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

IVD

Rx Only

Contents

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 PCR-DNA microarray hybridization. The Detect^X-Rv is designed for 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 PCR-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; 4 (four) SARS-CoV-2probes targeting each N1, N2, and N3 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

Biological Principles of The Procedure

Viral and host nucleic acids are isolated and purified from nasopharyngeal swabs and aspirate using 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 either Promega AccessQuick™ RT-PCR System. Following RT-PCR, two microliter of that primary RT-PCR (amplified cDNA) product is then PCR amplified in a second, nested and biased, 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 a 1" x 3" glass microscope slide containing 12 identical wells containing 144 synthetic ssDNA probes in a 12 x 12 array configuration. 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 one hour, 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

PathogenDx Detect^{X}-Rv Kit Components

- **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 1** for gene specific amplification of cDNA during the RT-PCR reaction
- **SARS-CoV-2 Positive Control** RNA for internal process control
- **PCR Master Mix** containing Molecular Biology Grade Water, PCR Buffer, $MgCl_2$, BSA and dNTPs for PCR amplification
- **Primer Set 2** for amplification and fluorescent labeling of the RT-PCR product
- **Taq Polymerase** for attaching nucleotides to a DNA template, thus copying the DNA
- **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. Slides are provided with barcode numbers of which the first 4 digits correspond to the probe map and the last 6 digits are the unique lot and print identifier for the slides.
- **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

- **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, Labeling 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

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.
- **Cautions:** 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.
- **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 1** should be stored at -20°C± 5˚C and may be freeze-thawed
- **SARS-CoV-2 Positive Control** should be stored at -20°C± 5˚C and may be freeze-thawed
- **PCR Master Mix** should be stored at -20°C± 5˚C and may be freeze-thawed
- **Primer Set 2** should be stored at -20°C± 5˚C and may be freeze-thawed. Must be protected from light
- **Taq Polymerase** should be stored at -20°C. Taq Polymerase is thermostable and does not need to thaw, only remove from freezer when starting PCR process
- **Positive Control** should be stored at -20°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.

Indication of Instability or Deterioration of Reagents

- If salt precipitation has occurred in Sample Prep Buffer or Buffer 1 after shipping or prolonged storage, re-dissolve by vortexing at room temperature (20-25°C).
- 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 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.
- If successful sample preparation did not take place, Sample Digestion Buffer may have been activated by excess heat during setup. The enzyme is inactive between 15°C to 25°C during setup, and then activated at 55°C during the 45 min incubation.

3 INSTRUMENT PROCEDURE

PathogenDx Detect^{X}-Rv Procedure

- This product insert contains instructions for running the PathogenDx Detect^{X}-Rv assay.

- ***Viral RNA Extraction:***
  - **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 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.

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.

Step		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	35x
	Annealing	55	30 sec	
	Extension	68	1 min	
	Final Extension	68	7 min	1x

6. Proceed to Labeling PCR Amplification

<u>Labeling PCR Reaction</u>

1. Thaw PCR Master Mix, Primer Set 2, and remove the Taq Polymerase from the freezer just prior to making the master mix
2. Mix and briefly centrifuge each component before use
3. Mix the indicated reagent volumes (calculated from **Table 3**) in a microfuge tube to prepare Labeling PCR Master Mix (made fresh each run)
4. Briefly vortex Labeling PCR master mix and centrifuge at 1000 g for 3-5 seconds
5. Store all reagents at -20°C after use

Table 3. Labeling PCR Reaction Master Mix.

Number of Reaction	PCR Master Mix	Primer Set 2	Taq Polymerase	Final Volume
6	372 µl	16 µl	4 µl	392 µl
12	697 µl	30 µl	7.5 µl	735 µl
24	1255 µl	54 µl	13.5 µl	1323 µl
36	1860 µl	80 µl	20 µl	1960 µl
48	2418 µl	104 µl	26 µl	2548 µl

Reaction volumes have been scaled to account for a negative control and to account for pipetting losses and volume lost on reservoir/tube walls. If a reservoir is not used for multichannel pipetting, there will be extra volume remaining in the PCR Master Mix tube.

1. Pipette 48 µL of the Labeling PCR Master Mix into the bottom of PCR tubes or PCR plate
2. In the Post PCR area, pipette 2 µL of the RT-PCR Product into the bottom of the corresponding tube or well for a final volume of 50 µL per PCR reaction. Pipet up and down to mix
   - **Always check pipette tip volumes before and after to ensure accuracy and release.**
   - **Warning: Add the Loci Enhancement PCR template into the Labeling PCR reaction outside of the PCR area to prevent contamination**
3. The Loci Enhancement PCR plate may be re-sealed and returned to 4°C.
4. Cap tubes, or seal plates with PCR film ensuring every well is completely sealed.
5. Centrifuge for 30 seconds.
6. Place tubes or plate into the thermal cycler with a pressure pad if necessary, before closing the thermal cycler lid.
7. Refer to **Table 4** to run the Labeling PCR Program.
8. Labeling PCR product may be stored for 7 days at 4°C protected from light.

Table 4: Labeling PCR program

	Labeling PCR		
Steps	**Temp.**	**Time**	**Cycles**
1	95°C	4 minutes	1
2	95°C	20 seconds	30
3	55°C	20 seconds	
4	72°C	30 seconds	
5	72°C	7 minutes	1
6	15°C	Hold Ready for Next Step	1

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.***

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 75µL (200 µL) of Molecular Biology Grade Water to each well of the 12-well (96-well) while being careful to avoid contact with the array.
5. Aspirate and then again, dispense 75µL (200 µL) of Molecular Biology Grade Water to each well of the 12-well or 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

12-Well Slides	**Volumes corresponding to the number of slides being hybridized**							
Pre-hybridization Buffer reagents	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Molecular biology grade water (µL)	640	1186	1816	2404	3034	3622	4210	4840
Buffer 1 (µL)	190	346	526	694	874	1042	1210	1390
Buffer 2 (µL)	100	178	268	352	442	526	610	700
96-Well Plate	**Volumes corresponding to a full 96 well plate being hybridized**							
Molecular biology grade water (µL)	16761							
Buffer 1 (µL)	4976							
Buffer 2 (µL)	2618							

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

Table 6: Reagent volumes for preparation of Hybridization Buffer

12-Well Slides	**Volumes corresponding to the number of slides being hybridized**							
Hybridization cocktail reagents	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Buffer 1 (µL)	190	346	526	694	874	1042	1210	1390
Buffer 2 (µL)	100	178	268	352	442	526	610	700
96-Well Plate	**Volumes corresponding to a full 96 well plate being hybridized**							
Buffer 1 (µL)	1390							
Buffer 2 (µL)	700							

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

7. Aspirate the water wash and add 50µL (200 µL) of Pre-hybridization Buffer to each well of the 12-well or 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 50µL (68 µL – Total Volume of PCR Reaction + Hyb Buffer) of the Hybridization

Cocktail to each array of the 12-well or 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

	Volumes corresponding to the number of 12 well slides being hybridized							
Wash Buffer reagents	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Buffer 1 **(µL)**	32.4	64.8	97.2	129.6	162	194.4	226.8	259.2
Molecular biology grade water **(*mL*)***	4.23 mL	8.58 mL	12.86 mL	17.15 mL	21.44 mL	25.73 mL	30 mL	34.30 mL
Total volume (mL)*	***4.32 mL***	***8.64 mL***	***12.96 mL***	***17.28 mL***	***21.60 mL***	***25.92 mL***	***30.24 mL***	***34.56 mL***
	Volumes corresponding to a full 96 well plate being hybridized							
Buffer 1 **(µL)**	**777.6 µL**							
Molecular biology grade water **(*mL*)***	**102.9 *mL***							
Total volume (mL)*	**103.7 *mL***							

**Volumes are measured in mL*

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

2. Aspirate Hybridization Cocktail from the slides.
3. Add 75µL (200 µL) of Wash Buffer to each well of the 12-well or 96-well, mix by pipetting 5 times, then aspirate.
4. Add 75µL (200 µL) of Wash Buffer to each well of the 12-well or 96-well, mix by pipetting 5 times, then aspirate.
5. Add 75µL (200 µL) of Wash Buffer to each well of the 12-well or 96-well, mix by pipetting 5 times, then aspirate.
6. Add 75µL (200 µL) of Wash Buffer to each well of the 12-well or 96-well, mix by pipetting 5 times, allow buffer to remain on the slides for 3 minutes, aspirate
7. Add 75µL (200 µL) of Wash Buffer to each well of the 12-well or 96-well, mix by pipetting 5 times, allow buffer to remain on the slides for 3 minutes, aspirate
8. Add 75µL (200 µL) of Wash Buffer to each well of the 12-well or 96-well, mix by pipetting 5 times, allow buffer to remain on the slides for 3 minutes, aspirate

Note: *Steps 6-8 need to occur quickly to ensure no drying occurs.*

9. Perform a final wash by dispensing and aspirating 75µL (200 µL) of Wash Buffer 2 times to each well of the 12-well or 96-well.
10. Following the last aspiration step, remove the slides from the Hybridization Chamber.

Note: *Do not allow drying to occur on slide surface. Therefore, if running more than two slides, aspirate the two and move those to Step 8 - allow the final to dispense of Wash Buffer to remain on the additional slides until the Slide Spinner is available for use. This will ensure no drying occurs on the slide surface.*

11. Load the slides into the Labnet Reusable Laboratory Micro Array Slide Spinner. (30 seconds is adequate to completely dry the slides.)
12. PathogenDx slides 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 12-well slide – PDx, 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	N3 CoV1/2	RNAse P	Status	Results	Action
Two or more N1 CoV-2, N2 CoV-2, and/or N3 CoV1/2 = POS			POS or NEG	Valid	SARS-CoV-2 Positive [2]	Report results to a healthcare provider and appropriate health authorities.

NEG	NEG	NEG	POS	Valid	SARS-CoV-2 Negative [2]	Report results to healthcare providers. Consider testing for other viruses.
NEG	NEG	NEG	NEG	Invalid	N/A	Repeat Test. If the repeat results remain invalid, consider collecting a new specimen.
NEG	NEG	POS	POS or NEG	Valid	SARS-CoV-2 Inconclusive [1 and 2]	Repeat Test to confirm that the patient is not positive for SARS-CoV-2. If the repeat results remain negative for SARS-CoV-2 the patient is negative for SARS-CoV-2. Report results to a healthcare provider and additional confirmation testing should be conducted if clinically indicated.

[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
  - Cross contamination during DNA hybridization
- 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.
- Both Primer Set 2 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

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 9) was determined by testing at three input concentrations 250, 100, and 50 copies per reaction 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 50 and 25 copies per reaction (125 to 62.5 copies/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-N3) 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 25 copies/reaction (62.5 copies/mL) which is at or near the theoretical limit of detection for post RNA extraction at ~2 copies/reaction.

Table 9: Initial Dilution Series LoD

Input Concentration	Total Valid Replicates	Positive Replicates				% Positive
		N1	N2	N3	RNase P	
250 Copies/Reaction (625 copies/mL)	12	12	12	12	12	100%
100 Copies/Reaction (250 copies/mL)	24	24	24	24	24	100%
50 Copies/Reaction (125 copies/mL)	24	24	24	24	24	100%

Table 10: Limit of Detection Results

Input Concentration	Total Valid Replicates	Positive Replicates				% Positive
		N1	N2	N3	RNase P	
25 Copies/Reaction/ 62.5 copies/mL	20	19	19	19	20	95%

The final LoD was confirmed by testing 20 replicates at the estimated LoD concentration of 25 cp/reaction (62.5 cp/mL) input RNA achieving 95% positives.

Clinical Evaluation:

The clinical performance of the DetectX-Rv assay was established using fresh clinical NP specimens obtained from Tricor 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)***	***30/30 (100% Agreement)***
Negative	***30/30 (100% Agreement)***	***30/30 (100% Agreement)***

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

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, N2, and N3 gene 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 11: Primer sequences for SARS-CoV-2 N1, N2 and N3

Database	**Identity to N1**		**Identity to N2**		**Identity to N3**	
	Primers (%)	**Probes (%)**	**Primers (%)**	**Probes (%)**	**Primers (%)**	**Probes (%)**
GISAID	>99.9%	>99.9%	>99.9%	100%	>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 12) and by testing pooled organisms spiked into negative NP clinical samples using (Exact Diagnostics RP Positive Run Control – RPPOS) based on five replicates (Table 13). The results from the cross-reactivity, both in silico and wet testing, are summarized below.

Table 12: Organisms tested for In silico Exclusivity Analysis

Microorganism	**In silico Analysis for % Identity target: N1**	**In silico Analysis for % Identity target: N2**	**In silico Analysis for % Identity target: N3**
Adenovirus (taxid:1643649)	No alignment found	No alignment found	No alignment found
Bordetella pertussis (taxid:520)	No alignment found	No alignment found	No alignment found
Candida albicans (taxid:5476)	No alignment found	No alignment found	No alignment found
Chlamydia pneumonia (taxid:83558)	No alignment found	No alignment found	No alignment found
Enterovirus (taxid:12059)	No alignment found	No alignment found	No alignment found
Haemophilus influenza (taxid:157239)	No alignment found	No alignment found	No alignment found
HCoV-SARS (taxid:694009)	No alignment found	No alignment found	100%

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

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

Table 13: Laboratory Tested Cross Reactivity Analysis

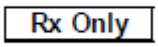
Analyte	Manufacturing Targets Concentration	Results	Final Result
Adenovirus	500,000 cp/mL	0/5	Negative
Bordetella parapertussis	5,000 cp/mL	0/5	Negative
Bordetella pertussis	500,000 cp/mL	0/5	Negative
Chlamydophila pneumoniae	1,000 cp/mL	0/5	Negative
Coronavirus 229E	500,000 cp/mL	0/5	Negative
Coronavirus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

cp/mL = copies/mL

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