw" requested. Unacceptable inpatient specimens will be reported to the requesting physician or nurse (R.N.) in charge of patient's care. The Laboratory Director will have responsibility for ensuring that specimen collection and delivery are appropriate.

Specimen Handling

Overall manipulation of respiratory specimens that might contain this new pathogen should be done in a BSL-2 laboratory with enhanced practices, where appropriate (certified BSC, appropriate physical containment devices such as a centrifuge with safety buckets or sealed rotors, eye and face protection, double gloves, and fit-tested N95 respirator or surgical mask if N95 respirator is not available).

Reagents

Note: All reagents are ready-to-use and labeled by the manufacturer. The labeling includes content and quantity, lot number, expiration date and storage requirements.

ePlex SARS CoV-2 (EA008212)
- ePlex SARS CoV-2 Test Cartridge (12) labelled as EUA
- Sample Delivery Device – SARS CoV-2 (12)

Kit Storage and Handling
- Kits are shipped at room temperature and stored at 2-8°C.
- Do not use kit components beyond the expiration date, currently 1 year past manufacture date on kit box.
- Do not open a cartridge pouch until you are ready to perform testing. Once sample is loaded into the cartridge, testing should commence as soon as possible.
- Verify the sample delivery device vial contains adequate liquid prior to use by tapping on the benchtop and visually inspecting volume of liquid.

Materials and Equipment

- GenMark ePlex instrument and software with printer

- Pipettes calibrated to deliver 200 µL (Rainen Pipet-Lite XLS+)
- Pipette tips (aerosol resistant, RNase/DNase-free) extended length tips (Pipette tips RT LTS 200ul Rainin FX768A/8)
- Vortex mixer
- 10% bleach for decontamination of appropriate surfaces-Healthcare Pull top Bleach solution
- 70% ethanol or isopropyl alcohol
- Gauze 4x4 size to deliver cleaning agents above

Quality Control

Internal Controls

Each cartridge includes internal controls that monitor performance of each step of the testing process.

1. RNA controls verify amplification and detection of RNA targets.

Each amplification reaction on the cartridge has at least one internal control. Within each reaction, either the internal control or a target must generate signal above the defined threshold for a valid test result. Internal control results are interpreted by the ePlex software and displayed on ePlex SARS-CoV-2 Test Reports.

Table 1: Internal Control Results

Internal Control Result	Explanation	Action
PASS	The internal control or a target from each amplification reaction has generated signal above the threshold. The test was completed and internal controls were successful, indicating valid results were generated.	All results are displayed on the Detection Report. Test is valid, report results.
FAIL	Neither the internal control nor any target in at least one amplification reaction generates signal above the threshold. The test was completed but at least one internal control was not detected, indicating that results are not valid.	No results are displayed on the Detection Report. Test is **not** valid, repeat the test using a new cartridge.
N/A	The internal control in every amplification reaction does not generate signal above the threshold, but a target in every amplification reaction does generate signal above the threshold. The test was completed and internal controls were not successful, however detection of signal above the threshold for a target in every amplification reaction indicates valid results were generated.	All results are displayed on the Detection Report. Test is valid, report results.

INVALID	An error has occurred during processing that prevents analysis of signal data. The test has not successfully completed and results for this test are not valid. This is often due to an instrument or software error.	No results are displayed on the Detection Report. Test is **not** valid, repeat the test using a new cartridge.

External Controls: C0V-2

- Note: Under EUA approval positive and negative controls are run each day of use.

Positive Control

- Zeptometrix SARS-CoV-2 recombinant material (#0831042 RUO) is positive control. Comes in 1 ml vials and is used until consumed.
  - Stored at 2-8^{o}C until expiration date on vial.
  - Add X on top of vial when opened along with opened date on vial.
- Alternative Positive QC can include control spiked samples previously identified as positive on the GenMark ePlex.
  - Stored in -80^{o}C freezer when available and identified as positive control material on box.

Negative Control

- viral transport medium (VTM) or universal transport medium (UTM) is used as the negative control.
  - Aliquot a vial of transport media using sterile techniques into 225-250ul aliquots to avoid contamination of original vial for use as negative control.
  - Samples are labeled as negative control with lot number and expiration of UTM
  - Aliquots are stored at 2-8^{o}C.

The SARS-CoV-2 Test External Control Report is generated for an external control that has been pre-defined in the ePlex RP Panel software. Results are reported as Detected, Not Detected, or Invalid. A target is reported as Pass if the actual result matches the expected result (as defined for that control); a target is reported as Fail (in Red) if the actual result does not match the expected result. A target is reported as Invalid if the test has not been successfully completed (often instrument or software error or failure of internal control). If the actual result for any target does not match the expected result, the overall result for the external control is reported as Fail in the Summary section.

Positive and Negative Controls are run on a rotational basis throughout the bays. A1/B1 are run on Monday morning, A2/B2 on Tuesday, A3/B3 on Wednesday, A4/B4 on Thursday, A5/B5 on Friday, and A6/B6 on Saturday. On Sunday any set of bays may be used.

Proficiency Testing

UC Davis Health Pathology Laboratory is enrolled in the CAP proficiency-testing program. The CAP Survey required for this assay is "CAP: SARS-COV-2, MOLECULAR - COV2 Multiplex Testing". The CAP proficiency testing is performed by assigned technologist.

Procedure

Loading Specimen into Cartridge

Note: PCR setup to be performed one sample/cartridge at a time to avoid contamination.

1. Decontaminate workbench in BSCII hood with 10% bleach followed by 70% alcohol.
   Note: Change gloves following decontamination and prior to handling reagents.
2. Remove one SARS CoV-2cartridge pouch and one Sample Delivery Device from kit packaging.
   Note: Do not touch electrodes on bottom of cartridge
   Note: Be sure to keep cartridge FLAT throughout handling.
   Note: Reagents and cartridge can be used immediately upon removal from 2-8 °C storage. There is no need to equilibrate to room temperature before use. Return reagents immediately to 2-8 °C for storage.
3. Vortex the sample for 3-5 seconds.
4. Gently tap the Sample Delivery Device on the counter or benchtop surface to collect liquid that may have adhered to the sides of the vial and visually inspect level of liquid.
5. Unscrew the purple cap from the Sample Delivery Device.
6. Use a calibrated pipette to aspirate 200 µL of vortexed sample and pipette into the Sample Delivery Device.
7. Replace purple cap on Sample Delivery Device. Ensure that cap is securely fastened on the Sample Delivery Device.
8. Vortex the Sample Delivery Device for 10 seconds.
   Note: This step should be done immediately before loading sample onto cartridge.
9. Open the cartridge pouch by tearing the edge rapidly and firmly towards you. Place the opened cartridge on top of the foil pouch packaging.
   Note: Once cartridge is removed from foil pouch, it should be used within 2 hours. Do not open the cartridge pouch until the sample is ready to be tested.
10. Place a barcode label with accession ID on the test cartridge in the designated area so both barcodes (sample and cartridge) can be scanned by instrument.
11. Remove the white cover from the tip of the Sample Delivery Device cap.
12. Invert the Sample Delivery Device and squeeze to dispense 8-10 drops into the sample loading port of the cartridge until bubbles occur at the tip of the sample transfer device.
    Note: Minimize dispensing of bubbles into sample loading port by dispensing drop by drop. Allow large bubbles to pop before closing device.
13. Close the sample loading port by sliding the cap over the port and firmly pushing down on the cap to securely seal the sample delivery port.
    Note: A few bubbles can be present when closing the cap.
    Note: Change gloves after loading cartridge and before handling new specimens.

Note: Be sure to keep the cartridges flat when transferring to the instrument holding by sides of cartridge.

14. Samples are saved in designated rack in coag reagent refrigerator for at least one week.

Loading cartridge onto the ePlex

1. Scan the cartridge using the barcode reader provided with the ePlex instrument holding the barcodes approximately 6 inches from scanner in a diagonal fashion so both sample and cartridge barcode are scanned together.

   Note: The barcode scanner will read both the accession ID barcode and the 2D barcode printed on the cartridge label; however, the barcode scanner will only beep once to indicate that both barcodes have been read. The LED on the scanner will blink green.
2. Insert the cartridge into any available bay, indicated by a blinking white LED light. The test will begin automatically when the cartridge has been inserted into the bay and the pre-run check (cartridge initialization) is completed, indicated by a blue LED light.
3. Do not insert a wet cartridge into the ePlex instrument. If the cartridge or sample has leaked, dispose of cartridge in accordance with all federal, state, and local regulations. A Kim wipe may be used to remove small amounts of moisture or condensation from the bottom of the device.

Results

Reports

A detection report will be prepared automatically following completion of testing and will print automatically if the user is logged into the system. If the user is not logged in upon completion of testing, click View Sample Report to view the detection report. Print sample report. This is only available if you have not removed the cartridge from the bay.

If cartridge has been removed or you need to reprint results, go to Menu >Reports>Sample Results tab. Scroll the up or down arrows to find your specimen or change the search criteria to include the desired sample. Select Specimen for the Sample Type and print the desired report. The Summary section of the report indicates the overall test result and lists detected target in that sample. Sample results are reported as Detected, Not Detected, or Invalid. Results for the Internal Control are reported as PASS, FAIL, INVALID, or N/A.

Reporting Results

Results will be reported Detected, Not Detected or Invalid.
Samples and controls will need to be approved on the ePlex prior to transmission to LIS.

- Choose sample from bay or reports menu and touch approve key. Wand barcode and signature on file will populate approved field.

Invalid Results

Invalid results should be repeated with a new test cartridge. In the event of a *repeat* Invalid for an internal control failure, the specimen will be resulted as "Invalid". The following comment should be added: "Specimen internal control did not amplify. Submit a new specimen."

Reference Range:
Not Detected (no COVID-19 detected)

Reportable range:
Not Detected (no COVID-19 detected)
Detected (COVID-19 detected)
Invalid (Internal control failure)

Instrument Downtime
In the event that the instrument is inoperable OR when daily sample quota has been reached, samples are pack listed out to STC Molecular department for running in batch mode.

Limitations of Procedure:

- This test has not been validated by GenMark for samples other than nasopharyngeal swabs in viral transport media or universal transport media. BAL and Bronchial Washings have not been validated.
- The performance of this test has not been established for immunocompromised individuals or for patients without signs or symptoms of respiratory infection.
- There is a risk of false negative values due to the presence of sequence variants in the viral target of the test, the presence of inhibitors or technical error. Test results can be affected by concurrent antiviral therapy or levels of virus in the sample that are below the limit of detection for the test. A result of Not Detected on the ePlex SARS-CoV-2 Test should not be used as the sole basis for diagnosis, treatment or other patient management decisions.
- Interfering Substances
  All substances and organisms tested for interference were shown to be compatible with the ePlex SARS-CoV-2 Panel. SARS-CoV-1 was the only organism to show cross reactivity and belongs to the same subgenus. No potentially interfering substances were found to inhibit the ePlex at the concentrations tested except for high concentrations of Tobramycin. See GenMark ePlex SARS-CoV-2 Assay manual page 13-14 for details.
- Limits of Detection for ePlex testing have been documented by manufacturer to be 1×10^5 copies/ml whereas the Cobas 6800 in Molecular has been documented to be 50 copies/ml. Internal studies for LOD testing in Molecular determined the LOD for the ePlex to be 20,000 copies/ml. Therefore, some samples with lower viral loads may test as negative for virus on the ePlex when present in low enough numbers.
- If clinical suspicion is still very high in the case of a reported negative result sample should be sent to Molecular lab for retesting with a more sensitive method.

Maintenance

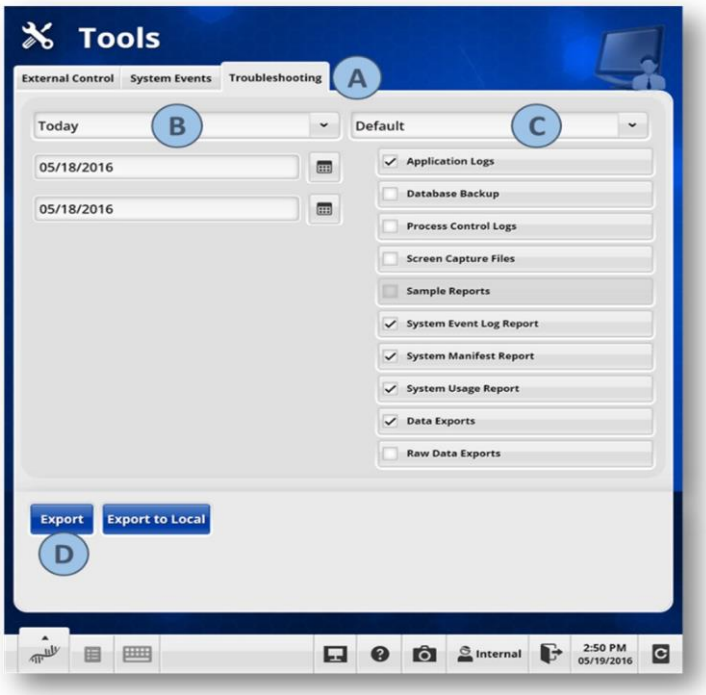
- Outer cover and display are wiped with 10% Bleach followed by 70% isopropyl alcohol on a monthly basis.
- Preventative maintenance is performed by GenMark service on a twice-yearly basis and involves cleaning and evaluating bay status.

Troubleshooting

Creating a Troubleshooting Package

A trouble shooting package will aid Genmark in capturing instrument or panel related issues. To generate a troubleshooting package, follow the steps below.

a) Navigate to the "Troubleshooting" tab located in the Tools menu of the instrument
b) Select a date range to include the dates on which the issues occurred.
c) Confirm that "Default" is the selected troubleshooting package type.
d) Press "Export" and save package to USB drive. Wait for the message "Troubleshooting package was successfully created" and select OK.
Note: Creating and compiling a troubleshooting package may take greater than 10-15 minutes depending on date range and content selected.



Submitting a Troubleshooting Package

After generating a troubleshooting package, follow the steps below.

a) E-mail TechnicalSupport@genmarkdx.com with a short description of the issue. Title the heading of the e-mail "UC Davis ePlex SARS-CoV-2".
b) Upload the troubleshooting file from the USB by navigating to the link provided by technical support and dropping the file into the location provided.

References:

1. Genmark Diagnostics, Inc., https://www.genmarkdx.com, ePlex SARS-CoV-2 Assay Manual, PI1108-A, March 2020
2. Zeptometrix NATrol™ SARS-CoV-2 (recombinant) Stock Catalog Number 0831042 package insert, PCA# 20-128, Revision 01, 03/11/2020
3. Centers for Disease Control and Prevention (CDC). Interim laboratory biosafety guidelines for handling and processing specimens associated with coronavirus disease 2019 (COVID-19). https://www.cdc.gov/coronavirus/2019-nCoV/lab/lab-biosafetyguidelines.html.

4. Iwen P., Stiles, K., Pentella, M.: Safety Considerations in the Laboratory Testing of Specimens Suspected or Known to Contain the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV2); Am J Clin Pathol 2020;XX;1-4

PROCEDURE HISTORY

Date	Reviewed by	Revision / Biennial Review	Approved Date	Approved by
4/2020	L. Freeman	New	04/30/2020	L Howell (onbase)
5/2020	L Freeman	Added specimen handling update		

Date	Reviewed by	Revision / Biennial Review	Approved Date	Approved by