Detect^{X}-Rv
Instructions for Use

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

Corporate Office
9375 E. Shea Blvd, Ste.100
Scottsdale, AZ 85260
www.pathogendx.com

Research & Development
1230 E. Pennsylvania St. Suite 102
Tucson, AZ 85714

IVD

Rx Only

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GENERAL INFORMATION

Intended Use

Detect^{X}-Rv is a RT-PCR and DNA microarray hybridization test intended for the qualitative detection of nucleic acids from SARS-CoV-2 in nasopharyngeal (NP) swabs, oropharyngeal (OP) swabs, mid-turbinate nasal swabs, anterior nasal swabs, nasal aspirates, nasopharyngeal wash/aspirates and bronchoalveolar lavage (BAL) specimens from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet requirements to perform high complexity tests.

Results are for the identification of SARS-CoV-2 RNA. SARS-CoV-2 RNA is generally detectable in upper respiratory specimens and bronchoalveolar lavage (BAL) specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out an accompanying 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 test 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.

Detect^{X}-Rv is intended for use by qualified and trained clinical laboratory personnel specifically instructed and trained in the techniques of RT-PCR, microarray hybridization, and *in vitro* diagnostic procedures. Detect^{X}-Rv is only for use under the Food and Drug Administration's Emergency Use Authorization.

Summary and Explanation of the Test

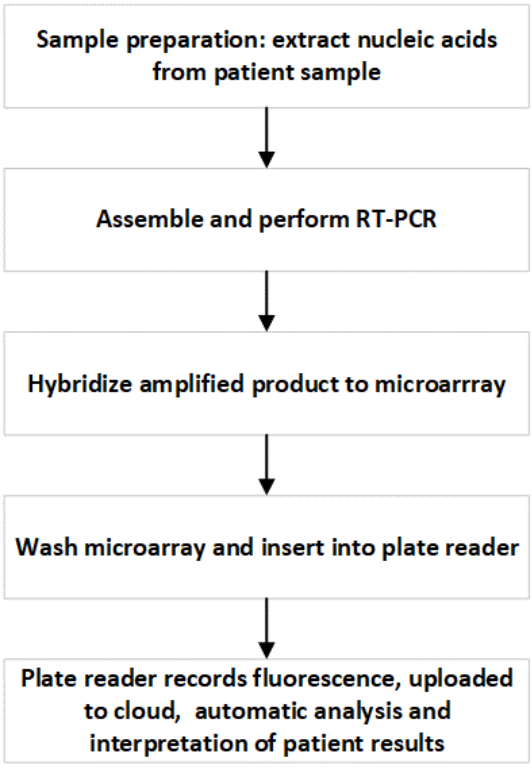
Detect^{X}-Rv is a qualitative test to detect the presence of SARS-CoV-2 RNA in nasopharyngeal swabs, oropharyngeal (OP) swabs, mid-turbinate nasal swabs, anterior nasal swabs, nasal aspirates, nasopharyngeal wash/aspirates and bronchoalveolar lavage (BAL). The test involves extraction of nucleic acids from samples followed by reverse transcription (RT) and subsequent polymerase chain reaction (PCR) performed in a single reaction well. The PCR amplification product is then hybridized to a DNA microarray. Detection of SARS-CoV-2 is determined by fluorescence value of specific spots in the microarray when visualized in a plate reader.

Principles of the Procedure

Overview

Detect^{X}-Rv targets the N1 and N2 nucleocapsid gene regions of the SARS-CoV-2 virus. Nucleic acids are extracted from patient upper respiratory samples using magnetic hydrogel beads. A portion of the extraction is removed and used to set up a reverse transcription and polymerase chain reaction (RT-PCR), which is performed in a single reaction well. The PCR is an asymmetric amplification that labels viral targets and control target amplicons with a cyanine (Cy3) end-labeled primer. The fluorescently labeled PCR amplification products are directly hybridized to a DNA microarray printed on the bottom of each well in a 96-well plate. A plate reader will record the fluorescent intensity of the PCR product hybridized to specific spots within the microarray, and data from the plate reader will then be transferred to the laboratory computer with Augury™ software. Detection of the presence of SARS-CoV-2 and amplification control is automatically determined and reported by software analysis.

WORKFLOW



Sample Preparation

The nucleic acids present in 500 µL samples are extracted using 200 µL of PathogenDx Detect^{X}-Rv Ceres beads (Ceres beads). A 500 µL portion of the patient sample is added to the Ceres beads and the viral particles present in the sample are captured in the hydrogel surface on magnetic beads. The beads are washed and the nucleic acids are directly liberated from the beads using 100 µL of lysis buffer. A 5 µL portion of the extract is then used for RT-PCR.

Assemble and perform RT-PCR

5 µL of the extracted nucleic acid sample is added to 45 µL of the complete One Step Reverse Transcription Master Mix (One Step RTMM) and placed in a ThermoFisher MiniAmp A37834 (PCR machine). Once placed in the PCR machine, the One Step RTMM performs three sequential functions with the sample extract (in one closed well) to prepare it for analysis. First, Cod UNG enzyme is activated at 25 °C before the one-step RT-PCR reactions are initiated. Second, when the temperature is raised to 55 ^{o}C, the UNG enzyme is irreversibly inactivated, and the reverse transcription of the nucleic acids is performed. Third, after RT is complete, 40 cycles of PCR are performed where the target viral and control nucleic acids are asymmetrically amplified, and the amplicons become labeled with the Cy3 fluorophore that is attached to the PCR amplification primers.

Hybridize amplified product to microarray
The entire amplified PCR reaction is ready for room-temperature hybridization to the DNA microarray without purification or heat denaturation steps. The DNA microarray used to detect SARS-CoV-2 is printed at the bottom of each well in a 96-well configuration (i.e., one plate can test and provide results for up to 94 patient samples plus one positive and one negative control sample).

Wash microarray and insert into plate reader
Following hybridization, the microarrays (wells) that were used in testing samples are washed in a series of short rinses with PathogenDx Detect^{X}-Rv Plate Wash buffer (HYB Buffer 1 diluted in MBG water) to remove unbound and unused Cy3 labeled primers or amplification products. After the final wash, the user ensures that the microarray plate wells used for patient sample testing have no residual Plate Wash Buffer, and the dried plate is inserted into the plate reader.

Plate reader performs analysis and automatic interpretation of results
Each well in the 96-well microarray plate contains a series of DNA oligonucleotide probes printed in an ordered array on the bottom of the well. The oligonucleotides are designed to identify N1 and N2 regions of SARS-CoV-2, external amplification control, RNase P (internal extraction and amplification control present in all human cell types), and a negative hybridization (background) signal.

Upon completion of the washing of the microarray plate, RT-PCR products bound to specific probe spots in the microarray generate fluorescent signals, which are used to determine if SARS-CoV-2 viral RNA is present in the original patient sample. The control data, obtained from the same microarray, are used to evaluate the specificity and validity of the hybridization data.

The fluorescently labelled hybridization signals from each microarray are scanned and analyzed without human intervention, using Sensospot^{TM} (Sensovation Inc) scanner (plate reader). The fluorescent data are loaded onto a laboratory computer and automatically quantified and interpreted using Augury^{TM} software (PathogenDx Inc.). The results are reported to the user for assignment to patient sample records.

REAGENTS and EQUIPMENT

Materials Required (Provided in Kit)

Detect^{X}-Rv components

- **Ceres Viral Collection and Lysis Kit** consisting of:
  - Ceres Beads (PN 10145), 24 mL, store at 2-8 °C
  - Ceres Wash Buffer (PN 10148), 50 mL, store at 15-30 °C
  - Ceres Lysis Buffer (PN 10146), 12 mL, store at 15-30 °C
- **One Step Reverse Transcription Master Mix**:
  - **PathogenDx Detect^{X}-Rv Master Mix 2X Concentration** (PN 10009) (Buffer, dNTPs [dATP, dCTP, dGTP, dTTP], MgSO4, DNA Polymerase) 2.5 mL, store at -20 °C
  - **PathogenDx Detect^{X}-Rv AMV Reverse Transcriptase** (PN 10010), 100 µL, store at -20 °C
  - **PathogenDx Detect^{X}-Rv RT-PCR Nuclease Free Water w/ dUTP** (PN 10003), 1.7 mL, store at -20 °C
  - **PathogenDx Detect^{X}-Rv Cod UNG** (PN 10025), 200 µL, store at -20 °C

- **PathogenDx Detect^{X}-Rv Primers** (PN 10055), 225 µL, store at -20 °C. For gene specific amplification of DNA during RT-PCR. Contains 2 (two) SARS-CoV-2 primer sets targeting each N1 and N2 region of SARS-CoV-2 nucleocapsid genes.
- **PathogenDx 96-Well Microarray Plate** (PN 10013), store at 18-30 °C. Each well has sequence-specific oligonucleotide probes attached to the surface labelled via a cyanine 5 (Cy5) co-print to facilitate accurate alignment for subsequent image analysis. Plates are provided with barcode numbers.
- **PathogenDx HYB Buffer 1** (PN 10011), 6.75 mL, store at 15-30 °C. For binding (hybridization) of PCR amplified DNA to sequence-specific microarray spots and washing of microarray to remove unbound fluorescence before analysis.
- **PathogenDx HYB Buffer 2** (PN 10012), 3.5 mL, store at -20 °C. For equilibration and hybridization of microarrays.
- **PathogenDx -HYB Molecular Biology Grade Water** (PN 10063), 160 mL, store at 15-30 °C. For equilibration and hybridization of microarrays and used in Plate Wash Buffer.
- **PathogenDx Detect^{X}-Rv External Positive Control** (PN 10007), 10 µL, store at -20 °C. Plasmid including the complete nucleocapsid gene from 2019-nCoV.

Equipment and Software

Materials Required (but not provided)

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

Equipment	Catalog #	Vendor
Sensovation Sensospot	Sensospot	Sensovation
Barcode Scanner	B00LE5VV1C	Amazon
MiniAmp Thermal Cycler	A37834	ThermoFisher Scientific
PCR Plate Spinner Centrifuge	C2000	Light Labs
Mini Centrifuge w/ 8 Place Tube Rotor and 4 Place PCR Strip Tube Rotor	C1601	Light Labs
Fisherbrand Analog Vortex Mixer	02-215-414	Fisher Scientific
MagStand	P1005	Zymo Research
Optical Film Compression Pad	43-126-39	Fisher Scientific
Plate-Sealing Paddle	2928-7355	USA Scientific
Augury™ Software (Version 4.6.11)	N/A	N/A
Lab Oven 10GC Gravity Convection Oven	10GC	Hogentogler
Plate Shaker S4P-D Four Plate Shaker (SP4-D) or equivalent	20P-S4P-D	Oxford BenchMate
Pipettors		
Fisherbrand Elite Pipettors 2 to 20 µL	FBE00020	Fisher Scientific
Fisherbrand Elite Pipettors 20 to 200 µL	FBE00200	Fisher Scientific
Fisherbrand Elite Pipettors 100 to 1000 µL	FBE01000	Fisher Scientific
Fisherbrand Elite Multichannel Pipette 1 to 10µL	FBE800010	Fisher Scientific
Fisherbrand Elite Multichannel Pipette 10 to 100µL	FBE800100	Fisher Scientific
Eppendorf Research Plus, 8-channel 120 – 1,200 µL	ES-8-1200	Pipette.com
Fisherbrand Multichannel 30-300ul	FBE800300	Fisher Scientific
Aspiration System		
Drummond Dispensing Manifold (Long)	EW-04396-07	Cole Parmer
Cole-Parmer Air diaphragm vacuum/pressure pump, 0.37 cfm, 115 VAC	EW-79202-00	Cole Parmer
Argos Technologies MiniVac Polystyrene Bottle with Transfer Cap, 1 L	SK-04396-17	Cole Parmer
Argos Technologies MiniVac Barbed Fitting Cap, Polypropylene, 45mm	SK-04396-15	Cole Parmer
Argos Technologies MiniVac Vacuum Tubing, Instrument to Bottle Lid, 700 mm	SK-04396-18	Cole Parmer

Consumables Required (but not provided)

Part Description	Vendor	Vendor Catalog No.
10ul barrier tips	USA Scientific	1121-3810
200ul barrier tips	USA Scientific	1120-8810
1000ul barrier tips	USA Scientific	1122-1832
5mL sterile tubes	Argos Tech	EW-04395-48
Disposable pipette basins	Fisher Scientific	13-681-501
50 mL conical polypropylene sterile tubes	Fisher Scientific	12-565-271
96 Well Deep Well Plate	Zymo Research	C2002
Cover Foil	Zymo Research	C2007
Microamp optical 96-well PCR plate	ThermoFisher	N8010560
Rnase Free Cleaning Reagent	Argos Technologies	04397-24
Nunc plate seal	Fisher Scientific	276014
15mL polypropylene sterile tubes	Thermo Fisher Scientific	12-565-268

Warnings and Precautions

- For *In Vitro* Diagnostic Use.
- For Use Under an Emergency Use Authorization (EUA) Only.
- For Prescription Use Only.
- This product has not been FDA cleared or approved, but has been authorized for emergency use by FDA under an EUA for use in laboratories certified under the Clinical Laboratory Improvement Amendments (CLIA) of 1988, 42 U.S.C. §263a, that meet requirements to perform high complexity tests.
- This product has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.
- This emergency use of this product is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug, and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated or authorization is revoked sooner.
- Laboratories within the United States and its territories are required to report all test results to the appropriate public health authorities.
- Handle all specimens as if infectious using safe laboratory procedures. Refer to Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with SARS-CoV-2 https://www.cdc.gov/coronavirus/2019-nCoV/lab-biosafety-guidelines.html
- Do not use kits or reagents after the expiration dates shown on kit labels.
- Do not use UTM-RT or UTM with Gelatin for collection of patient samples. DetectX-Rv is not compatible with transport media containing gelatin.
- **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 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:** Primers are light sensitive and must be stored away from light.

- **Caution:** Carefully inspect the storage conditions for all frozen reagents. Master Mix components and positive control must be stored at -20 ± 5˚C.
- **Caution:** PathogenDx microarrays are light and moisture sensitive and should be stored in the moisture barrier bag away from light.
- **Caution:** Buffer 1 and Buffer 2 may cause irritation upon contact. Always wear gloves and eye protection when handling these buffers.
  - Skin contact Rinse skin with water. Immediate medical attention is not required.
  - Eye contact Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
  - Ingestion Not expected to present a significant ingestion hazard under anticipated conditions of normal use. If you feel unwell, seek medical advice.
  - Inhalation Not expected to be an inhalation hazard under anticipated conditions of normal use of this material.
- **Caution:** In the post-hybridization/washing protocol, centrifuge speed for the microarray plate should not exceed 70 x g. Excessive force may cause the glass bottom of the plates to break.
- **Caution:** Refer to the Safety Data Sheets on the PathogenDx company website https://pathogendx.com/covid-19/.
- **Caution:** Kit components from different lot numbers should not be mixed.
- **Caution:** If there is no detectable Cy5 signal on the PathogenDx microarrays pre- or post-hybridization, either the plate may have been stored incorrectly or the plate reader settings are not correct. Please refer to the Troubleshooting Guide in this product insert and/or contact Technical Support.
- **Caution:** If the RT-PCR reaction did not amplify, the Master Mix or Reverse Transcriptase may have been stored incorrectly. Please refer to the Troubleshooting Guide in this product insert and/or contact Technical Support.

Storage Instructions

- **PathogenDx Detect^{X}-Rv One Step Reverse Transcription Kits** should be stored at -20 ± 5˚C Mix gently after thawing before use.
- **PathogenDx Detect^{X}-Rv Primers** should be stored at -20± 5˚C but must be protected from light. Mix gently after thawing before use.
- **PathogenDx Detect^{X}-Rv External Positive Control** should be stored at -20 ± 5˚C Mix gently after thawing before use.
- **PathogenDx Detect^{X}-Rv Cod UNG** should be stored at -20 ± 5˚C. Avoid freeze-thaws past six cycles. Mix gently before use.
- **PathogenDx Detect^{X}-Rv 96-Well Microarray Plate** is provided in a moisture barrier bag with desiccant. The plates should be stored in this manner to protect them from light and moisture. If only a partial plate was used, the plates should be stored in the provided case inside the moisture barrier bag if the remainder of the plate is to be used for testing additional patient samples. Store the microarray plate at room temperature.
- **PathogenDx HYB Buffer 1** should be stored at room temperature (15-30 ˚C).
- **PathogenDx HYB Buffer 2** should be stored at -20°C ± 5˚C Mix gently after thawing before use.
- **Ceres Wash Buffer** should be stored at room temperature (15-30 ˚C).
- **Ceres Lysis Buffer** should be stored at room temperature (15-30 ˚C).
- **Ceres Beads** should be stored at 4 °C

Work Areas and Best Practices

Detect^{X}-Rv Workflow to Minimize the possibility of amplicon contamination

Detect^{X}-Rv process requires four (4) dedicated areas, which are as follows:

- Sample Preparation and RT-PCR reaction set-up
- PCR Amplification
- Microarray Hybridization & Imaging

It is strongly recommended that each process be conducted in a dedicated negative pressure hood to minimize transfer of aerosols. Alternatively, processes 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 SARS-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 vortex mixers 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 products, tips, pipettes, 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.

RT-PCR reaction set-up area is dedicated to making RT-PCR Master mix before adding patient sample RNA and control templates to the reactions. Before taking reagents out from freezer to thaw, all the items including the bench, microcentrifuge rack, and all the pipettes must be surface cleaned with 10% bleach, then 70% ethanol. All the microcentrifuge tubes, pipette tips with aerosol barrier, and 96-well PCR plates must be RNase free.

PCR Amplification Area is dedicated to the RT-PCR steps of the Detect^{X}-Rv workflow. This area is solely dedicated to centrifuges for spinning down RT-PCR reaction in 96-well plates and to PCR machine. Laboratory coats, gloves, and equipment used in the PCR Amplification Area must remain in this area and never be allowed to enter the other areas.

- 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 reaction(s) are terminated, dispose of all commodities and reagents according to these instructions. 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 microarray hybridization and post-processing. Processes conducted in this area include the preparation of the microarray for hybridization, hybridization of the RT-PCR products to the Detect^{X}-Rv microarray plates, preparation of the microarray plates for imaging, and imaging of the 96-well plate in the Sensovation Imager.

- 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 or 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 Sample Preparation Area section above in these instructions. 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 PCR Amplification 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 reagents and commodities in 10% bleach and/or in a biohazard bin.
- 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 aerosols and cross-contamination

As with any test procedure, good laboratory practice is essential to the proper performance of this assay. Care should be taken to keep reagents and amplification mixtures free of contamination. The following precautions should be observed to minimize the risks of RNase contamination, sample contamination, and amplified DNA cross-contamination between samples:

- 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 materials and surfaces which may contain a high copy number of amplicons such as the post-PCR reactions plates and items in 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 use of extended aerosol barrier pipette tips is recommended. Care must also be taken when pipetting to avoid creating or introducing bubbles into reaction mixes.

- To prevent contamination of the pipette barrel, the length of the aerosol barrier pipette tip should be sufficient to reach the bottom of the reactions well and prevent contact of the pipette barrel with PCR surfaces. While pipetting, care should be taken to avoid touching the pipette barrel to the inside of the sample well or container.
- Change aerosol barrier pipette tips between ALL manual liquid transfers.
- The reagents in the **Ceres Viral Collection and Lysis Kit** are single use only. Use new reaction vessels, and newly opened reagents for every new Detect^{X}-Rv 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.

Specimen Collection, Storage, and Transport to the Test Site

Human nasopharyngeal (NP) swabs, oropharyngeal (OP) swabs, mid-turbinate swabs, anterior nasal swabs, nasal aspirates, nasopharyngeal wash/aspirates and bronchoalveolar lavage (BAL) specimens may be used with the Detect^{X}-Rv. Refer to the CDC Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Persons Under Investigation (PUIs) for Coronavirus Disease 2019 (COVID-19).

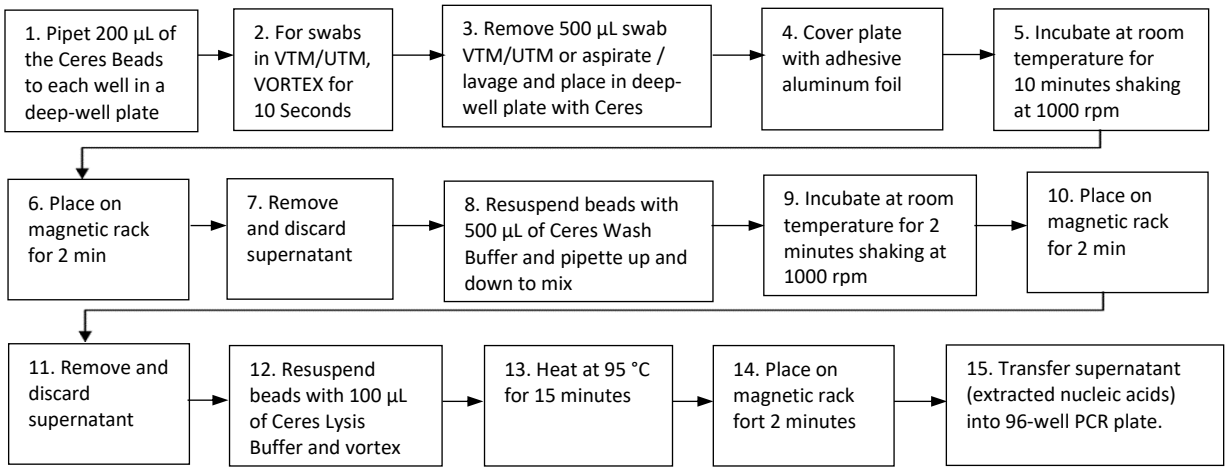
CAUTION – DO NOT USE UTM-RT or UTM with Gelatin for collection of patient samples. Detect^{X}-Rv is not compatible with transport media containing gelatin.

Store specimens at 2-8°C for up to 72 hours after collection. If a delay in testing or shipping is expected, store specimens at -70°C or below.

ASSAY PROCEDURE

Sample Preparation

Extract Viral RNA and Nucleic Acids from Patient Sample Using Ceres beads

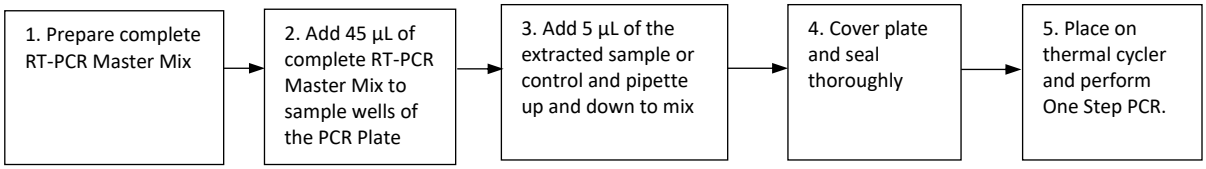


1. The nasopharyngeal (NP), oropharyngeal (OP), mid-turbinate and anterior nasal swabs should be received in VTM or UTM (minimum 500 µL). Vortex swabs in VTM or UTM for 10 seconds to dislodge the viral particles from the swab material.
2. Nasal aspirates, nasopharyngeal wash/aspirates and bronchoalveolar lavage (BAL) samples should be vortexed for 10 seconds.
3. Set up/label a 96-well deep-well plate (a deep well plate that can hold between 1.5 -2.5 mL per well) to test all patient samples and include 2 wells for the external Negative (no template) and Positive Controls. External controls must give expected results or the test will be interpreted as Invalid.
4. Vortex and/or invert repeatedly to mix the Ceres Beads to ensure that the beads are uniformly resuspended prior to use.
5. Add 200 µl of the Ceres Beads to the deep well plate to each well needed prior to the addition of patient samples or the two controls.
6. For swabs transported in VTM, vortex the patient sample for 10 seconds to help release viral particles from the swab. Vortex wash, aspirates or lavage samples for 10 seconds. Remove 500 µL of the patient sample and place each one in one well of the wells containing the Ceres beads.
7. Seal the plate with an adhesive aluminum foil cover. Use the paddle to ensure each well is thoroughly sealed.
8. Place the sealed plate on the plate shaker and incubate the patient samples with Ceres Beads at room temperature for 10 minutes, shaking at 1000 RPM.
9. Place the plate in the magnetic rack for 2 minutes to separate the Ceres Beads from the transport media.
10. Carefully remove and discard all of the transport media supernatant without disturbing the bead pellet
11. Add 500 µL of Ceres Wash Buffer to wash the pelleted magnetic beads.
12. Seal the plate with an adhesive aluminum foil cover.
13. Place the sealed plate on the plate shaker and shake the plate for 2 minutes at 1000 RPM

(alternatively, if isolating a small number of samples, the Ceres beads may be resuspended by gently pipetting the sample up and down).

14. Place the plate in the magnetic rack for 2 minutes to separate the Ceres Beads from the Ceres wash buffer.
15. Carefully remove and discard all of the Ceres wash buffer supernatant without disturbing the pellet. This can be performed by placing the pipette tip along the wall opposite the bead pellet and sliding down the well to the bottom surface, then slowly aspirating the supernatant into the pipette tip.
16. Resuspend the pellet in 100 µl of Ceres Lysis Buffer. Pipette up and down to mix thoroughly or until the bead pellet has been completely dispersed.
17. Seal the plate with an adhesive aluminum foil cover.
18. Place the sealed plate on the plate shaker and mix the sample thoroughly by shaking at 1000 RPM for 2 minutes.
19. Heat the samples at 95°C for 15 minutes (This is a critical step for viral RNA elution and collection. Ensure that your plate is being evenly heated).
20. Place the plate in the magnetic rack for 2 minutes to separate the Ceres Beads from the lysis buffer which contains the viral and patient nucleic acids.
21. Remove the supernatant (about 100 µL) and transfer to a clean tube/plate for storage; the patient sample extract is now ready for RT-PCR.
22. Once complete the extracted nucleic acids can be used immediately.

Assemble and Perform One Step RT-PCR Using Detect^{X}-Rv



The following procedure is used to prepare the RT-PCR reaction and then perform three sequential steps in the single plate in a PCR machine with no intervention. In Step 1 the UNG enzyme is activated. Step 2 performs reverse transcription and converts the RNA in the extracted nucleic acids into DNA. Step 3 is the end-point PCR step which uses gene-specific end-labeled primers provided in the Detect^{X}-Rv Kit to perform an asymmetric PCR amplification resulting in the generation of fluorescently labeled probe directly ready for hybridization to the microarray.

1. Thaw (as necessary), mix, and briefly centrifuge each component shown in **Table 1** before use. AMV and Cod UNG are enzymes and should be stored at -20^{o}C or on ice until they are ready to be added to the RT-PCR Master Mix
2. Determine the number of tests being performed. Each run should include two (2) additional tests: a 'No Template Control' and a 'Positive Control'.
3. Combine the components into a complete reaction master mix. If testing multiple samples, multiply the volumes shown in **Table 1** by the number of tests being performed (be sure to include an extra reaction volume to account for variance when performing multiple pipetting steps out from a common source). Remember to include the two (2) additional control test samples**:**

Table 1. Prepare PathogenDx Detect^{X}-Rv Complete RT-PCR Reaction Master Mix.

Component	Volume (µL) per RT-PCR Reaction
PathogenDx Detect^{X}-Rv RT-PCR Master Mix 2X	**25 µL**
PathogenDx Detect^{X}-Rv Primers 25X	**2 µL**
PathogenDx Detect^{X}-Rv AMV Reverse Transcriptase (5 Units/µL)	**1 µL**
PathogenDx Detect^{X}-Rv RT-PCR Nuclease Free Water (containing dUTP (2.67 mM))	**15 µL**
Cod UNG (1 Unit/µL)	**2 µL**
Total volume of Complete RT-PCR Reaction Master Mix (per test sample)	**45 µL**

4. After preparing the Complete Reaction Master Mix, invert the tube several times to ensure that the contents are mixed and briefly centrifuge to collect the liquid at the bottom of the tube.
5. Referring to Table 2, dispense 45 µL of the Complete Reaction Master Mix to each well that is required for testing.
6. Carefully add 5 µL of the extracted patient nucleic acid samples or control template individually to each designated well and pipette up and down gently to mix.

CAUTION: AVOID CREATING AIR BUBBLES
CAUTION: ALWAYS CHANGE THE PIPETTE TIP BETWEEN EACH SAMPLE ADDITION

Table 2. Add extracted nucleic acids (or controls) to Complete RT-PCR Reaction Master Mix.

Component	Volume (µL) per RT-PCR Reaction
Total volume of Complete RT-PCR Reaction Master Mix (per test sample)	**45 µL**
Extracted patient nucleic acid sample or control sample volume (added individually to each well AFTER dispensing Complete RT-PCR Reaction Master Mix into each well needed)	**5 µL**
Final RT-PCR Volume	**50 µL**

7. Cover the PCR plate with clear adhesive film and seal.
8. Centrifuge the plate in a plate spinner for 30 seconds.
9. Place the plate in the thermal cycler and cover with the optical film compression pad before closing the thermal cycler cover.
10. Program the PCR machine to perform the UNG treatment, Reverse Transcription, and PCR cycling in a single program as shown in **Table 3**:

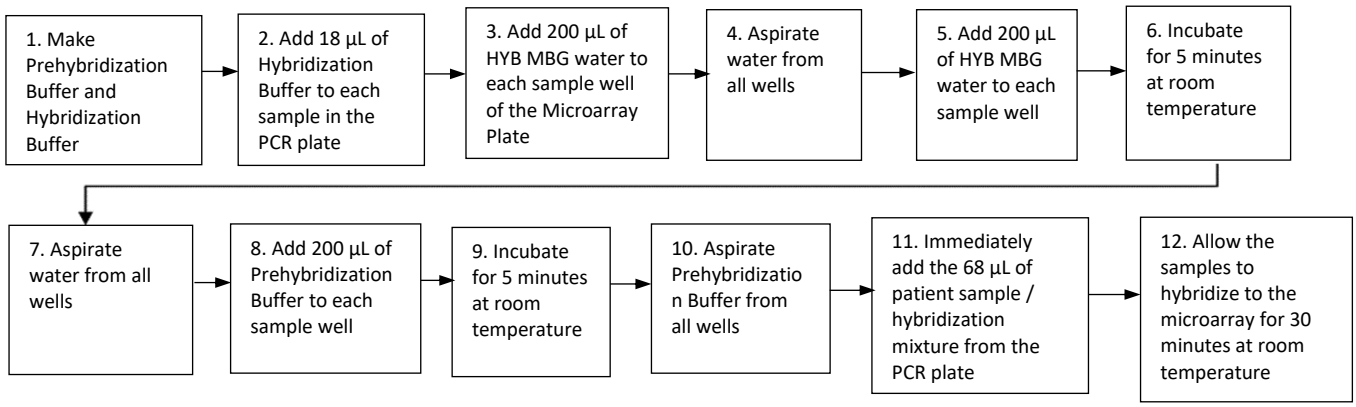
Table 3. Detect^{X}-Rv Reaction Conditions.

UNG/RT-PCR Reaction Steps		Temperature	Time	Cycle Number
UNG Treatment	UNG treatment	25ºC	5 min	1x
First Strand cDNA Synthesis	Reverse Transcription	55 ºC	20 min	1x
	Initial denaturation	94 ºC	2 min	1x
PCR Amplification	Denaturation	94 ºC	30 sec	40x
	Annealing	55 ºC	30 sec	
	Extension	68 ºC	30 sec	
	Final Extension	68 ºC	7 min	1x
	Hold	4 ºC	∞	

11. After the thermal cycling is complete, the amplified PCR product may be used directly to hybridize to the DNA microarray.

Hybridize PCR Amplified Product to Microarray

After each step be sure to cover the microarray plate with a low lint nonabrasive towel.



GENERAL GUIDELINES TO FOLLOW FOR THE HYBRIDIZATION AND WASHING STEPS:

CAUTION: When pipetting with the (multichannel) pipette into the 96-well microarray plate, only dispense to the first stop. Do not depress the (multichannel) pipette to the second stop, or full evacuation of the tips, to avoid creating air bubbles which may create aerosols that can result in cross-contamination.

CAUTION: when pipetting with the (multichannel) pipette into the 96-well microarray plate, avoid contact of the tip(s) with the bottom of the well since the bottom of the well contains the printed microarray. Perform all actions using the (multichannel) pipette directed to the sidewall or corner of the well near the bottom.

CAUTION: Avoid contact with the entire bottom surface (outside glass plate) of the microarray 96-well plate during processing as this may adversely affect the clarity of the image acquisition. Use only the 96-well plate edges or barcoded area for handling.

Prepare the 96-well microarray plate for hybridization

1. Thaw HYB Buffer 2 at room temperature and gently mix to ensure uniformity.
2. Based on the number of samples being tested, prepare the Pre-hybridization Buffer and Hybridization Buffers in clean tubes for the number of microarray wells that will be used for hybridization as shown in **Tables 4 and 5**. Vortex briefly to mix.

*Table 4: 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**
PathogenDx HYB Molecular biology (MB) grade water (mL)	5.587	11.174	16.761
PathogenDx HYB Buffer 1 (mL)	1.658	3.317	4.976
PathogenDx HYB Buffer 2 (mL)	0.872	1.745	2.618

**Indicated volumes include 20% extra volume to account for pipetting variance.*

*Table 5: 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**
PathogenDx HYB Buffer 1 (mL)	0.463	0.926	1.390
PathogenDx HYB Buffer 2 (mL)	0.233	0.466	0.700

**Indicated volumes include 20% extra volume to account for pipetting variance.*

3. Place a low lint nonabrasive paper towel on table and position the microarray plate on top of the paper towel.
4. Dispense 200 µL of PathogenDx Detect^{X}-Rv Molecular Biology Grade Water to each well of the 96-well microarray plate that is needed for testing. Avoid contact of the pipette tip(s) with the bottom of the well. Cover unused wells with a seal.
5. Aspirate the water from each well by directing the tip(s) into the corner of the well. All aspiration steps should be carefully performed in this manner.

   NOTE: If using the vacuum manifold, the tips should be directed to the bottom left (or right) corners of the wells. All aspirations should be performed in the same manner.
6. Dispense 200 µL of PathogenDx Detect^{X}-Rv Molecular Biology Grade Water to each well of the 96-well microarray that is needed for testing and allow 5 minutes before aspirating water from each well. Place the low lint nonabrasive paper towel on top of the plate.
7. Remove the low lint nonabrasive paper towel to aspirate the water from each well.
8. Add 200 µL of Pre-hybridization Buffer prepared according to Table 4 to each well of the 96-well microarray that is needed for testing without touching the pipette tip to the array surface.
9. Place the low lint nonabrasive paper towel on top of the plate. Allow Pre-hybridization Buffer to stay on the arrays for 5 minutes while performing steps 10 through 12 below.

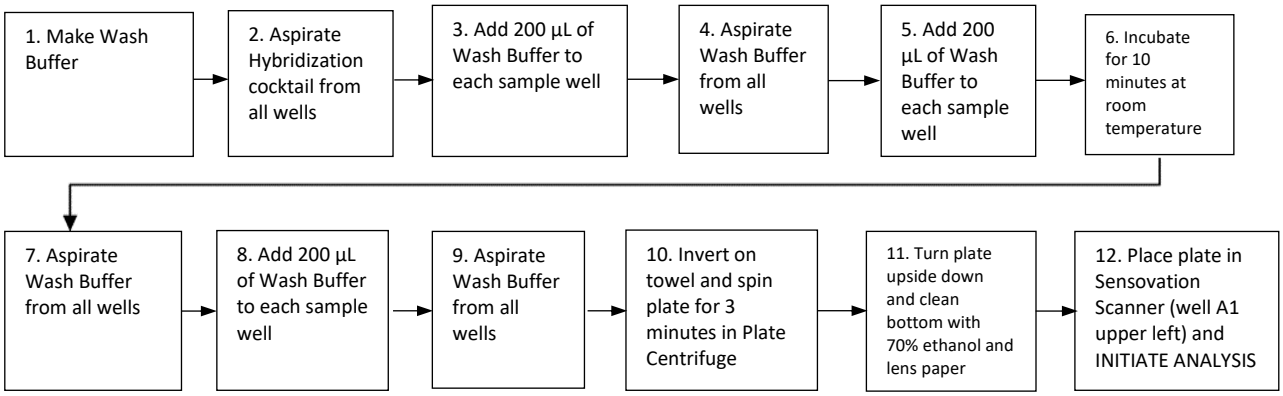
Hybridize the PCR amplified product to the microarrays.

CAUTION: During the following hybridization and wash steps, do not allow the microarray wells used for testing to air dry.

CAUTION: Avoid contacting the microarray surface with the pipette tip(s).

10. Briefly centrifuge the PCR plate containing the amplified PCR product for 20 seconds to collect the samples in the bottom of the wells.
11. Add 18 µL of Hybridization buffer prepared according to Table 5 to each well of amplified PCR product and gently pipette up and down to mix. Avoid creating bubbles or aerosols to reduce the likelihood of cross-contamination during this step. The amplified PCR product (50 µL) mixed with the PathogenDx Detect^{X}-Rv HYB Buffer 1 (18 µL) constitute the Hybridization Cocktail (68 µL).
12. Remove the low lint nonabrasive paper towel. Aspirate Pre-hybridization Buffer (from Step 8) from the wells in the microarray plate.
13. Immediately add 68 µL of the Hybridization Cocktail to each well of the 96-well microarray being tested. Be careful not to touch the array surface (bottom of the well) with the pipette tip. Ensure that the sample ID and location in the 96-well plate are recorded.
14. Place the low lint nonabrasive paper towel on the array to prevent dust from entering the wells. Allow the amplified product to hybridize to the microarray for 30 minutes at room temperature.

Post-Hybridization Wash of Microarray



NOTE: Washing must be performed according to the protocol to ensure robust signal-to-noise. Adequate washing also reduces elevated background fluorescent signals.

CAUTION: During the following wash steps, do not allow the microarray wells used for testing to air dry until all washing steps are completed.

CAUTION: Avoid contacting the microarray surface with the pipette tip(s).

1. Prepare Plate Wash Buffer (diluted HYB Buffer 1) according to the number of wells used. (Table 6).

Table 6: Reagent volumes for preparation of Plate Wash Buffer

96-Well Plate	**Volumes corresponding to the number of test wells being washed in the plate***		
	32-Wells	**64-Wells**	**96-Wells**
PathogenDx HYB Buffer 1 (mL)	0.333	0.667	1.0
PathogenDx Detect^{X}-Rv Molecular Grade Water (mL)	46.0	93.0	139.0

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

2. Aspirate Hybridization Cocktail from the microarray wells used for testing.
3. Add 200 µL of Plate Wash Buffer to each well used for testing.
4. Aspirate the Plate Wash Buffer.
5. Add 200 µL of Plate Wash Buffer to each well used for testing.
6. Allow buffer to remain in the wells for 10 minutes at room temperature.
7. Aspirate the Plate Wash Buffer.
8. Perform a final wash by dispensing 200 µL of Plate Wash Buffer to each well used for testing.
9. Aspirate the Plate Wash Buffer.
10. **Place the 96-well plate face down, over a paper towel, into the Laboratory Micro Array Plate Centrifuge and spin for 3 minutes to remove residual Plate Wash Buffer from the bottom of the wells.**
11. Proceed immediately to prepare the plate for scanning by turning plate upside down and spraying the glass underside with 70% ethanol and wipe dry with lens tissue.
12. Visually inspect the plate and remove any dust particles with the lens tissue before scanning. Do not place plate down after cleaning.

    NOTE: PLATES ARE LIGHT SENSITIVE. If not scanned immediately, Detect^{X}-Rv microarray plates should be placed back into a moisture barrier bag until scanning may be performed to protect the plates from light.

Scanning the Microarray and Data Acquisition

1. Access the Sensovation scanner desktop, select the application “Array Reader”.
2. Open the tray, select “Open Tray”.
3. Place the microarray into the tray oriented with the plate face up and aligned with A1 in the top left corner.
4. Close the tray, select “Close Tray”.
5. Select “Scan”.
6. From the dropdown menu for “Rack Layout” select the Full Plate (96 wells) PDx.
7. From the dropdown menu for assay layout, select “PathogenDx Assay 002.
8. Click on the three dots icon to the right of “Scan Position”.
9. To scan a full plate, double click the asterisk at the top left of the plate image.
10. To scan a partial plate, click the desired wells or click on the column number.

    Note: *All other information on this screen is pre-programmed – do not alter.*
11. Select the Blue Arrow to begin the scanning process.

12. While the plate is being scanned, select “Result Overview” to review the images of the wells.
13. When the plate is finished scanning and the screen displays the digital image of a plate with all green wells, select the Red X to exit the scanning process.
14. Open the tray, select “Open Tray”.
15. Remove the microarray and store inside the moisture barrier bag with the desiccant packets.
16. Close the tray, select “Close Tray”.
17. Exit the Array Reader application, select “Exit”.
18. On the Sensovation Scanner desktop, select the folder “Scan Results”.
19. Locate the folder associated with your plate and rename the folder with the plate barcode number by scanning the barcode located either on the outside of the barrier bag or on the plate itself.
    a. If a full plate was scanned, rename the scan file to reflect the plate barcode. For example, rename “ScanJob-191108130334_1” to “7024001001”.
    b. If a partial plate was scanned, add the wells scanned to the end of the barcode. For example, if the first two columns were scanned rename “ScanJob-191108130334_1” to “7024001001.well001-well016”.
20. Submit the whole barcode labeled folder to the “C:\Augury_Files\ImageFolders\” on the laboratory computer loaded with Augury.
21. Once the folder is submitted, the Augury™ Software will analyze the data and directly deposit the reports into the “C:\Augury_Files\Reports\” folder on the laboratory computer loaded with Augury™.

Quality Control and Validity of Results

External and internal test controls are examined prior to interpretation of patient results. If the controls are not valid, the patient results cannot be interpreted. Validation of the results is performed automatically by the Augury™ Software based on the performance of the external controls run on each plate and the internal positive and negative controls included in the arrays of each well.

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

The External Positive Control ensures that the complete process including the extraction and amplification of the nucleic acids was successful.

The No Template Control (NTC) ensures that there were no systematic errors introduced during the isolation and amplification of the nucleic acids and that no contamination has occurred. If hybridization signal is detected in the NTC, new reagents should be used.

Interpretation of Patient Results

Table 7. Interpretation of Patient Results

N1 SARS-CoV-2	N2 SARS-CoV-2	RNase P	Status	Result	Action
POS	POS	POS or NEG	Valid	SARS-CoV-2 Positive	Report results to a healthcare provider and appropriate health authorities.
POS	NEG	POS or NEG	Valid	SARS-CoV-2 Positive	Report results to a healthcare provider and appropriate health authorities.
NEG	POS	POS or NEG	Valid	SARS-CoV-2 Positive	Report results to a healthcare provider and appropriate health authorities.
NEG	NEG	POS	Valid	SARS-CoV-2 Negative	Report results to healthcare providers and appropriate health authorities.
NEG	NEG	NEG	Invalid[1]	N/A	Repeat Test. If the repeat results remain invalid, consider collecting a new specimen.

[1] Samples with a result of SARS-CoV-2 invalid results should be retested.

For Invalid results, following steps should be taken:

For First repeat: Start procedure at under step 1 of **Assemble and perform one step RT-PCR**
If second repeat is required: Start procedure from step 1 under **Sample preparation: Extraction Viral RNA and nucleic acids from patient sample using the Ceres Viral Collection and Lysis Kit**

Limitations of The Procedure

- This assay is for *in vitro* diagnostic use under FDA Emergency Use Authorization (EUA) only.
- For use only by personnel trained in the techniques of RT-PCR, microarray hybridization and *in vitro* diagnostic procedures. A thorough understanding of the instructions for use is necessary for successful use of the product. Reliable results will only be obtained by using precise laboratory techniques and accurately following these instructions for use.
- Laboratories are required to report all results to the appropriate public health authorities.
- Performance of Detect^{X}-Rv has only been established in nasopharyngeal swab specimens. Use of the Detect^{X}-Rv with other specimen types has not been assessed and performance characteristics are unknown.
- 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.
- The clinical performance has not been established in all circulating variants but is anticipated to be reflective of the prevalent variants in circulation at the time and location of the clinical evaluation. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of SARS-CoV-2 and their prevalence, which change over time.
- As with any molecular test, mutations within the target regions of SARS-CoV-2 could affect primer and/or probe binding resulting in failure to detect the presence of virus.
- The primers and the Detect^{X}-Rv plates are light sensitive and should be protected to avoid photobleaching.
- All equipment should be properly calibrated according to manufacturer's guidelines.
- The performance of this test has not been established for monitoring treatment of SARS- CoV-2 infection.
- The performance of this test has not been established for screening of blood or blood product for the presence of SARS-CoV-2.
- Based on the *in-silico* analysis, SARS-related CoV may cross-react with the primers/probes of Detect^{X}-Rv. The presence of other coronaviruses in the *Sarbecovirus* subgenus may cause false positive results. None of these other coronaviruses is known to currently circulate in the human population.
- This test cannot rule out diseases caused by other bacterial or viral pathogens.
- The effect on the test performance of administration of intranasal influenza vaccines (e.g., FluMist) has not been evaluated

Conditions of Authorization for Laboratories

The PathogenDx Detect^{X}-Rv Assay 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/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/in-vitro-diagnostics-euas. 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 Detect^{X}-Rv must 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 Detect^{X}-Rv must 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 Detect^{X}-Rv must notify the relevant public health authorities of their intent to run your product prior to initiating testing.
- Authorized laboratories using Detect^{X}-Rv must have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.

- Authorized laboratories must collect information on the performance of Detect^{X}-Rv and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUAReporting@fda.hhs.gov) and PathogenDx, Inc. (via email: IVDsupport@PathogenDx.com) for any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of which they become aware.
- All laboratory personnel using Detect^{X}-Rv must be appropriately trained in RT-PCR techniques and use appropriate laboratory and personal protective equipment when handling and using this kit in accordance with the authorized labeling.
- PathogenDx, Inc., authorized distributors, and authorized laboratories using your product must 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, that meet the requirements to perform high complexity tests" as "authorized laboratories".

Performance Characteristics

Detect^{X}-Rv – Limit of Detect (LoD) – Analytical Sensitivity:

The LoD studies establish the lowest SARS-CoV-2 viral concentration (copies per mL) that can be detected by the Detect^{X}-Rv in a specimen at least 95% of the time. The initial LoD was determined by testing six input concentrations (8000, 4000, 2000, 1000, 500 and 250 copies per mL) of gamma irradiated SARS-CoV-2 (BEI Resources, NR-52287, 1.16 x 10^{9} copies/mL) spiked into nasopharyngeal swab matrix in VTM (Table 8).

Table 8: Preliminary LoD Testing

Input Concentration	SARS-CoV-2 N1	SARS-CoV-2 N2	Positive Rate	% Positive
8000 cp/mL*	6/6	6/6	6/6	100%
4000 cp/mL	6/6	6/6	6/6	100%
2000 cp/mL	6/6	5/6	6/6	100%
1000 cp/mL	6/6	1/6	6/6	100%
500 cp/mL	4/6	0/6	4/6	66%
250 cp/mL	6/6	1/6	6/6	100%

** copies/mL*

To determine the final LoD an additional 20 samples at 250, 500 and 1,000 copies/mL were tested (Table 9). The final assay LoD was confirmed at 1,000 copies/mL.

Table 9: LoD Confirmation Testing

Input Concentration	SARS-CoV-2 N1	SARS-CoV-2 N2
1,000 cp/mL*	19/20 = 95%	9/20 = 45%

** copies/mL.*

Reactivity/ Inclusivity

All primer sets designed for the detection of the N1 and N2 were analyzed *in silico* against the complete available SARS-CoV-2 genome sequence as of March 12, 2021, (437,987 GISAID sequences) and March 9, 2021 (94,117 Genbank sequences). With the exception of the UK Clade Variant B.1.1.7, the analysis (Table 10) demonstrated that the regions recognized by the Detect^{X}-Rv primers and probes have 100% homology with >99% of all available SARS-CoV-2 sequences from the Global Initiative on Sharing Avian Influenza Data (GISAID) databases/databanks. Of the remaining <1%, none showed greater than a single base change in either the N1 or N2 primer binding region. All the single changes were positioned at least 5 bases from the 3' (initiation) terminus, which should not produce a measurable change in PCR efficiency. The UK Clade Variant B.1.1.7 includes a single base change in the 5' region of the N1 target, which is not expected to affect PCR efficiency.

Table 10: Percentage of primer and probe sequences for SARS-CoV-2 N1 and N2 with 100% complementarity

Database	Identity to N1		Identity to N2	
	Primers (%)	Probes (%)	Primers (%)	Probes (%)
GISAID	>75.6%*	>99.8%	>99.1%	>99.8%
Genbank	99.1%	99.9%	99.8%	99.1%

** The UK B.1.1.7 variant sequences represent 24.03% of the GISAID database as of March 12, 2021*

Cross-reactivity (Analytical Specificity)

Cross-reactivity of the Detect^X-Rv primers and probes was evaluated by both *in silico* analysis (Table 11) and by testing pooled analytes (Exact Diagnostics RP Positive Run Control – RPPOS and Integrated DNA Technologies plasmid encoding nucleocapsid gene from bat SARS-CoV) directly in the RT-PCR and hybridization steps (Table 12).

Table 11: Microorganisms tested for in silico Exclusivity Analysis

Microorganism	*In silico* Analysis for % Identity target: N1 Probe	*In silico* Analysis for % Identity target: N2 Probe
Adenovirus (taxid:1643649)	< 80% homology	< 80% homology
Bordetella pertussis (taxid:520)	< 80% homology	< 80% homology
Candida albicans (taxid:5476)	< 80% homology	< 80% homology
Chlamydia pneumonia (taxid:83558)	< 80% homology	< 80% homology
Enterovirus (taxid:12059)	< 80% homology	< 80% homology
Haemophilus influenza (taxid:157239)	< 80% homology	< 80% homology
SARS-related CoV (taxid:694009)*	94%	82%
Human coronavirus 229E (taxid:11137)	< 80% homology	< 80% homology
Human coronavirus HKU1 (taxid:290028)	< 80% homology	< 80% homology
Human coronavirus NL63 (taxid:277944)	< 80% homology	< 80% homology
Human coronavirus OC43 (taxid:31631)	< 80% homology	< 80% homology
Human genome (taxid:9606)	82%	94%
Human Metapneumovirus (taxid:162145)	< 80% homology	< 80% homology
Human parainfluenza virus 2 (taxid:11214)	< 80% homology	< 80% homology
Influenza A (taxid:11320)	< 80% homology	< 80% homology
Influenza B (taxid:11520	< 80% homology	< 80% homology
Legionella pneumophila (taxid:446)	< 80% homology	82%
MERS-coronavirus (taxid:1335626)	< 80% homology	< 80% homology
Mycobacterium tuberculosis (taxid:1773)	< 80% homology	< 80% homology
Mycoplasma pneumonia (taxid:2104)	< 80% homology	< 80% homology
Parainfluenza virus 1 (taxid:11210)	< 80% homology	< 80% homology
Parainfluenza virus 3 (taxid:11216)	< 80% homology	< 80% homology
Parainfluenza virus 4a (taxid:11224)	< 80% homology	< 80% homology
Parainfluenza virus 4a (taxid:1124)	< 80% homology	< 80% homology
Parainfluenza virus 4b (taxid:11226)	< 80% homology	< 80% homology
Parainfluenza virus 4b (taxid:1126)	< 80% homology	< 80% homology
Pneumocystis jirovecii (taxid:42068)	< 80% homology	< 80% homology
Pseudomonas aeruginosa (taxid:287)	82%	< 80% homology
Respiratory syncytial virus (taxid:11250)	< 80% homology	< 80% homology
Rhinovirus (taxid:12059)	< 80% homology	< 80% homology
Staphylococcus epidermis (taxid:1282)	< 80% homology	< 80% homology
Streptococcus pneumonia (taxid:1313)	< 80% homology	82%
Streptococcus pyogenes (taxid:1314)	< 80% homology	< 80% homology
Streptococcus salivarius (taxid:1304)	< 80% homology	< 80% homology

**Only SARS-related CoV showed cross reactivity for both the N1 and N2 primers and is expected to produce positive results in Detect^X-Rv (see Limitations). Neither the N1 of N2 primers showed cross-reactivity with any other microorganisms.*

Four potentially cross-reactive probe sequences were revealed by *in silico* analysis: SARS-related CoV (tax id:694009), human genome (taxid:9606), *Legionella pneumophila* (taxid:446) and *Pseudomonas aeruginosa* (taxid:287). Based on in silico analysis, only SARS-related CoV (taxid:694009) may cross-react with the Detect^{X}-Rv SARS-CoV-2 primers/probes due to high homology with N2 primers/probes. The risk that any of the remaining three candidates will cross-react is negligible due to low homology (<80%) of N1 and N2 primers. The probes are present only in the microarray and will only hybridize to products amplified during the RT-PCR step.

Wet testing was performed by adding 5 µL the Exact Biosciences RPPOS panel (with Bat SARS-CoV added) directly to 45 µL of the Master Mix in the PCR step to ensure that the full complement of microorganisms was present for all the analytical steps. The panel contained all the microorganisms listed in Table 12 and was run in 15 replicate wells from the RT-PCR step through the Hybridization and analysis steps.

Table 12. Laboratory Tested Cross Reactivity Analysis

Analyte	Manufacturing Target Concentrations	Results	Final Result
Adenovirus Type 3 strain GB.	500,000 cp/mL[1]	0/15	Negative
Bordetella parapertussis	5,000 cp/mL	0/15	Negative
Bordetella pertussis	500,000 cp/mL	0/15	Negative
Chlamydophila pneumoniae	1,000 cp/mL	0/15	Negative
Human Coronavirus 229E	500,000 cp/mL	0/15	Negative
Human Coronavirus HKU1 (RNA IVT) [2]	20,000,000 cp/mL	0/15	Negative
Human Coronavirus NL63 (RNA IVT)	2,000,000,000 cp/mL	0/15	Negative
Human Coronavirus OC43	25,000 cp/mL	0/15	Negative
Human metapneumovirus (RNA IVT)	20,000,000 cp/mL	0/15	Negative
Influenza A H1N1 (RNA IVT)	100,000 cp/mL	0/15	Negative
Influenza A H1N1-09	500,000,000 cp/mL	0/15	Negative
Influenza A H3N2 (RNA IVT)	500,000,000 cp/mL	0/15	Negative
Influenza B	500,000 cp/mL	0/15	Negative
Mycoplasma pneumoniae	1,000 cp/mL	0/15	Negative
Parainfluenza 1	50,000 cp/mL	0/15	Negative
Parainfluenza 2	5,000 cp/mL	0/15	Negative
Parainfluenza 3	5,000 cp/mL	0/15	Negative
Parainfluenza 4a	500,000 cp/mL	0/15	Negative
Rhinovirus 1A	500,000 cp/mL	0/15	Negative
RSV A	10,000 cp/mL	0/15	Negative
RSV B	10,000 cp/mL	0/15	Negative
MERS-coronavirus (RNA IVT)	5,000,000 cp/mL	0/15	Negative
Bat SARS-CoV[3]	200,000,000 cp/mL	0/15	Negative

[1]cp/mL = copies/mL. [2](RNA IVT) = samples consisting of in vitro transcribed RNA.

[3]SARS-CoV Control Plasmid, IDT Ref # 10006624: nucleocapsid gene from bat SARS-like coronavirus isolate bat-SL-CoVZC45, (GenBank: MG772933.1)

From the wet testing summarized in Table 12 it can be concluded that there is minimal risk of cross reactivity with common pathogens tested.

Interfering Substances

Interfering substances which could be found in the respiratory samples endogenously or exogenously were tested to evaluate potential cross-reactivity or interference with the detection of SARS-CoV-2 in the Detect^{X}-Rv. Potential interfering substances in the concentrations listed below (Table 13) were spiked into negative nasopharyngeal swab clinical matrix in the absence or presence (3x LoD) of SARS-CoV-2 (BEI Resources, NR-52287) and tested in triplicate. None of the tested substances demonstrated cross-reactivity or interference at concentrations tested (Table 13).

Table 13. Interfering Substances

Substance	Active Ingredients	Concentration of Interfering Substance	No SARS-CoV-2 (Negative/Total= % Agreement)	3X LOD SARS-CoV-2 (Positive/Total= % Agreement)
Nasal Spray	Sodium Chloride 0.65%, Disodium Phosphate, Phenylcarbinol, Monosodium Phosphate, Benzalkonium Chloride	15% v/v	3/3 = 100%	3/3 = 100%
ACT Dry Mouth Lozenges	Isomalt, Xylitol, Glycerin	3 mg/mL	3/3 = 100%	3/3 = 100%
Nasal Allergy Relief	Fluticasone propionate (glucocorticoid)	5% v/v	3/3 = 100%	3/3 = 100%
Nasal Spray	Oxymetazoline hydrochloride	5% v/v	3/3 = 100%	3/3 = 100%
Petroleum Jelly	Petroleum Jelly	1% w/v	3/3 = 100%	3/3 = 100%
Nicotine	Nicotine Polacrilex Lozenge	0.03 mg/mL	3/3 = 100%	3/3 = 100%
Sore Throat Phenol	Phenol	5% v/v	3/3 = 100%	3/3 = 100%
NyQuil	Acetaminophen, Doxylamine succinate, Dextromethorphan HBr	5% v/v	3/3 = 100%	3/3 = 100%
Listerine Mouthwash	Eucalyptol, Menthol, Methyl Salicylate, Thymol	5% v/v	3/3 = 100%	3/3 = 100%
Zinc Tablets	Zinc	5% v/v	3/3 = 100%	3/3 = 100%
Hall Relief Cough Drops	Menthol	5% v/v	3/3 = 100%	3/3 = 100%
Emergen-C	Zinc, Magnesium, Riboflavin, Vitamin C	5% v/v	3/3 = 100%	3/3 = 100%
Human Genomic DNA	Double Stranded Genomic DNA	10 ng/mL	3/3 = 100%	3/3 = 100%
Mucin, bovine submaxillary gland	Mucin, Bovine Submaxillary Gland	2.5 mg/mL	3/3 = 100%	3/3 = 100%
Whole Human Blood	N/A	1%	3/3 = 100%	3/3 = 100%
Tobramycin	Tobramycin	4 µg/mL	3/3 = 100%	3/3 = 100%
Mupirocin	Mupirocin	2% w/v	3/3 = 100%	3/3 = 100%
Oseltamivir	Oseltamivir	3.3 mg/mL	3/3 = 100%	3/3 = 100%
Nasal Corticosteroids Homeopathic Allergy Medicine	Triamcinolone Acetonide Nasal Spray, 55 mcg per spray	5% v/v	3/3 = 100%	3/3 = 100%

Nasal influenza vaccines (e.g. FluMist) were not evaluated.

Detect^{X}-Rv– Clinical Evaluation:

A clinical validation was performed to evaluate the performance of Detect^{X}-Rv. A total of 41 positive and 37 negative prospectively collected leftover nasopharyngeal (NP) swab in transport media samples were tested by a high sensitivity FDA authorized reference assay. The samples were then tested in a blinded fashion on the Detect^{X}-Rv. One positive sample returned an invalid result and there was insufficient sample available for retesting, leaving 40 positive samples with both reference and candidate results. Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) were determined by comparing the results of Detect^{X}-Rv relative to the expected results. Results of the clinical NP swab specimens are shown below. The PPA was 95.0% (95% CI: 83.5% - 98.6%) and NPA was 100% (95% CI: 90.6% - 100%). The results are summarized in Table 14.

Table 14. Detect^{X}-Rv Performance Results

Detect^{X}-Rv Clinical Performance	FDA authorized molecular assay		
	Positive	Negative	Total
Positive	38	0	38
Negative	2	37	39
Total	40*	37	77
Positive Percent Agreement (PPA)	95.0% (95% CI: 83.5% - 98.6%)		
Negative Percent Agreement (NPA)	100% (95% CI: 90.6% - 100%)		

** One positive sample returned an invalid result and there was insufficient sample available for retesting.*

Troubleshooting

ISSUE OBSERVED	POSSIBLE CAUSE	RESOLUTION
REAGENTS		
Salt precipitation occurs after rehydration and prolonged storage of Buffer 1	Salt concentration in hybridization cocktail will be too low	Re-dissolve by vortexing at room temperature (20-25°C)
Photobleaching of Primer Set	Primer Set contains CY3 and can photo bleach with extended exposure to light	Keep Primer Set in a tinted tube and do not allow prolonged light exposure
NO SIGNAL IS DETECTED		
There are no positive signals in samples known to be contaminated	Photobleaching of microarrays due to incorrect storage	Obtain a new PathogenDx plate that has been stored correctly, contact PathogenDx to determine if the co-print is compromised
	PCR Master Mix was not thawed completely	Allow to thaw completely and rerun samples at RT-PCR stage
	PCR Master Mix and reagents were not vortexed before use	Sedentary reagents need to be vortexed for proper use, rerun samples
	2 µL sample prep was not transferred to RT-PCR Master Mix plate	Rerun from RT-PCR stage, be sure to pipette up and down to mix and check tips for full evacuation
	New plastics (i.e., PCR plates, seals) are inhibiting PCR or causing evaporation	Check catalog number of plates; run QA on any new materials being used
	Evaporation from plate seal lifting	Always use seal applicator and thermocycler optical film compression pad for PCR
	Thermal Cycler conditions have been changed	Thermal Cycler conditions should match provided PCR cycling program
	PCR plate not spun down	After application of seal, spin down PCR plates to ensure all DNA and reagents are contained at bottom of plate
	PCR plates being left out before being set in Thermal Cycler	After application of seal, spin down and immediately place in Thermal Cycler to run PCR program
	Photobleaching of Primer Set due to light exposure	Repeat PCR with a new Primer Set
	Hybridization washes are not following time protocol	Washes need to be left in the wells for full time as given in the protocol and no longer or less
	Pre-Hybridization and Hybridization buffers were premade and left out or frozen overnight	Buffers should only be made during Hybridization process, remake new buffers and run
	Wash Buffer was made incorrectly	Follow table provided to make wash buffer in correct volumes, rerun
	Aspirator manifold is being washed with bleach or ethanol and used without adequate rinsing	If using either to decontaminate the manifold, adequate rinsing of 100mL MB Grade Water is required to completely remove residue

ISSUE OBSERVED	POSSIBLE CAUSE	RESOLUTION
	Pipette basins are being washed with bleach and reused	Utilize sterile pipette basins for each hybridization run, rerun from RT PCR stage
	Sensovation settings were changed	Refer to Sensovation user manual to reset settings and contact PathogenDx Technical Team for further instruction
	Sensovation imager was dropped, jostled, misaligned etc.	Refer to the Sensovation user manual to refocus imager and contact the PathogenDx Technical Team for further instruction
	Arrays were scanned in the incorrect orientation	Check images and orientation of the array in the scanner and rescan
	Arrays were scanned in the incorrect orientation	Check images and orientation of the array in the scanner and rescan
	Post-hybridization wash was not stringent due to incorrect dilution of wash buffer	Prepare wash buffer again and repeat hybridization with new array plate.
	Contamination	Decontaminate all areas with 10% bleach then 70% ethanol. Replace all consumable items. Follow all protocol and notes listed within the Product Insert
	Contaminated template added to NTC	Run the NTC as 45 µl of RT-PCR Master Mix ONLY, do not add a blank
SIGNAL IS DETECTED IN NEGATIVE CONTROLS		
Background Signal is high	Popping bubbles left in liquid in wells	**Do Not** pop any bubbles that may be on the liquid in the wells. This will cause cross contamination or removal of probes.
	Dust or fibers were present	Use Kimwipe to clean bottom of plate
	Compressed air was used to remove fibers from plate	Compressed air must not be used on the array plate at any time. Residue can be deposited that may affect the integrity of the probes
	Arrays were scanned in the incorrect orientation	Check images and orientation of the array in the scanner and rescan

If repeat results are invalid, call Technical Service at 1-800-641-2102

Technical Assistance

Visit pathogendx.com for the latest service and support information for immediate support please call 1-800-641-2102; 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

CONTACT INFORMATION AND ORDERING SUPPORT
Contact: PathogenDx Technical Support
Email: IVDsupport@pathogendx.com
Phone: 1-800-641-2102
Website: www.pathogendx.com

Product support information
- **Product FAQs**
- **Technical support**
- **Order and web support**

Product documentation
- **User guides, manuals, and protocols**
- **Fact Sheet for Healthcare Providers**
- **Fact Sheet for Patients**
- **Safety Data Sheets (SDSs; also known as MSDSs)**

Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.