

Bio-Rad SARS-CoV-2 ddPCR Test

Qualitative assay for use on the QX200™ and
QXDx™ Droplet Digital™ PCR Systems

Instructions For Use

For Rx Only

For Emergency Use Authorization Only

REF

2019-nCoV CDC ddPCR™ Triplex Probe Assay	(PN 12008202)
One-Step RT-ddPCR Advanced Kit for Probes	(PN 1864021)
Exact Diagnostic SARS-CoV-2 Standard	(PN COV019)
Exact Diagnostic SARS-CoV-2 Negative	(PN COV000)

IVD





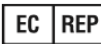












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Translations:

Product documents may be provided in additional languages on electronic media.

Symbols Lexicon:

 European Conformity	 Manufacturer	 Authorized Representative in the European Union
 Lot Number	 Use by	 For In Vitro Diagnostic Use
 Temperature Limit	 Catalog Number	 Consult Instructions for Use
 Number of Tests	 For use with	 Serial Number
 Prescription Use Only	 Unique Device Identification-Device Identifier	 Contains Latex

Bio-Rad Technical Support

For help and technical advice, please contact the Bio-Rad Technical Support department. In the United States, the Technical Support department is open Monday–Friday, 5:00 AM–5:00 PM, Pacific time.

Phone: 1-800-2BIORAD (1-800-224-6723)

Online technical support and worldwide contact information are available at www.consult.bio-rad.com.

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Bio-Rad SARS-CoV-2 ddPCR Test



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Safety and Regulatory Compliance

QXDx™ AutoDG™ ddPCR™ System has been tested and found to be in compliance with all applicable requirements of the following safety and electromagnetic standards:

- IEC 61010-1:2010 (3rd ed.), EN61010-1:2010 (3rd ed). Electrical Equipment for Measurement, Control, and Laboratory Use — Part 1: General requirements
- EN 61326-1:2006 (Class A). Electrical equipment for measurement, control, and laboratory use. EMC requirements, Part 1: General requirements
- UL 61010-1:2004, Laboratory equipment, Test & Measurement Equipment and Industrial Process Controls
- CAN/CSA 22.2 No 61010-1-04, Safety Requirements for Electrical. Equipment for Measurement, Control, and Laboratory Use, Part I: General. Requirements

This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference, in which case the user will be required to correct the interference at his own expense.

This equipment has been tested and found to comply with the limits for a Class A digital device pursuant to Part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment.



The CE mark indicates that the manufacturer ensures the product conforms to the essential requirements of the European Directive for in-vitro diagnostic medical devices 98/79/EC.



The CSA mark indicates that a product has been tested to Canadian and U.S. standards, and it meets the requirements of those applicable standards.



The Waste Electrical and Electronic Equipment Directive symbol indicates that when the end- user wishes to discard this product, it must be sent to separate collection facilities for recovery and recycling.

Bio-Rad SARS-CoV-2 ddPCR Test Warnings and Precautions

For in vitro diagnostic use under Emergency Use Authorization. For healthcare professional use.

This test kit should be handled only by qualified personnel trained in laboratory procedures and familiar with their potential hazards. Wear appropriate protective clothing, gloves and eye/face protection and handle appropriately with the requisite Good Laboratory Practices.

Position the equipment near a grounded outlet with the plug of the power supply accessible. The plug of the power supply is the disconnect device.

Two people are required to lift the QX200 or QXDx Automated Droplet Generator. Grip from the underside, one person on each side.

No serviceable parts inside.

PPE (Personal Protective Equipment) Training

Proper use of gloves is recommended with use of oils and sample plates. OSHA requirements for PPE are set forth in the Code of Federal Regulations (CFR) at 29 CFR 1910.132 (General requirements); 29 CFR 1910.138 (Hand protection); 29 CFR 1926.95 (Criteria for standard personal protective equipment). Any gloves with impaired protective ability should be discarded and replaced. Consider the toxicity of the chemicals and factors such as duration of exposure, storage, and temperature when deciding to reuse chemically exposed gloves. Features to aid glove selection for handling of machines, assays, oils, and cleaning solvents:

- Butyl gloves are made of a synthetic rubber and protect against peroxide, hydrofluoric acid, strong bases, alcohols, aldehydes, and ketones
- Natural (latex) rubber gloves are comfortable to wear and feature outstanding tensile strength, elasticity, and temperature resistance
- Neoprene gloves are made of synthetic rubber and offer good pliability, finger dexterity, high density, and tear resistance; they protect against alcohols, organic acids, and alkalis
- Nitrile gloves are made of copolymer and provide protection from chlorinated solvents such as trichloroethylene and tetrachloroethene; they offer protection when working with oils, greases, acids, and caustic substances.



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Intended Use

The Bio-Rad SARS-CoV-2 ddPCR Test is a partition-based endpoint RT-PCR test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in nasopharyngeal, anterior nasal and mid-turbinate nasal swab specimens as well as nasopharyngeal wash/aspirate and nasal aspirate specimens collected 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, to perform high complexity tests.

Results are for the identification of SARS-CoV-2 RNA which is generally detectable in upper respiratory specimens during the acute phase of the 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 bacterial infection or other co-infection with other viruses. Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.

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

Bio-Rad SARS-CoV-2 ddPCR Test is intended for use by qualified clinical laboratory personnel specifically trained and instructed in ddPCR techniques and *in vitro* diagnostic procedures.

Bio-Rad SARS-CoV-2 ddPCR Test 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 identified and reported to the WHO on December 31, 2019. The rapid spread of SARS-CoV-2 to numerous areas throughout the world necessitates preparedness and response in healthcare and lab facilities. 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 Bio-Rad SARS-CoV-2 ddPCR Test is a molecular *in vitro* diagnostic test containing the reagents required to perform a reverse transcription digital droplet polymerase chain reaction (RT-ddPCR) test. The SARS-CoV-2 primer and probe sets are designed to detect RNA from the SARS-CoV-2 virus in nasopharyngeal, anterior nasal and mid-turbinate swab specimens as well as nasopharyngeal wash/aspirate and nasal aspirate specimens from patients with signs and symptoms of infection who are suspected of COVID-19. Additional testing and confirmation procedures should be performed in consultation with public health and/or other authorities to whom reporting is required. Positive results should also be reported in accordance with local, state, and federal regulations. Performance is unknown in asymptomatic patients.

Principle of the Procedure

The Bio-Rad SARS-CoV-2 ddPCR Test is a reverse transcription (RT) droplet digital polymerase chain reaction (ddPCR) test designed to detect RNA from SARS-CoV-2 in nasopharyngeal, anterior nasal and mid-turbinate swab specimens as well as nasopharyngeal wash/aspirate and nasal aspirate specimens collected from individuals who are suspected of COVID-19 by their healthcare provider.

The oligonucleotide primers and probes for detection of SARS-CoV-2 are the same as those reported by CDC and were selected from regions of the virus nucleocapsid (N) gene. The panel is designed for specific detection of the 2019-nCoV (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. The Bio-Rad SARS-CoV-2 ddPCR Test includes these three sets of primers/probes into a single assay multiplex to enable a one-well reaction.

RNA isolated and purified from nasopharyngeal, anterior nasal and mid-turbinate swab specimens as well as nasopharyngeal wash/aspirate and nasal aspirate specimens is added to the mastermix comprised of reverse transcriptase whereby RNA is converted into cDNA and then amplified, using the Bio-Rad One-Step RT-ddPCR Advanced Kit for Probes.

The sample and mastermix RT-ddPCR mixtures are fractionated into up to 20,000 nanoliter-sized droplets in the form of a water-in-oil emulsion in the Automated Droplet Generator. The emulsions are then thermocycled to achieve reverse transcription to generate cDNA followed by target amplification plus probe hydrolysis in each droplet. Subsequent to PCR, the fluorescence intensity of each droplet is measured in two channels (FAM and HEX) in the Droplet Reader. The fluorescence data is then analyzed by the QuantaSoft v1.7 Software and QuantaSoft Analysis Pro v1.0 Software to determine the presence of SARS-CoV-2 N1 and N2 in the specimen.

Twenty-two microliters (22µl) of each RT-ddPCR ready sample are loaded into the wells of a 96-well PCR plate. The plate and required consumables are loaded into the QX200/QXDx Automated Droplet Generator. The consumables required are: Automated Droplet Generation Oil, Automated Droplet Generator plates, cartridges, and pipet tips. The QX200/QXDx Automated Droplet Generator uses microfluidics to combine oil and an aqueous sample to generate the nanoliter-sized droplets required for ddPCR analysis.

The 96-well RT-ddPCR ready plate containing droplets from the QX200/QXDx Automated Droplet Generator is sealed with foil using a plate sealer, and subjected to thermocycling using either the Bio-Rad C1000 Touch or S1000 Thermocyclers).

After thermocycling is complete, the 96-well RT-ddPCR ready plate is loaded into the QX200/QXDx Droplet Reader. The Droplet Reader singulates the droplets and flows them past a two-color fluorescence detector. The detector reads the droplets to determine which contain target (positive) and which do not (negative) for each of the targets identified with the SARS-CoV-2 ddPCR Test: N1, N2 and RP. The ddPCR system uses the QuantaSoft 1.7 and QuantaSoft Analysis Pro 1.0 for analysis software.






Bio-Rad SARS-CoV-2 ddPCR Test Workflow

The SARS-CoV-2 ddPCR Test is intended for use on Bio-Rad QX200 or QXDx AutoDG Droplet Digital PCR (ddPCR) System (Table 1) and the workflow consists of five steps (Figure 1).

Table 1: Instruments Required

Status	Catalog Number	Product Name
CE marked IVD System	17002229 (Top level part number)	Automated Droplet Generator, IVD system consisting of:
	12001045	QX200 Droplet Reader, IVD
	12001630	Automated Droplet Generator, IVD
FDA Cleared System	17005351 (Top level part number)	QXDx AutoDG ddPCR System consisting of:
	12008020	QXDx Droplet Reader
	12008019	QXDx Automated Droplet Generator
RUO System	1864100 (Top level part number)	QX200 AutoDG Droplet Digital PCR System consisting of:
	1864003	QX200 Droplet Reader
	1864101	Automated Droplet Generator
RUO Lab Equipment	1851197	C1000 Touch Thermal Cycler with 96-Deep Well Reaction Module
	1852197	S1000 Thermal Cycler with 96-Deep Well Reaction Module
	1814000	PX1 PCR Plate Sealer


Figure 1. SARS-CoV-2 ddPCR Test Workflow for 96 samples

Workflow		Instrument	Estimated Hands-on Time	Estimated Instrument Time
Step 1	<ul style="list-style-type: none"> Isolation of viral RNA from anterior nasal and mid-turbinate swab specimens as well as nasopharyngeal wash/aspirate and nasal aspirate specimens swabs 	ThermoFisher MagMAX Viral/Pathogen Nucleic Acid Isolation Kit or QIAamp Viral Mini Kit	<60 min.	N/A
Step 2	<ul style="list-style-type: none"> RT-ddPCR plate setup Droplet generation 		<10 min.	~45 min
Step 3	<ul style="list-style-type: none"> One-step reverse transcription and PCR amplification 	 or 	<10 min.	~210 min
Step 4	<ul style="list-style-type: none"> Droplet reading 		<5 min.	~120 min
Step 5	<ul style="list-style-type: none"> Analysis 		< 5 min	< 1 min

Reagents and Instruments

Materials Provided

Table 2. Materials Required for the SARS-CoV-2 ddPCR Test

<div>  The SARS-CoV-2 ddPCR Test contains sufficient reagents to process a total of 200 samples and quality control specimens. </div>				
Bio-Rad Catalog #	Name	QTY (Tubes)	Volume	Storage Conditions
12008202	2019-nCoV CDC ddPCR Triplex Probe Assay	1	250 µL	-25°C to -15°C
1864021	One-Step RT-ddPCR Advanced Kit for Probes			
	• Supermix	1		-25°C to -15°C
	• Reverse Transcriptase	1		
	• 300 mM Dithiothreitol (DTT)	1		
COV019	Exact Diagnostic SARS-CoV-2 Standard	5	300 µL	-25°C to -15°C
COV000	Exact Diagnostic SARS-CoV-2 Negative	5	300 µL	-25°C to -15°C
12013769	SARS-CoV-2 ddPCR Test Instructions For Use	1	—	—

Note: Safety Data Sheets (SDS) are available at www.bio-rad.com

Materials Required but Not Provided

Reagents & Consumables:

Reagents for RNA Purification

The ThermoFisher MagMAX™ Viral/Pathogen Nucleic Acid Isolation Kit (Cat No. A48310, 1000 reactions) and the QIAamp Viral Mini Kit (Cat No. 52906, 250 reactions) were validated for use with the SARS-CoV-2 ddPCR Test per the manufacturer's instructions.

Generic Reagents and Consumables for Droplet Digital PCR

Table 3. Materials Required But Not Provided for Running on the QXDx ddPCR System

Bio-Rad Catalog #	Name	QTY (each)	Volume	Storage Conditions
12001922	QXDx AutoDG Consumable Pack	1	N/A	15°C to 30°C
12003015	ddPCR Pierceable Foil Seals	50	N/A	15°C to 30°C
12003185	ddPCR 96 Well Plates	15	N/A	15°C to 30°C
12003016	DG32 Cartridges w/ Gaskets	15	N/A	15°C to 30°C
12003010	Pipet Tips for AutoDG (Racks)	10	N/A	15°C to 30°C
12003017	AutoDG Oil for Probes	1	50 mL	15°C to 30°C
12002526	QXDx Droplet Reader Oil Pack	1	1L	15°C to 30°C

Bio-Rad SARS-CoV-2 ddPCR Test



Bio-Rad Catalog #	Name	QTY (each)	Volume	Storage Conditions
1863052	ddPCR Buffer Control for Probes	1	2 x 4.5 mL	15°C to 30°C

Table 4. Materials Required but not Provided for running on the QX200 ddPCR System

Bio-Rad Catalog #	Name	QTY (each)	Volume	Storage Conditions
1814040	ddPCR Pierceable Foil Seals	50	N/A	15°C to 30°C
12001925	ddPCR 96 Well Plates	15	N/A	15°C to 30°C
1864108 1864109	DG32 AutoDG Cartridges w/ Gaskets	15	N/A	15°C to 30°C
1864120 1864121	Pipet Tips for AutoDG (Racks)	10	N/A	15°C to 30°C
1864125	Waste Bins for AutoDG		N/A	15°C to 30°C
1864110	AutoDG Oil for Probes	1	50 mL	15°C to 30°C
1864008 1864007	DG8 Cartridges	1	N/A	15°C to 30°C
1863009 1864007	DG8 Gaskets	1	N/A	15°C to 30°C
1863005	DG Oil for Probes	1	50 mL	15°C to 30°C
1863004	Droplet Reader Oil Pack	1	1L	15°C to 30°C
1863052	ddPCR Buffer Control for Probes	1	2 x 4.5 mL	15°C to 30°C

Instrumentation, Software and General Laboratory Equipment:

Table 5. Instruments Required but not Provided in the Bio-Rad SARS-CoV-2 ddPCR Test

Status	Product Name	Bio-Rad Catalog #
CE marked IVD System	Automated Droplet Generator, IVD system consisting of:	17002229
	• QX200 Droplet Reader, IVD	12001045
	• Automated Droplet Generator, IVD	12001630
FDA Cleared System	QXDx AutoDG ddPCR System consisting of:	17005351
	• QXDx Droplet Reader	12008020
	• QXDx Automated Droplet Generator	12008019
RUO System	QX200 AutoDG Droplet Digital PCR System consisting of:	1864100
	• QX200 Droplet Reader	1864003
	• Automated Droplet Generator	1864101
RUO Lab Equipment	C1000 Touch Thermal Cycler with 96-Deep Well Reaction Module	1851197
	S1000 Thermal Cycler with 96-Deep Well Reaction Module	1852197
	PX1 PCR Plate Sealer	1814000
Software (all systems)	QuantaSoft 1.7	10026368
	QuantaSoft Analysis Pro 1.0	N/A

Table 6. General Laboratory Equipment Required but not Provided in the Bio-Rad SARS-CoV-2 ddPCR Test

Description
Adjustable pipettors (Rainin or Eppendorf)
• 1 – 10 µL
• 10 – 100 µL
• 20 – 200 µL
• 100 – 1000 µL
Adjustable 8-channel pipettors (Rainin or Eppendorf)
• 10 – 50 µL, 10 – 100 µL or 20 – 200 µL
Microcentrifuge
Microwell plate Centrifuge
Vortex mixer

General Precautions & Warnings

1. For In Vitro Diagnostic (IVD) Use under Emergency Authorization only.
2. For professional use only.
3. Positive results are indicative of the presence of SARS-CoV-2 RNA.
4. Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.
5. All biological specimens should be treated as if they are capable of transmitting infectious agents using safe laboratory procedures, such as those outlined in HHS Publication (CDC) 21-1112, Biosafety in Microbiological and Biomedical Laboratories and in CLSI Document M29-A4, Protection of Laboratory Workers from Infectious Disease Transmitted by Blood, Body Fluids, and Tissue.[1, 2]
6. Thoroughly clean and disinfect all work surfaces with a freshly prepared solution of 0.5% sodium hypochlorite (10% bleach) in deionized or distilled water, followed by 70% alcohol.
7. To minimize nucleic acid contamination routinely decontaminate bench space, pipettors and equipment, and separate the specimen and RNA/DNA handling area from the assay preparation area.
8. Optimize workflow and space to minimize risk of carry-over contamination from completed PCR reactions.
9. Ensure that Automated Droplet Generator and Droplet Reader have a dedicated space in separate areas to avoid amplicon contamination.
10. Perform assay setup and template addition in different locations, with dedicated pipettors.
11. Use proper laboratory safety procedures for working with chemicals and handling specimens.
12. Change gloves frequently when transporting and working with different reagents.
13. Failure to follow the procedures and conditions described in this document can cause incorrect results and adverse effects.
14. Do not substitute Bio-Rad SARS-CoV-2 ddPCR Test reagents with other reagents.
15. Setup and template addition must be performed under RNase/DNase-free conditions.
16. Ensure that regular maintenance and calibration is performed on all equipment according to manufacturer's recommendations.
17. Use nuclease-free tips and reagents, and routinely clean pipettors.
18. Ensure that only the recommended thermal cycling protocol is used.
19. Do not use DEPC treated water for PCR amplification.
20. Closely follow procedures and guidelines provided to ensure that the test is performed correctly. Any deviation from the procedures and guidelines may affect optimal test performance.
21. False positive results may occur if carryover of samples is not adequately controlled during sample handling and processing.

Specimen Collection, Transport and Storage

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.

1. Sample acceptance criteria
 - Samples should be collected into sterile, labeled tubes, and shipped at 2°C to 8°C on frozen gel packs.
2. 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.
3. Collecting the Specimen
 - Refer to Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Patients Under Investigation (PUIs) for 2019 Novel Coronavirus (2019-nCoV) <https://www.cdc.gov/coronavirus/2019-nCoV/guidelines-clinical-specimens.html>
 - Follow specimen collection devices manufacturer instructions for proper methods.
 - Swab specimens should be collected using only swabs with a synthetic tip, such as nylon or Dacron® and an aluminum or plastic shaft. Calcium alginate swabs are unacceptable and cotton swabs with wooden shafts are not recommended. Place swabs immediately into sterile tubes containing 2-3 ml of viral transport media or universal transport media.
4. Transporting Specimens
 - Specimens must be packaged, shipped, and transported according to the current edition of the International Air Transport Association (IATA) Dangerous Goods Regulation. Follow shipping regulations for UN 3373 Biological Substance, Category B when sending potential 2019-nCoV specimens to the testing laboratory.
 - Store specimens at 2-8°C and ship overnight to the testing laboratory on ice pack. If a specimen is frozen at -70°C or lower, ship overnight to the testing laboratory on dry ice.
5. 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 4°C if it is to be used within 4 hours, or -70°C or lower if stored longer than 4 hours.

Reagent Preparation and Storage

2019-nCoV CDC ddPCR Triplex Probe Assay

- Store at 4°C, long term storage at -20°C is recommended.
- Prepare aliquot based on the number of reactions required (Table 7).

One-Step RT-ddPCR Advanced Kit for Probes

- The kit contains supermix, reverse transcriptase (RT), and 300 mM dithiothreitol (DTT) solution.
- Thaw all components on ice for 30 min.
- Mix thoroughly by vortexing each tube at maximum speed for 30 sec to ensure homogeneity because a concentration gradient may form during -20°C storage.
- Centrifuge briefly to collect contents at the bottom of each tube.
- Prepare the reaction mix aliquot for the number of reactions required (Table 7).
- Assemble all required components except the sample, dispense equal aliquots into each reaction well, and add the sample to each reaction well as the final step.
- The reactions should be set up on ice before droplet generation and the RT enzyme must be stored at -20°C at all times or on ice when being used.

Control Materials

Controls to be used with the Bio-Rad SARS-CoV-2 ddPCR Test:

1. A “no template” (negative) control is needed to detect reagent and/or environmental contamination, in which RNase/DNase-free water is used in lieu of a clinical specimen sample, per each sample extraction batch, with a minimum of one well per every 96-well plate.
2. A positive template control is needed to detect substantial reverse transcriptase process and/or reagent failure including primer and probe integrity. The test will utilize Exact Diagnostic’s SARS-CoV-2 Standard which is manufactured with synthetic RNA transcripts containing five gene targets: E, N, ORF1ab, RdRP and S Genes of SARS-CoV-2, each quantitated at 200,000 cp/ml along with human genomic DNA background. The material is spiked into a sample-like matrix that requires nucleic acid extraction, identical to a clinical patient specimen. The positive control will be used at a concentration close to the limit of detection (<5x LoD) of the test and is run like a clinical specimen (100 µL aliquot per extraction). One positive template control must be included per each sample extraction batch, with a minimum of one well per every 96-well plate.
3. An extraction control is needed to detect extraction step failure. The test will utilize Exact Diagnostic’s SARS-CoV-2 Negative which is manufactured with human genomic RNA and DNA spiked into a sample-like matrix that requires nucleic acid extraction, identical to a clinical patient specimen (100 µL aliquot per extraction). One extraction control must be included per each sample extraction batch, with a minimum of one well per every 96-well plate.

Nucleic Acid Extraction

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-ddPCR 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)

General Handling

Bio-Rad SARS-CoV-2 ddPCR Test



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.

Performance of the Bio-Rad SARS-CoV-2 ddPCR Test is dependent upon the amount and quality of template RNA purified from human specimens. The following commercial extraction kits and procedures have been qualified and validated for recovery and purity of RNA for use with the test:

ThermoFisher MagMAX Viral/Pathogen Nucleic Acid Isolation Kit (Cat No. A48310, 1000 reactions)

- Add patient sample to an input volume of 100µl and elute with 75µL of RNase/DNase-free water following the manufacturer's instructions.

QIAamp Viral RNA Mini Kit (Cat No. 52906, 250 reactions)

- Add patient sample to an input volume of 140µl and elute with 75µL of AVE buffer or RNase/DNase-free water following the manufacturer's instructions.

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.

Manufacturer's recommended procedures (except as noted in recommendations above) are to be followed for sample extraction. No template control (NTC) and extraction control must be included in each extraction batch.

Bio-Rad SARS-CoV-2 ddPCR Test Protocol

Overview:

The SARS-CoV-2 ddPCR Test is intended for the qualitative detection of nucleic acid from the SARS-CoV-2 in respiratory specimens (nasopharyngeal swabs, anterior nasal and mid-turbinate swabs as well as nasopharyngeal wash/aspirate and nasal wash/aspirate). The assay targets regions of the virus nucleocapsid gene (N1 and N2) and is designed for the specific detection of SARS-CoV-2. Detection of viral RNA not only aids in the diagnosis of illness but also provides epidemiological and surveillance information.

The test is composed of three principal steps: (1) extraction of RNA from patient specimens, RT-PCR reaction preparation and droplet generation, (2) one-step reverse transcription and PCR amplification with SARS-CoV-2 specific primers and ddPCR detection with the SARS-CoV-2 specific Taqman probes and (3) ddPCR droplet reading on the QX200/QXDX Droplet Reader.

Description of Test Steps:

Nucleic acids are isolated and purified from nasopharyngeal, anterior nasal and mid-turbinate swabs, nasopharyngeal wash/aspirate and nasal wash/aspirate specimens using the ThermoFisher MagMAX Viral/Pathogen Nucleic Acid Isolation Kit or the QIAamp Viral RNA Mini Kit, following the manufacturer's instructions for use. The purified nucleic acids are reverse transcribed and amplified using Bio-Rad's One-Step RT-ddPCR Advanced Kit for Probes, which includes three components: Supermix, Reverse Transcriptase and DTT. The reaction mastermix preparation is described in Table 7. The 2019-nCoV CDC ddPCR Triplex Probe assay contains the primers and probes for SARS-CoV-2 targets N1 and N2 as well as the Human RNase P primers and probes in a single tube so that it can be used as a single well test.



Table 7. Preparation of the Reaction Mastermix

Component	Volume per Reaction, μ l	Final Concentration
Supermix	5.5	1x
Reverse transcriptase	2.2	20U/ μ l
300 mM DTT	1.1	15mM
2019-nCoV CDC ddPCR Triplex Probe Assay (20x)	1.1	1x
RNA sample	5.5	100 fg-100 ng per reaction
RNase/DNase free water	6.6	--
Volume per reaction*	22	

*Volumes include 10% excess in setup.

Preparation of One-Step RT-ddPCR Reactions

Note: Ensure that the bench space has been properly cleaned with 10% bleach and 70% alcohol.

Note: Repeated freezing and thawing of the supermix is not recommended. DTT should be aliquoted to multiple tubes and stored at -20°C to minimize freezing and thawing.

1. Ensure extracted RNA sample(s) are thawed.

Note: Do not vortex RNA samples. RNA samples may be mixed by flicking the tubes, followed by brief centrifugation to collect the contents to the bottom of the tubes.

2. Place the Reverse Transcriptase on ice or cold block.
3. Bring the 2019-nCoV CDC ddPCR Triplex Probe Assay to room temperature.
4. Thaw the following components at room temperature for up to 15 minutes.
 - a. One-Step Supermix
 - b. 300 mM DTT
 - c. Nuclease-free water
5. Vortex each component in step 4, and briefly centrifuge to collect contents to the bottom of the tube.
6. Place the components on ice or cold block.
7. RT Master Mix Preparation:
 - a. Prepare Master Mix according to the number of patient samples and controls to be tested (Table 8).
 - b. Vortex the Master Mix for 30 seconds, and centrifuge briefly to collect the contents to the bottom of the tube.

Note: The One-Step RT-ddPCR Supermix is extremely viscous. It is critical to vortex at high speed for at least 30 seconds after thawing and before beginning the assay mix preparation. It is recommended to vortex the assay mix as it is being prepared after each addition of reagent. Quick-centrifuge to spin down and collect the solution before adding the next component. Finally, vortex the prepared reaction mix for 30 seconds at high speed before plating.

Table 8. RT-ddPCR Master Mix Component Volumes by Number of Patient Samples

Component	# of Samples* (samples + overage) Volume μ L				
	1x	(16+2)x	(32+3)x	(48+4)x	(96+6)x
Supermix	5.5	99	192.5	286	561
Reverse transcriptase	2.2	39.6	77	114.4	224.4
300 mM DTT	1.1	19.8	38.5	57.2	112.2

Bio-Rad SARS-CoV-2 ddPCR Test



Component	# of Samples* (samples + overage) Volume μ L				
	1x	(16+2)x	(32+3)x	(48+4)x	(96+6)x
2019-nCoV CDC ddPCR Triplex Probe Assay	1.1	19.8	38.5	57.2	112.2
Nuclease-free water	6.6	118.8	231	343.2	673.2
Total	16.5	297	577.5	858	1683

8. Dispense 16.5 μ L of the Master Mix into the appropriate wells of the RT-ddPCR plate. Add 5.5 μ L of the extracted RNA sample per the suggested plate layout (Figure 2).
9. Add 5.5 μ L of the ExactDx Standard and ExactDx Negative (Figure 2)

Note: The RT-ddPCR plate may be placed on ice while loading.

Note: RT-ddPCR plates may not contain incomplete columns. If any columns are partially used, follow step 10.

10. If all eight wells in a column are not used for controls or samples, ddPCR Buffer Control (ddPCR™ Buffer Control for Probes diluted 1:1 with water) must be added to the unused wells. The Automated Droplet Generator requires all wells in a column to contain a PCR reaction or buffer. Droplets will not form if any wells in a column are empty.

Figure 2. RT-ddPCR Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	NTC	PtSample6	PtSample14	PtSample22	PtSample30	PtSample38	PtSample46	PtSample54	PtSample62	PtSample70	PtSample78	PtSample86
B	ExactDxNegExtCtrl	PtSample7	PtSample15	PtSample23	PtSample31	PtSample39	PtSample47	PtSample55	PtSample63	PtSample71	PtSample79	PtSample87
C	ExactDxPosExtCtrl	PtSample8	PtSample16	PtSample24	PtSample32	PtSample40	PtSample48	PtSample56	PtSample64	PtSample72	PtSample80	PtSample88
D	PtSample1	PtSample9	PtSample17	PtSample25	PtSample33	PtSample41	PtSample49	PtSample57	PtSample65	PtSample73	PtSample81	PtSample89
E	PtSample2	PtSample10	PtSample18	PtSample26	PtSample34	PtSample42	PtSample50	PtSample58	PtSample66	PtSample74	PtSample82	PtSample90
F	PtSample3	PtSample11	PtSample19	PtSample27	PtSample35	PtSample43	PtSample51	PtSample59	PtSample67	PtSample75	PtSample83	PtSample91
G	PtSample4	PtSample12	PtSample20	PtSample28	PtSample36	PtSample44	PtSample52	PtSample60	PtSample68	PtSample76	PtSample84	PtSample92
H	PtSample5	PtSample13	PtSample21	PtSample29	PtSample37	PtSample45	PtSample53	PtSample61	PtSample69	PtSample77	PtSample85	PtSample93

11. After adding template to the master mix plate, cover the plate with a foil seal using the PX1 Plate Sealer.
 - a. Set the PX1 plate sealer to 180°C for 5 seconds (not the default conditions)
 - b. Keep the aluminum block at room temperature while the sealer is not in use.
 - c. Place the ddPCR plate on the room temperature block.
 - d. Cover the plate with one sheet of pierceable foil seal. The red stripe should be visible.

Note: Do not use the metal frame.

- e. Touch the Seal button to seal the plate (this will close the door, and initiate heat sealing).
- f. Remove the plate and aluminum block from PX-1 plate sealer.
- g. Check that all of the wells on the plate are sealed by confirming the depressions of the wells are visible on the foil.

Note: For more detailed instructions refer to PX1 Plate Sealer Instruction Manual.

Note: Remove the aluminum block from PX1 plate sealer while not in use, in order to avoid over-heating the block.

12. Vortex the plate for 30 seconds at high speed.
13. Centrifuge the ddPCR plate for 30 seconds at 1000 rcf to remove any air bubbles and allow the RT-ddPCR reaction mix to pool at the bottom of the wells. If bubbles remain, spin the plate again.
14. Proceed with droplet generation.

Droplet Generation:

Note: The instructions included in this section are applicable for QX200 and QXDx AutoDG Droplet Digital PCR Systems.

Note: Ensure that the bench space and instrument surface area have been properly cleaned with 10% bleach and 70% alcohol.

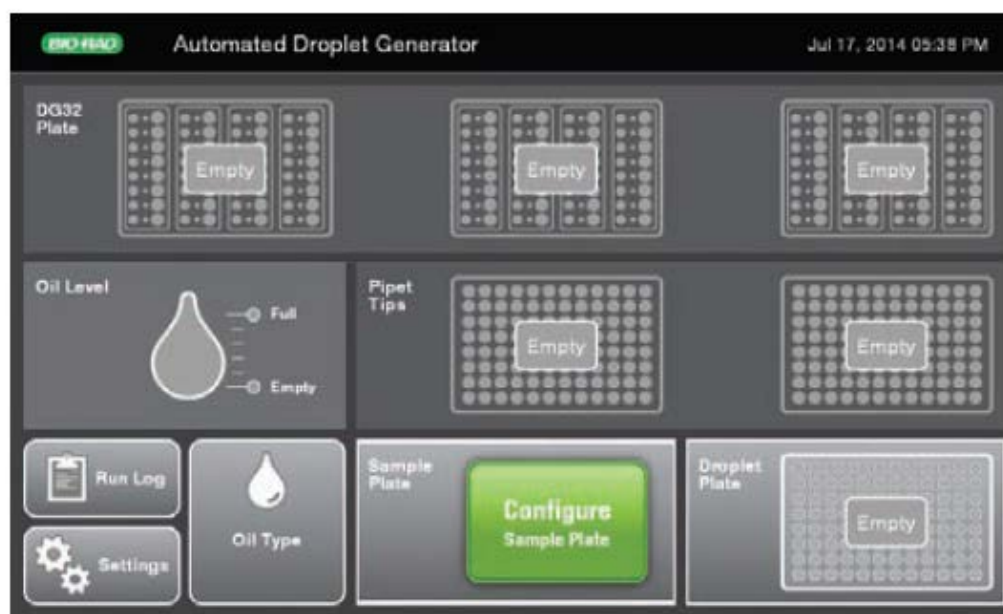
Note: Ensure that the Trash container is empty and is sitting in the appropriate location on the AutoDG.

Note: Ensure that the cooling block accessory is stored upside down at -20°C for at least 2 hours before starting droplet generation.

Note: Periodically inspect the cooling block accessory to ensure that the foil seal is intact.

1. Gather all of the consumable materials needed to setup the QX200 or QXDx Automated Droplet Generator.
 - a. DG32 Cartridges
 - b. Pipet Tips for AutoDG (racks)
 - c. AutoDG Oil for probes
 - d. Sample Plate (RT-ddPCR plate containing the reactions)
 - e. Droplet Plate (new ddPCR plate into which the generated droplets will be dispensed)
2. Open the AutoDG door and load the Sample plate onto the Automated Droplet Generator in the **Sample Plate** position. The indicator light should turn green (Figure 3).

Figure 3. Automated Droplet Generator System User Interface

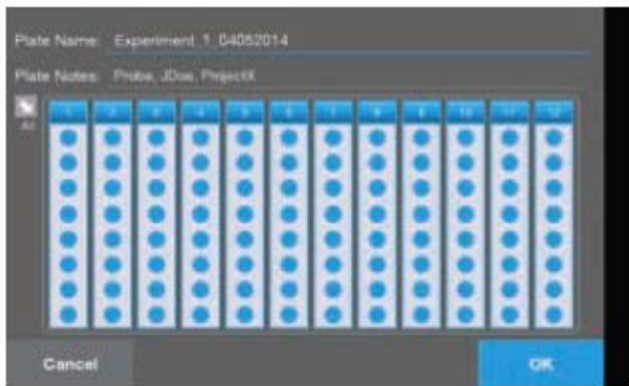


3. Touch the **Configure Sample Plate** button on the AutoDG interface, select the columns in which the samples are located, and touch "OK." Plate name and plate notes may be entered, but are not required (Figure 4).

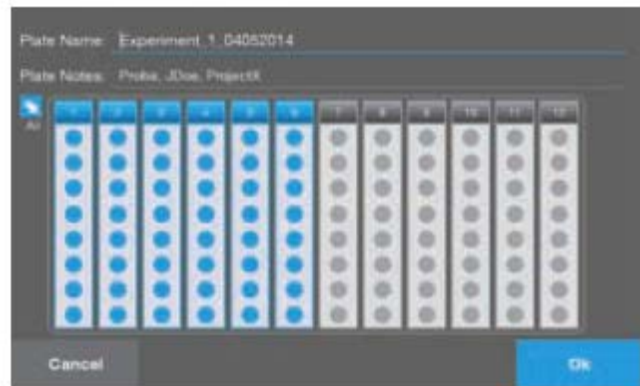
Note: Wells can only be selected in columns of 8 on the AutoDG Configure Screen (Figure 4).

Figure 4. Sample Plate Configuration on the AutoDG Interface

A. 12 columns selected (96 wells, 93 samples, 3 controls)



B. 6 columns selected (48 wells, 45 samples, 3 controls)



4. Based on the number of columns selected on the sample plate, yellow indicator lights will light up indicating consumables that need to be loaded on the instrument.
5. Remove the cooling block accessory from the freezer (-20°C) and place in the Droplet Plate location on the Automated Droplet Generator (Figure). The indicator light should turn green.
6. Load appropriate consumables until associated indicator lights are green.

- a. Load an empty ddPCR 96 well plate onto the cooling block accessory in the Droplet Plate location.
- b. Load the AutoDG Oil for Probes on the left side of the instrument by removing the cap, and twisting the bottle into the tower. Select **Probes** in the Oil Type area of the AutoDG User Interface, and select **OK**. The Oil Level icon on the screen will turn blue and display the current oil level in the bottle.

Note: This step is required only if the oil level is not sufficient for the run.

- c. Load AutoDG pipet tips along the center row of the instrument after removing the plastic wrap and box lid.

Note: Only full tip boxes should be loaded on the system.

- d. Load the DG32 cartridges (with green gaskets to the right) along the back row of the instrument into the plate holders. The holders are keyed for proper orientation, and when placed correctly, the light will turn green.

7. Once all of the indicators on the AutoDG are green, touch the blue **Start Droplet Generation** button.

Warning: Stand clear of the instrument, the door closes automatically prior to run initialization.

8. The AutoDG user interface will ask the user to confirm starting the droplet generation run by touching **Start Run**.

Note: The screen will indicate the time remaining until droplet generation is complete. If droplet formation stops for any reason, a "Run Terminated" message will appear on the AutoDG screen. If the run is terminated, determine the cause of the failure and follow the instructions in the troubleshooting section of the QX200 or QXDx AutoDG Droplet Digital PCR System Instruction Manual.

9. Upon successful completion of droplet generation, remove the Droplet Plate, seal it with a foil seal, and start the PCR run.

Note: PCR amplification should begin within 30 minutes of the completion of the AutoDG Droplet Generation run.



10. Cover the plate with a foil seal using the PX1 Plate Sealer.
 - a. Set the PX1 plate sealer to 180°C for 5 seconds (not the default conditions).
 - b. Keep the aluminum block at room temperature while the sealer is not in use.
 - c. Place the ddPCR plate on the room temperature block.
 - d. Cover the plate with one sheet of pierceable foil seal. The red stripe should be visible.

Note: Do not use the metal frame.

- e. Touch the Seal button to seal the plate (this will close the door, and initiate heat sealing).
- f. Remove the plate and aluminum block from PX-1 plate sealer.
- g. Check that all of the wells on the plate are sealed by confirming the depressions of the wells are visible on the foil.

Note: For more detailed instructions refer to PX1 Plate Sealer Instruction Manual.

Note: Remove the aluminum block from PX1 plate sealer while not in use, in order to avoid over-heating the block.

11. The Droplet Plate is now ready for the one step RT-PCR reaction.
12. Proceed immediately to RT-ddPCR setup, or store the Droplet Plate at 4°C up to 24 hours.

RT- ddPCR (C1000 Touch or S1000 with 96-Deep Well Reaction Module Thermal Cyclers)

1. Place the sealed Droplet Plate into the thermal cycler for PCR amplification and run the SARS-CoV-2 ddPCR Test Thermal Cycling Protocol (Table 9).

Table 9. SARS-CoV-2 RT-ddPCR Thermal Cycling Protocol

Cycling Step	Temperature (°C)	Time	Number of Cycles
Reverse Transcription	50	60 minutes	1
Enzyme activation	95	10 minutes	1
Denaturation	94	30 seconds	40
Annealing/Extension	55	60 seconds	
Enzyme Deactivation	98	10 minutes	1
Droplet Stabilization	4	30 minutes	1
Hold (optional)	4	24 hours	1

Warning: It is critical to set the ramp rate to 2°C/second, as default ramp rates differ for different cyclers.

Note: When using a Bio-Rad C1000 Touch or S1000 with 96-Deep Well Reaction Module Thermal Cyclers, set the sample volume to 40 µL and lid temperature to 105°C. Refer to the C1000 Touch or S1000 with 96-Deep Well Reaction Module Thermal Cycler Instruction Manual for additional information.

Droplet Reading, Data Acquisition and Analysis

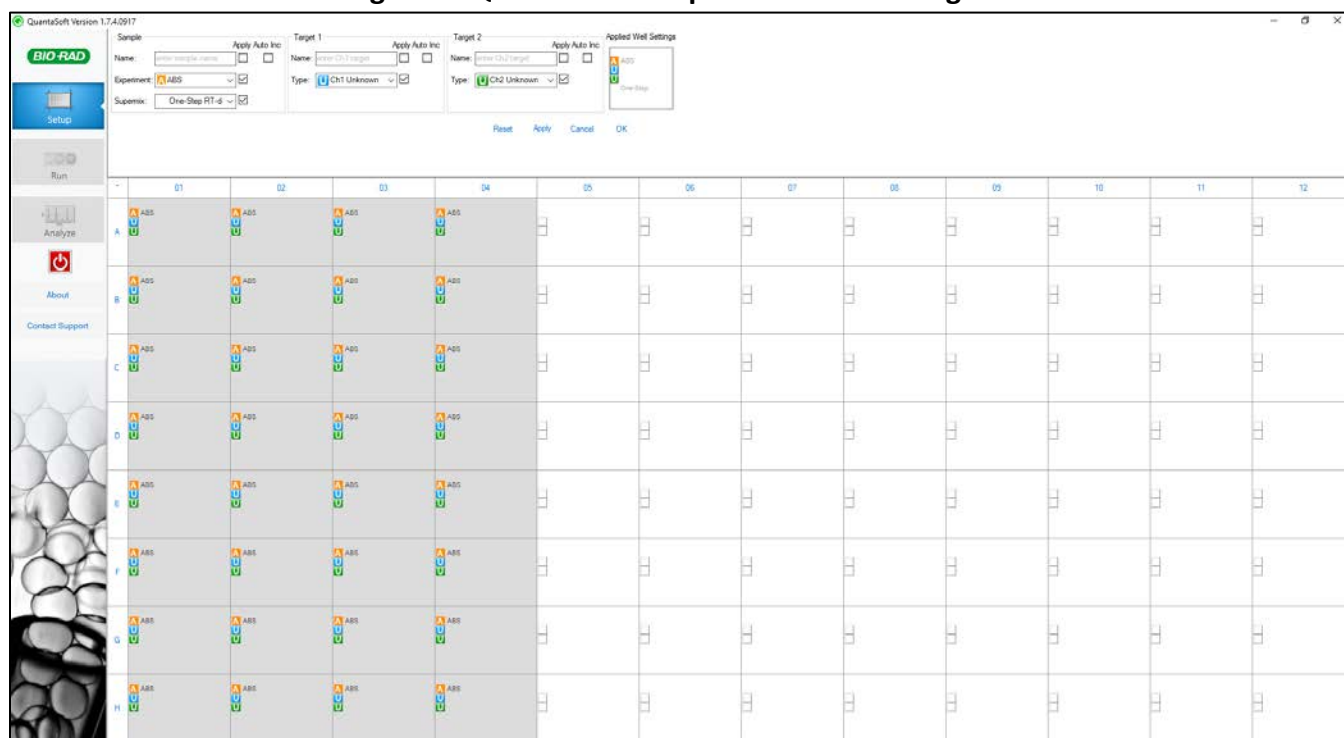
Setting up a run in QuantaSoft Software

1. Upon completion of thermal cycling, transfer the sealed Droplet Plate to the QX200 Droplet Reader, IVD, QX200 Droplet Reader or QXDx Droplet Reader.
2. From the computer connected to the Droplet Reader, open QuantaSoft Software in the setup mode and Select New Run --> Select Test Name --> Select New Plate.

Note: The instructions included in this section are applicable for both QX200 and QXDx AutoDG Droplet Digital PCR Systems.

3. Double-click on a well in the plate layout to open the **Well Editor** dialog box. Select the wells appropriate for the run and choose the following (Figure 5):
 - a. Experiment: ABS
 - b. Supermix: One-Step RT-ddPCR Kit for Probes
 - c. Target 1 Type: Ch1 Unknown
 - d. Target 2 Type: Ch2 Unknown
4. Designate the sample names by well based on the plate layout.

Figure 5. QuantaSoft Setup: Well Editor Dialog Box

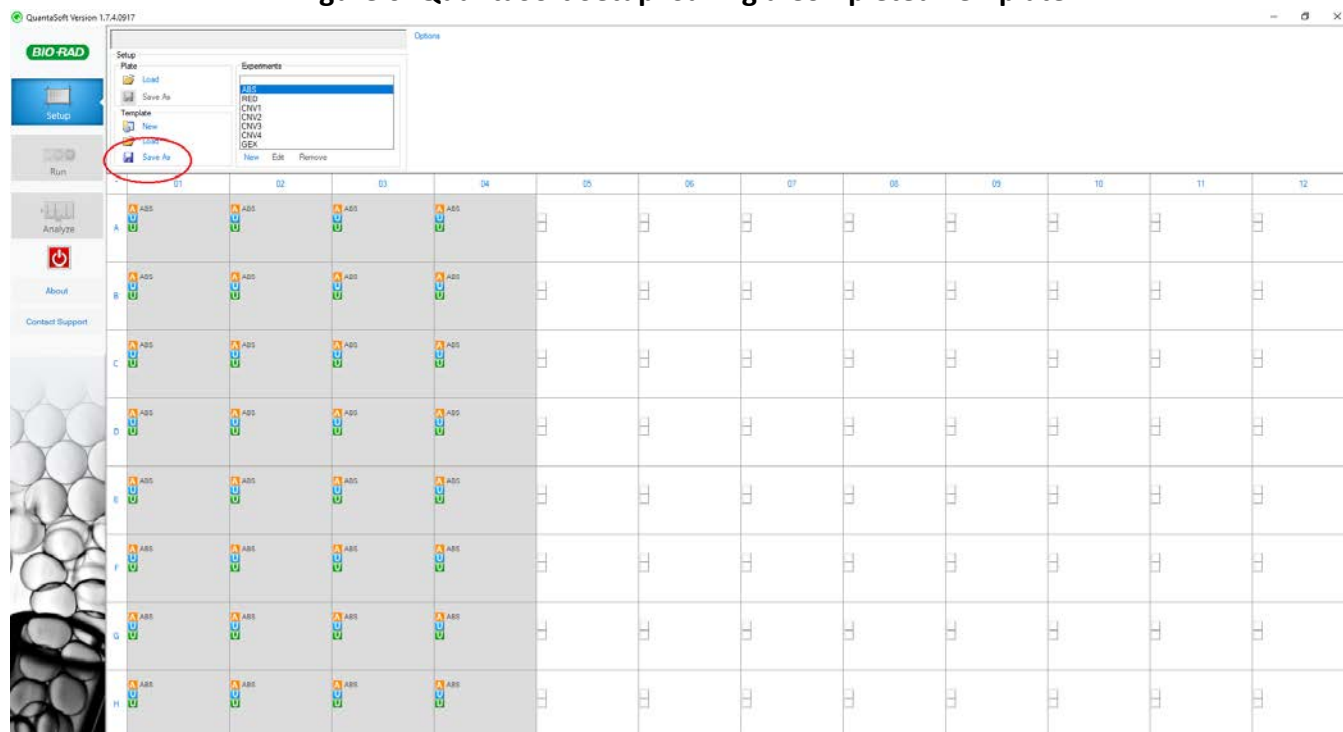


5. For each well on the plate, enter the Sample ID and select **Apply**.
6. Select the Sample Type for each of the wells.
 - a. For wells that contain buffer, rather than a sample, select **Buffer** and touch **Apply**.
7. After finishing with well-name designations and the plate setup is complete, select **OK**.
8. Save a name for the plate by clicking **Save As** under the Template heading in Setup (Figure 6).
9. **Prime** the Droplet Reader by clicking the button on the upper right-hand side of the Setup mode window. It is recommended to prime the instrument before the first run each day.
10. Click the **Run** button from the left-hand panel to begin the droplet reading process.
11. On the right side of the screen, instrument status indicators should display green checkmarks for the system mechanical checks.

Warning: If any of the instrument status lights are not green, follow the instructions provided on the tool tip of the status.

- During data acquisition the software will display information on preliminary quality of acquired data and expected time to completion.

Figure 6. QuantaSoft Setup: Saving a Completed Template



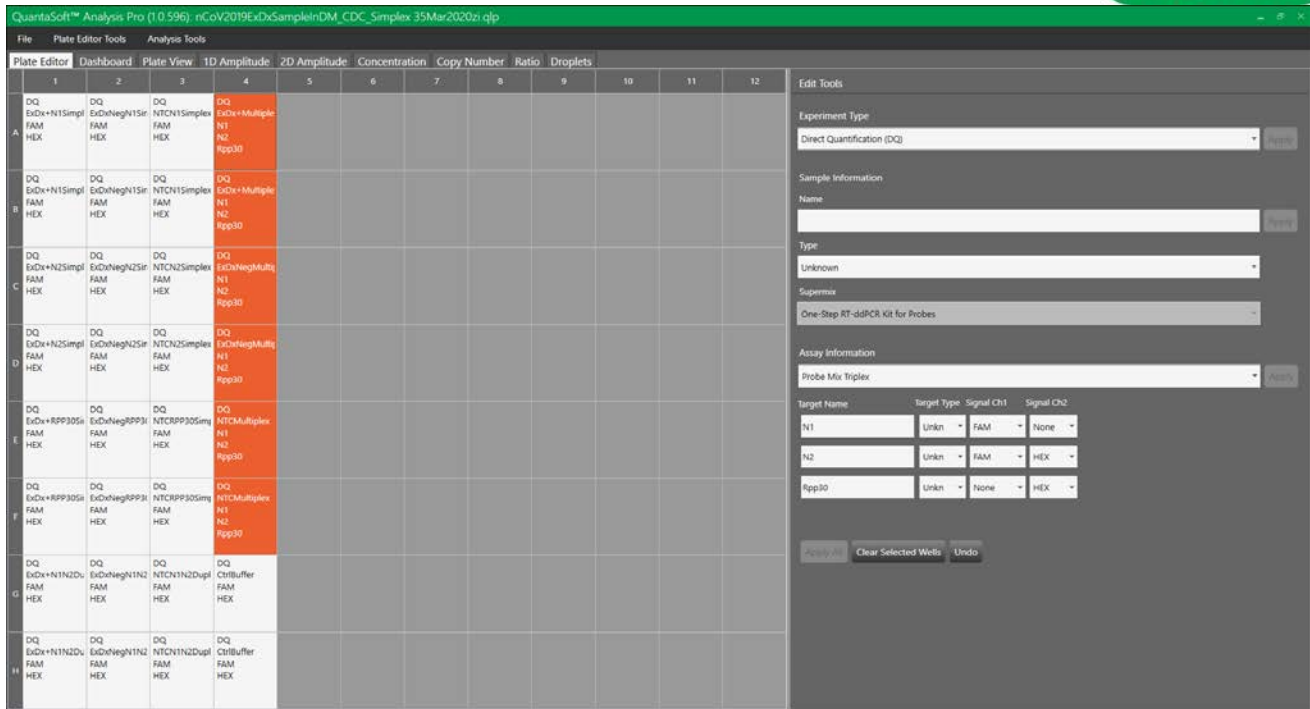
Data Analysis Using QuantaSoft Analysis Pro

- QuantaSoft Analysis Pro (AP) software is available to download and install at <http://www.bio-rad.com/en-us/product/qx200-droplet-digital-pcr-system>. Click on the Download tab. Please refer to the minimum system requirements before installation.
- QuantaSoft Analysis Pro (AP) software opens and analyzes files generated by QuantaSoft Software 1.4 and later. This software is for analysis only; it cannot be used to run the QX200 or QXDx Droplet Reader.
- After Droplet Reading has completed, right-click the file for analysis and select **Open with**. Select **QuantaSoftAnalysisPro**. Alternatively, open QSAP by double-clicking the shortcut on the desktop or through the start menu then drag and drop the .qlp files to be analyzed into the analysis pro window.
- In the **Plate Editor** tab, select all the wells to be analyzed. With **Experiment Type** as **Direct Quantification (DQ)**; select **Probe Mix Triplex** under **Assay Information** and then click **Apply**. Enter Target information (Table 10) and click **Apply**.

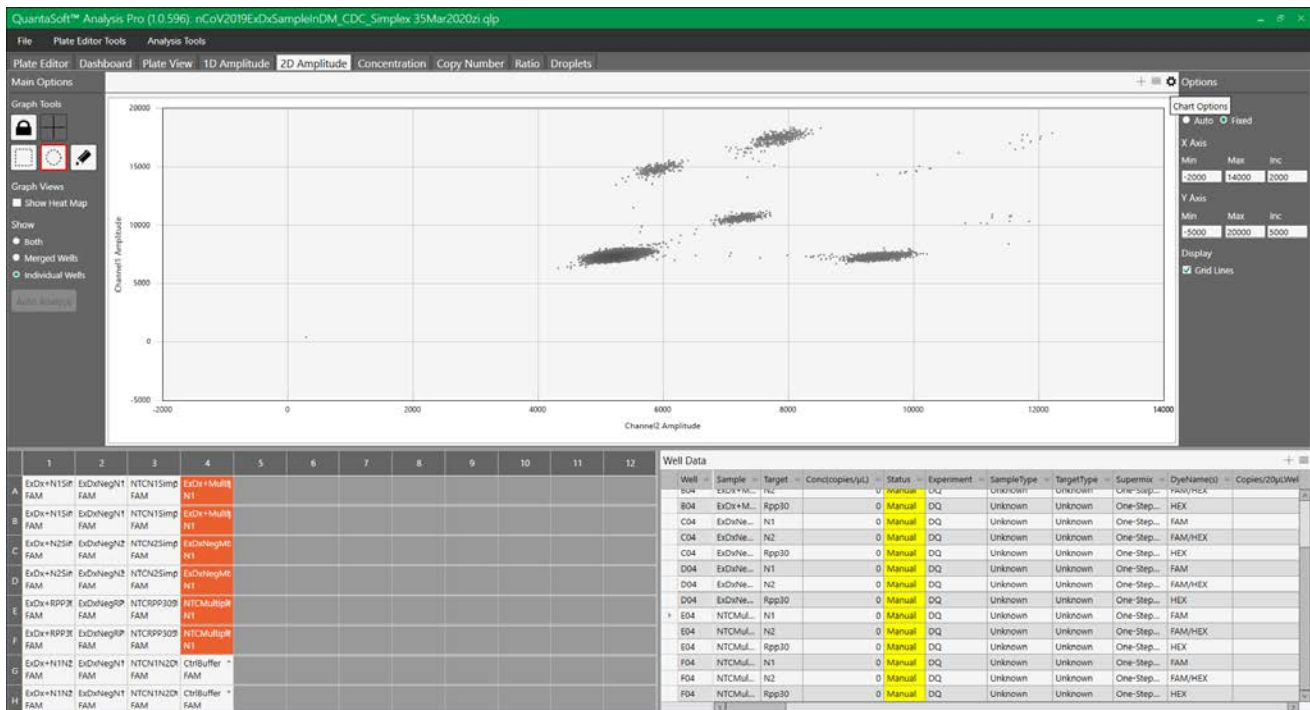
Table 10. QuantaSoft Analysis Pro Target Information

Target Name	Signal Ch1	Signal Ch2
N1	FAM	None
N2	FAM	HEX
RP	None	HEX

Bio-Rad SARS-CoV-2 ddPCR Test



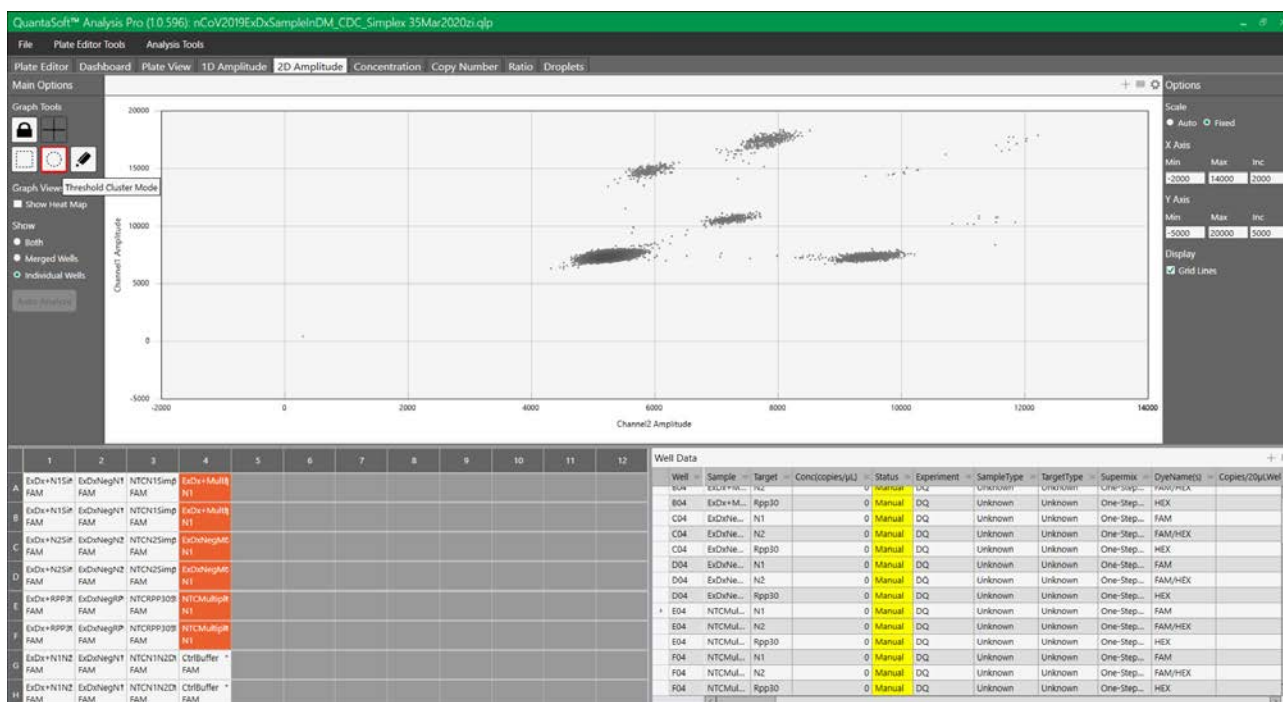
- In the 2D Amplitude tab, select all the wells to be analyzed. Click the small gear icon to the right of the 2D plot and under **Options** and select **Fixed**. The 2D plot graph axes minimums and maximums can be adjusted to remove peripheral white spaces.



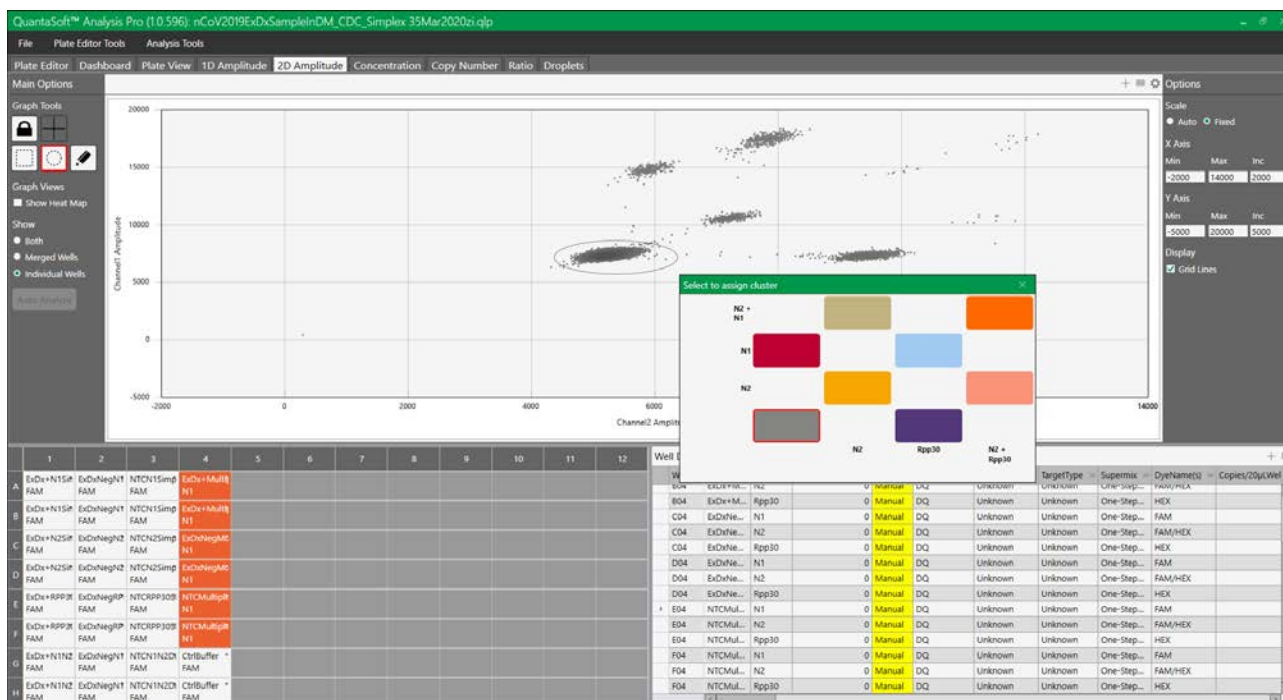
Bio-Rad SARS-CoV-2 ddPCR Test



- From the Graph Tools to the left of the 2D plot, select one of the **Threshold Cluster Modes**. Preferred mode can be selected based on user preferences; the Circular tool may be the most user-friendly. Keep **Graph View** at **Individual Wells**.



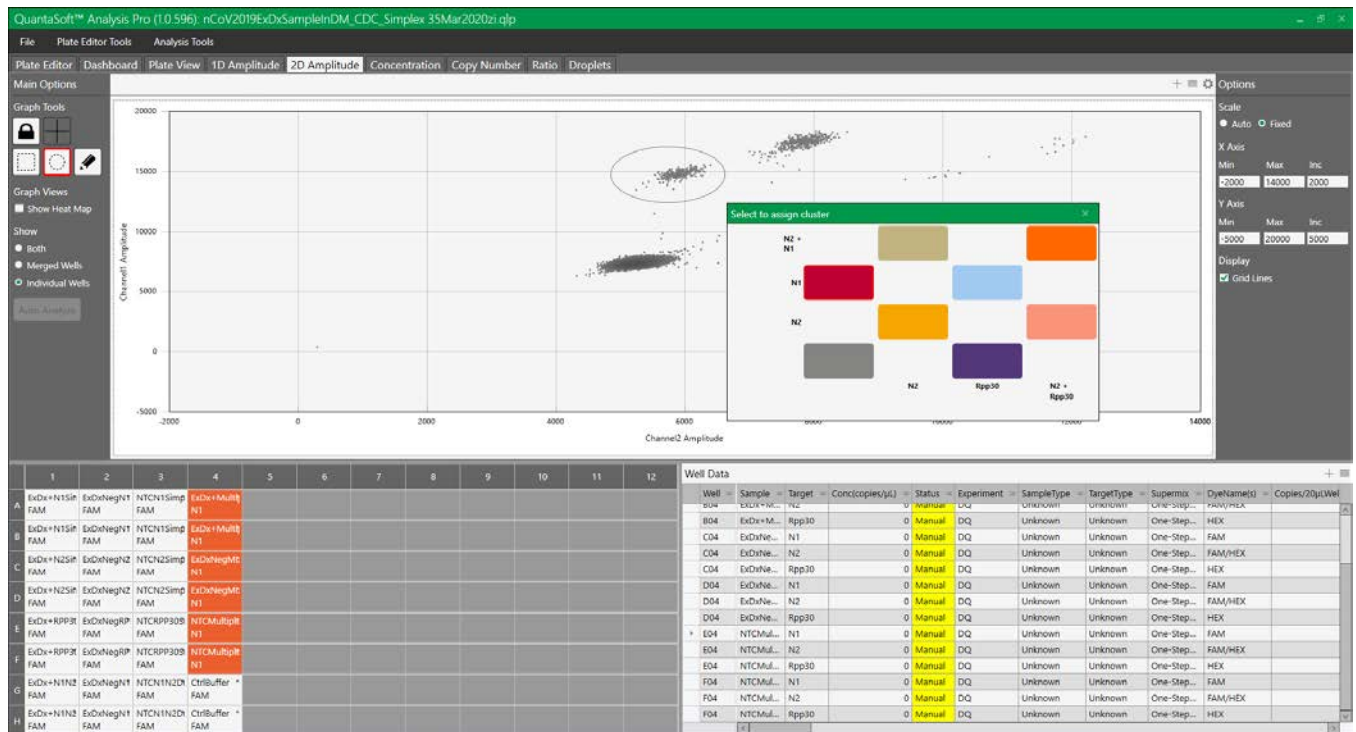
- Using the mouse cursor, select the bottom left (triple negative) cluster. The **Select to assign cluster** window pops up. Choose the bottom left, grey assignment.



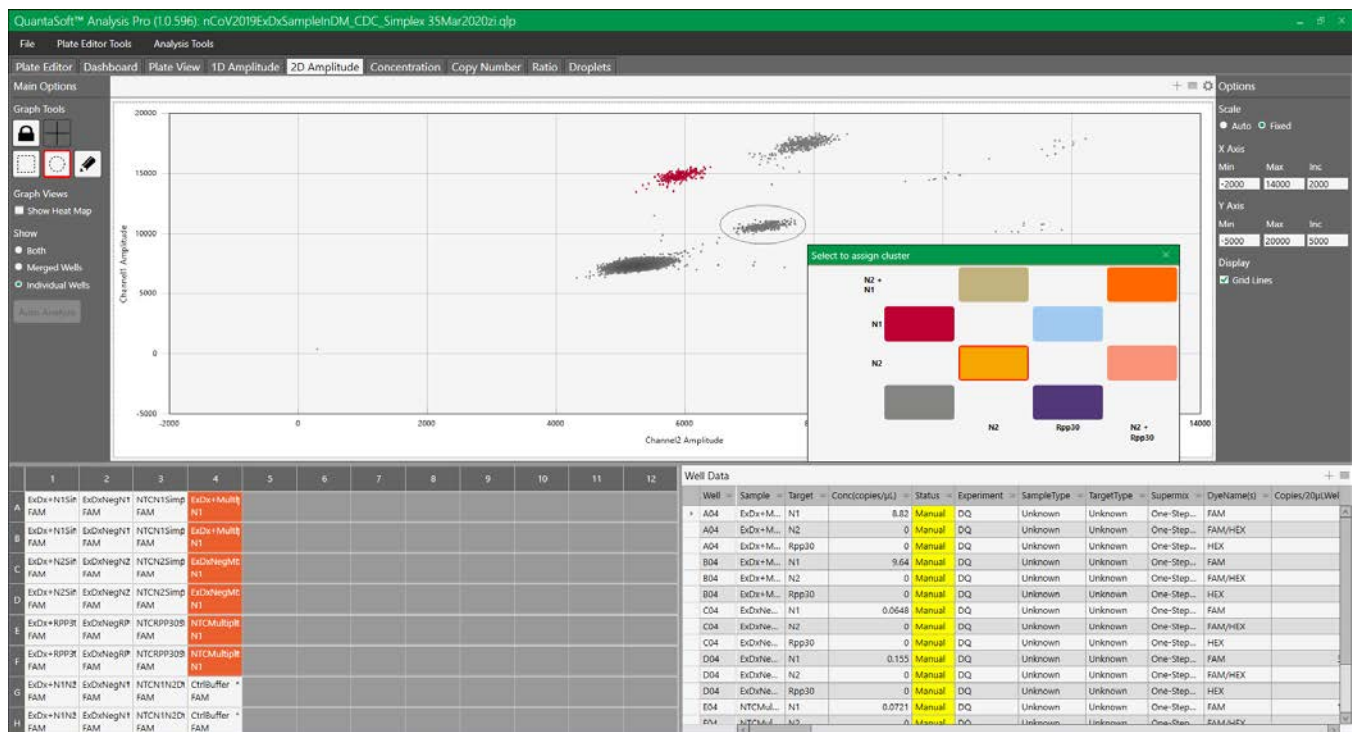
Bio-Rad SARS-CoV-2 ddPCR Test



8. Repeat circling and assigning cluster definitions to all the clusters.
9. Cluster immediately above the triple negative corresponds to **N1** (single-positive, FAM channel).



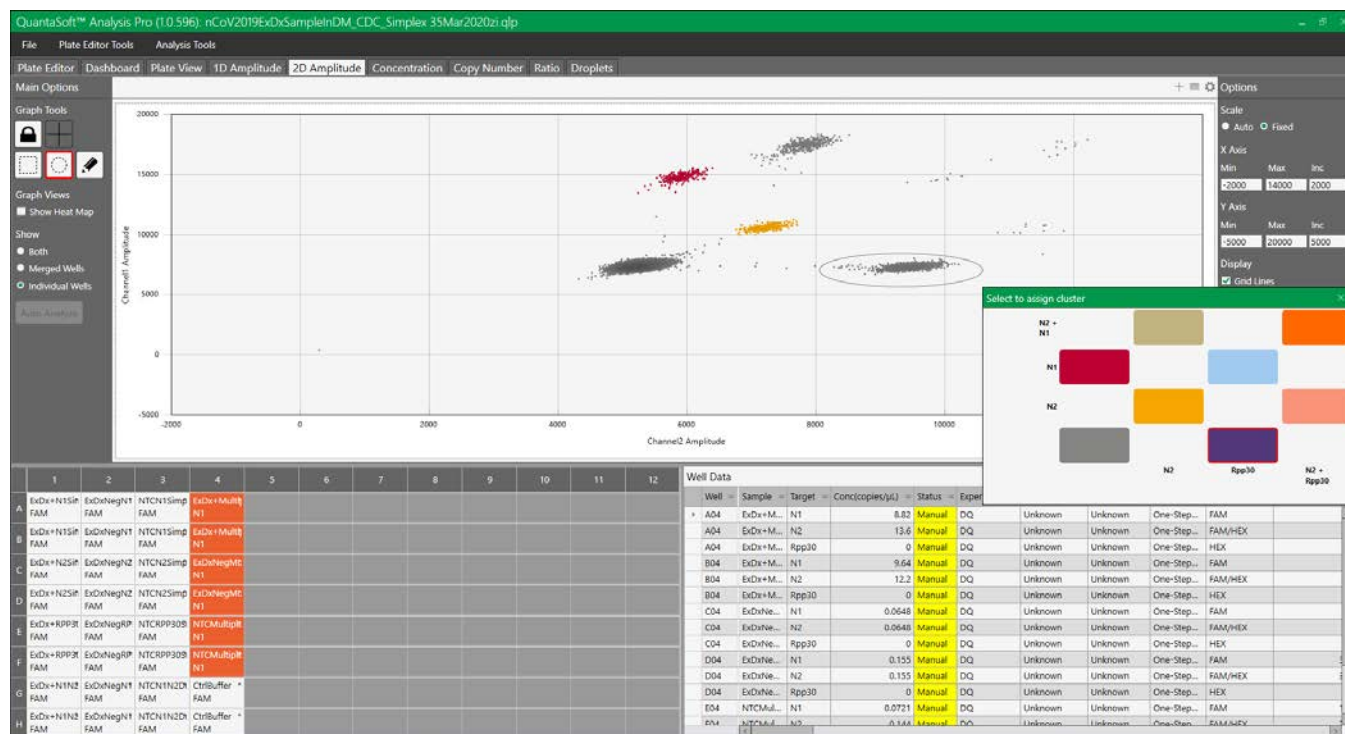
10. Cluster at a 45° angle from the triple negative corresponds to **N2** (single-positive; FAM and HEX channels).



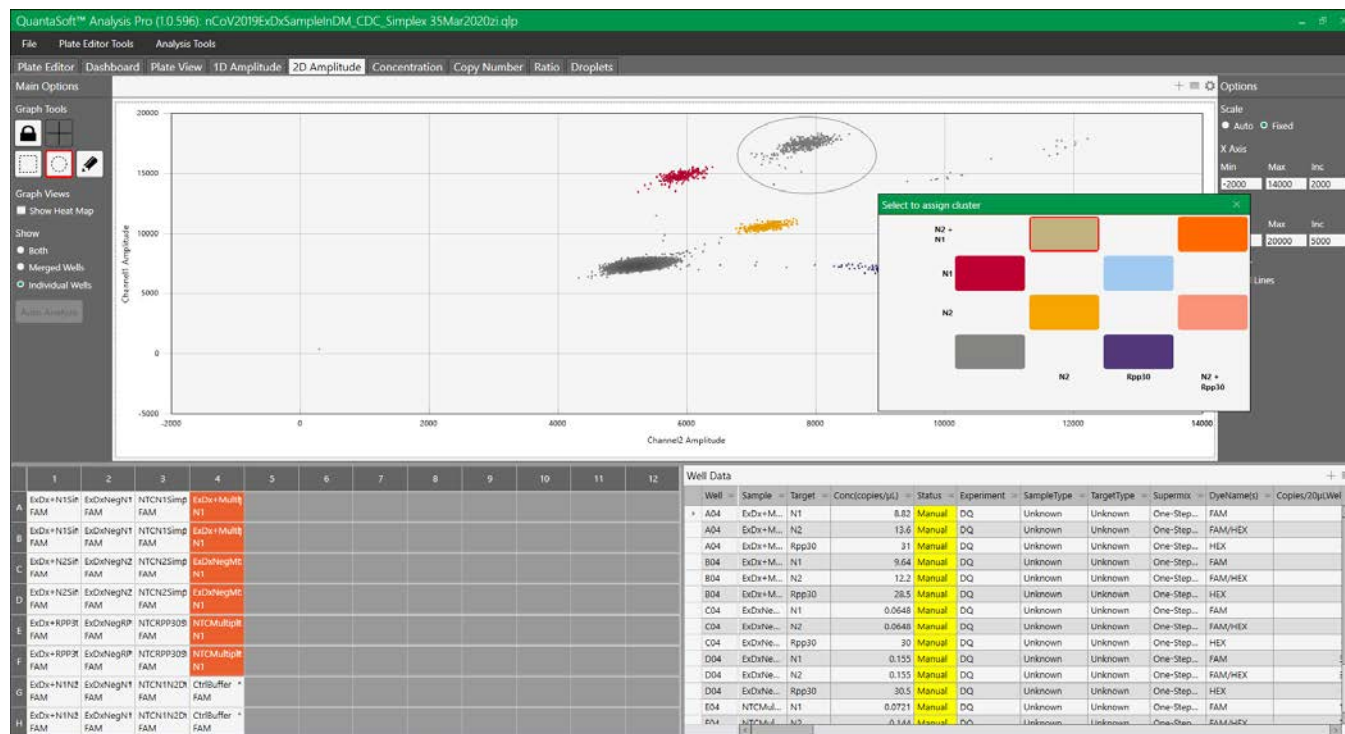
Bio-Rad SARS-CoV-2 ddPCR Test



11. The cluster immediately to the right of the triple-negative cluster corresponds to **RP** (single-positive, HEX channel).



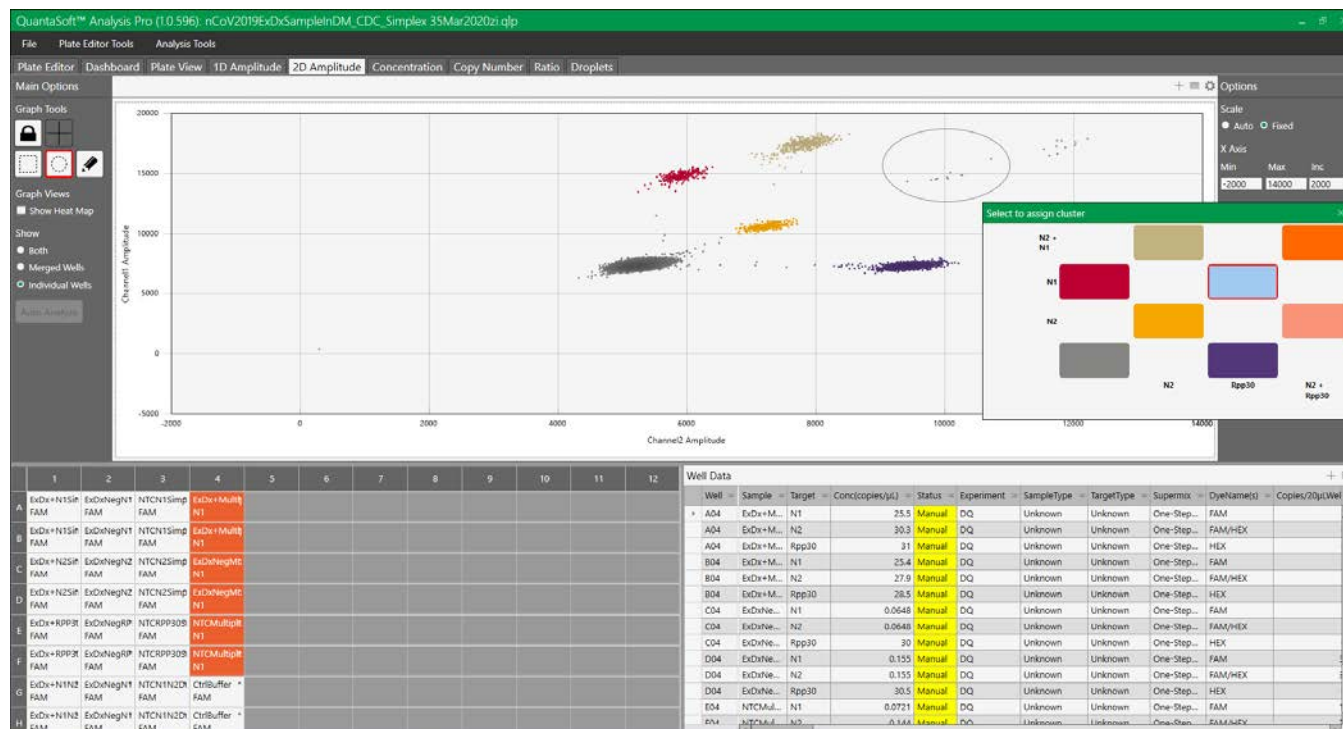
12. Above the N1 and N2 single-positive clusters is a double-positive cluster corresponding to droplets that are both **N1 and N2 positive**.



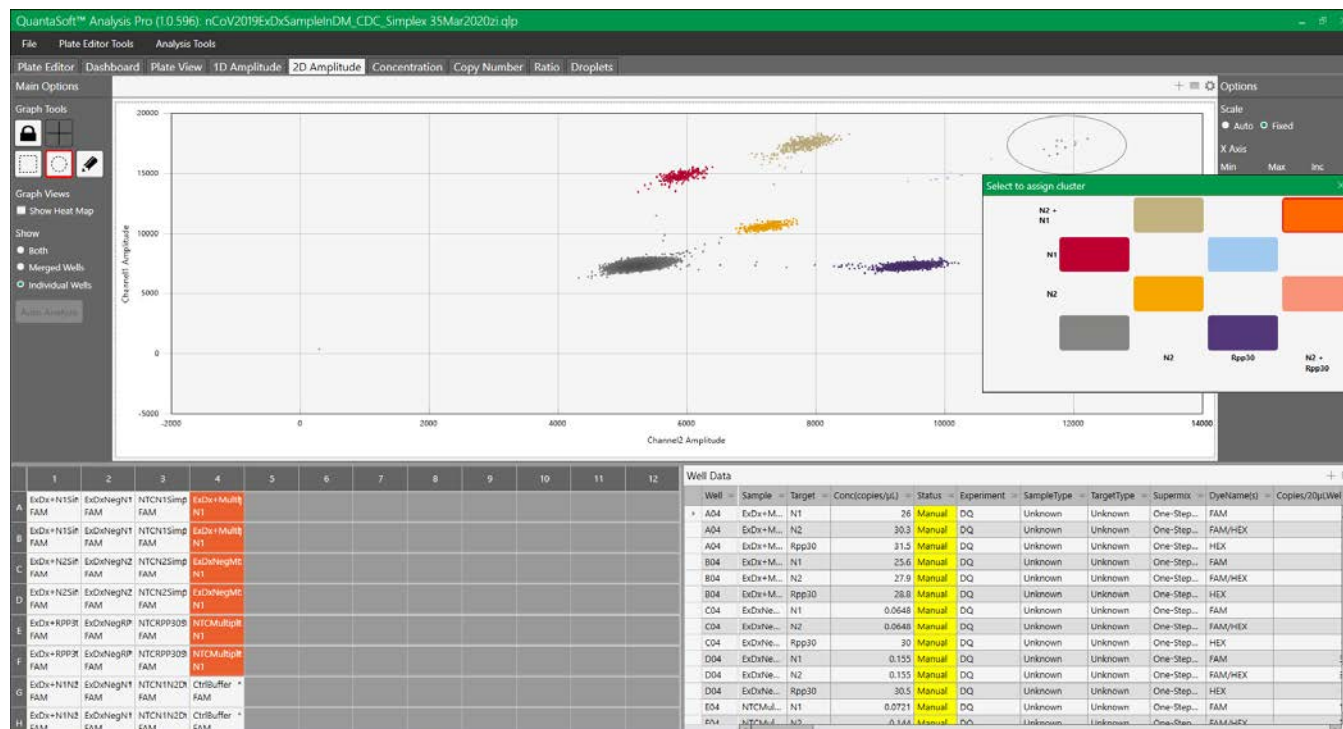
Bio-Rad SARS-CoV-2 ddPCR Test



13. Above the RP single-positive cluster, there is a double-positive cluster corresponding to droplets that are both **N1** and **RP** positive.



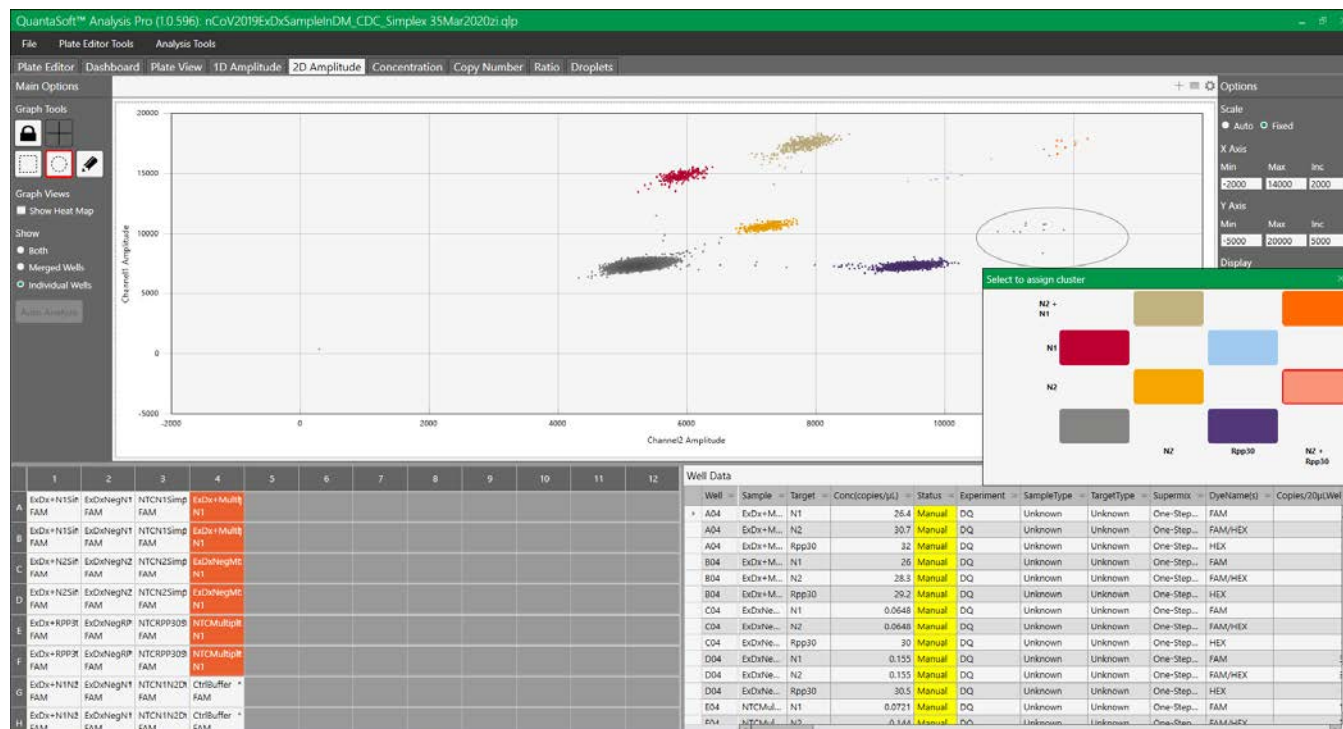
14. To the right of the N1 and N2 double-positive cluster there is an **N1, N2 and RP** triple-positive cluster corresponding to droplets that are positive for all three targets.



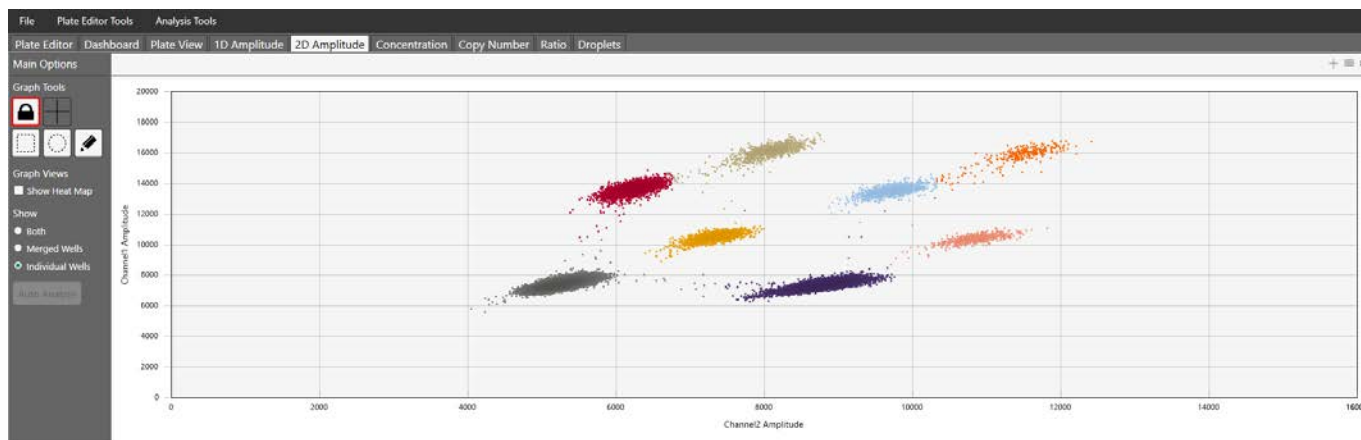
Bio-Rad SARS-CoV-2 ddPCR Test



15. Below the triple-positive cluster there is an **N2 and RP** double-positive cluster corresponding to droplets that are both N2 and RP positive



16. Cluster assignments are applied upon selection. The final configuration will look like the image below.



Note: After applying cluster designation on an entire plate basis, it is essential to select each well and inspect corresponding clusters. Use the circular to Threshold Cluster Mode tool to reassign tight clusters on a per-well basis and check for inaccurate clustering or clusters that weren't entirely selected.

17. Quantifications are provided in the **Well Data** window on the lower right. Use the triple-bar icon on the upper right hand of the Well Data table to **export data to Excel/csv**.
18. The concentration is stated in copies/μL of the final 1X ddPCR reaction for each of the targets (N1, N2 and RP).

Interpretation of Results

All test controls should be examined prior to interpretation of patient results. If the controls are not valid, the patient results cannot be interpreted.

1. Bio-Rad SARS-CoV-2 ddPCR Test Controls – Positive, Negative and Extraction

No Template Control (NTC)

The NTC consists of using nuclease-free water in the one step RT-ddPCR reaction instead of RNA. The NTC reactions for the triplex probe mix should not exhibit positive signal in any channel (HEX or FAM) for any of the three targets tested, N1, N2 or RP. If any of the NTC reactions exhibit positivity, sample contamination may have occurred. Invalidate the run and repeat the assay with the residual extracted nucleic acid with strict adherence to the guidelines. If the repeat test result is positive, re-extract and re-test all samples that were included in that batch.

SARS-CoV-2 Positive Control

The Exact Dx SARS-CoV-2 Standard is used as a positive control and consists of in vitro transcribed RNA and will yield a positive result with the following primer and probe sets: N1, N2 (≥ 0.8 cp/ μ L and ≥ 2 positive droplets for each, N1 and N2 targets) and RP (≥ 0.8 cp/ μ L and ≥ 4 positive droplets).

Extraction Control

The Exact Dx SARS-CoV-2 Negative Control is used as an extraction control. When the negative control is run with the Bio-Rad SARS-CoV-2 ddPCR Test, it is used as an RNA extraction procedural control to demonstrate successful recovery of RNA as well as extraction reagent integrity. The negative control consists of total nucleic acids extracted from a noninfectious cultured human cell material. Purified nucleic acid should yield a positive result with the RP primer and probe set (≥ 0.8 cp/ μ L and ≥ 4 positive droplets) and negative results with all SARS-CoV-2 markers (≤ 0.1 cp/ μ L and ≤ 1 positive droplet).

2. Examination and Interpretation of Patient Specimen Results

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. A control is considered valid if it meets both the expected copies/ μ L concentration and the number of positive droplets as outlined in Table 11. If the controls are not valid, the patient results cannot be interpreted. The software calculates the copies/ μ L value as well as the number of positive droplets. Given that all controls perform as expected, the user then utilizes these parameters to manually determine whether the N1 and N2 targets are present in the sample per the Test Results Interpretation (Table 12). The final test interpretation is qualitative.

The expected performance of the Bio-Rad SARS-CoV-2 ddPCR assay controls is in Table 11.

Table 11. Expected Performance of Controls Required for the Bio-Rad SARS-CoV-2 ddPCR Test

Control Type	External Control Name	Used to Monitor	SARS-CoV-2		Internal Control	Expected cp/ μ L (# of positive droplets)		
			N1	N2		N1	N2	RP
Positive	EDx SARS-CoV-2 Standard	Substantial reagent failure including primer and probe integrity	Positive	Positive	Positive	≥ 0.8 (≥ 2)	≥ 0.8 (≥ 2)	≥ 0.8 (≥ 4)

Bio-Rad SARS-CoV-2 ddPCR Test



Control Type	External Control Name	Used to Monitor	SARS-CoV-2		Internal Control	Expected cp/μL (# of positive droplets)		
			N1	N2		N1	N2	RP
Negative	NTC	Reagent and/or environmental contamination	Negative	Negative	Negative	None detected		
Extraction	EDx SARS-CoV-2 Negative	Failure in extraction procedure	Negative	Negative	Positive	≤0.1* (≤1)	≤0.1 (≤1)	≥0.8 (≥4)

*≤0.1 (≤1); values are below the LoB for the ddPCR assay and are considered negative for N1 and N2

RNase P (Internal Control)

All clinical samples should exhibit positive signal with the RNase P primers and probe, thus indicating the presence of the human RNase P gene. Failure to detect RNase P in any clinical specimens may indicate:

- Improper extraction of nucleic acid from clinical materials resulting in loss of RNA and/or RNA degradation
- Absence of sufficient human cellular material due to poor collection or loss of specimen integrity
- Improper assay set up and execution
- Reagent or equipment malfunction

If the RP assay does not produce a positive result for human clinical specimens, interpret as follows:

- If the SARS-CoV-2 N1 and/or N2 is/are positive even in the absence of a positive RP, the result should be considered valid. It is possible that some samples may fail to exhibit RNase P positive droplets due to low cell numbers in the original clinical sample. A negative RP signal does not preclude the presence of SARS-CoV-2 virus RNA in a clinical specimen.
- If all SARS-CoV-2 markers AND RNase P are negative for the specimen, the result should be considered invalid for the specimen. If residual specimen is available, repeat the extraction procedure and repeat the test. If all markers remain negative after re-test, report the results as invalid and a new specimen should be collected if possible.

SARS-CoV-2 Markers (N1 and N2)

- When all controls exhibit the expected performance, a specimen is considered negative if all SARS-CoV-2 markers (N1, N2) do not present positive droplets AND the RNase P presents positive droplets.
- When all controls exhibit the expected performance, a specimen is considered positive for SARS-CoV-2 if the SARS-CoV-2 markers N1 and/or N2 exhibit positive droplets. The RNase P may or may not be positive as described above, but the SARS-CoV-2 result is still valid.
- When all controls exhibit the expected performance but there are no positive droplets for the SARS-CoV-2 markers (N1, N2) OR for the RNase P, the result is invalid. The extracted RNA from the specimen should be retested. If residual RNA is not available, re-extract RNA from residual specimen and re-test. If the retested sample is negative for all markers and RNase P, the result is invalid and collection of a new specimen from the patient should be considered.
- If the extraction control (SARS-CoV-2 Negative) is positive for N1 or N2, then contamination may have occurred during extraction or sample processing. Invalidate all results for specimens extracted alongside the extraction control. Re-extract specimens and extraction control and re-test.

A tabularized version of the Interpretation Guide is in Table 12. The concentration (cp/μL) and the number of positive droplets are displayed in QuantaSoft Analysis Pro for each well tested. For ease of interpretation, the user can refer to this table.

Table 12. Bio-Rad SARS-CoV-2 ddPCR Test Results Interpretation Guide

SARS-CoV-2 N1 Expected cp/μL (# of positive droplets)	SARS-CoV-2 N2 Expected cp/μL (# of positive droplets)	Internal Control RP Expected cp/μL (# of positive droplets)	Interpretation	Actions
Negative <0.1 (<2)	Negative <0.1 (<2)	Positive ≥0.2 (≥4)	SARS-CoV-2 not detected	None
Positive ≥0.1 (≥2)	Positive ≥0.1 (≥2)	Positive or Negative	SARS-CoV-2 detected	Store samples at -70°C and refer to the appropriate Public Health laboratory if requested
Negative <0.1 (<2)	Negative <0.1 (<2)	Negative <0.2 (<4)	Invalid	Repeat extraction and RT-ddPCR. If the repeated result remains invalid, consider collecting a new specimen from the patient.
If only one of the two targets is positive ≥0.1 (≥2)		Positive or Negative	SARS-CoV-2 detected	Store samples at -70°C and refer to the appropriate Public Health laboratory if requested

Limitations

1. Bio-Rad SARS-CoV-2 ddPCR Test has been evaluated only for use in combination with the 2019-nCoV CDC ddPCR Triplex Probe Assay, One-Step RT-ddPCR Advanced Mix for Probes Kit and the Exact Diagnostic SARS-CoV-2 Standard and Negative reagents for use on the QX200 and QXDx Digital Droplet PCR Systems.
2. Reliable results depend on proper sample collection, storage and handling procedures.
3. This test is intended to be used for the detection of SARS-CoV-2 RNA in nasopharyngeal, anterior nasal and mid-turbinate swab specimens as well as nasopharyngeal wash/aspirate and nasal aspirate specimens collected in a Universal Transport Medium (UTM) or Universal Viral Transport System (UVT). Testing of other sample types with the SARS-CoV-2 ddPCR Test may result in inaccurate results.
4. Detection of SARS-CoV-2 RNA may be affected by sample collection methods, patient factors (e.g., presence of symptoms), and/or stage of infection.
5. Nasopharyngeal wash/aspirate or nasal aspirates and self-collected or healthcare provider collected nasal and mid-turbinate nasal swabs are additional acceptable upper respiratory specimens that can be tested with the SARS-CoV-2 ddPCR Test; however, performance with these specimen types have not been determined.
6. The outputs of the SARS-CoV-2 ddPCR Test are quantitative including copies/μL and number of positive droplets. The user assesses these cut-off values for both sample concentration and positive droplets to make a qualitative call of SARS-CoV-2 detected or not detected; however, the values reported should not be used or interpreted as quantitative.
7. As with any molecular test, mutations within the target regions of the Bio-Rad SARS-CoV-2 ddPCR Test could affect primer and/or probe binding resulting in failure to detect the presence of virus.
8. Due to inherent differences between technologies, it is recommended that, prior to switching from one technology to the next, users perform method correlation studies in their laboratory to qualify technology differences. One hundred percent agreement between the results should not be expected due to aforementioned differences between technologies. Users should follow their own specific policies/procedures.



9. False negative or invalid results may occur due to interference. The Internal Control is included in the Bio-Rad SARS-CoV-2 ddPCR Test to help identify the specimens containing substances that may interfere with nucleic acid isolation and PCR amplification.

Conditions of Authorization for the Laboratory

The Bio-Rad SARS-CoV-2 ddPCR Test 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/emergency-situations-medical-devices/emergency-use-authorizations#covid19ivd>.

However, to assist clinical laboratories using the Bio-Rad SARS-CoV-2 ddPCR Test ("your product" in the conditions below), the relevant Conditions of Authorization are listed below:

- A. Authorized laboratories¹ using your product will include with result reports of your product, all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.
- B. Authorized laboratories using your product will use your product as outlined in the Instructions for Use. Deviations from the authorized procedures, including the authorized instruments, authorized extraction methods, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to use your product are not permitted.
- C. Authorized laboratories that receive your product will notify the relevant public health authorities of their intent to run your product prior to initiating testing.
- D. Authorized laboratories using your product will have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.
- E. Authorized laboratories will collect information on the performance of your product and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and Bio-Rad Technical Support at 1-800-2BIORAD (1-800-224-6723) any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of your product of which they become aware.
- F. All laboratory personnel using your product must be appropriately trained in PCR techniques and use appropriate laboratory and personal protective equipment when handling this kit, and use your product in accordance with the authorized labeling.
- G. You, authorized distributors, and authorized laboratories using your product will ensure that any records associated with this EUA are maintained until otherwise notified by FDA. Such records will be made available to FDA for inspection upon request.

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

Analytical Performance Characteristics

Analytical Sensitivity

Limit of detection (LoD) studies determine the lowest detectable concentration of SARS-CoV-2 at which greater or equal to 95% of all (true positive) replicates test positive. To determine the LoD, a synthetic virus from a commercial source (SeraCare SARS-CoV-2 5,000 cp/mL) was serially diluted in a background of pooled nasopharyngeal swab matrix. A total of 8 concentration levels, with 2-fold serial dilutions between the levels, were tested with 3 extraction replicates per concentration and 3 RT-ddPCR replicates per extraction for a total of 9 replicates per level. This was performed in two sets, one for each extraction kit validated for use with the Bio-Rad SARS-CoV-2 ddPCR Test. LoD testing was completed on the C1000 Touch Thermal Cycler with 96-Deep Well Reaction Module. The results from these serial dilutions is summarized in Table 13.

A confirmation of the LoD was then determined using the three 2-fold serial dilutions that were positive for N1 and N2 in at least 8/9 replicates. These three concentrations were each tested with 20 extraction replicates. The LoD was determined as the lowest concentration tested where $\geq 95\%$ (19/20) of the replicates were positive.

The LoD for the samples extracted with the Thermo MagMAX Viral/Pathogen Nucleic Acid Isolation kit was 625 cp/mL for N1 and N2, as summarized in Table 14. The LoD for the samples extracted with the QIAamp Viral RNA Mini kit was also 625 cp/mL for N1 and N2, as summarized in Table 15.

Table 13. Initial Limit of Detection Evaluation of the Bio-Rad SARS-CoV-2 ddPCR Test

SARS-CoV-2 copies/mL	Samples extracted with Thermo MagMAX		Samples extracted with QIAamp Viral RNA	
	N1 Positive Replicates / Total Number of Replicates	N2 Positive Replicates / Total Number of Replicates	N1 Positive Replicates / Total Number of Replicates	N2 Positive Replicates / Total Number of Replicates
5000	9/9	9/9	9/9	9/9
2500	9/9	9/9	9/9	9/9
1250	9/9	9/9	9/9	9/9
625	9/9	9/9	8/9	8/9
312.5	4/9	7/9	3/9	1/9
156.25	3/9	5/9	4/9	1/9
78.12	0/9	1/9	0/9	0/9
39.06	2/9	1/9	0/9	0/9

Table 14. Limit of Detection* Confirmation of the Bio-Rad SARS-CoV-2 ddPCR Test

Target	2019 nCoV N1			2019 nCoV N2		
SARS-CoV-2 Concentration (cp/mL)	2500	1250	625	2500	1250	625
Positive Replicates/Total Number of Replicates	20/20	20/20	19/20	20/20	20/20	19/20

*Samples extracted with Thermo MagMAX Viral/Pathogen Nucleic Acid Isolation kit

Table 15. Limit of Detection* Confirmation of the Bio-Rad SARS-CoV-2 ddPCR Test

Target	2019 nCoV N1			2019 nCoV N2		
SARS-CoV-2 Concentration (cp/mL)	2500	1250	625	2500	1250	625
Positive Replicates/Total Number of Replicates	20/20	20/20	20/20	20/20	20/20	19/20

*Samples extracted with QIAamp Viral RNA Mini Kit

Inclusivity

Bio-Rad SARS-CoV-2 test uses the same sequences for N1, N2 and RP as the CDC authorized assay. CDC performed an alignment with the oligonucleotide primer and probe sequences of the CDC 2019 nCoV Real Time RT-PCR Diagnostic Panel with all publicly available nucleic acid sequences for 2019-nCoV in GenBank as of February 1, 2020 to demonstrate the predicted inclusivity of the CDC 2019 nCoV Real-Time RT-PCR Diagnostic panel. All the alignments showed 100% identity of the CDC panel to the available 2019-nCoV sequences with the exception of one nucleotide mismatch with the N1 forward primer in one deposited sequence.

Since the alignment performed on February 1, 2020 there have been additional sequences deposited in GenBank and GISAID. For this reason, in silico analysis was performed using sequences that have been deposited after February 1, 2020. A total of 4456 complete or partial genomes were downloaded from GISAID and NCBI nucleotide databases, from February 1, 2020- April 6 2020. 4431 of these genomes cover the target region of the N1 assay and 4413 of them cover the target region of the N2 assay. For the N1 assay, 4353 (98.2%) of these genomes perfectly match all three assay components; 77 genomes contain one total mismatch from all three assay components; 1 (one) genome (hCoV-19/Iceland/68/2020|EPI_ISL_417858|2020-03-08) has a single base insertion in the forward primer. No genomes were found to have more than one total mismatch in the oligo binding sites. For the N2 assay, 4392 (99.5%) of the genomes perfectly match all binding sites of the assay oligos; 21 genomes contain one total mismatch from all three assay components. No genomes were found to have more than one total mismatch in the oligo binding sites.

The risk of a single mismatch resulting in a significant loss in reactivity, and false negative result, is low due to the design of the primers and probes with melting temperatures > 60°C and run conditions of the assay with annealing temperature at 55°C to tolerate one to two mismatches.

Analytical Specificity (Cross-reactivity)

In silico analysis for the pathogens listed in Table 16 was performed by downloading one GenBank reference sequence per genome for each of the organisms. The reference sequences were compared against the Bio-Rad SARS-CoV-2 targets, N1 and N2 for all possible combinations (forward primer, reverse primer, probe and the reverse complements for all of these) to determine homology percent. If any of these primer combinations were mapped to a sequence on opposite strands with a homology of >80% on the same target within a short distance (≤100bp) apart, potential amplifications were flagged. No potential unintended cross reactivity is expected based on this in silico analysis except for SARS-coronavirus with the N2 target.

As reported under the CDC EUA, the in silico analysis for the N1 primer/probe set showed high sequence homology of the N1 probe with SARS coronavirus and Bat SARS-like coronavirus genome. However, forward and reverse primers showed no sequence homology with SARS coronavirus and Bat SARS-like coronavirus genome. Combining the primers and probe results, there is no significant homologies with human genome, other coronaviruses or human microflora that would predict potential false positive RT-ddPCR results.

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*Analysis of the forward primer of the N2 target showed high homology to Bat SARS-like coronaviruses. However, the reverse primer and probe sequences showed no significant homology with human genome, other coronaviruses or human microflora was observed that would predict potential false positive RT-ddPCR results. Combining the primers and probe results, there is no prediction of potential false positive RT-ddPCR results.

Table 16. In silico analysis for SARS-CoV-2

Pathogens tested in-silico	Unintended Cross-reactivity to N1	Unintended Cross-reactivity to N2
SARS-coronavirus	None detected	Homology match 92%*
MERS-coronavirus	None detected	None detected
Human adenovirus A	None detected	None detected
Human adenovirus B1	None detected	None detected
Human adenovirus B2	None detected	None detected
Human adenovirus C	None detected	None detected
Human adenovirus D	None detected	None detected
Human adenovirus E	None detected	None detected
Human adenovirus F	None detected	None detected
Human Metapneumovirus (hMPV)	None detected	None detected
Parainfluenza virus 1	None detected	None detected
Parainfluenza virus 2	None detected	None detected
Parainfluenza virus 3	None detected	None detected
Parainfluenza virus 4	None detected	None detected
Influenza A H3N2	None detected	None detected
Influenza A H2N2	None detected	None detected
Influenza A H7N9	None detected	None detected
Influenza A H1N1	None detected	None detected
Influenza B	None detected	None detected
Human enterovirus A	None detected	None detected
Human enterovirus B	None detected	None detected
Enterovirus E, Bovine enterovirus	None detected	None detected
Enterovirus F	None detected	None detected
Enterovirus G, Porcine enterovirus 9	None detected	None detected
Enterovirus H, Simian enterovirus A	None detected	None detected
Enterovirus J strain 1631	None detected	None detected
Enterovirus J strain N203	None detected	None detected
Respiratory syncytial virus	None detected	None detected
Rhinovirus A, Human rhinovirus 89	None detected	None detected
Rhinovirus A, Human rhinovirus 1 strain ATCC VR-1559	None detected	None detected
Rhinovirus B	None detected	None detected

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Pathogens tested in-silico	Unintended Cross-reactivity to N1	Unintended Cross-reactivity to N2
Rhinovirus C, Human rhinovirus C	None detected	None detected
Rhinovirus C, Human rhinovirus NAT001	None detected	None detected
<i>Haemophilus influenzae</i>	None detected	None detected
<i>Legionella pneumophila</i>	None detected	None detected
<i>Mycobacterium tuberculosis</i>	None detected	None detected
<i>Streptococcus pneumoniae</i>	None detected	None detected
<i>Streptococcus pyogenes</i>	None detected	None detected
Enterovirus (e.g. EV68)	None detected	None detected
<i>Pneumocystis jirovecii</i>	None detected	None detected

Clinical Evaluation

The performance of Bio-Rad SARS-CoV-2 ddPCR Test with nasopharyngeal swab clinical samples was evaluated using 40 individual negative clinical samples and 39 confirmed positive clinical samples collected from patients with signs and symptoms of an upper respiratory infection. Clinical samples were collected by qualified personnel according to the package insert of the collection device. Samples were stored frozen at -80°C until use. Samples were tested for COVID-19 at CerbaXpert (Paris, France) with the Seegene Allplex™ 2019-nCoV Assay (EUA authorized 4-21-2020) and designated positive or negative based on the manufacturer's instructions.

The Allplex assay is an in vitro diagnostic (IVD) real-time reverse transcriptase polymerase chain reaction (RT-PCR) test intended for the qualitative detection of nucleic acid from severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2) in human nasopharyngeal swab, oropharyngeal swab, anterior nasal swab, midturbinate and sputum specimens from individuals with signs and symptoms of infection who are suspected of COVID-19 by their health care provider.

Nucleic acid extraction was performed twice on the set of 79 samples, once with the ThermoFisher MagMAX Viral/Pathogen Nucleic Acid Isolation Kit and once with the QIAamp Viral RNA Mini Kit. Negative samples and a range of low, moderate and high positive clinical samples were randomized, blinded and tested with the Bio-Rad SARS-CoV-2 ddPCR Test was completed on the C1000 Touch Thermal Cycler with 96-Deep Well Reaction Module. Both the positive percent agreement (PPA) and negative percent agreement (NPA) was 94.87%, 95% CI [81.37, 99.11] for the sample set extracted with Thermo MagMAX, as shown in Table 17. The PPA was 94.59%, 95% CI [80.47, 99.06] and the NPA was 95.00%, 95% CI [81.79, 99.13] for the sample set extracted with QIAamp Viral RNA Mini, as shown in Table 19. **Discordant analysis for the 2 false positives and 2 false negatives was performed with the CDC 2019-Novel Coronavirus Real Time RT-PCR Diagnostic Panel and these results were 100% concordant with the Bio-Rad SARS-CoV-2 ddPCR Test (Tables 18 and 20).** The Bio-Rad SARS-CoV-2 ddPCR Test demonstrated equivalent performance regardless of the extraction method used.

Table 17. PPA and NPA of the Bio-Rad SARS-CoV-2 (Thermo MagMAX extraction) against comparator test

	Comparator Test Positive	Comparator Test Negative
Bio-Rad SARS-CoV-2 Positive	37	2
Bio-Rad SARS-CoV-2 Negative	2	37

PPA = 94.87%, 95% CI [81.37, 99.11]

Bio-Rad SARS-CoV-2 ddPCR Test



NPA = 94.87%, 95% CI [81.37, 99.11]

A total of 78 samples were available for analysis when using the Thermo MagMAX extraction method because one sample was excluded due to an invalid test result (the internal control was negative, e.g. no RP signal was detected and there were no N1 or N2 positive droplets). There were a total of 4 discordant cases which were adjudicated with a third test – the CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel. The detail information for the excluded case, the discordant cases and the adjudication results are described in Table 18.

Table 18. Description of excluded and discordant cases

Case ID	Bio-Rad SARS-CoV-2 ddPCR Test	Comparator Test (Seegene EUA Assay)	Adjudication Test (CDC EUA Assay)
132	Invalid	Negative	Negative N1 Cq = N/A N2 Cq = N/A RP Cq = 25.41
619	Positive N1 concentration = 0.182 cp/μL N2 concentration = 0.364 cp/μL RP concentration = 71.6 cp/μL	Negative	Positive N1 Cq = 33.33 N2 Cq = 35.6 RP Cq = 26.83
127	Positive N1 concentration = 0.171 cp/μL N2 concentration = 0.514 cp/μL RP concentration = 277 cp/μL	Negative	Positive N1 Cq = 35.52 N2 Cq = 41.48 (negative) RP Cq = 26.65
818	Negative N1 concentration = 0 cp/μL N2 concentration = 0 cp/μL RP concentration = 71.7 cp/μL	Positive	Negative N1 Cq = N/A N2 Cq = N/A RP Cq = 27.05
398	Negative N1 concentration = 0 cp/μL N2 concentration = 0 cp/μL RP concentration = 49.1 cp/μL	Positive	Negative N1 Cq = N/A N2 Cq = N/A RP Cq = 26.6

Table 19. PPA and NPA of the Bio-Rad SARS-CoV-2 (QIAamp Viral RNA extraction) against comparator test

	Comparator Test Positive	Comparator Test Negative
Bio-Rad SARS-CoV-2 Positive	35	2
Bio-Rad SARS-CoV-2 Negative	2	38

PPA = 94.59%, 95% CI [80.47, 99.06]

NPA = 95.00%, 95% CI [81.79, 99.13]

A total of 77 samples were available for analysis when using the QiaAmp extraction method because two samples were excluded due to insufficient specimen available for extraction. The same 4 discordant cases were identified. The results for the excluded case and the discordant cases is described in Table 20.

Table 20. Description of excluded and discordant cases

Bio-Rad SARS-CoV-2 ddPCR Test



Case ID	Bio-Rad SARS-CoV-2 ddPCR Test	Comparator Test (Seegene EUA Assay)	Adjudication Test (CDC EUA Assay)
998	Not enough specimen to test	Positive	N/A
44	Not enough specimen to test	Positive	N/A
619	Positive N1 concentration = 0.166 cp/μL N2 concentration = 0.498 cp/μL RP concentration = 59.1 cp/μL	Negative	Positive N1 Cq = 33.33 N2 Cq = 35.6 RP Cq = 26.83
127	Positive N1 concentration = 0.383 cp/μL N2 concentration = 0.230 cp/μL RP concentration = 124 cp/μL	Negative	Positive N1 Cq = 35.52 N2 Cq = 41.48 (negative) RP Cq = 26.65
818	Negative N1 concentration = 0 cp/μL N2 concentration = 0 cp/μL RP concentration = 59.6 cp/μL	Positive	Negative N1 Cq = N/A N2 Cq = N/A RP Cq = 27.05
398	Negative N1 concentration = 0 cp/μL N2 concentration = 0 cp/μL RP concentration = 51.1 cp/μL	Positive	Negative N1 Cq = N/A N2 Cq = N/A RP Cq = 26.6

References:

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2. Clinical and Laboratory Standards Institute (CLSI). Protection of laboratory workers from occupationally acquired infections. Approved Guideline-Fourth Edition. CLSI Document M29-A4; Wayne, PA; CLSI, 2014. Siegel, R.L., K.D. Miller, and A. Jemal, *Cancer statistics, 2018*. CA Cancer J Clin, 2018. **68**(1): p. 7-30.