Clarifi COVID-19 Test Kit

For Real Time qRT-PCR Test

Instructions for Use

Catalog #1105/1176 and 1110 – Individual Test
Catalog #1154/1178 and 1155 – Pooled Test

For Use under Emergency Use Authorization Only

Rx Only
For *in vitro* diagnostic use

Quadrant Biosciences • 505 Irving Ave, Suite 3100AB, Syracuse, NY 13210 • 866-205-7336
quadrantbiosciences.com

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Intended Use

The Clarifi COVID-19 Test Kit (Clarifi Test) is a real-time qRT-PCR test intended for the qualitative detection of RNA from SARS-CoV-2 in saliva specimens collected in a healthcare setting from individuals who are suspected of COVID-19 by their healthcare provider using the ORAcollect•RNA (OR-100/ORE-100) or the OMNIgene•ORAL (OM-505/OME-505) collection device. Testing is limited to laboratories which are certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, and meet the requirements to perform high complexity tests.

This test is also for the qualitative detection of nucleic acid from the SARS-CoV-2 in pooled samples containing up to twelve (12) individually collected saliva specimens. Negative results from pooled testing should not be treated as definitive. If a patient's clinical signs and symptoms are inconsistent with a negative result or results are necessary for patient management, the patient should be considered for individual testing. Specimens included in pools with a positive, presumptive positive, or invalid result must be tested individually prior to reporting a result. Specimens with low viral loads may not be detected in sample pools due to decreased sensitivity in pooled testing.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in saliva specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out 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 results to the appropriate public health authorities.

Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information. Negative results for SARS-CoV-2 RNA from saliva should be confirmed by testing of an alternative specimen type if clinically indicated.

The Clarifi COVID-19 Test Kit is intended for use by qualified clinical laboratory personnel, specifically instructed and trained in the techniques of qRT-PCR and *in vitro* diagnostic procedures. The Clarifi COVID-19 Test Kit 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 (SAR-CoV-2) in Wuhan City, Hubei Province, China was initially reported to the World Health Organization (WHO) in early December 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.

Principles of the Procedure

The Quadrant Biosciences Clarifi COVID-19 Test Kit is a real-time reverse transcription polymerase chain reaction (qRT-PCR) test. The SARS-CoV-2 primer and probe set(s) are designed to detect two specific sequences of the RdRp gene of the SARS-CoV-2 genome. RNA is extracted from saliva swabs collected from patients suspected of COVID-19 by their healthcare provider. Saliva is collected using the ORAcollect•RNA (OR-100/ORE-100) saliva collection device or the OMNIgene•ORAL (OM-505/OME-505) collection device. Collection is performed following the Instructions for Use included on the pouch of the OR-100/ORE-100 and OM-505/OME-505 collection devices.

Once received by the laboratory, the ORAcollect•RNA (OR-100/ORE-100) and OMNIgene•ORAL (OM-505/OME-505) collection devices containing saliva samples are incubated at 60 °C for 2 hours for viral inactivation. After incubation, samples are either tested individually or up to 12 samples can be pooled with equal volumes, as desired. Subsequently, RNA is extracted from either 100 µL of the individual sample or 100 µL of the pooled sample using the Clarifi COVID-19 Extraction Kit, for which 100 µl sample input volume is used. RNA is eluted in 30 µL of DNase/RNase-Free Water and 1.5 µL of the eluted RNA is used for the down-stream qRT-PCR reaction.

The test consists of three processes in a single-well assay:

- Reverse transcription of total RNA to cDNA
- PCR amplification of target and Internal Control cDNA
- Simultaneous detection of PCR amplicons by fluorescent dye labeled probes

Components and Storage

The Clarifi COVID-19 Test kit is provided in two versions: Part number 1110/1176 & 1105 (Individual Kit) are used for individually tested samples and Part number 1154/1178 & 1155 (Pooled Kit) are used for sample testing in pools. The versions contain different amounts of reagents, but reagents are identical.

Components - Individual Kit (Catalogue Number 1110/1176 & 1105)

Note: The Clarifi COVID-19 Kit is shipped at different temperatures each with a unique part number. See table below to indicate the kit part number the component is contained within. The collection devices are packaged separately from the lab components.

Name	Part Number	Quantity	Storage Conditions
Part Number 1110 (shipped at ambient temperature)			
Clarifi COVID-19 Extraction Kit	1114	6 boxes	15°C to 30°C
Saliva collection device*	1110 or 1176	1 swab/test, 2300/kit	15°C to 25°C
Instructions for Use	1106	1	N/A
Part Number 1105 (shipped on dry ice)			
4x Reliance One-Step Multiplex Supermix**	12010176	10 x 1mL	-20°C
Positive Control	1111	200 copies/µL	-20°C
Clarifi COVID-19 Primer/Probe Set	1108	10 x 780µL	-20°C

* The ORAcollect•RNA saliva swab collection device lot (P/N 1110) or OMNIgene•OM-505/OME-505 collection devices (P/N 1176) provided with the Clarifi COVID-19 Test Kit should not be exchanged with ORAcollect•RNA saliva swabs or OMNIgene•OM-505/OME-505 collection devices from lots that were procured from other sources or with different swabs as this may impact the performance of this test.
**The BioRad enzyme mix lot provided in this test kit was specifically qualified for use with this assay. Do not exchange the enzyme lot provided in this test kit with different lots of the same enzyme or with different enzymes as this may impact the performance of this test.

Components - Pooled Kit (Catalogue Number 1154/1178 & 1155)

Note: The Clarifi COVID-19 Kit is shipped at different temperatures each with a unique part number. See table below to indicate the kit part number the component is contained within. The collection devices are packaged separately from the lab components.

Name	Part Number	Quantity	Storage Conditions
Part Number 1154 (shipped at ambient temperature)			
Clarifi COVID-19 Extraction Kit	1114	1 box	15℃ to 30℃
Saliva collection device*	1154 or 1178	1 swab/test, 4100/kit	15℃ to 25℃
Instructions for Use	1106	1	N/A
Part Number 1155 (shipped on dry ice)			
4x Reliance One-Step Multiplex Supermix**	12010176	2 x 1mL	-20℃
Positive Control	1111	200 copies/µL	-20℃
Clarifi COVID-19 Primer/Probe Set	1108	2 x 780µL	-20℃

* The ORAcollect•RNA saliva swab collection device lot (P/N 1154) or OMNIgene• OM-505/OME-505collection devices (P/N 1178) provided with the Clarifi COVID-19 Test Kit should not be exchanged with ORAcollect•RNA saliva swabs or OMNIgene• OM-505/OME-505collection devices from lots that were procured from other sources or with different swabs as this may impact the performance of this test.
**The BioRad enzyme mix lot provided in this test kit was specifically qualified for use with this assay. Do not exchange the enzyme lot provided in this test kit with different lots of the same enzyme or with different enzymes as this may impact the performance of this test.

Components Required but Not Provided with The Kit – Pooled and Individual

Name	Manufacturer	Part Number	Storage Conditions
PCR Grade Water	Any	Any	15°C to 30°C
Deep 96 well plate (1.0 mL)	Nest	501062	N/A
Ethanol	Fisher Scientific	BP2818500	15°C to 30°C
Beta-mercaptoethanol	Fisher Scientific	03446I-100	15°C to 30°C

Components Required but Not Provided with The Kit (Continued)

Name	Manufacturer	Part Number	Storage Conditions
MicroAmp™ Optical 96-Well Reaction Plate with Barcode, or equivalent	ThermoFisher Scientific	4306737	N/A
Hard-Shell 96-Well PCR Plates, low profile, thin wall, skirted, white/white, or equivalent	BioRad	HSP9655	N/A
Hard-Shell 384-Well PCR Plates, thin wall, skirted, clear/white, or equivalent	BioRad	HSP3805	N/A
Microseal 'B' PCR Plate Optical Sealing Film, or equivalent	BioRad	MSB1001	N/A
Single-Channel Pipette	Ranin	P-10, P-20, P-200, P-1000	N/A
Multi-Channel Pipette	Ranin	P-10, P-20, P-50, P-200	N/A
Aerosol Barrier Pipette Tips	Ranin	P-10, P-20, P-50, P-200, P-1000	N/A
MicroCentrifuge tube, 1.5 or 2mL, PCR grade	N/A	N/A	N/A

Equipment/Instrumentation Required

Name	Manufacturer	Part Number	Software Version
BioRad-CFX96 Touch Real Time Detection System[2]	BioRad	1855195	Version 1.4.1
BioRad-CFX384 Touch Real Time Detection System[2]	BioRad	1855485	Version 1.4.1
QuantStudio 5[2]	Applied Biosystems	A28139	Version 1.5.1
QuantStudio 7[2]	Applied Biosystems	4485695	Version 1.7.1
7500 Fast Real Time PCR System[2]	Applied Biosystems	4351107	Version 1.4.1
Swing Bucket Plate Centrifuge	N/A	N/A	N/A
Incubator	N/A	N/A	N/A

[2] Please refer to Appendix B of this IFU for EUO labeling that should be affixed to select instruments.

Reagent Storage, Handling, and Stability

- Clarifi COVID-19 Test Kit is shipped at two temperatures (ambient temperature and on dry ice).
- All components of the kit must be stored at the appropriate storage conditions as listed in the section *Kit Components*.
- The Clarifi COVID-19 Primer/Probe Set should be stored at -20°C and protected from light.
- Do not use kit components after expiration date printed on the tube label.
- If there is damage to the packaging inside and outside or kit contents have been tempered with or storage condition failed to meet above -20°C do not use.
- Dispose of unused reagent and waster in accordance with country, federal, state, and local regulations.
- Repeated freezing and thawing may lead to inaccurate results

Sample Collection, Handling and Storage

Proper collection of specimens is the most important step in the laboratory diagnosis of infectious disease. A specimen that is not collected correctly may lead to false negative test results. All testing for SARS-CoV-2 should be conducted in consultation with a healthcare provider. Specimens should be collected as soon as possible once a decision has been made to pursue testing, regardless of the time of symptom onset. Training in specimen collection is highly recommended due to the importance of specimen quality.

- **Collecting the Sample**
  - Refer to Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Persons for Coronavirus Disease 2019 (COVID-19). See https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html
  - Follow the instructions of the sample collection device for proper collection methods.
- **Transporting Samples**
  - Label each sample container with patient's ID number (e.g. medical record number), date and time sample were collected.
  - Specimens should be shipped only by the collection site.
  - Specimens must be packaged, shipped, and transported according to the current edition of the International Air Transport Association (IATA) Dangerous Goods Regulations. Follow shipping regulations for UN 3373 Biological Substance, Category B when sending potential SARS-CoV-2 specimens. Store specimens at ambient temperatures and ship overnight.
- **Storing Samples**
  - Upon receipt, samples can be stored at ambient temperatures for up to 6 days. Within this 6-day period the ORAcollect•RNA (OR-100/ORE-100) or OMNIgene•ORAL (OM-505/OME-505) collected saliva samples should be incubated at 60 °C for 2 hours.
  - Store purified nucleic acids at -80°C or below.

Warning and Precautions

All procedures should be performed in a laboratory of adequate Bio Safety Level (BSL) as recommended by CDC and specimens handled within a Biological Safety Cabinet (BSC). Samples should always be considered potentially infectious and handled in accordance with safe laboratory procedures. Refer to Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with Coronavirus disease 2019 (COVID-19). https://www.cdc.gov/coronavirus/2019-ncov/lab/lab-biosafety-guidelines.html

Separate work areas should be used for:

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

General Handling:

Proper molecular biological, 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 when 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 RNase.

Cleaning working surfaces, pipettes, with cleaning reagents that destroy nucleic acids and RNase. To eliminate accelerated deterioration of any plastics and metals, wipe down surfaces with 70% ethanol.
As with any testing procedure, good laboratory practices are essential to the proper performance of this assay.

- For *In Vitro* diagnostic use (IVD)
- For use under Emergency Use Authorization only
- For prescription Use only
- This test has not been FDA cleared or approved; but has been authorized for emergency use by FDA under an EUA for use by authorized laboratories certified under the Clinical Laboratory Improvement Amendments (CLIA) of 1988, 42 U.S.C. §263a, that meet the requirements to perform high complexity tests.
- This test has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.
- The emergency use of this test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food,

Drug and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated or authorization is revoked sooner.

- Positive results are indicative of the presence of SARS-CoV-2 RNA
- Laboratories within the United States and its territories are required to report all results to the appropriate public health authorities.
- All human-sourced materials should be considered potentially infectious and should be handled with universal precautions. If spillage occurs, immediately disinfect with freshly prepared solution of 0.5% sodium hypochlorite (20% v/v bleach). Dispose of cleaning materials in a biohazard waste container.
- Do not use bleach in areas where DNA RNA Shield is used.
- Proper personal protective equipment including lab coats, gowns, gloves, eye protection, and biological safety cabinet are recommended for manipulation of clinical specimens. Refer to Microbiologic 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 Coronavirus Disease 2019 (COVID-19). See https://www.cdc.gov/coronavirus/2019-ncov/lab/lab-biosafety-guidelines.html
- Specimen processing should be performed in accordance with national biological safety recommendations. See https://www.cdc.gov/labs/BMBL.html
- 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.
- Process human clinical specimens within a Class II (or higher) biological safety cabinet (BSC).
- Closely follow procedures and guidelines provided to ensure that the test is performed correctly. Any deviation from the procedures and guidelines may affect the test performance.
- Avoid over exposure of the primer-probe mixes to light for optimal fluorescent signal.
- If exposure of biological materials to skin or mucous membranes occurs, immediately wash the area with large amounts of water. Seek medical advice immediately.
- Do not use components beyond the expiration the date printed on the kit boxes.
- Reagents supplied are formulated specifically for use with this kit. Make no substitutions in order to ensure optimal performance of the kit. Do not mix reagents from different lots.
- Return all components to the appropriate storage conditions after preparing working reagents.
- Keep all the materials on ice when in use.

Quality Control

Patient samples must be collected according to CDC guidelines.

The following controls are required to be included in every run to accurately interpret patient test results.

a) A negative control (processing blank) is needed to monitor contamination of reagents with amplifiable material. For individual tests, the processing blank is 100µL PCR Grade RNase-Free Water in each extraction run. For pooled testing the volume for the processing blank is 550 µL PCR Grade RNase-Free Water in each extraction run. This control is then included as template at least once per qRT-PCR plate (i.e. for each qRT-PCR run). The processing blanks should be included in the sample RNA extraction step (individual and pooled testing).

b) A positive control is needed to monitor integrity of reagents, screen for improper assay set up and qRT-PCR reagent failure. Recommended concentration for the control included in the Kit is 30 copies per reaction (1.5µL per reaction). The control needs to be pre-diluted by the user with PCR-grade RNase-Free water to a concentration of 20 copies/µL of PCR reaction. At least one well should be used per qRT-PCR run.

c) A no template (negative) control containing PCR grade RNase-Free water is needed to determine if amplicon contamination occurred during the qRT-PCR step. The control consists of 1.5µL of water (in place of sample) least once per plate (i.e. for each qRT-PCR run).

d) An internal (sample) control is used to monitor poor specimen quality, extraction, reverse transcription and PCR processes and reagent failures. The internal control is the amplification of the human Peptidylprolyl Isomerase A (PPIA) gene with its own primer and probe set included in the Clarifi COVID-19 Test Kit. Failure to amplify the human gene indicates a failed test.

Clarifi COVID-19 Positive Control Preparation:

Caution: The positive control should be handled in a separate area from samples and from PCR set up and amplification with caution to prevent possible contamination. Freeze-thaw cycles should be avoided. Maintain on cold block when thawed.

The positive control is needed to monitor integrity of reagents, screen for improper assay set up and PCR reagent failure. Recommended concentrations for the control included in the Clarifi COVID-19 Test Kit is 30 copies per reaction. The control is provided as 100 µL at a concentration of 200 copies/µL and needs to be diluted by adding 900 µL nuclease free water to the positive control for a concentration of 20 copies/µL (10:1 dilution). At least one well should be used per batch of specimens.

Procedure

Sample Collection:

The workflow begins with saliva collection using the ORAcollect•RNA (OR-100/ORE-100) or OMNIgene•ORAL (OM-505/OME-505) saliva collection device provided with the Clarifi COVID-19 Test Kit. Saliva collection is performed by a healthcare provider according to the Instructions for Use of the collection device.

These collection devices contain a stabilizing solution that stabilizes collected RNA at room temperature when used according to the provided instructions.

Assay:

After samples are received in the laboratory for processing, the following steps are to be followed:

Note: Each Extraction Run requires one negative control to be included.

Sample Preparation

a) Incubation

Clarifi COVID-19 Test Kit saliva collection devices containing saliva samples should be incubated at 60 °C for 2 hours. After incubation, vials can be stored at room temperature prior to downstream processing.

b) Pooled Testing

NOTES: Pooling can be done for up to 12 samples, combining equal volumes (minimally 50µL of stabilized saliva from each sample). If samples are tested individually without pooling, proceed directly to the RNA Extraction – Individual Sample Extraction in step c.

1. From each sample to be pooled, aliquots 100µL of saliva sample into a sterile pooling vial or tube (≥2mL).
2. After all samples are added to the pooling vial, mix well by pipetting.
3. Proceed to RNA Extraction – Pooled Sample Extraction.

Reagent Preparation

NOTE: Prepare the following reagents fresh on the day of use. Calculate the appropriate volume of reagents needed based on the number of samples to be processed.

1. Prepare a Beta-mercaptoethanol/Viral RNA Buffer solution by adding beta-mercaptoethanol to the Viral RNA Buffer (Cat. No: 1119) to a final dilution of 0.5 % (v/v) (i.e. 500 µL per 100 mL). The final volume should be enough to ensure 400 µL of the solution can be added to each sample or 1100 µL for pooled samples.

2. Prepare an Ethanol/Viral Wash Buffer Solution by adding 4 mL of 100% ethanol to every 1 mL Viral Wash Buffer (Cat. No: 1117) for a final volume of 5 mL. Volumes may be adjusted to ensure there is enough volume to add 1mL to every sample.

RNA Extraction

a. Individual Sample Extraction

NOTE: Pooled samples are extracted per separate extraction procedure, found in the "Pooled Sample Extraction" section below.

NOTE: Perform all steps at room temperature. All centrifugation steps are performed on swinging bucket plate centrifuge at 3000-5000g for 5 minutes. Use 96- deep well plates or PCR tubes (1.0-2.0mL per well) for steps 1 and 2 since the total volume of each well can be up to 600 µL.

NOTE: Each Extraction Run requires one negative control to be included.

1. Add 100µL of each saliva sample or negative control to each well.

   NOTE: Add 100µL of nuclease free water to one well to be used as a negative control and process it in parallel with each batch of samples.
2. Add 100µL of DNA/RNA Shield™ (Cat. No: 1118) to each 100µL of saliva sample. Mix well by pipetting.
3. Add 400µL of Beta-mercaptoethanol /Viral RNA Buffer solution prepared above to each 200µL sample solution. Mix well by pipetting.
4. Transfer the sample mixture into each well of the I-96 Plate (Cat. No: 1124) mounted on a Collection Plate (Cat. No: 1123) and centrifuge. Discard the flow-through from the collection plate.
5. Add 500µL of Ethanol/Viral Wash Buffer Solution prepared above in Reagent Preparation to each well and centrifuge. Discard the flow-through from the collection plate. Repeat this step on each well.
6. Add 500µL ethanol (95-100%) to each well and centrifuge. Discard the flow-through from the collection plate.
7. Mount the plate onto an Elution Plate (Cat. No: 1122).
8. To elute RNA, add 30 µL of DNase/RNase-Free Water (Cat. No: 1120) directly to the column matrix of each well and centrifuge. Once RNA is eluted it should be kept on ice during processing.
9. Cover the elution plate with cover foil (Cat. No: 1121) to prevent evaporation.
   NOTE: The eluted RNA can be used immediately or stored frozen at -70°C.

b. Pooled Sample Extraction

NOTE: Individual samples are extracted per separate extraction procedure, found in the "Individual Sample Extraction" section above.

NOTE: Perform all steps at room temperature. Centrifugation steps are performed on a swinging bucket plate centrifuge at 3000-5000×g for either 5 or 7 minutes. It is recommended to use a deep 96-well plate (2.0mL per well) or individual 2.0 mL microcentrifuge tubes for steps 1 and 2 since the total volume of each well can be up to 1.6mL.

NOTE: Pooled Sample Extraction does not require the use of the DNA/RNA Shield™ (Cat. No: 1118) that is provided in the Clarifi COVID-19 Extraction Kit.

1. Add 550µL of each pooled saliva sample to each well in a deep 96-well plate.

   NOTE: Add 550µL of nuclease free water to one well to be used as a negative control and process it in parallel with each batch of samples.

2. Add 1100µL of Beta-mercaptoethanol /Viral RNA Buffer solution prepared above to each 550µL sample solution. Mix well by pipetting slowly up and down 5X.
3. Transfer half of the sample mixture (825 µL) into each well of the I-96 Plate (Cat. No: 1124) mounted on a Collection plate (Cat. No: 1123) and centrifuge for 7 minutes. Discard the flow-through from the collection plate. Repeat this step on each well for the remaining volume of each pooled sample mixture (approximately 825µL).
4. Add 700µL of Ethanol/Viral Wash Buffer Solution and centrifuge for 5 minutes. Discard the flow-through from the collection plate. Repeat this step on each well.
5. Add 500µL ethanol (95-100%) to each well and centrifuge. Discard the flow-through from the collection plate.
6. Mount the plate onto an Elution Plate (Cat. No: 1122).
7. To elute RNA, add 15 µL of DNase/RNase-Free Water (Cat. No: 1120) directly to the column matrix of each well and centrifuge after a minimum of 1 minute. Once RNA is eluted it should be kept on ice during processing.
8. Cover the elution plate with cover foil (Cat. No: 1121) to prevent evaporation.

   NOTE: The eluted RNA can be used immediately or stored frozen

PCR Reagent Preparation

1. Place BioRad Reliance (4x Reliance One-Step Multiplex Supermix) and Clarifi Probe/Primer Mix (both provided in the kit) on a lab bench on ice and protected from light and equilibrate until they reach ambient temperature, vortex for 2-3 seconds, and then spin briefly to collect the reagent
2. Prepare Clarifi qRT-PCR Mix in bulk according to the following table. This should be done on ice.
    1. To determine the total volume to prepare, use the volume per test below multiplied by the number of tests plus 2 (positive and negative control)

2. It is recommended that 10-15% extra qRT-PCR Mix is prepared to account for loss that may occur during pipetting of mix to individual tubes/plate wells, numbers in the table are actual volumes required for each sample
3. Add appropriate reagent amounts in the order of: PCR Grade Water, BioRad Reliance and Clarifi Probe/Primer Mix.
4. Mix reagents by pipetting up and down 10 times.
5. Centrifuge at 3000g for 10 seconds.
6. Keep reagents on ice and protected from light until ready to use

Clarifi qRT-PCR Mix

Reagent	Volume/test
BioRad Reliance (4x Reliance One-Step Multiplex Supermix)	3.75 µL
Clarifi Probe/Primer Mix	7.0 µL
PCR Grade Water	2.75 µL
Total volume	13.5 µL

qRT-PCR Run

Plate setup:

1. The assay has been optimized for 15µL reactions in either 96-well or 384-well plates. For the total number of specimens and controls to be run, add 13.5µL Clarifi qRT-PCR Mix to a separate PCR reaction well on the PCR plate.

   Note: Each PCR plate requires 1 no template control (water), 1 negative control (from extraction run) and 1 positive control well.

2. Add 1.5µL of each sample, sample pool, and each control from the sample RNA preparation to each well on the PCR plate. ***Note: The final volume in each well is 15 µL/test.***
3. Seal the plate with optical film.
4. Using a plate spinning rotor, Centrifuge the plate for approximately 1 minute at 2000 - 3000 rpm.
5. Load the plate onto Bio-Rad CFX96 or CFX384 Touch Real-Time PCR Detection System, or Applied Biosystems™ QuantStudio 5 Real-Time PCR System, as desired

qRT-PCR Instrument Setup and Operation

Initial setup:

1. Follow instructions on qRT-PCR equipment to create the following new qRT-PCR protocol for the Clarifi Test:

Step	Cycles	Temp.	Time
Reverse transcription (RT)	1	50℃	20 minutes
Enzyme Inactivation, Polymerase activation	1	95℃	10 minutes
Amplification	45	95℃	10 seconds
		59℃	20 seconds* (24 seconds if using ABI7500)

*ABI7500 will not allow setting less than 24 seconds

1. Select 15µL reaction volume
2. Select 99℃ lid temperature
3. Save the protocol for future runs
4. Load the plate on the instrument
5. Start run. Note: when using BioRad CFX96 or CFX384, you will receive a prompt, select "all channel"

Repeat setup:

1. Open the previously saved setup file
2. Load the plate on the instrument
3. Start run. Note: when using BioRad CFX96 or CFX384, you will receive a prompt, select "all channel"

qRT-PCR Results Export - BioRad CFX96 or CFX384

1. Export the run file from the instrument. Files are stored in a folder named "Real-Time Data"
2. Using BioRad CFX Maestro Software, open the BioRad compressed file (.zpcr) for the desired run
3. Go to "Settings" > "Plate Setup" > "View/Edit Plate". Label positive control, negative control and test samples according to your plate layout. Choose HEX and Cy5 channels and give them "Target Name" as HEX and Cy5 respectively. Hit "OK>Apply" to finish your plate setup
4. Threshold setup: under the "Quantification" tab, drag and set both threshold bars to 500 RFU

qRT-PCR Results Export - Applied Biosystems QuantStudio 5 and QuantStudio 7

1. Export the run file from the instrument
2. Using QuantStudio software, open the .eds file for the desired run
3. Under the "Plate" tab > "Advanced Setup", add HEX and Cy5 channels to the "Targets" section and give them names as HEX and Cy5 respectively. Label positive control, negative control and test samples according to your plate layout in the "Samples" section. Hit "Next" > "Next" > "Analyze" to view your results

4. Export your results to excel files under the "Export" tab.

qRT-PCR Results Export - Applied Biosystems 7500 Fast Real-Time

1. Select File > Open > Select the data file to be analyzed
2. Select the Result tab at upper left corner of software.
3. Click on the Amplification Plot Tab.
4. Highlight all the samples from the run to view all amplification curves.
5. Set Data to Delta Rn vs. Cycle on the right side of the panel.
6. Set Detector to FAM
7. Set Line Color to Detector Color
8. Select Manual Ct and Manual Baseline under Analysis settings. Do not change the Manual Baseline default numbers.
9. Click and drag the threshold line until it lies within the exponential phase of the fluorescence curves and above any background signal.
10. Click the Analyze button in the lower right corner of the window. The red threshold will turn green, indicating the data is analyzed.
11. Repeat step 6-10 to analyze results for HEX and Cy5.

Interpretation of Results

All test controls must be examined prior to interpretation of patient results. If the controls are not valid, the patient results are invalid and cannot be interpreted. If the external controls are invalid a root-cause investigation should be performed and the entire run has to be repeated after the root cause is eliminated. Results are interpreted based on a cutoff of Ct ≤ 40.00.

Examination and Interpretation of Control Results:

1. **Positive control review:** To be valid, the positive control should demonstrate amplification below the designated Ct cutoff (≤40) in the HEX channel. If the positive control is not valid, stop here. The entire plate is invalid and the assay will need to be repeated.
2. **No template Control review:** To be valid, the new template control should demonstrate no amplification (exceeding background levels) below the designated Ct cutoff (≤40) in the HEX channel and no amplification (exceeding background levels) below the designated Ct cutoff (≤35) in the Cy5 channel.
3. **Negative control review:** To be valid, the negative control should demonstrate no amplification (exceeding background levels) below the designated Ct cutoff (<40) in the HEX channel. If the negative control is not valid, stop here. The entire plate is invalid and the assay will need to be repeated.
4. **Internal control review:** once both the positive and negative control pass, examine each sample for the internal control. Each sample should demonstrate amplification below the designated Ct cutoff (≤35) in the Cy5 channel. If the internal control is not valid, the sample should be excluded from further interpretation. The sample is invalid and will need to be repeated. If the internal control fails a second time, the sample will need to be recollected.

Expected performance of the controls in the Clarifi COVID-19 Test Kit

Control	Excepted Ct Value (SARS-CoV-2; HEX)	Expected Ct Value (Internal Control; Cy5)
Positive Control	≤ 40.00	N/A
Negative Control (processing blank)	> 40.00	N/A
No Template Control	> 40.00	> 35.00
Internal Control	N/A	≤ 35.00

Examination and Interpretation of Patient Specimen Results:

Assessment of clinical specimen test results should be performed after the positive, negative and internal controls have been examined and determined to be valid and acceptable. If the controls are not valid, the patient results cannot be interpreted.

a. Individual Sample Results:

1. **Internal Control:** The results of the sample control (internal control) should be examined prior to the release of clinical specimen results. The internal control should produce a positive within each sample analysis. If the internal control is not valid, the clinical specimen results cannot be released, and the sample will need to be rerun. If the internal control fails a second time, the sample needs to be recollected.

2. **SARS-CoV2 targets**: To interpret clinical specimen results, the Ct cutoff value for SARS-CoV-2 is 40.00 or less, and the amplification curve shape must be typical in appearance.

   Positive: A Ct value ≤ 40.00 indicates that the specimen contains the target nucleic acid sequence and is positive (+) for SARS-CoV-2.

   Negative: A Ct value > 40.00 indicates that the specimen does not contain the target nucleic acid sequence and is negative (-) for SARS-CoV-2.

HEX channel (SARS-CoV-2)	Cy5 channel (Internal Control)	Result
Early amplification below Ct cutoff (Ct≤35)	Amplification below Ct cutoff (Ct≤35)	Positive for SARS-CoV-2
	No amplification below Ct cutoff (Ct>35)	Positive for SARS-CoV-2
Amplification below Ct cutoff (35≤Ct≤40)	Amplification below Ct cutoff (Ct≤35)	Positive for SARS-CoV-2
	No amplification below Ct cutoff (Ct>35)	SARS-CoV-2 presumptive positive; retest specimen*
No amplification below Ct cutoff (Ct>40)	Amplification below Ct cutoff (Ct≤35)	Negative for SARS-CoV-2
	No amplification below Ct cutoff (Ct>35)	Invalid - failed internal control, retest specimen**

*Results for SARS-CoV-2 RNA is Presumptive Positive. Samples with lower target signal (Ct > 35.00, ≤ 40.00) and no Cy5 signal, may be retested by re-extracting RNA from the same specimen. For samples with a repeated Presumptive Positive result will be reported with a note of the internal control failure and the request of a new sample for additional confirmatory testing.

**First conduct the repeat test by re-isolating RNA phase from the same specimen. If the test fails again, collect a new specimen from the patient and repeat the test.

b. Pooled Sample Results:

1. **Internal Control:** The results of the sample control (internal control) should be examined prior to the release of clinical pool result. The internal control targeting human PPIA should produce a positive result within each pool analysis. If the internal control is not valid, the pool results cannot be released, and all samples in that pool should be tested individually prior to reporting.

2. **SARS-CoV-2 targets**: To interpret clinical pool results, the Ct cutoff value for SARS-CoV-2 for pooled samples is detailed below:

   Positive: A Ct value ≤ 40.00 indicates that the pool contains the target nucleic acid RdRp gene sequence and is positive (+) for SARS-CoV-2. All samples in that pool should be tested individually prior to reporting.

   Presumptive Positive: A Ct value > 41.00 and ≤ 42.00 indicates that the pool likely contains low levels of SARS-CoV-2 nucleic acid and may contain low positive (+) samples. All samples in that pool must be tested individually prior to reporting.

   Negative: A Ct value > 42.00 indicates that the pool does not contain the target nucleic acid sequence and is negative (-) for SARS-CoV-2.

HEX channel (SARS-CoV-2)	Cy5 channel (Internal Control)	Result
Amplification below Ct cutoff (Ct ≤41)	ANY	Positive for SARS-CoV-2, test all samples individually*
Amplification below Ct cutoff (41<Ct ≤42)	ANY	SARS-CoV-2 presumptive positive; test all samples individually*
No amplification below Ct cutoff (Ct>42)	Amplification below Ct cutoff (Ct≤35)	Pool is Negative for SARS-CoV-2 and results can be reported as negative for each specimen in the pool.
	No amplification below Ct cutoff (Ct >35)	Invalid - failed internal control, test all samples individually

*All individual samples from positive, presumptive positive, or invalid pools should be run through the individual sample workflow, starting with RNA extraction since the saliva specimens have already been incubated.

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. Quadrant Biosciences will limit the distribution of this device to only those users who have documented successful completion of appropriate training.
- Saliva testing is limited to individuals suspected of COVID-19 by their healthcare provider.
- The test was validated for use only with saliva samples. Performance with other specimen types is unknown.
- Performance with other sample matrices is unknown.
- 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. Additional testing of other sample types 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.
- 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 product a false negative result. An interference study evaluating the effect of common cold medications was not performed.
- Samples should only be pooled when testing demand exceed laboratory capacity and/or when testing reagents are in short supply.
- 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 blood or blood product for the presence of SARS-CoV-2.
- This test cannot rule out diseases caused by other bacterial or viral pathogens.
- The clinical performance has not been established in all circulating variants but is anticipated to be reflective of the prevalent variants in circulation at the time and location of the clinical evaluation. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of SARS-CoV-2 and their prevalence, which change over time.

Conditions of Authorization for the Laboratory

The Clarifi COVID-19 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/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/vitro-diagnostics-euas.

However, to assist clinical laboratories running the Clarifi COVID-19 test, the relevant Conditions of Authorization are listed below.

a) Authorized laboratories[1] using the Clarifi COVID-19 test must include with test result reports, all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating the Fact Sheets may be used, which may include mass media.

b) Authorized laboratories using the Clarifi COVID-19 test must use the Clarifi COVID-19 test as outlined in the authorized labeling. 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 perform the Clarifi COVID-19 are not permitted.

c) Authorized laboratories that receive the Clarifi COVID-19 test must notify the relevant public health authorities of their intent to run the test prior to initiating testing.

d) Authorized laboratories using the Clarifi COVID-19 test must have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.

e) Authorized laboratories must collect information on the performance of the Clarifi COVID-19 test and report the DMD/OHT7-OIR/OPEQ/CDRH (via email:CDRH-EUA-Reporting@fda.hhs.gov) and Quadrant Biosciences Technical Support (via email: operations@quadrantbiosciences.com or by phone: 1-866-205-7336) 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.

f) All laboratory personnel using the Clarifi COVID-19 test must be appropriately trained in RT-PCR techniques, the specific processes and instruments used in the Clarifi COVID-19 Test Kit and use appropriate laboratory and personal protective equipment when handling this kit and use the test in accordance with the authorized labeling.

g) Authorized laboratories using specimen pooling strategies when testing patient specimens with the Clarifi COVID-19 test must include with test result reports for specific patients whose specimen(s) were the subject of pooling, a notice that pooling was used during testing and that "Patient specimens with low viral loads may not be detected in sample pools due to the decreased sensitivity of pooled testing."

[1] For ease of reference, this letter will refer to, "laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. § 263a, that meet requirements to perform high complexity tests" as "authorized laboratories."

h) Authorized laboratories implementing pooling strategies for testing patient specimens must use the "Laboratory Monitoring Plan for Pooling" recommendations available in the authorized labeling to evaluate the appropriateness of continuing to use such strategies based on the recommendations in the protocol.

i) Authorized laboratories must keep records of specimen pooling strategies implemented including type of strategy, date implemented, and quantities tested, and test result data generated as part of the Protocol for Monitoring of Specimen Pooling Strategies. For the first 12 months from the date of their creation, such as records will be made available to FDA within 48 business hours for inspection upon request and will be made available within a reasonable time after 12 months from the date of their creation.

j) Quadrant Biosciences, its authorized distributor(s) and authorized laboratories using the Clarifi COVID-19 test must ensure that any records associated with this EUA are maintained until otherwise notified by FDA. Such records will be made available to FDA for inspection upon request.

Performance Characteristics

Limit of Detection:

LoD of OR-100 collected saliva

Limit of detection (LoD) studies determine the lowest detectable concentration of the SARS-CoV-2 virus at which approximately 95% of all (true positive) replicates test positive. LoD estimates were first determined using a dilution series with 5 different concentrations of heat-inactivated virus (BEI Resources, NR-52286). Saliva specimens were collected from confirmed negative subjects using the ORAcollect•RNA (OR-100) saliva swab collection device (approximately 250µl are absorbed by this swab). The swabs are then inserted into the transport media (1mL) included in the ORAcollect•RNA Kit which stabilizes the sample. For testing, 100 µL aliquots of this sample matrix were prepared and individually spiked with a range of 0, 20, 50, 100, 150, and 200 copies of heat-inactivated virus (BEI Resources, NR-52286) for final virus concentrations of 0, 0.2, 0.5, 1, 1.5 and 2 copies per µL. Each concentration was tested in 5 individually processed replicates following the Clarifi COVID19 Test Kit procedure (RNA eluted in 30 µL RNase-Free Water, qPCR on the BioRad CFX96 Touch Real Time Detection System) using 1.5 µL of extracted RNA per qPCR reaction.

Target Level [cp/µL*]	**Valid tested replicates**	**SARS-CoV-2 Positive (Ct ≤ 40.0 cycles)**			**Internal Control Positive (Ct ≤ 35.0 cycles)**		
		n	**Mean Ct**	**Detection Rate**	**n**	**Mean Ct**	**Detection Rate**
2.0	10	10	33.23	100%	10	28.22	100%
1.5	10	10	33.75	100%	10	27.61	100%
1.0	**10**	**10**	**34.42**	**100%**	**10**	**27.95**	**100%**
0.5	10	9	36.04	90%	10	28.00	100%
0.2	10	6	36.63	60%	10	27.31	100%
0	10	0	NA	0%	10	17.49	100%

* copies/µL original saliva specimen

The tentative LoD was identified as 1 copy/µL original patient saliva. To confirm that the LoD of the Clarifi COVID-19 Test Kit is 1 copy/µL, saliva specimens were collected from 20 clinical negative subjects with ORAcollect•RNA (OR-100) saliva collection swabs. 100 µL of each sample was spiked with 100 copies of heat-inactivated SARS-CoV-2 virus. Samples were processed according to the Clarifi COVID-19 Test Kit procedure using 1.5 µL of eluted RNA. This was examined in all 20 samples reactions using the Clarifi COVID-19 Test Kit on five instruments: the BioRad-CFX96 Touch Real Time Detection System, the BioRad CFX384 Touch Real Time Detection System, and the Applied Biosystems QuantStudio 5, Applied Biosystems QuantStudio 7 and Applied Biosystems ABI7500 Fast instruments.

This study confirmed the LoD of the Clarifi Test to be 1 copy/µL with saliva swab specimen collected using the OR-100 collection device for all tested instruments.

Confirmatory LoD on Multiple Instruments (OR-100 Collection)

Instrument	Target Level* [copies/µl SALIVA]	Valid tested replicates	SARS-CoV-2 Positive			Internal Control Positive		
			n	Mean Ct	Detection Rate	n	Mean Ct	Detection Rate
CFX96	1 cp/µL	20	20	33.85	100%	20	27.49	100%
QS5	1 cp/µL	20	19	34.31	95%	20	25.11	100%
CFX384	1 cp/µL	20	20	35.92	100%	20	29.26	100%
QS7	1 cp/µL	23	23	36.15	100%	23	27.13	100%
ABI7500 Fast	1 cp/µL	20	20	35.06	100%	20	26.22	100%

* copies/µL original saliva specimen

Confirmatory LoD with OME-505 Collected Saliva in comparison to OR-100

Limit of detection (LoD) studies determine the lowest detectable concentration of the SARS-CoV-2 virus at which approximately 95% of all (true positive) replicates test positive. To determine the LoD of the Clarifi COVID-19 Test Kit with the OME-505 saliva collection device in comparison to the OR-100 collection device, LoD was analyzed at 3 different concentrations (0.3, 1, and 3 copies/µL) based on the LoD of the OR-100 device. Saliva specimens were collected concurrently from confirmed negative subjects using the OMNIgene•ORAL OM-505 collection device and ORAcollect•RNA collection device. Three 100 µL aliquots of this sample matrix from each device were individually spiked with 30, 100, and 300 copies of heat-inactivated virus (BEI Resources, NR-52286) for final virus concentrations of 0.3, 1, and 3 copies per µL. Each concentration was tested in 5 individually processed replicates for each saliva collection device following the Clarifi COVID19 Test Kit procedure using the BioRad CFX96 Touch Real Time Detection System. The study confirmed the LoD of the Clarifi COVID-19 Test Kit with the OME-505 collection device to be equal to the LoD with the OR-100 collection device - 1 copy/µL original patient saliva.

LoD Bridging Study Comparing OR-100 and OME505 collected Saliva

Target Level [cp/µL*]	Valid tested replicates	OR-100						OME-505					
		SARS-CoV-2 Positive (Ct ≤ 40.0 cycles)			Internal Control (PPIA) Positive (Ct ≤ 35.0 cycles)			SARS-CoV-2 Positive (Ct ≤ 40.0 cycles)			Internal Control (PPIA) Positive (Ct ≤ 35.0 cycles)		
		n	Mean Ct	Detection Rate	n	Mean Ct	Detection Rate	n	Mean Ct	Detection Rate	n	Mean Ct	Detection Rate
3.0	5	5	34.18	100%	5	27.85	100%	5	35.93	100%	5	26.91	100%
1.0	**5**	**5**	**35.88**	**100%**	**5**	**27.33**	**100%**	**5**	**38.00**	**100%**	**5**	**25.95**	**100%**
0.3	5	5	37.92	40%	5	27.55	100%	5	38.68	20%	5	25.69	100%

* copies/µL original saliva specimen

Inclusivity (Analytical Sensitivity):

Analytical inclusivity of the primer probe sets was assessed through *in silico* analysis of the Clarifi COVID-19 Test primers and probes. The complete set of available SARS-CoV-2 sequences as of June 30, 2020 was downloaded from the NCBI sequence repository. All sequences longer than 500 bases (n=8,282, average length=29,052 bases) were subsequently aligned with the BWA aligner (version 0.7.15) to the SARS-CoV-2 reference sequence (NC_045512) using the BWA-MEM algorithm. This resulted in 100% alignment of all sequences, with an average coverage depth of 8,040 bases across 100% of the genome and an average alignment length of 27,666 bases.

Sequence mismatches between the sequence set of the BWA aligner and the assays primer and probe sequences were then identified using the LoFreq program (version 2.1.3a) This resulted in the identification of 531 total sequence variants with quality scores ranging from 68 - 49,314. Within the RdRp Target 1 primer and probe regions, we identified a total of 2 sequences which had single base pair mismatched located in the RdRp Target 1 reverse primer region as shown in the table below, and none of them occurred in the same location.

SARS-CoV-2 Region	Mismatch	Position	Sequences	Frequency	Effect on Tm
Forward Primer 1	None	-	-	-	-
Probe 1	None	-	-	-	-
Reverse Primer 1	C/T G/A	12,781 12,795	11/8045 9/8044	0.001367 0.001118	- 5.5 ℃ -5.0 ℃
Forward Primer 2	None	-	-	-	-
Probe 2	None	-	-	-	-
Reverse Primer 2	None	-	-	-	-

Notably, none of the mutations that were found in the RdRP Target 1 reverse primer are located in the 3' terminal base. Thus, the PCR amplification component of the assay is tolerant to all identifiable variants in publicly available sequence data. Even if these rare variants occurred, the RdRp Target 1 reverse primer, it will still be possible for PCR to continue for that amplicon, albeit with less efficiency.

Given that no sequence changes were found for the primers and probes covering the RdRp Target 2 amplicon, and the rare frequency of variants seen in the Clarifi RdRp Target 1 primer and probe binding sites in database, it is highly unlikely that subject samples would incur a signal loss for the SARS-CoV-2 should they contain these variants. Moreover, the dual target design of the Clarifi Test completely mitigates the likelihood of assay failure due to random mutations in the target sequences by having both fluorescent probes in the same channel.

The *in silico* analysis was updated on March 4, 2021 for the test's inclusivity of the emerging UK, South Africa, and Brazilian variants as displayed in the table below. The targets of the Clarifi COVID-19 test are outside of the sequence variations characteristic of the UK (B.1.1.7), South African (B.1.351), and Brazilian (P.1) SARS-CoV-2 variants. Therefore, reduced reactivity to these variants is not expected based on this *in silico* analysis.

***In silico* Analysis for IP2/IP4 Primer and Probe Sets on Variants**

Description	Target 1			Target 2		
	FWD P	REV	PROBE	FWD P	REV	PROBE
Isolate SARS-CoV-2/human/England/2/2020	18/18 100%	18/18 100%	21/21 100%	19/19 100%	20/20 100%	19/19 100%
20I/501Y.V1 (South Africa)	18/18 100%	18/18 100%	21/21 100%	19/19 100%	20/20 100%	19/19 100%
20H/501Y.V2 (Brazil)	18/18 100%	18/18 100%	21/21 100%	19/19 100%	20/20 100%	19/19 100%

Cross-Reactivity (Analytical Specificity):

The sequences for the IP2 and IP4 primers/probes used in the Clarifi COVID-19 Test Kit are the same as those designed by the Institute Pasteur, Paris, and have been shown to have equal sensitivity and specificity as other validated primer and probe sequences[2]. Wet testing of cross-reactivity of the SARS-CoV-2 primers and probes of the Clarifi COVID-19 Test Kit were described in the referenced publication for specimens known to be positive for various other respiratory viruses (human coronaviruses 229E, OC43, HKU1, and NL63, human influenza A and B viruses, rhinovirus, respiratory syncytial virus, parainfluenza virus, adenovirus, metapneumovirus, and picornavirus)[2]. Testing for some of these organisms was performed by Quadrant Biosciences in the context of the Microbial Interference study per the table below.

All of the Clarifi COVID-19 Test Kit primer and probe sequences were queried individually against all organisms listed in the FDA EUA template, shown below. This was performed using NCBI BLASTN with default alignment parameters.

The *in silico* results indicate that, across all organisms analyzed, there were 11 hits obtained that had >80% homology in one of the primers or probes in the Clarifi COVID-19 test. These 11 hits were obtained from 6 full length sequences that belong to 3 distinct organisms, namely human coronavirus OC43 strain, *Candida albicans*, and *Streptococcus salivarius* ASM78551v1. Of the 11 total hits, six sequences had 3' terminal mismatches with the primers with no risk of amplification since DNA polymerase cannot extend on a mismatch. The remaining 5 sequence hits all belong to the organism *Candida albicans*. However, the primers would align poorly with these sequences and binding would be between 6.5 kilobases to 1.7 mega basepairs away from any upstream or downstream match; based on the 20 second extension time per cycle and 59°C annealing temperature the primers of the Clarifi COVID-19 Test Kit would be unable to

[2] Etievent et al (2020) "Performance Assessment of SARS-CoV-2 PCR Assay Developed by WHO Referral Laboratories"

exponentially amplify these sequences of the non-targeted organism. Alignments data is shown in the table below.

High priority organisms	%Homology Forward and Reverse Primers and Probes*
Severe acute respiratory syndrome coronavirus 2 isolate Wuhan-Hu-1, complete genome	100%
Human coronavirus 229E, complete genome	None
Human coronavirus OC43 strain ATCC VR-759, complete genome	**(84%)**
Human coronavirus HKU1, complete genome	None
Human coronavirus NL63, complete genome	None
SARS coronavirus Tor2, complete genome	None
Middle East respiratory syndrome-related coronavirus isolate HCoV-EMC/2012, complete genome	None
Human adenovirus type 1, complete genome	None
Human metapneumovirus isolate 00-1, complete genome (hMPV)	None
Human parainfluenza virus 4a viral cRNA, complete genome, strain: M-25	None
Influenza A virus (A/California/07/2009(H1N1))	None
Influenza B virus	None
Human enterovirus, complete	None
Respiratory syncytial virus, complete genome	None
Human rhinovirus A, strain ATCC VR-1559, complete genome	None
Human rhinovirus B, complete genome	None
Human rhinovirus C, complete genome	None
Chlamydia pneumoniae TW-183	None
Haemophilus influenzae ASM76707v1	None
Legionella pneumophila ASM194158v1	None
Mycobacterium tuberculosis H37Rv	None
Streptococcus pneumoniae R6	None
Streptococcus pyogenes M1 GAS	None
Bordetella pertussis 18323	None
Mycoplasma pneumoniae FH	None
Pneumocystis jirovecii (PJP) RU7	None
Candida albicans SC5314, complete genome	**(89%)**
Staphylococcus epidermidis, complete genome	None
Pseudomonas aeruginosa strain PA0750 chromosome, complete genome	**(89%)**

*For organism with multiple homologies, only the worst homology is presented in this table

Microbial Interference Studies:

Because this test is a dual target assay the risk of false negative results due to competitive interference of the identified organisms with the primers of this assay is mitigated. In addition, because of the lack of substantial homology (>80%) of organisms noted above, a comprehensive microbial interference study was not performed. However, the ability to detect SARS-CoV-2 in a low titer sample (Ct > 36.0 cycles) was examined using the Clarifi COVID-19 Test Kit for some selected organisms. This was determined by adding 1.5 µL of purified RNA from saliva samples containing SARS-CoV-2 alone or in combination with hCoV-NL63, HRV/enterovirus, *Mycoplasma pneumoniae*, hMPV/metapneumovirus, or *Chlamydia pneumoniae*. Results indicate no difference in the cycles to threshold for detection of SARS-CoV-2, and thus no interference in the assay performance.

Sample ID	SARS-CoV-2 patient sample	Known Pathogen Present	COVID (HEX)	PPIA (Cy5)
379-0907	-	Human coronavirus NL63 (hCoV-63)	NA	27.48
470-326	-	Rhinovirus	NA	29.08
132-8996	-	*Mycoplasma pneumoniae*/Human metapneumovirus	NA	28.67
106-2898	-	*Chlamydia pneumoniae*	NA	29.56
379-0907	+	Human coronavirus NL63 (hCoV-63)	36.10	26.99
470-326	+	Rhinovirus	36.34	27.48
132-8996	+	*Mycoplasma pneumoniae*/Human metapneumovirus	36.00	27.54
106-2898	+	*Chlamydia pneumoniae*	37.13	27.74

Interfering Substances Study:

The potential interfering effects of 7 substances on the RT-PCR reactions were tested in accordance with FDA guidance. These substances included: Mucin (bovine submaxillary gland, type I-S, 2.5 mg/ml), human blood (2.5% v/v), Afrin original nasal spray (15% v/v), Basic Care allergy relief nasal spray (Glucocorticoid, 5% v/v), Cepacol Sore Throat (benzocaine/menthol lozenges, 5mg/mL), toothpaste (5% v/v), and mouthwash (5% v/v). Saliva specimens were collected from three confirmed negative subjects with the ORAcollect•RNA (OR-100) saliva collection swabs. Each subject was tested with the substances indicated in Table 17 below. Eight aliquots (100 µl) were made from each subject's sample and spiked with one of the 7 potential interfering substances or PCR grade water, as well as 200 copies of heat-inactivated SARS-CoV-2 (2x LOD). The samples were processed according to the Clarifi COVID-19 Test Kit procedure. Results indicated no differences in the cycles to threshold for detection of SARS-CoV-2.

Potential Interfering Substance	Valid Tested replicates	Valid Positive replicates	Mean Ct SARS-Cov-2	Mean Ct Internal Control
Mucin: bovine submaxillary gland, type I-S	3	3	36.62	28.55
Blood (human)	3	3	37.36	29.38
Afrin Original nasal spray	3	3	37.63	29.61
Basic Care allergy relief nasal spray (Gluococorticoid)	3	3	36.74	26.94
Cepacol Sore Throat (benzocaine/menthol lozenges)	3	3	36.53	27.07
Toothpaste	3	3	35.53	32.02
Mouthwash	3	3	35.67	26.91
PCR grade water	3	3	36.16	27.22

Clinical Evaluations

NOTE: For both studies (Individual sample testing and Pooled sample testing), sample processing was performed according to the IFU including the heat inactivation step. No frozen storage of the saliva samples occurred between the individual and the pooled testing of samples.

Individual Sample Testing:

A total of 63 Clarifi COVID-19 saliva swab specimens were collected with the OR-100 collection device within 0-5 days following collection of the comparative nasopharyngeal swabs (32 negative, 31 positive). Clinical nasopharyngeal specimen results were obtained from an EUA authorized SARS-CoV-2 RT-PCR assay. Saliva samples were processed using the Clarifi COVID-19 Test Kit as per the Instructions for Use. The Clarifi COVID-19 Test Kit resulted in a positive agreement of 100% (31/31) and a negative agreement of 100% (32/32).

		EUA authorized Comparator (NP Swab)		**Total**
		POSITIVE	NEGATIVE	
Clarifi COVID-19 Test Kit (Saliva)	POSITIVE	31	0	**31**
	NEGATIVE	0	32	**32**
	Total	**31**	**32**	**63**
Positive Percent Agreement (PPA): 31/31 = 100% (95% CI) **Negative Percent Agreement (NPA): 32/32 = 100% (95%CI)**				

Clinical comparison of OME-505 Collection Device to ORA-100 Swab Collection Device:

A total of 32 saliva specimens were collected using the OME-505 collection device across three sites within 0-3 days following collection of the comparative saliva swab specimens collected with the OR-100 collection device (16 negative and 16 positive as determined by the EUA authorized Clarifi COVID-19 Test Kit). Saliva samples collected with the OME-505 were also processed and tested using the Clarifi COVID-19 Test Kit. All samples were individually tested. The Clarifi COVID-19 Test Kit resulted in a positive agreement of 100% (16/16) and a negative agreement of 100% (16/16) against the comparator results.

The clinical study contained approximately 5 low positive samples (31%) based on the mean Ct value of the EUA authorized Clarifi COVID-19 Test at the established LoD.

Comparison of Clinical Samples when tested with the OR-100 and the OME505

		Clarifi COVID-19 (OR-100 Saliva Swab)		**Total**
		POSITIVE	NEGATIVE	
Clarifi COVID-19 Test Kit (OME-505 Saliva)	POSITIVE	16	0	**16**
	NEGATIVE	0	16	**16**
	Total	**16**	**16**	**32**
Positive Percent Agreement (PPA): 16/16 = 100% (95% CI) **Negative Percent Agreement (NPA): 16/16 = 100% (95%CI)**				

Pooled Sample Testing:

A total of 600 (572 negative, 28 positive) saliva swab specimens were prospectively collected using OR-100 as part of back to work/school testing at more than 10 New York State colleges across the state. SARS-CoV-2 status of these samples was determined by individual testing with the Clarifi COVID-19 Test Kit. All specimens were self-collected from asymptomatic individuals (confirmed with a symptoms questionnaire) under healthcare provider supervision per the instructions provided with the authorized OR-100 saliva swab collection device. Each specimen was analyzed individually and as part of a pool of 12 samples (28 pools with 1 positive and 11 negatives, and 22 pools with 12 negatives). Saliva samples were processed using the Clarifi COVID-19 Test Kit procedure for individual and pooled samples (pools of 12 samples). All samples (both pooled and individual) were run on the BioRad CFX96 Touch Real Time Detection System instrument.

Eleven of the positive individual results were derived from low positive samples with Ct values that were within 0-4 Ct of the observed mean Ct at the established LoD. Ct-distribution of the 28 positive individual samples was as follows:

Ct Range*	**Number of Samples**
27.1 - 30.0	5
30.1 – 35.0	11
35.1 – 40.0	12

(*Note: Mean Ct for the CFX96 instrument at LoD is ~34)

The Clarifi COVID-19 Test Kit pooled procedure resulted in a positive agreement of 100% (28/28) and a negative agreement of 100% (22/22) compared to the individual testing results using the Clarifi COVID-19 Test Kit individual testing procedure.

		Clarifi COVID-19 Test Kit Individual		**Total**
		POSITIVE	NEGATIVE	
Clarifi COVID-19 Test Kit Pooled (12 samples)	POSITIVE (1 POSITIVE plus 11 NEGATIVES)	28	0	**28**
	NEGATIVE (12 NEGATIVES)	0	22	**22**
	Total	**28**	**22**	**50**

Additionally, 15 independent positive clinical saliva samples collected using the OR-100 saliva swab collection devices were processed using the Clarifi COVID-19 Test Kit procedure for individual and pooled samples in pools of 3, 6, 9, and 12 samples. A total of 165 individual unique negative samples were used (11 negatives for each pool x 15 sets of pools). The 11 negatives within each n=12 pool set were also used for the pool of 3, 6, and 9 with the same positive sample; however, each pool set of 3, 6, 9, 12 had its unique, independent negative samples set. SARS-CoV-2 status was determined by individual testing with the Clarifi COVID-19 Test Kit. All samples (both pooled and individual) were run on the BioRad CFX96 Touch Real Time Detection System instrument. Each pool consisted of 100 µL of a verified single positive sample added to a negative saliva pool matrix consisting of 100µL of verified negative subjects equal in number to the targeted pool size minus 1; n x 100µL – 1 x 100µL (i.e., 3 sample pool = 100µL positive sample and 200uL of negative subjects, 8 sample pool = 100 µL positive sample combined with 800 µL of negative subjects). The 15 individual positive specimens had Ct values between 25.29 – 37.18. The performance of testing sample pools of 3, 6, 9 and 12 samples containing 1 positive sample each, compared to testing individual samples is shown in Table 35 with the mean of each pool size shown in Table 35.

			Individual Testing			
			Expected Positive			**Total**
			37<Ct≤40	**34<Ct≤37**	**Ct≤34**	
Pooled Testing (n=12)	**Positive**	**37<Ct≤40**		2	1	15
		34<Ct≤37		1	2	
		Ct≤34	1		8	
Pooled Testing (n=9)	**Positive**	**37<Ct≤40**	1			15
		34<Ct≤37		3	2	
		Ct≤34			9	
Pooled Testing (n=6)	**Positive**	**37<Ct≤40**		1		15
		34<Ct≤37	1	2	2	
		Ct≤34			9	

Pooled Testing (n=3)	**Positive**	**37<Ct≤40**				15
		34<Ct≤37	1	2		
		Ct≤34		1	11	
Total			15			
Positive Agreement (95% CI)			100% (78.2% - 100%)			

Pooled sample Results

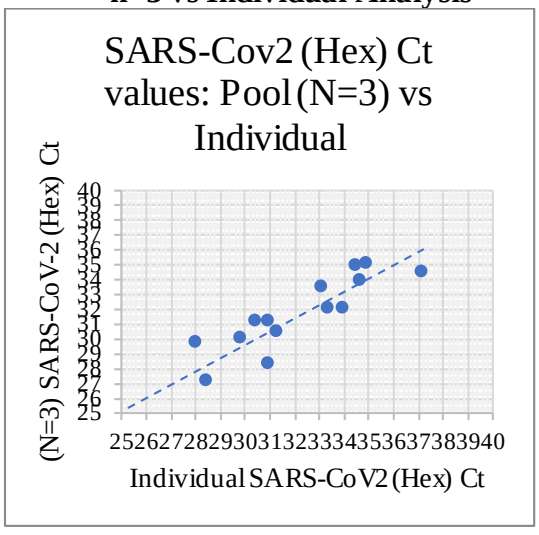
Results	SARS-CoV-2 Ct					Internal Control (PPIA) Ct				
	Individual n=1	Pool n=3	Pool n=6	Pool n=9	Pool n=12	Individual n=1	Pool n=3	Pool n=6	Pool n=9	Pool n=12
Average	31.80	31.20	32.34	32.59	33.78	26.19	24.33	23.60	23.79	24.22
SEM	0.810	0.795	0.895	0.881	0.773	0.283	0.342	0.193	0.279	0.472

To confirm there was no analytical bias due to pooling, a Passing-Bablock regression analysis was performed to compare individual Cts to Pooled Cts for N=3, N=6, N=9, and N=12. Results of the analysis can be found in the table and figures.

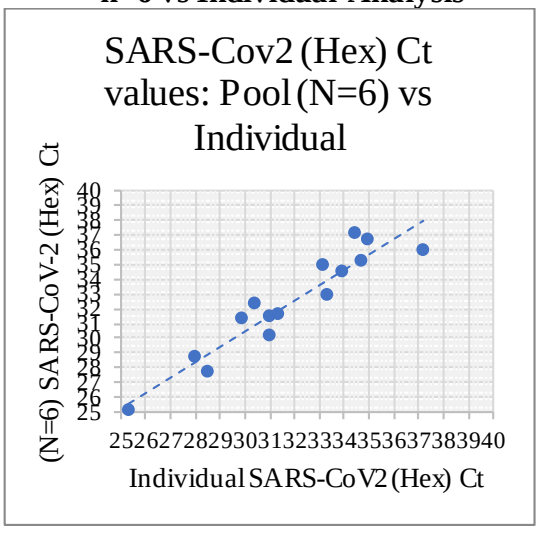
Passing Bablok Regression Estimates and Ct Shifts

Pool Size	Slope	LCI Slope	UCI Slope	Intercept	LCI	UCI	eqn solved for y=40.0	Ct Shift
3	1.046	0.765	1.395	-1.893	-13.002	7.337	40.06	-0.06
6	1.235	0.983	1.575	-6.667	-17.280	1.081	37.79	2.21
9	1.161	0.993	1.429	-4.033	-12.616	1.263	37.92	2.08
12	0.975	0.785	1.390	3.501	-9.914	9.376	37.42	2.58

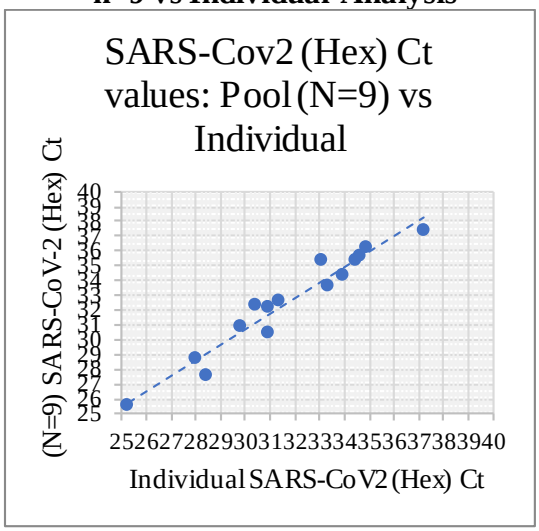
n=3 vs Individual Analysis



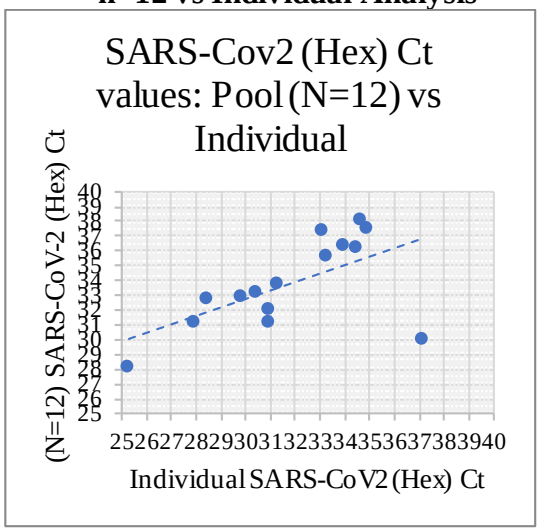
n=6 vs Individual Analysis



n=9 vs Individual Analysis



n=12 vs Individual Analysis



In silico Analysis

An *in silico* analysis was performed to estimate the impact of 12 sample pooling on the test performance across a sample cohort encountered in clinical practice. Historical data from a 11-day period from March 12 to March 23, encompassing the most recent 204 consecutive, positive specimens from 6 sites were compiled. All sites used the BioRad CFX96 Touch Real Time instrument. The Ct shift calculated from the Passing-Bablok regression analysis above was applied to the historic data set of individually tested.

The historic cohort contained no samples above the cutoff of 41 and 46% (93/204) of saliva samples were low positive samples with Ct values at or above the mean Ct value at the Clarifi COVID-19 test's LoD. However, only 4 of these samples would generate Ct values of ≥41 when tested pooled based on applying the theoretical Ct-shift to the historic cohort. Of these 4 samples, one would still return a positive result based on the pooling result interpretation; two samples would return presumptive positive results based on the implemented equivocal zone for pooled samples; only one of the samples would result in a Ct value that would theoretically generate a false negative pool.

Based on these data, a PPA of 99.5% was determined in Table 37.

Table 37. Summary of *in silico* regression analyses from sample pooling (n = 12)

N	204
Interval [X*, 41] for SARS-CoV-2	[38.44, 41]
No. samples with Ct value in the interval [38.44, 41]	4
No. samples with Ct value in the presumptive interval [41-42]	2
No. samples above the thresholds for SARS-CoV-2 pooled sample testing [Ct 42]	1
Positive	201
Presumptive Positive	2
Negative	1
Percent Positive Agreement (95% CI)	99.5% (97.3% - 99.9%)

Laboratory Monitoring Plan for Pooling

Laboratories should evaluate the appropriateness of the pooling and pool size using the FDA recommended monitoring procedure described below. Laboratories may also consider the sensitivity of pooled testing based on the assay's Limit of Detection.

After implementing a n-sample strategy, calculate the percent positivity rate (P_{pool}) based on the n sample pooling strategy periodically using the data pooled from the previous 7-10 days.

Ongoing assessment of positivity rate during application of the initial selected n-sample pooling strategy:

a. If historical data on testing individual samples from the laboratory is available:
   - The percent positivity rate, $P_{pools,}$ should be updated daily using a moving average of the data from pooled samples from the previous 7-10 days. If the P_{pools} is less than 85% of $P_{individual}$ ($P_{pools} < 0.85 * P_{individual}$), then it is recommended that the pool size be adjusted to maximize pooling efficiency according to the criteria in the table.
   - If P_{pool} is greater than 25%, pooling of patient samples is not efficient and should be discontinued until the percent positivity rate decreases.
   - It is recommended that nmaxefficiency, using Ppools and the table, be reassessed periodically while sample pooling is implemented by the laboratory to ensure maximum pooling efficiency.
   - If historical data on testing individual samples from the laboratory is unavailable:
   - After initiating the pooling strategy, calculate the initial pooling positivity rate (Ppools-initial) for the first 7-10* days using a moving average of the data from n pool testing results.
   - If Ppools-initial is greater than 25%, then pooling of patient specimens is not efficient and should cease.
   - If Ppools-initial is less than or equal to 25%, pooling of patient specimens can be discontinued.
   - Following the first 7-10* day period of sample pooling, calculate the pooling positivity rate (Ppools-x) for the next 7-10* day period on n pool testing results. If Ppools-x is less than 90% of Ppools-initial (Ppools-x < 0.90 * Ppools-initial), it is recommended that the pool size be adjusted to maximize pooling efficiency, according to the criteria in Table 39.
   - It is recommended that the nmaxefficiency, using Ppools-x and in the table, be re-assessed periodically while sample pooling is implemented by the laboratory to ensure maximum pooling efficiency.

- It is recommended that $P_{individual}$ be calculated from the previous 7-10 days, while $P_{pools\ and}$ $P_{pools\text{-}x}$ are calculated from data collected during a 7-10 day time frame. However, when determining if 7-10 days is appropriate, take into consideration the laboratory testing volume and percent positivity, among other factors. Note that if the number of individual or pooled positive results collected during a given time frame in less than 10, $P_{individual,}$

P_{pools} and $P_{pools\text{-}x}$ may not be representative of the percent positivity in the testing population and the laboratory may want to consider extending the testing time period to increase the chance of capturing positives.

Efficiency of Pooling

P, percent of positive subjects in the tested population	$n_{maxefficiency}$ (n corresponding to the maximal efficiency)	Efficiency (F) of n-sample pooling (a maximum increase in the number of tested patients when Dorfman n-pooling strategy used)
1%-4%	12	5.08 - 2.12
5%-6%	12	1.84 - 1.65
7%-12%	12	1.50 - 1.15
13%-25%	12	1.12 - 0.95
1%-4%	9	5.06 - 2.39
5%-6%	9	2.08 - 1.86
7%-12%	9	1.69 - 1.26
13%-25%	9	1.21 - 0.97
1%-4%	6	4.44 - 2.60
5%-6%	6	2.32 - 2.10
7%-12%	6	1.92 - 1.42
13%-25%	6	1.36 - 1.01
1%-4%	3	2.75 - 2.23
5%-6%	3	2.10 - 1.99
7%-12%	3	1.89 - 1.53
13%-25%	3	1.48 - 1.10

Laboratory Monitoring of Sample Pooling - Re-Assessment

i) Option 1: Stop n-sample pooling and return to individual testing

- Patient samples should be tested individually until 10 consecutive positive samples have been collected. The total number of samples, tested individually, depends on the positivity rate.
- Using these samples, 10 pools should be created and tested with 1 positive and (n-1) negative samples and the PPA between testing sample pools and individual samples should be calculated.

ii) Option 2: Continue n-sample pooling

- Re-assessment study should start from time T0 and should consist of individual sample testing in parallel with the pooled testing. However, since all non-negative sample pools require individual testing of all individual samples included in the pool as a part of the n-sample pooling and deconvoluting workflow, the re-assessment study essentially consists of testing individual samples from the negative n-sample pools.
- Re-assessment study may pause at time T1 when a minimum of 10 consecutive positive individual results are obtained, including both positive individual results generated from individual testing of samples from the non-negative sample pools following the n-sample pooling and deconvoluting workflow, and positive individual results obtained from individual testing of samples from the negative sample pools for the time period from T0 to T1 [T0, T1].
- Considering that number of positive individual sample results among negative pools is K, PPA between testing n-sample pools and assaying single specimens using the candidate test should be calculated as PPA (EUA Test $_{pool}$ vs. EUA Test $_{individual}$) = 100% x (10-K)/10. It is critical that all consecutive positive samples from time period [T0, T1] are included in the PPA calculations. With regard to calculating the PPA, all non-negative results testing pooled samples should be counted as in agreement with positive individually tested results.

Re-assessment Acceptance Criteria for Option 1 and Option 2

- If the PPA (EUA Test $_{pool}$ vs. EUA Test $_{individual}$) is $\geq$ 90% (9 out of 10 or 10 out of 10), then implementation of testing using n-sample pooling is acceptable.
- If the PPA between pooled-testing results and individual-testing results is less than 90%:
  - If PPA $\leq$70% (7 out of 10), reduce the pool size (consider a new n as n-1)
  - If PPA is 80% (8 out of 10), collect an additional 10 consecutive individually positive samples. Then, calculate the PPA from the combined data of 20 samples, between pooled testing results and individual testing results. If the PPA is $\geq$ 85%, then implementation of testing using n-sample pooling is acceptable. Or, to compensate for lost sensitivity, reduce the pool size (consider a new n as n-1) and continue with the re-assessment testing until PPA of pooled compared to individual testing is $\geq$ 90%.

- If PPA of at least 85% cannot be reached for any pool size evaluated in the re-assessment, cease pooling patient specimens.

If n-sample pooling is acceptable based on re-assessment, re-establish $P_{individual}$ in your laboratory by estimating the positivity rate from individual testing in the population from which the 10 (or 20) consecutive individual positive samples were collected. If the total number of samples (N*) that needed to be tested to obtain the 10 (or 20) consecutive positive samples is stopped at the 10th (or 20th) positive sample, then the positivity rate of 10/N* (or 20/N*) is overestimated. The positivity rate should be corrected by the following corresponding multiplier:

- Positivity rate for 10 samples is (10/N*) × (10/11)
- Positivity rate for 20 samples is (20/N*) × (20/21).

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 and OR-100 collected negative sample matrix. 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 instrument used were the Clarifi COVID-19 Extraction kit and the BioRad CFX96. The results are summarized in the table below.

Reference Materials Provided by FDA	Specimen Type	Product LoD	Cross-Reactivity
SARS-CoV-2	Saliva	600 NDU/mL	N/A
MERS-CoV		N/A	ND

NDUmL = RNA NAAT detectable units/mL.
N/A = Not applicable.
ND = Not Detected.

Additional Information

Symbols

The following symbols are used in labeling for Quadrant Biosciences PCR diagnostic products.

Symbols used in labeling for Quadrant Biosciences PCR diagnostic products.

	In Vitro Diagnostics
	Manufactured By
	Batch Code
	Consult Instruction for use
	Catalog Number
	Temperature Limitation
	Single Use
	Date of Manufacturer
DD-MM-YYYY	Date Format (Date-Month-Year)

Manufacturer and distributors

Quadrant Biosciences Inc.
505 Irving Ave.
Suite 3100AB
Syracuse, NY 13210
www.quadrantbiosciences.com

Technical Support: operations@quadrantbiosciences.com

To Report an Adverse Event: 1-866-205-7336

References

1. Center for Disease Control and Prevention. Biosafety in Microbiological and Biomedical Laboratories, 5th ed. U.S. Department of Health and Human Services, Public Health Services, Centers for Disease Control and Prevention, National Institutes of Health HHS Publications No. (CDC) 21-112, revised December 2009.

2. Clinical and Laboratory Standards Institute (CLSI). Protection of laboratory workers from occupationally acquired infections. Approved Guidelines – Fourth Edition. CLSI Document M29-A4: Wayne, PA; CLSI, 2014

Document History

Version No.	Version Date	Description of Change
B	05/07/2021	• Intended Use: modified to include pooled testing for up to twelve (12) individually collected saliva specimens • Principles of the Procedure: added OM-505/OME-505 device, self-collection, and pooling • Components and Storage: added Pooled Kit components; IFU to individual kit • Equipment/Instrumentation Required: added QS 7 and ABI7500 PCR instruments • Storing Samples: modified sample storage to up to six (6) days at ambient temperatures • Quality Control: modified negative control to include different volume for pooled testing • Sample Collection: OM-505/OME-505 device and self-collection • Sample Preparation, Reagent Preparation, and RNA Extraction: added instructions for pooled testing • qRT-PCR Instrument Setup and Operation and qRT-PCR Results Export: added instructions for QS7 and ABI7500 • Examination and Interpretation of Patient Specimen Results: added Pool Sample Results section • Limitations: added sample pooling requirements • Conditions of Authorization for the Laboratory: added pooling strategy record keeping • Limit of Detection: added QS7 and ABI7500 to instruments, OME-505 to collection device • Inclusivity: updated *In silico* analysis to include variants • Clinical Evaluations: added clinical OM-505/OME-505 data; added clinical pooled sample data; added *In silico* pool analysis • Laboratory Monitoring Plan for Pooling: added section • Laboratory Monitoring of Sample Pooling - Re-Assessment: added section • FDA SARS-CoV-2 Reference Panel Testing: added section • Appendix A: added section and label • Instrument Operation Manual Addendum: added section • Updated OR-100 with OR-100/ORE-100 throughout the document • Added OM-505/OME-505 as a collection device throughout the document
A	09/22/2020	First Issuance

Appendix A: Additional Label

For CFX96 Touch, CFX384 Touch, QuantStudio 5, QuantStudio 7, and Qualified* AB7500 Fast Real-Time PCR Detection Systems:

Please print and place this label on the front panel of the instrument. If the instruments include labeling indicating "For Research Use Only", please cover with the below "Emergency Use Only" labeling. The instrument should retain this labeling throughout the EUA use of the Clarifi COVID-19 Test Kit.

Emergency Use Only

This instrument is authorized for use with the Clarifi COVID-19 Test Kit

Warnings:

- This product has not been FDA cleared or approved but has been authorized for emergency use by FDA under an EUA for use by authorized laboratories.
- This product has been authorized only for the detection of nucleic acid from SARS-CoV-2, not from any other viruses or pathogens.
- The emergency use of this product is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug, and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated, or authorization is revoked sooner.