New York SARS-CoV-2 Real-time Reverse Transcriptase (RT)-PCR Diagnostic Panel

For Emergency Use Only

Instructions for Use

Catalog # LVD SOP-151.2

1,000 reactions

For *In-vitro* Diagnostic (IVD) Use

Rx Only

Wadsworth Center
New York State Department of Health
Empire State Plaza, PO Box 509
Albany, NY 12201-0509

WC/DID/Laboratory of Viral Diseases

Table of Contents

Intended Use

The New York SARS-CoV-2 Real-time Reverse Transcriptase (RT)-PCR Diagnostic Panel is a real-time RT-PCR test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in nasopharyngeal/oropharyngeal swabs and sputa collected from individuals who meet COVID-19 clinical and/or epidemiological criteria (for example, clinical signs and symptoms associated with COVID-19, contact with a probable or confirmed COVID-19 case, history of travel to a geographic locations where COVID-19 cases were detected, or other epidemiologic links for which COVID-19 testing may be indicated as part of a public health activity). Testing is limited to qualified laboratories designated by Wadsworth Center, NYSDOH and, in the United States, certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. § 263a, to perform high complexity tests.

Results are for the detection and identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in nasopharyngeal/oropharyngeal swabs and sputa during the acute phase of infection. Positive results are indicative of active infection with SARS-CoV-2 but do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.

Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient treatment or other patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

Testing with the New York SARS-CoV-2 Real-time Reverse Transcriptase (RT)-PCR Diagnostic Panel is intended for use by trained and competency-certified clinical laboratory personnel, specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures. The New York SARS-CoV-2 Real-time Reverse Transcriptase (RT)-PCR Diagnostic Panel is only for use under the Food and Drug Administration's Emergency Use Authorization.

Summary and Explanation

An outbreak of pneumonia caused by a novel coronavirus (SARS-CoV-2) in Wuhan City, Hubei Province, China was initially reported to WHO on December 31, 2019. On January 31, 2020, Health and Human Services Secretary Alex M. Azar II declared a public health emergency (PHE) for the United States to aid the nation's healthcare community in responding to SARS-CoV-2. The emergence and rapid spread of SARS-CoV-2 to numerous areas throughout the world, has necessitated preparedness and response in laboratories, as well as health care and other areas of society in general. The availability of specific and sensitive assays for the detection of the virus are essential for accurate diagnosis of cases, assessment of the extent of the outbreak, monitoring of intervention strategies and surveillance studies.

The New York SARS-CoV-2 Real-time Reverse Transcriptase (RT)-PCR Diagnostic Panel is a molecular *in vitro* diagnostic test that aids in the detection and diagnosis of 2019-nCoV and is based on widely used nucleic acid amplification technology. The product contains oligonucleotide primers and dual-labeled hydrolysis probes (TaqMan®) and control material used in RT-PCR for the *in vitro* qualitative detection of SARS-CoV-2 RNA in respiratory specimens.

Principles of the Procedure

The oligonucleotide primers and probes for detection of SARS-CoV-2 were selected from regions of the virus nucleocapsid (N) gene. The panel is designed for specific detection of the SARS-CoV-2 (two primer/probe sets). An additional primer/probe set to detect the human RNase P gene (RP) in control samples and clinical specimens is also included in the panel.

RNA isolated and purified from upper and lower respiratory specimens is reverse transcribed to cDNA and subsequently amplified in the Applied Biosystems® 7500 Fast Dx Real-Time PCR Instrument with SDS version 1.4 software. In the process, the probe anneals to a specific target sequence located between the forward and reverse primers. During the extension phase of the PCR cycle, the 5' nuclease activity of Taq polymerase degrades the probe, causing the reporter dye to separate from the quencher dye, generating a fluorescent signal. With each cycle, additional reporter dye molecules are cleaved from their respective probes, increasing the fluorescence intensity. Fluorescence intensity is monitored at each PCR cycle by Applied Biosystems® 7500 Fast Dx Real-Time PCR System with SDS version 1.4 software.

Materials Required (Provided)

Assay Primers and Probes

Reagent	Part #	Description	Quantity / Tube	Reactions / Tube
SARS-CoV-2 N1 Forward Primer	WC20.0001	SARS-CoV-2 N1 forward primer	10 nmol	1,000
SARS-CoV-2 N1 Reverse Primer	WC20.0002	SARS-CoV-2 N1 reverse primer	10 nmol	1,000
SARS-CoV-2 N1 Probe	WC20.0003	SARS-CoV-2 N1 probe	5 nmol	1,000
SARS-CoV-2 N2 Forward Primer	WC20.0004	SARS-CoV-2 N2 forward primer	10 nmol	1,000
SARS-CoV-2 N2 Reverse Primer	WC20.0005	SARS-CoV-2 N2 reverse primer	10 nmol	1,000
SARS-CoV-2 N2 Probe	WC20.0006	SARS-CoV-2 N2 probe	5 nmol	1,000
Human RNase P (RP) Forward Primer	WC20.0007	Human RNase P (RP) forward primer	10 nmol	1,000
Human RNase P (RP) Reverse Primer	WC20.0008	Human RNase P (RP) reverse primer	10 nmol	1,000
Human RNase P (RP) Probe	WC20.0009	Human RNase P (RP) probe	5 nmol	1,000

Controls

Reagent	Description	Quantity	NY Wadsworth Catalog No.
No Template Control (NTC)	Molecular grade, DNase and RNase-free water.	20 x 1 ml	WC20.0010
Positive Template Control (SARS2PC)	Manufactured by Bio-Synthesis, Lewisville, TX. Synthetic RNA transcript contains SARS-CoV-2 N1 and N2 RT-PCR amplicon sequences diluted to 100,000 gene copies/µl in RNA storage solution. For use, this stock is diluted 1:100 (to 1,000 gene copies/µl) with extracted nucleic acid from human embryonic lung cells which contain RNase P RNA.	1 x 100 µl	WC20.0012
Extraction Control	Manufactured by NY Wadsworth. Non-infectious, human embryonic lung cells (HEL) diluted 1:10 with Gelatin Tris Hanks (GTH) media. To be used for the negative extraction control in the assay. Additionally, extracted HEL is used as the diluent for the working strength SARS2PC.	20 x 1 ml	WC20.0011

Materials Required (But Not Provided)

RNA Extraction

Instrument / Manufacturer	Extraction Kit	Catalog No.
Qiagen EZ1 Advanced XL	EZ1 DSP Virus Kit	48 extractions Catalog # 62724
		Buffer AVL Catalog # 19073
		EZ1 Advanced XL DSP Virus Card Catalog # 9018703
bioMerieux easyMAG	NucliSENS	Magnetic silica Catalog # 280133
		easyMAG Lysis Buffer Catalog # 200292 Catalog # 280134
		Buffer 1 Catalog # 280130
		Buffer 2 Catalog # 280131
		Buffer 3 Catalog # 280132
		Disposables Catalog # 280135
bioMerieux EMAG	NucliSENS	Magnetic silica Catalog # 280133
		easyMAG Lysis Buffer Catalog # 200292 Catalog # 280134
		Buffer 1 Catalog # 280130
		Buffer 2 Catalog # 280131
		Buffer 3 Catalog # 280132
		Disposables Catalog # 280135
		eMAG tips Catalog # 418922

Sputum digestion

Reagent Manufacturer	Reagent	Catalog No.
Scientific Device Laboratory, Des Plaines, IL	Snap n digest	SDL668 150ml

RT-PCR Enzyme Master Mix

Manufacturer	Reagent	Quantity	Catalog No.
Applied Biosystems	TaqPath™ 1-Step RT-qPCR Master Mix, GC	1000 reactions	A15299
		2000 reactions	A15300

Dilution of Positive Template Control SARS2PC

Instrument / Manufacturer	Description	Catalog No.
Ambion	THE RNA Storage Solution To be used to dilute SARS2PC when received from manufacturer, to 100,000 copies/ul stock, dispense to 100 µl aliquots for the kit and freeze to -70°C or below.	AM7001

Real-Time RT-PCR Amplification

Instrument / Manufacturer	Description	Catalog No.
Applied Biosystems® 7500 Fast Dx Real-time PCR System with SDS 1.4 software	The Applied Biosystems® 7500 Fast Dx Real-Time PCR Instrument with SDS Software is a real-time nucleic acid amplification instrument and five-color fluorescence detection system available for in vitro diagnostic use. The Applied Biosystems® 7500 Fast Dx Real-Time PCR Instrument delivers the performance required for high-quality results in a 96-well format	4406984

Equipment and Consumables Required (But Not Provided)

- Biological safety cabinet
- Refrigerated microcentrifuge
- Cold blocks
- Vortex mixer
- 1.5 ml screw-capped microcentrifuge tubes
- Tube racks
- Dedicated adjustable P-10, P-20, P-100, P-200, P-1000, and P-5000 pipettes and aerosol barrier tips
- Disposable Plasticware: The use of sterile, disposable polypropylene tubes is recommended throughout the procedure. These tubes are generally RNase-free and do not require pretreatment to inactivate RNases
- Dedicated laboratory coat for each area
- Disposable booties
- Biohazard bag for tip and tube disposal
- Powder-free latex, vinyl or nitrile gloves
- 20% (v/v) bleach solution (2.0% w/v sodium hypochlorite in water)
- 70% ethanol
- 96-well PCR plates
- Optical caps
- Applied Biosystems 7500 Fast DX Real-Time PCR Instrument
- TaqPath™ 1-Step RT-qPCR Master Mix, CG (Applied Biosystems, cat no. A15299)
- Ambion THE RNA Storage Solution (Catalog # AM7001)
- Qiagen extractor EZ1 Advanced XL and EZ1 DSP Virus kit (62724)
- Buffer AVL (19073)
- Nuclease-free water

Warnings and Precautions

- For *in vitro* diagnostic use (IVD).
- For emergency use only.
- Follow standard precautions. All patient specimens and positive controls should be considered potentially infectious and handled accordingly.
- Proper personal protective equipment including lab coats, gowns, gloves, eye protection, and a biological safety cabinet are recommended for manipulation of clinical specimens. Refer to Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition - CDC.
- Do not eat, drink, smoke, apply cosmetics or handle contact lenses in areas where reagents and human specimens are handled.
- 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.
- Specimen processing should be performed in accordance with national biological safety recommendations.
- If infection with SARS-CoV-2 is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions.
- Perform all manipulations of human clinical specimens within a Class II (or higher) biological safety cabinet (BSC).
- Immediately clean up any spill containing potentially infectious material with 0.5-1% (w/v) sodium hypochlorite (20% v/v bleach). Dispose of cleaning materials in a biohazard waste stockpot.
- Report incident to supervisor, fill in Wadsworth Accident Report and consult a physician immediately in the event that infectious materials are ingested or come into contact with mucus membranes, open lacerations, lesions or other breaks in the skin.
- Amplification technologies such as PCR are sensitive to accidental introduction of PCR product from previous amplifications reactions. Incorrect results could occur if either the clinical specimen or the real-time reagents used in the amplification step become contaminated by accidental introduction of amplification product (amplicon). Workflow in the laboratory should proceed in a unidirectional manner.
    - Maintain separate areas for assay setup and handling of nucleic acids.
    - Always check the expiration date prior to use. Do not use expired reagent. Do not substitute or mix reagent from different kit lots or from other manufacturers.
    - Change aerosol barrier pipette tips between all manual liquid transfers.
    - During preparation of samples, compliance with good laboratory techniques is essential to minimize the risk of cross-contamination between samples, and the inadvertent introduction of nucleases into samples during and after the extraction procedure. Proper aseptic technique should always be used when working with nucleic acids.
    - Maintain separate, dedicated equipment (e.g., pipettes, microcentrifuges) and supplies (e.g., microcentrifuge tubes, pipette tips) for assay setup and handling of extracted nucleic acids.
    - Perform work in a unidirectional workflow, from areas without specimen/nucleic acid or amplicon to areas with amplified nucleic acid

- Wear a clean lab coat and powder-free disposable gloves (not previously worn) when setting up assays.
- Change gloves between samples and whenever contamination is suspected.
- Keep reagent and reaction tubes capped or covered as much as possible.
- Primers, probes (including aliquots), and enzyme master mix must be thawed and maintained on a cold block at all times during preparation and use.
- Work surfaces, pipettes, and centrifuges should be cleaned and decontaminated with cleaning products such as 20% bleach, "DNA*Zap*™" or "RNase AWAY®" to minimize risk of nucleic acid contamination. Residual bleach should be removed using 70% ethanol.

- RNA should be maintained on a cold block during preparation and use to ensure stability.
- Dispose of unused kit reagents and human specimens according to local, state, and federal regulations.

Reagent Storage, Handling, and Stability

- Store all dried primers and probes and the positive control, SARS2PC, at 2-8°C until re-hydrated for use. Store liquid HEL control materials at ≤ -80°C.
- Always check the expiration date prior to use. Do not use expired reagents.
- Protect fluorogenic probes from light.
- Primers, probes (including aliquots), and enzyme master mix must be thawed and kept on cold block at all times during preparation and use.
- Do not refreeze probes.
- Controls and aliquots of controls must be thawed and kept on ice at all times during preparation and use.

Specimen Collection, Handling, and Storage

Sample acceptance criteria

- Samples should be collected into sterile, labeled tubes, and shipped at 2°C to 8°C on frozen gel packs.
- Submitters can reference the Virology section of the Wadsworth Center website for specimen collection, transportation shipping, labeling. https://www.wadsworth.org/programs/id/virology/services/specimen-collection
  - See SOP LVD-054 VL Specimen Receiving and Processing

Specimen rejection criteria

- Samples that have not been pre-approved for testing and those that are labeled improperly will not be tested until the required information is obtained.

Adequate, appropriate specimen collection, storage, and transport are important in order to obtain sensitive and accurate test results. Training in correct specimen collection procedures is highly recommended to assure good quality specimens and results. CLSI MM13-A may be referenced as an appropriate resource.

Collecting the Specimen

- Collection of nasopharyngeal/oropharyngeal swabs: Flocked swabs are preferred. Sterile dacron or rayon swabs with plastic or flexible metal handles may also be used. Do NOT use cotton or calcium alginate swabs or swabs with wooden sticks

as they may contain substances that inactivate some viruses and inhibit PCR. Place swabs immediately in tubes containing 2-3 ml of sterile virus transport medium. Label the specimen vials, store at 2-8°C and ship overnight on frozen cold packs.

- Sputum: place in sterile, labeled specimen collection leak-proof cup. Label the specimen vials, store at 2-8°C and ship overnight on frozen cold packs.

Transporting Specimens

- It is the shipper's responsibility to ensure that appropriate shipping materials are used. Please refer to IATA and NYSDOT regulations.
- If specimens cannot be shipped within 72 hours of collection, store at -70°C or below and ship overnight on dry ice.

Storing Specimens

- Specimens can be stored at 2-8°C for up to 72 hours after collection.
- If a delay in extraction is expected, store specimens at -70°C or lower.
- Extracted nucleic acid should be stored at -70°C or lower.

Specimen Referral to CDC

Referring a specimen to the CDC:

- In the event of an unusual test result needing clarification at CDC, ship specimens to CDC using the following guidelines.
- Ship frozen specimens on dry ice and non-frozen specimens on frozen cold packs.
- Refer to the International Air Transport Association (IATA - www.iata.org) for requirements for shipment of human or potentially infectious biological specimens.
- Prior to shipping, notify CDC Division of Viral Diseases (see contact information below) that you are sending specimens.
- Send all samples to the following recipient:

Centers for Disease Control and Prevention
c/o STATT
1600 Clifton Rd., Atlanta, GA 30329-4027
Phone: (404) 639-1587

The emergency contact number for CDC Emergency Operations Center (EOC) is 770-488-7100.

Reagent Controls and Preparation

SARS-CoV-2 Positive Control (SARS2PC) Preparation:

1) Precautions: This reagent should be handled with caution to prevent possible contamination. Freeze-thaw cycles should be avoided. Maintain on cold block when thawed.
2) Dilute one 10 µl aliquot of the SARS2PC with 990ul of HEL cell extract, to achieve the working concentration of the SARS2PC (1,000 gene copies/µl) and incorporated RP control. Make single use aliquots (approximately 20 µl each) and store at ≤ -70°C.
3) Thaw a single aliquot of the working strength positive control for each experiment and hold on cold block until adding to plate. Discard any unused portion of the aliquot.

Human Embryonic Lung Control (HEL)

1) Human Embryonic Lung Control (HEL) must be extracted and processed with each specimen extraction run.

No Template Control (NTC)

1) Sterile, nuclease-free water.
2) Used to check for contamination during specimen extraction and/or plate set-up.

Quality Control

- Quality control requirements must be performed in conformance with local, state, and federal regulations or accreditation requirements and the user's laboratory's standard quality control procedures. For further guidance on appropriate quality control practices, refer to 42 CFR 493.1256.
- Quality control procedures are intended to monitor reagent and assay performance.
- Test all positive controls prior to running diagnostic samples with each new kit lot to ensure all reagents and kit components are working properly.
- Good laboratory practice (cGLP) recommends including a positive extraction control in each nucleic acid isolation batch.
- The extraction control (HEL) must proceed through nucleic acid isolation per batch of specimens to be tested.
- Always include a negative control (NTC), and the appropriate positive control (SARS2PC) in each amplification and detection run. All clinical samples should be tested for human RNase P (RP) gene to control for specimen quality and extraction.

Nucleic Acid Extraction and Assay Set up

All procedures should be performed in a BSL2 laboratory, and specimens handled within a Biological Safety Cabinet. All necessary safety precautions should be taken according to the Laboratory guidelines. Precautions must also be taken to prevent cross contamination of samples.

Separate work areas should be used for:

- Nucleic acid extraction
- Reagent preparation (e.g., preparation of RT-PCR master mix; **NO** amplified reactions, target solutions, or clinical specimens should be brought into this area. After working in this area, laboratory coat and gloves should be changed before moving into the nucleic acid addition area)
- Nucleic acid addition
- Instrumentation (e.g., thermocyclers)

Fill out an extraction worksheet and a real-time PCR worksheet, to include specimen numbers, reagent lot numbers and dates of extraction and PCR.

General Handling: Proper microbiological, aseptic technique should always be used when working with RNA. Hands and dust particles may carry bacteria and molds and are the most common sources of RNase contamination. Always wear powder-free latex, vinyl, or nitrile gloves while handling reagents, tubes and RNA samples to prevent RNase contamination from the surface of the skin or from dusty laboratory equipment. Change gloves frequently and keep tubes closed. During the procedure, work quickly and keep everything on cold blocks when possible to avoid degradation of RNA by endogenous or residual RNases.

Clean working surfaces, pipettes, etc. with 20% bleach or other solution that can destroy nucleic acids and RNases. To eliminate accelerated deterioration of any plastics and metals, wipe down with 70% ethanol after using 20% bleach. Make sure all bleach is removed to eliminate possible chemical reactions between bleach and guanidine thiocyanate which is present in the extraction reagents.

In addition to patient samples, negative extraction control should be extracted for each run. The negative extraction control is uninfected cell culture medium (HELs).

The following commercially available RNA extraction kits and procedures have been qualified and validated for recovery and purity of RNA for use with the panel:

- Qiagen EZ1 Advanced XL: Kit: Qiagen EZ1 DSP Virus Kit and Buffer AVL (supplied separately) for offboard lysis Card: EZ1 Advanced XL DSP Virus Card. Recommendation(s): Add 120 µl of sample to 280 µl of pre-aliquoted Buffer AVL (total input sample volume is 400 µl). Proceed with the extraction on the EZ1 Advanced XL. Elution volume is 120 µl.
- bioMerieux easyMAG: NucliSENS Nucleic Acid Extraction Reagents: Add 110µL of sample to 2mL lysis buffer. Mix and incubate at room temperature for minimum 10 minutes. Proceed with extraction on easyMAG with Generic 2.0.1 protocol, according to manufacturer's instructions. Elution volume is 110 µL.
- bioMerieux EMAG: NucliSENS Nucleic Acid Extraction Reagents: Add 110µL of sample to 2mL lysis buffer. Mix and incubate at room temperature for minimum 10 minutes. Proceed with extraction on eMAG with Generic 3.0.4 protocol, according to manufacturer's instructions. Elution volume is 110 µL.
- Extracted nucleic acid should be stored at 4°C if it is to be used within 4 hours, or at -70°C if stored longer than 4 hours.

RT-PCR Master Mix Preparation:

Prepare sufficient quantity of the following reagent mix for the number of samples and controls being tested

Reagent	per reaction
4x TaqPath™ 1-Step RT-qPCR Master Mix, GC	5.0 µl
Forward primers (25 µM)	0.72 µl
Reverse primers (25 µM)	0.72 µl
Probes (25 µM)	0.08 µl
Nuclease-free water	8.48 µl

- In PCR Clean Room, add 15 µl of Master Mix to required wells of plate
- Add 5 µl of NTC to plate and cap
- Move to template addition room
- Thaw one tube of the SARS-CoV-2 working strength control
- Add 5 µl of nucleic acid from each patient sample to the test wells
- Add 5 µl of nucleic acid from HEL to the negative control well
- Add 5 µl of SARS2PC to the positive control well
- Cap the wells securely with optical caps

- Centrifuge plates to collect the liquid in the bottom if the wells using the tabletop refrigerated centrifuge
- Transfer the plate to an ABI 7500 FAST DX PCR Sequence Detection System Instrument

RT-PCR using the ABI 7500 FAST Dx PCR Detection System Instrument:

Standard Cycling conditions for 20 µl SARS-CoV-2 virus RT-PCR:

Step	Temperature/Duration
UNG incubation	25°C for 1 min
RT incubation	50°C for 15 min
Enzyme activation	95°C for 2 min
Amplification (45 cycles)	95°C for 3 sec 55°C for 0:30 (data collection)

- Save in the appropriate folder then start run

Interpretation of Control Results

A. No Template Controls (NTC): The NTC is molecular grade, DNase and RNase-free water used in place of sample nucleic acid. One well is run for each N1, N2, and RP assay. All NTC reactions should be negative, meaning no amplification curves cross the PCR threshold. If any of the N1, N2, or RP NTC reactions exhibit positive fluorescence that cross the PCR cycle threshold (Ct), it is possible that contamination occurred, or the assay was setup improperly. The run is rejected and must be repeated.

B. SARS-CoV-2 Positive Control (SARS2PC): The positive control includes quantified synthetic RNA transcript that contains the N1 and N2 real-time RT-PCR amplicon sequences, diluted with extracted nucleic acid from HEL cells. N1, N2 and RP primer/probe sets should yield a positive result with the positive control, with a Ct value < 40.0. Negative results with any of the primer/probe sets invalidates the run and suggests the assay may have been set up incorrectly, or the integrity of the primers/probes is compromised. The run should be repeated.

C. Negative Extraction Control (HEL control): The negative extraction control consists of uninfected HEL cells and is processed with every extraction batch run. RNA from the HEL should yield negative results with the N1 and N2 assays, and a positive result on the RP assay with a Ct value < 40.0. If positive results occur on the N1 or N2 primer/probe sets with the HEL control, the extraction run and the RT-PCR run are invalid and should be repeated. Possible contamination occurred during extraction or reaction plate setup.

D. Positive Extraction Control (RP): Detection of RNase P RNA in extracted nucleic acid serves as a positive extraction control for each sample. RNA extracted from each specimen, including the negative extraction control (HEL), should yield a positive result on the RP assay, with a Ct value < 40.0. If the RP assay is negative on a clinical sample interpret as follows:

- If the SARS-CoV-2 N1 and N2 assays are positive with a negative RP result, consider the run valid and proceed with next steps.
- If the SARS-CoV-2 N1 and N2 assays are negative in conjunction with a negative RP, the specimen results are considered invalid and should be repeated. If residual specimen is available, re-extract the specimen and perform testing again. If results remain invalid, a new specimen should be collected.

Expected Performance of Controls

Control Type	Control Name	Used to Monitor	Expected results and Ct Values		
			SARS-CoV-2 N1	SARS-CoV-2 N2	RP
Negative	NTC	Assay or extraction reagent contamination	Negative Ct ND*	Negative Ct ND	Negative Ct ND
Positive	SARS2PC	Improper assay setup, reagent failure including primer and probe degradation	Positive Ct < 40.0	Positive Ct < 40.0	Positive Ct < 40.0
Negative extraction control	HEL	Cross-contamination during extraction	Negative Ct ND	Negative Ct ND	Positive Ct < 40.0
Positive extraction control	RP	Inefficient lysis of specimen, poor specimen collection, improper assay setup, extraction failure, PCR inhibition	Negative Ct ND	Negative Ct ND	Positive Ct < 40.0

*ND = not detected

SARS-CoV-2 RT-PCR Diagnostic Panel Results Interpretation and Reporting Guide

Assessment of clinical specimen test results should be performed after the positive and negative controls have been examined and determined to be valid and acceptable. If the controls are not valid, the patient results cannot be interpreted. Please see table below for guidance on interpretation and reporting of results. If results are obtained that do not follow these guidelines, re-extract and re-test the sample.

SARS-CoV-2 N1	SARS-CoV-2 N2	RP	Result Interpretation	Report	Actions
+	+	±	SARS-CoV-2 detected	Positive SARS-CoV-2	Report result to sender and local or state department of health.
If only one of the two targets are positive		±	Inconclusive Result	Inconclusive	Repeat rRT-PCR of extracted nucleic acid and/or repeat from extraction. If result still inconclusive, report result to sender and local or state department of health and recommend collection of a new specimen(s) from the patient.
-	-	+	SARS-CoV-2 not detected	SARS-CoV-2 Not Detected	Report result to submitter.
-	-	-	Invalid Result	Invalid	Repeat from extraction. If the repeated result remains invalid, report result to sender and local or state department of health and recommend collection of a new specimen(s) from the patient.

Optimal timing of collection for upper and lower respiratory specimens during infections with SARS-CoV-2 have not been determined. Consider the collection of multiple specimens from the same patient, which may increase the probability of detecting the virus during active infection. If diagnostic tests for other respiratory illnesses are negative, and the patient's clinical presentation and epidemiological information suggest that SARS-CoV-2 infection is possible, then a false negative result should be considered, and re-testing of the patient should be discussed.

The Reference Range: negative or undetected is reported as "SARS-CoV-2 RNA not detected".

Performance Characteristics

Analytical Comparison:

Since the New York SARS-CoV-2 Real-time Reverse Transcriptase (RT)-PCR Diagnostic Panel utilizes two oligo sequences (N1 and N2), master mix, extraction and amplification instruments, and cycling conditions, identical to those used in the CDC 2019-Novel Coronavirus (2019-CoV) Real-Time RT-PCR Diagnostic Panel FDA-authorized under EUA200001, an alternative approach was used to evaluate the analytical assay performance. Limiting dilutions of the transcript that were used in the "Limit of Detection study" in the EUA200001 were prepared and tested in parallel with the New York SARS-CoV-2 Real-time Reverse Transcriptase (RT)-PCR Diagnostic Panel and the CDC 2019-Novel Coronavirus (2019-CoV) Real-Time RT-PCR Diagnostic Panel. Variation of < 2 Ct was observed between the two assays at a given dilution for both N1 and N2 targets.

Comparison of New York SARS-CoV-2 (RT)-PCR Panel and CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel using CDC RNA				
Gene copies/reaction	**New York SARS-CoV-2 (RT)-PCR Panel**		**CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel**	
	N1	N2	N1	N2
50,000	23.26	24.10	21.40	22.97
50,000	23.23	24.07	21.49	22.89
50,000	23.18	24.07	21.84	22.83
5,000	26.50	27.43	24.91	26.24
5,000	26.60	27.46	24.84	26.24
5,000	26.52	27.68	25.06	26.33
500	29.41	29.49	28.52	29.84
500	29.77	29.41	28.61	30.07
500	29.63	29.03	28.93	30.10
50	32.34	31.97	32.04	33.11
50	33.09	32.69	32.18	33.14
50	33.42	32.33	32.11	33.55
5	37.42	35.58	36.26	36.08
5	35.70	35.41	35.05	36.03
5	35.64	36.52	35.60	37.32
0.5	ND	ND	ND	37.28
0.5	38.10	ND	ND	ND
0.5	38.08	36.58	ND	ND

ND = Not Detected

For each analyte concentration level listed above, the mean Ct, standard deviation (SD), and percent of coefficient of variation (%CV) were calculated. Results for samples tested using the New York SARS-CoV-2 Real-time Reverse Transcriptase (RT)-PCR Diagnostic Panel and the CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel are shown in the tables below.

Results using the New York SARS-CoV-2 (RT)-PCR Panel

Gene copies/reaction	N1			N2		
	Mean Ct	SD	%CV	Mean Ct	SD	%CV
50,000	23.22	0.04	0.17	24.08	0.02	0.07
5000	26.54	0.05	0.20	27.52	0.14	0.50
500	29.60	0.18	0.61	29.31	0.25	0.84
50	32.95	0.55	1.68	32.33	0.36	1.11
5	36.25	1.01	2.79	35.84	0.60	1.67
0.5	38.09	0.01	0.04	NA	NA	NA

NA= Not available

Results using the CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel

Gene copies/reaction	N1			N2		
	Mean Ct	SD	%CV	Mean Ct	SD	%CV
50,000	21.58	0.23	1.08	22.90	0.07	0.31
5000	24.94	0.11	0.45	26.27	0.05	0.20
500	28.69	0.22	0.75	30.00	0.14	0.47
50	32.11	0.07	0.22	33.27	0.25	0.74
5	35.64	0.61	1.70	36.48	0.73	2.00
0.5	NA	NA	NA	NA	NA	NA

NA=Not available

***Limit of Detection (LoD)*:**

The LoD of the New York SARS-CoV-2 r-RT-PCR Panel (for N1 and N2 detection) was determined using quantified whole viral genomic RNA. A preliminary LoD was determined by testing in triplicate 10-fold serial dilutions of the whole viral genomic RNA spiked into pooled sputum samples. The approximate LoD was further fine-tuned by testing 2-fold dilutions in triplicate. The LoD was confirmed by testing 20 replicates extracted by each extraction method, i.e., the bioMerieux NucliSENS easyMAG and EMAG. Both extraction methods generated the same LoD of 25 genome copies/reaction. Additionally, a separate comparative clinical study demonstrated that the LoD obtained with Qiagen EZ1 was ~5-10 times higher (analytically less sensitive) than the LoDs observed with easyMAG and EMAG.

***Analytical Specificity*:**

The New York SARS-CoV-2 Real-time Reverse Transcriptase (RT)-PCR Diagnostic Panel utilizes identical oligo sequences, master mix, extraction and amplification instruments, and cycling conditions identical to those used in the CDC 2019-Novel Coronavirus (2019-CoV) Real-Time RT-PCR Diagnostic Panel.

A panel of more than 50 respiratory pathogens were tested with the New York SARS-CoV-2 r-RT-PCR Panel following extraction, using bioMerieux NucliSENS easyMAG extraction, at clinically relevant concentrations (generally at 10^6 genome copies/mL). All pathogens were tested in triplicate and none produced any detectable reactivity with the New York SARS-CoV-2 r-RT-PCR Panel.

Analytical Specificity of the New York SARS-CoV-2 r-RT-PCR Panel

Organism	Strain / Genotype	Source	SARS-CoV-2_N1	SARS-CoV-2_N2
Human coronavirus	OC43	Isolate	0/3	0/3
Human coronavirus	229E	Clinical specimen	0/3	0/3
Human coronavirus	HKU1	Clinical specimen	0/3	0/3
Human coronavirus	NL63	Clinical specimen	0/3	0/3
MERS coronavirus	N/A[a]	CDC, positive control material containing N gene targets	0/3	0/3
SARS-coronavirus	Unknown	cDNA[b]	0/3	0/3
Rhinovirus A	A1	Clinical isolate	0/3	0/3
Influenza A (H1pdm09)	N/A	Clinical isolate	0/3	0/3
Influenza A (H1N1)	N/A	Clinical isolate	0/3	0/3
Influenza A (H3N2)	N/A	Clinical isolate	0/3	0/3
Influenza B	N/A	Clinical isolate	0/3	0/3
Parainfluenza 1	N/A	Clinical isolate	0/3	0/3
Parainfluenza 2	N/A	Clinical isolate	0/3	0/3
Parainfluenza 3	N/A	Clinical isolate	0/3	0/3
Parainfluenza 4	4A	Clinical isolate	0/3	0/3

Parainfluenza 4	4B	Clinical isolate	0/3	0/3
Respiratory Syncytial Virus	A	Clinical isolate	0/3	0/3
Respiratory Syncytial Virus	B	Clinical isolate	0/3	0/3
Human metapneumovirus	1B	Clinical isolate	0/3	0/3
Enterovirus A	Coxsackie A16	Clinical isolate	0/3	0/3
Enterovirus A	Coxsackie A9	Clinical isolate	0/3	0/3
Enterovirus A	Enterovirus 71	Clinical isolate	0/3	0/3
Enterovirus B	Coxsackie B3	Clinical isolate	0/3	0/3
Enterovirus B	Coxsackie B4	Clinical isolate	0/3	0/3
Enterovirus B	Coxsackie B5	Clinical isolate	0/3	0/3
Enterovirus B	Echovirus 18	Clinical isolate	0/3	0/3
Enterovirus B	Echovirus 6	Clinical isolate	0/3	0/3
Enterovirus B	Echovirus 9	Clinical isolate	0/3	0/3
Enterovirus B	Echovirus 11	Clinical isolate	0/3	0/3
Enterovirus B	Echovirus 30	Clinical isolate	0/3	0/3
Enterovirus C	Coxsackie A21	Clinical isolate	0/3	0/3
Enterovirus D	Enterovirus D68	Clinical isolate	0/3	0/3
Measles virus	N/A	Clinical isolate	0/3	0/3
Mumps virus	G	Clinical isolate	0/3	0/3
Rhinovirus A	A1	Clinical isolate	0/3	0/3
Adenovirus-Group B	3	Clinical isolate	0/3	0/3
Adenovirus-Group E	4	Clinical isolate	0/3	0/3
Adenovirus-Group C	5	Clinical isolate	0/3	0/3
Adenovirus-Group D	8	Clinical isolate	0/3	0/3
Adenovirus-Group D	31	Clinical isolate	0/3	0/3
Human herpes virus 1 (HSV1)	N/A	Clinical isolate	0/3	0/3
Human herpes virus 2 (HSV2)	N/A	Clinical isolate	0/3	0/3
Human herpes virus 6 (HHV6)	B	Isolate	0/3	0/3
Epstein Barr Virus (EBV)	N/A	Isolate	0/3	0/3
Varicella Zoster virus (VZV)	N/A	Clinical isolate	0/3	0/3
Cytomegalovirus (CMV)	N/A	Clinical isolate	0/3	0/3
Lymphocytic choriomeningitis virus	N/A	Isolate	0/3	0/3
Haemophilus parainfluenzae	N/A	Clinical isolate	0/3	0/3
Haemophilus influenzae	N/A	Clinical isolate	0/3	0/3
Corynebacterium sp.	N/A	Clinical isolate	0/3	0/3
Neisseria meningitidis	Group B	Clinical isolate	0/3	0/3
Neisseria meningitidis	Group C	Clinical isolate	0/3	0/3
Neisseria meningitidis	Group Y	Clinical isolate	0/3	0/3
Streptococcus pneumoniae	10A	Clinical isolate	0/3	0/3

Group A *Streptococcus*	11A	Clinical isolate	0/3	0/3

[a] Not available
[b] cDNA derived from extracted RNA from cultured virus. Provided by the CDC in 2009.

FDA SARS-CoV-2 Reference Panel Testing:

The evaluation of sensitivity and MERS-CoV cross-reactivity was performed using reference material (T1), blinded samples and a standard protocol provided by the FDA. The study included a range finding study and a confirmatory study for LoD. Blinded sample testing was used to establish specificity and to corroborate the LoD. The extraction method and instruments used were bioMérieux easyMAG and Applied Biosystems™ 7500 Fast Real-Time PCR System. The results are summarized below.

Summary of LoD Confirmation Result Using the FDA SARS-CoV-2 Reference Panel

Reference Materials Provided by FDA	Specimen Type	Product LoD	Cross-Reactivity
SARS-CoV-2	NP	$1.8x10^4$ NDU/mL	N/A
MERS-CoV		N/A	ND

NDU/mL: RNA NAAT detectable units/mL
N/A: Not Applicable
ND: Not Detected

***Endogenous Interference Substances Studies*:**

The New York SARS-CoV-2 Real-time Reverse Transcriptase (RT)-PCR Diagnostic Panel uses conventional well-established nucleic acid extraction methods and based on experience, we do not anticipate interference from common endogenous substances using this method.

Clinical Evaluation:

The New York SARS-CoV-2 Real-time Reverse Transcriptase (RT)-PCR Diagnostic Panel is being performed with identical oligo sequences, master mix, extraction and amplification instruments, and cycling conditions, as those in the CDC assay already authorized by the FDA under EUA. A clinical evaluation of the CDC assay was performed under an EUA.
A limited contrived clinical study was performed with clinical matrices. SARS-CoV-2 RNA transcript was spiked at two different concentrations. The performance of the New York SARS-CoV-2 Real-time Reverse Transcriptase (RT)-PCR Diagnostic Panel in clinical matrices appeared comparable and demonstrated 100% agreement with the expected results in negative and contrived positive samples.

A second contrived clinical study was conducted to evaluate the performance of the New York SARS-CoV-2 r-RT-PCR Panel. A total of 73 individual sputum samples were used in this study. The sputum samples were spiked with the extracted whole virus genomic RNA at 2x, 3x, 4x, 14x, 40x, and 200x LoD observed with the bioMerieux easyMAG and EMAG extractions. The spiked sputum samples were blind coded, then, all samples were extracted on bioMerieux easyMAG and Qiagen EZ1 instruments and tested with the New York SARS-CoV-2 r-RT-PCR Panel. Due to limiting amount of sputum samples volume, not all contrived positive samples were extracted on EMAG. Since the LoD with Qiagen EZ1 was found to be ~5-10 times higher than the LoDs observed with easyMAG and EMAG extractions in a comparative LoD study, the lower positive percent agreements were expected at lower concentrations (2X, 3X, 4x LoD of easyMAG and EMAG). easyMAG generated one invalid result, EMAG generated two invalid and two inconclusive results, and EZ1 generated one

invalid result. The positive and negative percent agreements between the New York SARS-CoV-2 r-RT-PCR Panel and the expected results are shown below:

Clinical performance of the NY SARS-CoV-2 r-RT-PCR Panel on sputum

NY SARS-CoV-2 r-RT-PCR Panel	easyMAG		eMAG		EZ1	
	Results (Detected/tested)	Agreements (95% CIs)	Results (Detected/tested)	Agreements (95% CIs)	Results (Detected/tested)	Agreements (95% CIs)
2x LoD	7/7	100% (64.57%-100%)	NT[f]	NA[g]	1/7[e]	14.3% [e] (2.56%-51.31%)
3x LoD	3/3	100% (43.85%-100%)	NT	NA	0/3 [e]	0% [e] (-0.01%-56.15%)
4x LoD	3/3	100% (43.85%-100%)	NT	NA	0/3 [e]	0% [e] (-0.01%-56.15%)
14x LoD	19/20[a]	95%[a] (76.39%-99.11%)	18/20[c]	90% [c] (69.90% %-97.22%)	20/20	100% (83.89%-100%)

40x LoD	5/5	100% (56.56%-100%)	5/5	100% (56.56%-100%)	5/5	100% (56.56%-100%)
200x LoD	5/5	100% (56.56%-100%)	5/5	100% (56.56%-100%)	5/5	100% (56.56%-100%)
Negative	29/29[b]	100% (88.31%-100%)	28/28[d]	100 % (87.94%-100%)	30/30	100% (88.65%-100%)

[a] One low positive not detected on easyMAG. RP Ct value on easyMAG compared to that from EZ1 extraction, suggests that easyMAG extraction was inefficient for that sample.
[b] Results of one of the 30 samples was invalid (negative RP). Excluded from calculations.
[c] Results with EMAG for two samples were inconclusive, with positive Ct values on N2 and RP only. The RP Ct value on one sample indicates a possible extraction issue for that sample on EMAG. The results for the other sample suggest a pipetting error on the robotic liquid handler.
[d] Results of two of the 30 samples were invalid (negative RP values). Excluded from calculations.
[e] Expected results, as the LoD studies demonstrated the LoD on EZ1 to be ~5-10 times higher than that on easyMAG and EMAG.
[f] NT = not tested
[g] NA = not applicable

This evaluation was performed on sputum samples, a notably more challenging and variable specimen type than upper respiratory swabs. Overall, the clinical evaluation experiments demonstrated the easyMAG and EMAG automated extraction instruments to perform as well or better, than the EZ1, for sample extraction prior to the NY SARS-CoV-2 real-time RT-PCR panel, detecting more contrived samples at low concentrations.

Limitations

- All users, analysts, and any person reporting diagnostic results should be trained to perform this procedure by a person with documented competency. Ability to competently perform the test and interpret the results should be documented prior to performing the assay independently. Wadsworth Center, NYSDOH, will limit the distribution of this device to only those users who have documented successful completion of appropriate training.
- The test was validated for use only with upper and lower respiratory specimens.
- Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for treatment or other patient management decisions. Optimum specimen types and timing for peak viral levels during infections caused by SARS-CoV-2 have not been determined. Collection of multiple specimens (types and time points) from the same patient may be necessary to detect the virus.
- A false negative result may occur if a specimen is improperly collected, transported or handled. False negative results may also occur if amplification inhibitors are present in the specimen or if inadequate numbers of organisms are present in the specimen.
- Positive and negative predictive values are highly dependent on prevalence. False negative test results are more likely when prevalence of disease is high. False positive test results are more likely when prevalence is moderate to low.
- Do not use any reagent past the expiration date.
- If the virus mutates in the RT-PCR target region, SARS-CoV-2 may not be detected or may be detected less predictably. Inhibitors or other types of interference may produce

a false negative result. An interference study evaluating the effect of common cold medications was not performed.

- Test performance can be affected because the epidemiology and pathology of disease caused by SARS-CoV-2 is not fully known. For example, clinicians and laboratories may not know the optimum types of specimens to collect, and when during the course of infection these specimens are most likely to contain levels of virus that can be readily detected.
- Detection of viral RNA may not indicate the presence of infectious virus or that SARS-CoV-2 is the causative agent for clinical symptoms.
- 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.
- This test cannot rule out diseases caused by other bacterial or viral pathogens.

Conditions of Authorization for the Laboratory

The New York SARS-CoV-2 Real-time Reverse Transcriptase (RT)-PCR Diagnostic Panel 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/Safety/EmergencySituations/ucm161496.htm

Use of the New York SARS-CoV-2 Real-time Reverse Transcriptase (RT)-PCR Diagnostic Panel must follow the procedures outlined in these manufacturer's Instructions for Use and the conditions of authorization outlined in the Letter of Authorization. Deviations from the procedures outlined are not permitted under the Emergency Use Authorization (EUA). To assist clinical laboratories running the New York SARS-CoV-2 Real-time Reverse Transcriptase (RT)-PCR Diagnostic Panel, the relevant Conditions of Authorization are listed verbatim below, and are required to be met by laboratories performing the EUA test.

- Authorized laboratories[1] will include with reports of the results of the New York SARS-CoV-2 Real-time Reverse Transcriptase (RT)-PCR Diagnostic Panel, the authorized Fact Sheet for Healthcare Providers and the authorized Fact Sheet for Patients. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.
- Authorized laboratories will perform the New York SARS-CoV-2 Real-time Reverse Transcriptase (RT)-PCR Diagnostic Panel as outlined in the New York SARS-CoV-2 Real-time Reverse Transcriptase (RT)-PCR Diagnostic Panel Instructions for Use Package Insert. Deviations from the authorized procedures, including the authorized RT-PCR instruments, authorized extraction methods, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to perform the New York SARS-CoV-2 Real-time Reverse Transcriptase (RT)-PCR Diagnostic Panel are not permitted.
- Authorized laboratories will have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.
- Authorized laboratories will maintain records of test usage and will collect information on the performance of the test and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and Wadsworth Center NYSDOH (via email: NYS.CoV2.test.event.report@health.ny.gov) any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of the test of which they become aware.
- All laboratory personnel using the test must be appropriately trained in RT-PCR techniques and use appropriate laboratory and personal protective equipment when handling this kit and use the test in accordance with the authorized labeling.

[1]Authorized Laboratories: For ease of reference, this letter will refer to "Wadsworth Center, New York State Public Department of Health, and the New York City Department of Health and Mental Hygiene, Public Health Laboratories" as "authorized laboratories."

- Wadsworth Center, NYSDOH and authorized laboratories 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.

Disposal

Dispose of hazardous or biologically contaminated materials according to the practices of your institution.

References

1. Applied Biosystems 7300/7500 real time PCR system getting started guide
2. EZ1 User's Manual
3. TaqPath™ 1-Step RT qPCR Master Mix, CG kit product insert

Contact Information, Ordering, and Product Support

Information and product support can be obtained from NYS.CoV2.test.event.report@health.ny.gov