# PathogenDx Product Insert
# Detect$^{X}$-Rv

**For prescription use only under Emergency Use Authorization (EUA) only**

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***For Prescription Use Only***
***For In-Vitro Diagnostic (IVD) Use***
***For Emergency Use Authorization Only***

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# 1 INTRODUCTION

This Emergency Use Authorization (EUA) package insert must be read carefully prior to use. EUA package insert instructions must be followed accordingly. Reliability of Detect$^{X}$-Rv assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

## Intended Use

Detect$^{X}$-Rv diagnostic assay kit contains the assays and controls for RT-PCR and DNA microarray hybridization. The Detect$^{X}$-Rv is intended for the qualitative detection of nucleic acid from SARS-CoV-2 in nasopharyngeal swabs, nasal aspirate, and oropharyngeal swab specimens utilized in testing for upper respiratory viruses from individuals with signs and symptoms of infection who are suspected of COVID-19 by their healthcare provider. Testing in the United States is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform high complexity testing.

Results are for the identification of SARS-CoV-2, and internal positive RNase P RNA. The SARS-CoV-2 RNA is generally detectable nasopharyngeal swabs, nasal aspirate, and oropharyngeal swab specimens utilized in testing for respiratory viruses during the acute phase of infection. Positive results are indicative of active infection. 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.

The Detect$^{X}$-Rv test is intended for use by trained clinical laboratory personnel specifically instructed and trained in the techniques of RT-PCR and DNA microarray hybridization procedures. The Detect$^{X}$-Rv test is for prescription use only and is only for use under the FDA's Emergency Use Authorization.

## Summary and Explanation of the Test

The Detect$^{X}$-Rv test is a test based on end-point reverse transcription polymerase chain reaction (RT-PCR) coupled to DNA microarray hybridization for the detection of multiple genes within SARS-CoV-1 and SARS-CoV-2 viruses. The DNA microarray contains:

- Detect$^{X}$-Rv Kit –
  - Detect$^{X}$-Rv SARS-CoV-2 Multiplex Assay—contains 5 (five) SARS-CoV-2 primer sets; 2 (two) SARS-CoV-2 probes targeting each N1 and N2 genes
  - Detect$^{X}$-Rv RNase P Control—internal process control for nucleic acid extraction 2 (two) RNase P primer and probe sets as an internal positive control.
- Detect$^{X}$-Rv SARS-CoV-2 Control – RNA control that contains targets specific to the SARS-CoV-2 genomic regions that are targeted by the assay

## Principles of The Procedure

Viral and host nucleic acids are isolated and purified from nasopharyngeal swabs and aspirate using the CERES Nanosciences Nanotrap Virus Capture Kit (CERES Nanotraps) (44250) or the Zymo Research *Quick*-DNA/RNA™ Viral MagBead (R2140 or R2141) magnetic silica bead extraction kit. Subsequently, five microliters of the purified RNA product is reverse transcribed using Promega AccessQuick™ RT-PCR System. During the PCR reaction, in which the PCR target is labeled with a Cy3 fluorophore for detection. The resulting PCR product is then ready for hybridization to the DNA microarrays without additional

denaturation or purification. The DNA microarray is printed in a 96 well configuration mounted under a 96 well proplate. The array contains probes to identify genes in SARS-CoV-2 and RNase P. The labeled PCR product is hybridized to the DNA microarray, over the course of thirty minutes, to determine if viral RNA is present in the patient sample. Following the hybridization, the arrays are scanned to determine the fluorescence intensity of each using an FDA-Cleared Sensospot™ (Sensovation Inc) scanner. The microarray results are uploaded to a secure server, quantified, and interpreted automatically, using Augury™ software (PathogenDx Inc.).

## 2 REAGENTS AND EQUIPMENT

PathogenDx Detect$^{X}$-Rv Kit Components

- **RNA Extraction:** Please review the RNA extraction component in the kit and proceed with Ceres extraction or Zymo extraction as appropriate. Kit contains one of the following:
  - **CERES Nanosciences Nanotrap Virus Capture Kit (44250)**
  - **Zymo Research Quick-DNA/RNA Viral MagBead Kit (R2140 or R2141)**
- **One Step Reverse Transcription Kit** contains 2X Master Mix (Buffer, dNTPs, MgSO4, DNA Polymerase), Reverse Transcriptase, and water
- **RT-PCR Primer Set** for gene specific amplification of cDNA during the RT-PCR reaction
- **SARS-CoV-2 Positive Control** RNA for internal process control
- **PathogenDx microarrays** with sequence-specific oligonucleotide probes attached to the surface and a cyanine 5 (Cy5) co-print for accurate grid alignment for subsequent imaging. Plates are provided with barcode numbers.
- **Buffer 1** for binding of target DNA to sequence-specific oligonucleotide probes
- **Buffer 2** for equilibration of arrays and optimal binding of target DNA to sequence specific oligonucleotide probes

Material Required but Not Provided

- **CERES Nanosciences Nanotrap Virus Capture Kit (44250 – 96 Preps)**
  - ZR-96 MagStands – (P1005)
  - Collection Plate – (C2002)
  - Elution Plate – (C2003)
  - Cover Foil – (C2007)
  - DR NAse Free Cleaning Reagent – Recommended (Argos Technologies – 04397-24)
- **Quick DNA/RNA Viral MagBead Extraction Kit (R2140 – 96 Preps)**
  - ZR-96 MagStands – (P1005)
  - Collection Plate – (C2002)
  - 96-Well Block – (P1001)
  - Elution Plate – (C2003)
  - Cover Foil – (C2007)
  - Beta-mercaptoethanol
  - Isopropanol, molecular grade
  - Ethanol, molecular grade
  - DNase/RNase Free Water
  - DR NAse Free Cleaning Reagent – Recommended (Argos Technologies – 04397-24)
- **RT-PCR and DNA Hybridization**
  - 96-Well Reactions Plates – Recommended (MicroAmp Optical 96-Well Reaction Plate – N8010560)
  - Clear Adhesive Film – Recommended (MicroAmp Clear Adhesive Film – 4306311)
  - Microcentrifuge Tubes – DNase/RNase Free, Prelubricated 1.7 mL Tubes

− 15 mL Disposable Centrifuge Tube, Sterile, Polystyrene or Polypropylene Flat Cap
− Sterile, barrier Pipette Tips – Recommended (ThermoFisher-ART Universal Pipette Tips)
− Molecular Biology Grade Water

Equipment, Software and Other Materials

The following equipment and software are required to run the test and analyze results:

- Sensovation, SensoSpot Fluorescence Microarray Analyzer
- Augury Software (Version 4.5.11)

Warnings and Precautions

- **Caution: For Use Under an Emergency Use Authorization Only.**
    a. This assay is only for in vitro diagnostic use under the FDA Emergency Use Authorization.
    b. For Prescription Use Only.
- **Follow standard precautions.** All patient specimens and positive controls should be considered infectious and/or biohazardous and handled accordingly with safe laboratory procedures.
- **Caution:** This kit is designed to identify viral RNA from infected individuals. Follow necessary precautions when handling specimens. Use personal protective equipment consistent with current guidelines for the handling of potentially infectious samples. Handle all samples and controls as if they are capable of transmitting infectious agents.
- **Caution:** Always use pipette tips with aerosol barriers. Tips that are used must be sterile and free from DNases and RNases.
- **Caution:** Modifications to assay reagents, assay protocol, or instrumentation are not permitted, and are in violation of the product Emergency Use Authorization
- **Caution:** Primer Set 2 is light sensitive and must be stored away from light.
- **Caution:** All frozen reagents must be stored at -20°C± 5˚C. They must NOT be stored at -80°C as this will cause degradation of reagents.
- **Caution:** PathogenDx microarrays are light and moisture sensitive and should be stored in the moisture barrier bag with desiccant packet provided with the kit, away from light.
- **Caution:** Buffer 1 and Buffer 2 can cause irritation upon contact, always wear gloves and eye protection when handling this product. Upon contact, rinse with water.
- **Caution:** In the post-hybridization protocol, centrifuge speed should not exceed 70 x g or slides may break.
- **Caution:** Refer to the Safety Data Sheets on the PathogenDx company website.
- **Caution:** Kit components from different lot numbers should not be mixed.

Storage Instructions

- **One Step Reverse Transcription Kits** should be stored at -20°C± 5˚C and may be freeze-thawed
- **RT-PCR Primer Set** should be stored at -20°C± 5˚C and may be freeze-thawed, must be protected from light
- **SARS-CoV-2 Positive Control** should be stored at -20°C± 5˚C and may be freeze-thawed
- **PathogenDx Microarrays** are provided in slide cases in a moisture barrier bag with desiccant. The slides should be stored in this manner to protect them from light and moisture. Subsequent to hybridization, the slides should be stored in the provided slide case inside the moisture barrier bag with desiccant. Store at room temperature.
- **Buffer 1** should be stored at room temperature.
- **Buffer 2** should be stored at -20°C and may be freeze-thawed.
- **Extraction Buffer** should be stored at room temperature.

- **Magnetic Nanotrap Particles** should be stored at 4°C

Indication of Instability or Deterioration of Reagents

- If there is no detectable Cy5 signal on the PathogenDx microarrays pre- or post-hybridization there may have been bleaching during storage or imager settings are not correct. If needed, please refer to the Troubleshooting Guide in this product insert and/or contract Technical Support.
- If PCR did not take place, the Taq Polymerase or Reverse Transcriptase may have been activated by excess heat during PCR setup. The enzyme is inactive between 15°C to 25°C during PCR setup, and then activated at 95°C during initial denaturation.

## 3 INSTRUMENT PROCEDURE

PathogenDx Detect$^{X}$-Rv Procedure

- This product insert contains instructions for running the PathogenDx Detect$^{X}$-Rv assay.
- **Lab Practices to Minimize Contamination Detect$^{X}$-Rv Workflow**

**Work Areas and Best Practices.**

The Detect$^{X}$-Rv process requires 3 dedicated areas, which are as follows:

- Sample Preparation
- PCR Amplification
- Microarray Hybridization & Imaging

It is strongly recommended that each is enclosed in a dedicated negative pressure hood to minimize transfer of aerosols. Alternatively, they can each be isolated in a separate room maintained at negative pressure and/or physical separation.

Details for each work area and best practices to minimize contamination:

**Sample Preparation Area** is dedicated to processing all specimens and CoV-2 positive controls in preparation for Detect$^{X}$-Rv microarray analysis. The same area may also be used for adding processed samples and controls to the 96-Well PCR plates to be used for the initial RT-PCR reaction. Reagents to be used in the Sample Preparation Area should always remain in that dedicated area. *Laboratory coats, pipettes, pipette tips, and vortexers used in the Sample Preparation Area must remain in this area and not be moved to the PCR Amplification or Hybridization Area.* Never bring amplified product or tips or pipettors or PPE from the PCR Amplification Area or the Hybridization Areas back into the Sample Preparation Area. The use of aerosol barrier pipette tips is always recommended.

**PCR Amplification Area** is dedicated to the RT-PCR steps of the Detect$^{X}$-Rv workflow: RT-PCR reaction. The delivery of Mastermix should be performed in a dedicated area separate from any of the 3 areas used for Detect$^{X}$-Rv analysis. Laboratory coats and equipment used in the PCR Amplification Area must remain in this area and not be moved to the Sample Preparation Area or the Hybridization Area. The use of aerosol barrier pipette tips is recommended at all times.

- Components contained within a Detect$^{X}$-Rv PCR kit are intended to be used together. Do not mix components from different kit lots.
- Do not use kits or reagents after the expiration dates shown on kit labels.
- Work area and instrument platforms must be considered potential sources of contamination. Change gloves after contact with potential contaminants (specimens, eluates, and/or amplified product).

- If the Detect$^{X}$-Rv RT-PCR are aborted, dispose of all commodities and reagents according to the Detect$^{X}$-Rv IFU. They must be treated as a "High Copy Number" material during disposal and kept separate from equipment, tips and reagents used in the Sample Preparation Area.
- Decontaminate and dispose of all potentially biohazardous materials in accordance with local, state, and federal regulations. All materials should be handled in a manner that minimizes the chance of potential contamination of the work area.

**Hybridization & Imaging Area** is dedicated to the addition of Hybridization buffer to the product of the RT-PCR reaction, then to the pipetting of that product onto Detect$^{X}$-Rv microarray slides, followed by the removal of the sample from the slides upon completion of hybridization, to the addition and removal of Wash buffer from each well of the slide, centrifugation of the slides after washing to dry them prior to imaging, then loading of the slides onto the Sensovation Imager. 96 samples are processed without movement of the slides. These are then imaged on the Sensovation, which should be handled with a gloved hand in the same Hybridization Area.

- Reagents to be used in the Hybridization and Imaging Area should always remain in that dedicated area. Laboratory coats, pipettes, pipette tips, and the centrifuge used in the Hybridization and Imaging Area must remain in this area and not be moved to the Sample Preparation of PCR Amplification area. Never bring product materials from the Hybridization Area back into the Sample Preparation Area or PCR Amplification Area, as products from the Hybridization and Imaging area are "High Copy Number". Waste material obtained during hybridization and washing is also a "High Copy Number" material and should be disposed of to mitigate the risk that such "High Copy Number" fluids would come into contact with Equipment in or Personnel using the (Low Copy Number) Sample Preparation Area. See Detect$^{X}$-Rv Instructions for Use. The use of aerosol barrier pipette tips is recommended at all times.
- **Laboratory coats and equipment used in the Hybridization Area must remain in this area and not be moved to the Sample Preparation Area or the Hybridization Area.**
- Components contained within a Detect$^{X}$-Rv Hybridization kit are intended to be used together. Do not mix components from different kit lots.
- Do not use kits or reagents after the expiration dates shown on kit labels.
- Work area and instrument platforms of the Hybridization Area must be considered potential sources of contamination. Change gloves after contact with potential contaminants (specimens, eluates, and/or amplified product) in the Hybridization area.
- If the Detect$^{X}$-Rv Hybridization process is aborted, dispose of all commodities and reagents according to the Detect$^{X}$-Rv IFU.
- Decontaminate and dispose of all potentially biohazardous materials in accordance with local, state, and federal regulations. All materials should be handled in a manner that minimizes the chance of potential contamination of the work area.

**Additional precautions to reduce the risk of cross contamination**

The following precautions should be observed to minimize the risks of RNase contamination, and amplified DNA cross-contamination between samples and inhibition:

- Always wear appropriate personal protective equipment.
- Use powder-free gloves.
- Change gloves after having contact with potential contaminants (such as specimens, eluates, and/or amplified product) especially High Copy number materials and surfaces of the Hybridization & Imaging Area.
- To reduce the risk of nucleic acid contamination due to aerosols formed during pipetting, pipettes with aerosol barrier tips must be used for all pipetting. The length of the tip should be sufficient to prevent contamination of the pipette barrel. While pipetting, care should be taken to avoid touching the pipette barrel to the inside of the sample tube or container. The use of extended aerosol barrier pipette tips is recommended.
- Change aerosol barrier pipette tips between ALL manual liquid transfers.
- The Ceres Nanotraps and Zymo Sample Preparation reagents in the Detect$^{X}$-Rv kits are single use only. Use new reaction vessels, and newly opened reagents for every new Detect$^{X}$-Rv SARS-CoV-2 assay run. At the end of each run, discard all remaining reagents from the worktable.
- Clean the work area between each RNA extraction
- Decontaminate and dispose of all potentially biohazardous materials in accordance with local, state, and federal regulations.
- All materials should be handled in a manner that minimizes the chance of potential contamination of the work area

- ***Viral RNA Extraction:*** **Perform RNA extraction based on the extraction kits**
  - **Capture Virus and Extract RNA with the CERES Nanosciences Nanotrap Virus Capture Kit (44250):**
  - Place the nasopharyngeal swab into 400 µL – 2mL 1x VTM or UTM for storage prior to RNA extraction; Place the 200 µL of nasopharyngeal aspirate into 200 µL of 2x VTM.
  - If using a swab transported in VTM, vortex for 10 seconds to release viral particles and remove 500 µL and place in a 96-well deep well plate® particles and add 200 µl of the Magnetic Nanotrap® particles to each sample
  - Store any remaining samples at -20°C for up to two weeks or -80°C for long term storage.
  - Seal the plate with an adhesive aluminum foil cover
  - Incubate the samples with Magnetic Nanotrap® particles at room temperature for 10 minutes, shaking at 1000 RPM
  - Use the magnetic rack to separate the Magnetic Nanotrap® particle from the sample (2 minutes)
  - Carefully remove and discard all of the supernatant without disturbing the pellet
  - Resuspend the pellet in 100 µl of Extraction Buffer and vortex to mix
  - Heat the samples at 95°C for 10 minutes
  - Use the magnetic rack to separate the Magnetic Nanotrap® particles from the sample (2 minutes)
  - Collect the supernatant; the sample is now ready for RT-PCR
  - Once complete the extracted RNA can be used immediately or stored frozen at -20°C for up to two weeks or -80°C for long term storage.

- **Extract RNA with the Zymo Research Quick-DNA/RNA Viral MagBead Kit (R2140 or R2141):** For a complete description view the Zymo Research Quick-DNA/RNA Viral MagBead Kit product insert. Follow the manufacturer's guidelines for the proper use and procedure for this product.
- Place the nasopharyngeal swab into 400 µL – 2mL 1x VTM for storage prior to RNA extraction; Place the 200 µL of nasopharyngeal aspirate into 200 µL of 2x VTM.
- If using a swab transported in VTM, vortex for 10 seconds to release viral particles and remove 400 µL for Sample Preparation and DNA/RNA Purification procedures per the Zymo Research Quick-DNA/RNA Viral MagBead Kit.
- Store any remaining samples at -20°C for up to two weeks or -80°C for long term storage.
- Once complete the extracted RNA can be used immediately or stored frozen at -20°C for up to two weeks or -80°C for long term storage.

One Labeling Step Reverse Transcription-PCR.

Perform the RT-PCR on the AccessQuick™ RT-PCR System.

*Promega – AccessQuick RT-PCR System:*

For a complete description of the AccessQuick RT-PCR System please view the technical bulletin on the Promega website, product number A1702.

The following procedure can be used to convert total RNA into first-strand cDNA using gene-specific primers provided in the ***Detect$^{X}$-Rv*** Kit.

1. Mix and briefly centrifuge each component before use. Combine the following into a master mix, multiply per reaction as shown in **Table 1:**

*Table 1. AccessQuick RT-PCR System Master mix.*

| Component | Volume (µl) per Reaction | Final Concentration |
|---|---|---|
| AccessQuick Master Mix, 2X | 25 µl | 1X |
| RT-PCR Primer Set 1 | 2 µl | 1µM |
| AMV Reverse Transcriptase (5u/µl) | 1 µl | 0.1u/µl |
| *Purified RNA Sample* | *5 µl* | N/A |
| Nuclease-Free Water (Final volume 5µl) | 17 µl | N/A |
| **Total Volume per Reaction** | **50 µl** | **N/A** |

   - Determine the number of samples in the reaction and multiply each reagent to prepare the master mix, leaving out the Purified RNA Template from the master mix and add individually to each designated well
   - Mix by pipetting and add 45 µl of the master mix per well
   - Add 5 µl of the RNA template to each designated well making sure to change tips between samples
2. Cover the plate with clear adhesive film and seal.
3. Centrifuge the plate in a plate spinner for 30 seconds.
4. Place the plate in the thermal cycler and cover with a heat pad before closing the thermal cycler cover.

5. Input the Reverse Transcriptase and PCR cycling program as shown in **Table 2**:

*Table 2. AccessQuick RT-PCR System Reaction Conditions.*

| RT-PCR Reaction Steps | | Temperature (°C) | Time | Cycle Number |
|---|---|---|---|---|
| First Strand cDNA Synthesis | Reverse Transcription | 45 | 45 min | 1x |
| | AMV RT Inactivation and RNA/cDNA/primer denaturation | 94 | 2 min | 1x |
| PCR Amplification | Denaturation | 94 | 30 sec | 40x |
| | Annealing | 55 | 30 sec | |
| | Extension | 68 | 1 min | |
| | Final Extension | 68 | 7 min | 1x |

6. Proceed to Labeling PCR Amplification

DNA Hybridization

- General guidelines to follow for hybridization: When pipetting with the multichannel onto the microarray slide, only dispense to the first stop. DO NOT depress the multichannel to the second stop, or full evacuation of the tips to avoid cross contamination.

**Caution:** ***Avoid contact with the array surface of the slide during processing. Use slide edges or barcoded area for handling.***
***Caution: The directions below are for 96 well plates. Please note that the bracketed volumes refer to the volume that should be added to the 96 well plates.***

1. Before starting, thaw Buffer 2 at room temperature.
2. Cut paper towel to size to fit the bottom of the hybridization chamber provided.
3. Place the slides to be used in the Hybridization Chamber.
4. Apply 200 µL of Molecular Biology Grade Water to each well of the 96-well while being careful to avoid contact with the array.
5. Aspirate and then again, dispense 200 µL of Molecular Biology Grade Water to each well of the 96-well and allow to sit covered in the Hybridization Chamber for 5 minutes before aspirating water from the slides.
6. Prepare the Pre-hybridization Buffer and Hybridization Buffers in clean tubes for the number of microarrays that will be hybridized as per **Tables 5 and 6**. Vortex briefly to mix.

*Table 5: Reagent volumes for preparation of Pre-hybridization Buffer*

| ***96-Well Plate*** | **Volumes needed for the number of wells being pre-hybridized in the plate** | | |
|---|---|---|---|
| | **32 Wells** | **64 Wells** | **96 Wells** |
| Molecular biology grade water (mL) | 5.587 | 11.174 | 16.761 |
| Buffer 1 (mL) | 1.658 | 3.317 | 4.976 |
| Buffer 2 (mL) | 0.872 | 1.745 | 2.618 |

*Calculations include 20% extra volume to account for pipetting errors.*

*Table 6: Reagent volumes for preparation of Hybridization Buffer*

| 96-Well Plate | Volumes needed for the number of wells being hybridized in the plate | | |
|---|---|---|---|
| | 32 Wells | 64 Wells | 96 Wells |
| Buffer 1 (mL) | 0.463 | 0.926 | 1.390 |
| Buffer 2 (mL) | 0.233 | 0.466 | 0.700 |

*Calculations include 20% extra volume to account for pipetting errors.*

7. Aspirate the water wash and add 200 µL of Pre-hybridization Buffer to each well of the 96-well slides without touching the pipette tip to the array surface. Close the Hybridization Chamber box lid.
8. Allow Pre-hybridization Buffer to stay on the arrays for 5 minutes; do not remove slides from the Hybridization Chamber.
9. Briefly centrifuge the tubes or plate containing the Labeling PCR product.
10. Add 18µL of Hybridization Buffer to each well of the Labeling PCR product within the 96-well PCR plate or tubes, pipette up and down to mix. It is important that no cross- contamination occurs during this step. The PCR product and the Hybridization Buffer mix constitute the Hybridization Cocktail.
11. Aspirate Pre-hybridization Buffer from the arrays.
    **Caution:** ***Do not allow the arrays to air dry. Avoid contact with the array surface.***
12. Immediately add 68 µL – (Total Volume of PCR Reaction + Hyb Buffer) of the Hybridization Cocktail to each array of the 96-well being careful not to touch the array surface with the pipette tip. Ensure that the sample ID and location are recorded.
13. Close the Hybridization Chamber lid.
14. Allow to hybridize for 30 minutes at room temperature in the Hybridization Chamber.
    **Caution:** ***Avoid moving the Hybridization Chamber while hybridizing to avoid sample merging.***

Post hybridization PathogenDx slide processing

1. Prepare Wash Buffer according to the number of slides to be used. (Table 7). Washing must be performed according to the protocol to ensure detectable signal and adequate washing to prevent elevated background signals.

*Table 7: Reagent volumes for preparation of Wash Buffer*

| 96-Well Plate | Volumes corresponding to the number of wells being washed in the plate | | |
|---|---|---|---|
| | 32 Wells | 64 Wells | 96 Wells |
| Buffer 1 (mL) | 0.333 | 0.667 | 1.0 |
| Molecular Grade Water (mL) | 46.0 | 93.0 | 139.0 |

**Volumes are measured in mL*

*** Calculations include 20% extra volume to account for pipetting errors.*

2. Aspirate Hybridization Cocktail from the slides.
3. Add 200 µL of Wash Buffer to each well of the 96-well, then aspirate.
4. Add 200 µL of Wash Buffer to each well of the 96-well, allow buffer to remain on the slides for 10 minutes, aspirate

***Note:*** *Steps 3 and 4 need to occur quickly to ensure no drying occurs.*

5. Perform a final wash by dispensing and aspirating 200 µL of Wash Buffer to each well of the 96-well.
6. Following the last aspiration step, remove the plate from the Hybridization Chamber.

***Note:*** *Do not allow drying to occur on slide surface.*

7. Load the plate, face down, into the Reusable Laboratory Micro Array Plate Centrifuge. (1 minute is adequate to completely dry the plates.)
8. PathogenDx plates should be placed back into a slide case and moisture barrier bag with desiccant until scanning may be performed to protect the slides from light. Slides should be scanned within two weeks of hybridization.

Scanning conditions and Data Acquisition

1. Access the Sensovation scanner desktop, select the application “Array Reader”.
2. Open the tray, select “Open Tray”.
3. Place the slides in the tray oriented with the barcode towards the technician and face down.
    a. If scanning a 96-well plate, place the plate face up and aligned with A1 in the top left
4. Close the tray, select “Close Tray”.
5. Select “Scan”.
6. From the dropdown menu select the 96-well – PDx.
7. Note: *All other information on this screen is preprogrammed – do not alter.*
8. Select the Blue Arrow to begin the scanning process.
9. While the slides are being scanned, select “Result Overview” to review the images of the wells.
10. When the slides are finished scanning and the screen displays the digital image of a slide with all green wells, select the Red X to exit the scanning process.
11. Open the tray, select “Open Tray”.
12. Remove the slides and store in the slide case inside the moisture barrier bag with the desiccant packets.
13. Close the tray, select “Close Tray”.
14. Exit the Array Reader application, select “Exit”.
15. On the Sensovation Scanner desktop, select the folder “Scan Results”.
16. Locate the folder associated with your slide and rename the folder with the slide barcode number by scanning the barcode located either on the outside of the barrier bag or on the slide itself. (ex. rename: ScanJob-191108130334_1 to 7024001001)
17. Submit the whole barcode labeled folder to the “Image Folder” within Dropbox.
18. The folder will automatically begin uploading, the PathogenDx Augury© Software will analyze the data and directly deposit the reports into the “Reports” folder within Dropbox.

## 4 QUALITY CONTROL AND VALIDITY OF RESULTS

One Negative Control (No Template Control) and one Positive Process Control are processed with each run.

Validation of results is performed automatically by the Augury Software based on the performance of internal positive and negative controls in each well.

## 5 INTERPRETATION OF RESULTS

Table 8. Interpretation of Results

| N1 CoV-2 | N2 CoV-2 | RNAse P | Status | Results | Action |
|---|---|---|---|---|---|
| POS | POS | POS or NEG | Valid | SARS-CoV-2 Positive [2] | Report results to a healthcare provider and appropriate health authorities. |
| POS | NEG | POS or NEG | Valid | SARS-CoV-2 Positive [2] | Report results to a healthcare provider and appropriate health authorities. |

| | | | | | |
|---|---|---|---|---|---|
| NEG | POS | POS or NEG | Valid | SARS-CoV-2 Positive [2] | Report results to a healthcare provider and appropriate health authorities. |
| NEG | NEG | POS | Valid | SARS-CoV-2 Negative [2] | Report results to healthcare providers. Consider testing for other viruses. |
| NEG | NEG | NEG | Invalid | N/A | Repeat Test. If the repeat results remain invalid, consider collecting a new specimen. |

[1] Samples with a result of SARS-CoV-2 inconclusive should be retested one time.
[2] SARS-CoV-2 = COVID19

**For Invalid results, following steps should be taken:**

For First repeat: Start procedure over at ***One Step Reverse Transcription-PCR*** under Description of Test Steps.

If second repeat is required: Start procedure from ***Viral RNA Extraction*** under Description of Test Steps.

If repeat results are invalid: **Consider collecting a new patient specimen**.

## 6 LIMITATIONS OF THE PROCEDURE

- This assay is for an in vitro diagnostic use under FDA Emergency Use Authorization only.
- Laboratories are required to report all positive results to the appropriate public health authorities.
- The Detect$^{X}$-Rv assay was established using nasopharyngeal swab and aspirate samples only. Other specimen types have not been evaluated and should not be tested with this assay for clinical purposes.
- Samples must be collected, transported, and stored using appropriate procedures and conditions. Improper collection, transport, or storage of specimens may hinder the ability of the assay to detect the target sequences.
- Extraction and amplification of nucleic acids from clinical samples must be performed according to the specified methods listed in this procedure. Other extraction and processing systems have not been evaluated.
- False-negative results may arise from:
  - Improper sample collection
  - Degradation of the viral RNA during shipping and/or storage
  - Specimen collection after nucleic acid can no longer be found in the specimen matrix
  - Using unauthorized extraction or assay reagents
  - The presence of RT-PCR inhibitors
  - Mutation in the SARS-CoV-2 virus
  - Failure to follow instructions for use
- False-positive results may arise from:
  - Cross contamination during specimen handling or preparation
  - Cross contamination between patient samples
  - Specimen mix-up
  - RNA contamination during product handling
- The impacts of vaccines, antiviral therapeutics, antibiotics, chemotherapeutic or immunosuppressant drugs have not been evaluated

- It is recommended to run a no template negative control to ensure no contamination has occurred, if hybridization signal is detected, new reagents should be used.
- The Primers and the PathogenDx slides are light sensitive and should be protected to avoid photobleaching.
- All equipment should be properly calibrated according to manufacturer's guidelines.

## 7 CONDITIONS OF AUTHORIZATION FOR LABORATORIES

The PathogenDx Detect$^{X}$-Rv Kit Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Patients, and authorized labeling are available on the FDA website: https://www.fda.gov/medicaldevices/emergency-situations-medical-devices/emergency-useauthorizations#covid19ivd

However, to assist clinical laboratories using the SARS-CoV-2 Fluorescent PCR Kit the relevant Conditions of Authorization are listed below:

- Authorized laboratories[1] using PathogenDx Detect$^{X}$-Rv will include with result reports of your product, all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.
- Authorized laboratories using PathogenDx Detect$^{X}$-Rv will use your product as outlined in the Instructions for Use. Deviations from the authorized procedures, including the authorized instruments, authorized extraction methods, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to use your product are not permitted.
- Authorized laboratories that receive PathogenDx Detect$^{X}$-Rv will notify the relevant public health authorities of their intent to run your product prior to initiating testing.
- Authorized laboratories using PathogenDx Detect$^{X}$-Rv will have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.
- Authorized laboratories will collect information on the performance of PathogenDx Detect$^{X}$-Rv and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUAReporting@fda.hhs.gov) and PathogenDx, Inc. (via email: techsupport@PathogenDx.com) any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of your product of which they become aware.
- All laboratory personnel using PathogenDx Detect$^{X}$-Rv must be appropriately trained in RT-PCR techniques and use appropriate laboratory and personal protective equipment when handling this kit and use your product in accordance with the authorized labeling.
- PathogenDx, Inc., authorized distributors, and authorized laboratories using your product will ensure that any records associated with this EUA are maintained until otherwise notified by FDA. Such records will be made available to FDA for inspection upon request.

*[1] The letter of authorization refers to, "United States (U. S.) laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform high complexity tests" as "authorized laboratories.*

# 8 SPECIFIC PERFORMANCE CHARACTERISTICS

*Ceres – Limit of Detect (LoD) – Analytical Sensitivity:*

The limit of detection (LoD) of the ***Detect$^{X}$-Rv*** assay was performed using NP matrix from patients' samples that had tested negative for SARS-CoV-2. The LoD studies establish the lowest SARS-CoV-2 viral

concentration (Genomic Copies Per Reaction or copies per mL) that can be detected by the ***Detect$^{x}$-Rv*** Kit in a specimen at least 95% of the time. The study was conducted according to CLSI-EP17, Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures, 2nd Edition.

The heat inactivated SARS-CoV-2 from SARS-CoV-2 (BEI Resources, NR-52286, 3.75 x $10^8$ genome equivalents/mL) was diluted in SARS-CoV-2 Negative Human Nasopharyngeal swab samples in VTM from and extracted and purified using the CERES Nanosciences Nanotrap Virus Capture Kit (Ceres Nano, 44250).

The initial LoD (see Table 9) was determined by testing at six input concentrations 5000, 3000, 2000, 1000, 500 and 300 copies per mL of heat inactivated SARS-CoV-2 from SARS-CoV-2 (BEI Resources, NR-52286, 1.16 x $10^9$ copies/ml) in VTM. It was established through this first test that the LoD was between 1000 to 300 copies/mL of input heat inactivated SARS-CoV-2. For the initial three concentrations tested we obtained 100% positive results for all three concentrations tested. The final LoD was established by obtaining 19/20 (95% Positive) results for all probes (N1 and N2) in the LoD experiment as seen below in the results summary Table 10**.** therefore, for our final LoD we tested an additional 20 samples at 500 copies/mL.

**Table 9: Initial Dilution Series LoD (Ceres)**

| Input Concentration | Total Valid Replicates | % Positive |
|---|---|---|
| 5000 cp/mL | 6 | 100% |
| 3000 cp/mL | 6 | 100% |
| 2000 cp/mL | 6 | 100% |
| 1000 cp/mL | 6 | 100% |
| 500 cp/mL | 6 | 100% |
| 300 cp/mL | 6 | 50% |

**Table 10: Limit of Detection Results (Ceres)**

| Input Concentration | Total Valid Replicates | % Positive |
|---|---|---|
| 500 cp/mL | 20 | 95% |

The final LoD was confirmed by testing 20 replicates at the estimated LoD concentration of 500 cp/mL input RNA achieving 95% positives.

*Zymo - Limit of Detection (LoD) - Analytical Sensitivity:*

The limit of detection (LoD) of the ***Detect$^{x}$-Rv*** assay was performed using NP matrix from patients' samples that had tested negative for SARS-CoV-2. The LoD studies establish the lowest SARS-CoV-2 viral concentration (Genomic Copies Per Reaction or copies per mL) that can be detected by the ***Detect$^{x}$-Rv*** Kit in a specimen at least 95% of the time. The study was conducted according to CLSI-EP17, Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures, 2nd Edition.

The purified genomic RNA from SARS-CoV-2 (BEI Resources, NR-52285, 5.5 x $10^7$ copies/mL) was diluted in SARS-CoV-2 Negative Human Nasopharyngeal swab samples in VTM from and extracted and purified using the Quick DNA/RNA Viral MagBead (Zymo Research, R2140).

The initial LoD (see Table 11) was determined by testing at three input concentrations 625, 250, and 125 copies per mL of purified genomic RNA from SARS-CoV-2 (BEI Resources, NR-52285, 5.5 x $10^7$ copies/mL) in

VTM. It was established through this first test that the LoD was between 125 and 62.5 copies per mL of input RNA. For the initial three concentrations tested we obtained 100% positive results for all three concentrations tested. The final LoD was established by obtaining 19/20 (95% Positive) results for all probes (N1 and N2) in the LoD experiment as seen below in the results summary Table 12**.** therefore, for our final LoD we tested an additional 20 samples at 62.5 copies/mL which is at or near the theoretical limit of detection for post RNA extraction at ~2 copies/reaction.

**Table 11: Initial Dilution Series LoD (Zymo)**

| Input Concentration | Total Valid Replicates | % Positive |
|---|---|---|
| 625 copies/mL | 12 | 100% |
| 250 copies/mL | 24 | 100% |
| 125 copies/mL | 24 | 100% |

**Table 12: Limit of Detection Results (Zymo)**

| Input Concentration | Total Valid Replicates | % Positive |
|---|---|---|
| 62.5 copies/mL | 20 | 95% |

The final LoD was confirmed by testing 20 replicates at the estimated LoD concentration of 62.5 cp/mL input RNA achieving 95% positives.

Primer and Probe - Reactivity/ Inclusivity:

An *in silico* inclusivity analysis of the DetectX-Rv SARS-CoV-2 primers and probes was performed. All primer sets designed for the detection of the N1 and N2 were tested against the complete available SARS-CoV-2 genome sequence as of July 15, 2020 (>41,000 high quality genomes). The analysis demonstrated that the regions recognized by the designed primers and probes have 100% homology with all available SARS-CoV-2 sequences from the Global Initiative on Sharing Avian Influenza Data (GISAID) databases/databanks.

**Table 13:** Primer sequences for SARS-CoV-2 N1 and N2

| **Database** | **Identity to N1** | | **Identity to N2** | |
|---|---|---|---|---|
| | **Primers (%)** | **Probes (%)** | **Primers (%)** | **Probes (%)** |
| **GISAID** | >99.9% | >99.9% | >99.9% | 100% |

*In silico Cross-reactivity (Analytical Specificity):*

Cross-reactivity of the DetectX-Rv SARS-CoV-2 Assay was evaluated both *in silico* analysis (Table 14) and by testing pooled organisms spiked into negative NP clinical samples using (Exact Diagnostics RP Positive Run Control – RPPOS) based on six replicates (Table 15). The results from the cross-reactivity, both in silico and wet testing, are summarized below.

***Table 14:*** Organisms tested for In silico Exclusivity Analysis

| Microorganism | In silico Analysis for % Identity target: N1 | In silico Analysis for % Identity target: N2 |
|---|---|---|
| Adenovirus (taxid:1643649) | No alignment found | No alignment found |
| Bordetella pertussis (taxid:520) | No alignment found | No alignment found |
| Candida albicans (taxid:5476) | No alignment found | No alignment found |

| | | |
|---|---|---|
| Chlamydia pneumonia (taxid:83558) | No alignment found | No alignment found |
| Enterovirus (taxid:12059) | No alignment found | No alignment found |
| Haemophilus influenza (taxid:157239) | No alignment found | No alignment found |
| HCoV-SARS (taxid:694009) | No alignment found | No alignment found |
| Human coronavirus 229E (taxid:11137) | No alignment found | No alignment found |
| Human coronavirus HKU1 (taxid:290028) | No alignment found | No alignment found |
| Human coronavirus NL63 (taxid:277944) | No alignment found | No alignment found |
| Human coronavirus OC43 (taxid:31631) | No alignment found | No alignment found |
| Human genome (taxid:9606) | No alignment found | No alignment found |
| Human Metapneumovirus (taxid:162145) | No alignment found | No alignment found |
| Human parainfluenza virus 2 (taxid:11214) | No alignment found | No alignment found |
| Influenza A (taxid:11320) | No alignment found | No alignment found |
| Influenza B (taxid:11520 | No alignment found | No alignment found |
| Legionella pneumophila (taxid:446) | No alignment found | No alignment found |
| MERS-coronavirus (taxid:1335626) | No alignment found | No alignment found |
| Mycobacterium tuberculosis (taxid:1773) | No alignment found | No alignment found |
| Mycoplasma pneumonia (taxid:2104) | No alignment found | No alignment found |
| Parainfluenza virus 1 (taxid:11210) | No alignment found | No alignment found |
| Parainfluenza virus 3 (taxid:11216) | No alignment found | No alignment found |
| Parainfluenza virus 4a (taxid:11224) | No alignment found | No alignment found |
| Parainfluenza virus 4a (taxid:1124) | No alignment found | No alignment found |
| Parainfluenza virus 4b (taxid:11226) | No alignment found | No alignment found |
| Parainfluenza virus 4b (taxid:1126) | No alignment found | No alignment found |
| Pneumocystis jirovecii (taxid:42068) | No alignment found | No alignment found |
| Pseudomonas aeruginosa (taxid:287) | No alignment found | No alignment found |
| Respiratory syncytial virus (taxid:11250) | No alignment found | No alignment found |
| Rhinovirus (taxid:12059) | No alignment found | No alignment found |
| Staphylococcus epidermis (taxid:1282) | No alignment found | No alignment found |
| Streptococcus pneumonia (taxid:1313) | No alignment found | No alignment found |

| Streptococcus pyogenes (taxid:1314) | No alignment found | No alignment found |
|---|---|---|
| Streptococcus salivarius (taxid:1304) | No alignment found | No alignment found |

***Table 15:*** Laboratory Tested Cross Reactivity Analysis

| Analyte | Manufacturing Targets Concentration | Results | Final Result |
|---|---|---|---|
| Adenovirus | 500,000 cp/mL | 0/6 | Negative |
| *Bordetella parapertussis* | 5,000 cp/mL | 0/6 | Negative |
| *Bordetella pertussis* | 500,000 cp/mL | 0/6 | Negative |
| *Chlamydophila pneumoniae* | 1,000 cp/mL | 0/6 | Negative |
| Coronavirus 229E | 500,000 cp/mL | 0/6 | Negative |
| Coronavirus HKU1 | 20,000,000 cp/mL | 0/6 | Negative |
| Coronavirus NL63 | 2,000,000,000 cp/mL | 0/6 | Negative |
| Coronavirus OC43 | 25,000 cp/mL | 0/6 | Negative |
| Human metapneumovirus | 20,000,000 cp/mL | 0/6 | Negative |
| Influenza A H1N1 | 100,000 cp/mL | 0/6 | Negative |
| Influenza A H1N1-09 | 500,000,000 cp/mL | 0/6 | Negative |
| Influenza A H3N2 | 500,000,000 cp/mL | 0/6 | Negative |
| Influenza B | 500,000 cp/mL | 0/6 | Negative |
| *Mycoplasma pneumoniae* | 1,000 cp/mL | 0/6 | Negative |
| Parainfluenza 1 | 50,000 cp/mL | 0/6 | Negative |
| Parainfluenza 2 | 5,000 cp/mL | 0/6 | Negative |
| Parainfluenza 3 | 5,000 cp/mL | 0/6 | Negative |
| Parainfluenza 4a | 500,000 cp/mL | 0/6 | Negative |
| Rhinovirus 1A | 500,000 cp/mL | 0/6 | Negative |
| RSV A | 10,000 cp/mL | 0/6 | Negative |
| RSV B | 10,000 cp/mL | 0/6 | Negative |
| MERS-coronavirus | 5,000,000 cp/mL | 0/6 | Negative |
| SARS-CoV | 200,000,000 cp/mL | 0/6 | Negative |

*cp/mL = copies/mL*

*Ceres – Clinical Evaluation:*

The clinical performance of the DetectX-Rv assay was established using fresh clinical NP specimens obtained from Tricore Labs, 30 positive and 30 negatives, previously tested on the Roche Cobas 6800 FDA-EUA

approved platform. The clinical evaluation was done internally at PathogenDx. These samples were provided to the PathogenDx technician in a blinded format prior to RNA extraction. PathogenDx followed the protocol listed in Section 2, Description of test, in this document to extract RNA and analyze the 60 total clinical samples. The 60 total samples tested as well as the PathogenDx external positive and external negative controls (described in Section 3). The ***Detect$^{X}$-Rv*** assay demonstrated *100% concordance* with the thirty (30) samples called positive and *100% concordance* with the 30 samples called negative.

| ***Samples Tested Individually*** | ***Comparator Method Result*** | |
|---|---|---|
| ***Candidate Test Result*** | ***Positive*** | ***Negative*** |
| ***Positive*** | ***30/30 (100% Agreement)*** | ***0/30 (100% Agreement)*** |
| ***Negative*** | ***0/30 (100% Agreement)*** | ***30/30 (100% Agreement)*** |

**PathogenDx conducted this with on one run with our internal controls. Detailed analysis provided in the FDA EUA Data file.*

*Zymo - Clinical Evaluation:*

The clinical performance of the DetectX-Rv assay was established using fresh clinical NP specimens obtained from Tricore Labs, 30 positive and 30 negatives, previously tested on the Roche Cobas 6800 FDA-EUA approved platform. The clinical evaluation was done internally at PathogenDx. These samples were provided to the PathogenDx technician in a blinded format prior to RNA extraction. PathogenDx followed the protocol listed in Section 2, Description of test, in this document to extract RNA and analyze the 60 total clinical samples. The 60 total samples tested as well as the PathogenDx external positive and external negative controls (described in Section 3). The ***Detect$^{X}$-Rv*** assay demonstrated *100% concordance* with the thirty (30) samples called positive and *100% concordance* with the 30 samples called negative.

| ***Samples Tested Individually*** | ***Comparator Method Result*** | |
|---|---|---|
| ***Candidate Test Result*** | ***Positive*** | ***Negative*** |
| ***Positive*** | ***30/30 (100% Agreement)*** | ***0/30 (100% Agreement)*** |
| ***Negative*** | ***0/30 (100% Agreement)*** | ***30/30 (100% Agreement)*** |

**PathogenDx conducted this with on one run with our internal controls. Detailed analysis provided in the FDA EUA Data file.*

## 9 TECHNICAL ASSISTANCE

Visit pathogendx.com for the latest service and support information for immediate support please call 1-877-795-1153; IVDsupport@pathogendx.com

**Glossary:**

| | Manufacturer | Rx Only | Prescription use only |
|---|---|---|---|
| | Use by Date | | Keep Dry |
| LOT | Batch code | | Keep away from sunlight |
| | Do not use if package is damaged | | Temperature limitation |
| | Consult instructions for use | | Do not re-use |
| IVD | In vitro diagnostic medical device | CONTROL + | Positive control |
| CONTROL | Control | CONTROL - | Negative control |