

Instructions for use

Biosearch Technologies SARS-CoV-2 ultra-high-throughput End-Point RT-PCR Test

For Use Under Emergency Use Authorization Only. For In Vitro Diagnostic Use. For Prescription Use Only.

Instructions for use

Biosearch Technologies SARS-CoV-2 ultra-high-throughput End-Point RT-PCR Test

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1. Summary and explanation of test

1.1 Name/identification

LGC Biosearch Technologies SARS-CoV-2 ultra-high-throughput End-Point RT-PCR Test (BT-SCV2-UHTP-EP)

1.2 Intended use

The Biosearch Technologies SARS-CoV-2 ultra-high-throughput End-Point RT-PCR Test (BT-SCV2-UHTP-EP) is an end-point reverse transcription polymerase chain reaction (RT-PCR) assay that is intended for the qualitative detection of nucleic acids from SARS-CoV-2 in anterior nasal swab specimens collected dry from any individual, including from individuals without symptoms or other reasons to suspect COVID-19 when collected by a healthcare provider (HCP) or self-collected under the supervision of an HCP.

This test is also authorized for use with anterior nasal swab specimens that are collected using a collection kit that conforms to HealthPulse@home Fusion when used consistent with its authorization.

Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA) 42 U.S.C. §263a, that meet requirements to perform high complexity tests.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in anterior nasal swab 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.

The BT-SCV2-UHTP-EP test is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the techniques of end-point PCR and *in vitro* diagnostic procedures. The BT-SCV2-UHTP-EP test is only for use under the Food and Drug Administration's Emergency Use Authorization.

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1.3 Principles of the procedure

The Biosearch Technologies SARS-CoV-2 ultra-high-throughput End-Point RT-PCR Test is a molecular, reverse transcription polymerase chain reaction (RT-PCR), *in vitro* diagnostic test that is based on widely used nucleic acid amplification technology. The Biosearch Technologies SARS-CoV-2 ultra-high-throughput End-Point RT-PCR Test contains primers and probes and internal controls used in RT-PCR for the *in vitro* qualitative detection of SARS-CoV-2 RNA from anterior nares specimens. Sample collection is performed with an integrated polyester swab and tube from SteriPack Corporation (catalog# 60600). This test is also authorized for use with anterior nasal swab specimens that are self-collected using a home collection kit that conforms to HealthPulse@home Fusion when used consistent with its authorization.

Dry swab specimens are resuspended in 400 µL of a 1x Direct Lysis Buffer containing TCEP solution with EGTA, vortexed and heated at 90 °C to lyse and inactivate the samples. RNA extraction/purification from the lysis is not required and the lysed samples are used directly in the RT-PCR reaction. Aliquots of the lysed sample are transferred to 384-well plates for use on the [Nexar™](#) system. Prepared master mix (containing [RapiDxFire™ qPCR 5X Master Mix](#), [EpiScript™ RNase H- Reverse Transcriptase](#), [SuperROX™](#) and [BHQ™ Probes and primers](#)) is combined with the lysed specimens from the 384-well plates using the Biosearch Technologies Nexar platform in two replicate arrays. Using a one-step RT-PCR approach, the viral RNA template is converted to cDNA and subsequently amplified through PCR thermal cycling in the [Hydrocycler²™](#). Final end-point fluorescence is then detected using the Biosearch Technologies [Araya™](#) fluorescent detection instrument using the FAM channel for N1 and/or N2 gene detection and the CFO channel for RNase P detection.

A summary of the mixing/amplification/detection workflow is as follows:

1. Dry collection swab devices are registered with the lab.
2. 400 µL of direct lysis buffer is added to the collection device and is incubated/lysed/inactivated.
3. A sub-aliquot of 50-100 µL of the lysed sample are transferred into a 384-well plate.
4. 3.85 µL ± 6% samples volumes are transferred from barcoded 384-well source plates to barcoded 5 µL Nexar [Array Tape™](#) wells
5. 1.15 µL ± 6% of PCR reagent master mix is aspirated from barcoded assay plates and added to the Array Tape wells by Nexar.
6. Array Tape is sealed and transferred to Hydrocycler² for amplification
7. End-point fluorescent intensities are collected using the Araya and saved as Array Tape barcoded reading files.
8. Pairs of barcoded readings files that represent the paired replicate arrays are uploaded to the UgenTec FastFinder™ analysis software for determination of 'Detected', 'Not detected', or 'Void' status.

The assay uses the primers and probe sequences intended to amplify and detect two different regions of the SARS-CoV-2 nucleocapsid gene (N1 and N2), together with human RNase P

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nucleic acid as an endogenous internal control. The assay uses the same primers and probe sequences as those in the FDA-authorized CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel (EUA 200001/A004) and is configured as a multiplex assay in which probes detecting the N1 and N2 regions are labeled with the same fluorophore and the RNase P-based endogenous internal control is labeled with a second fluorophore. In the presence of the target, primers and probes will hybridize to the specific sequence and allow amplification by the polymerase. The different probes include fluorescent dyes and quenchers in sufficient proximity to each other that the quencher limits the fluorescence prior to amplification. However, during amplification, the polymerase extends the new strand and degrades the fluorescent dye-labeled probe using its exonuclease activity. This results in the separation of the quencher from the fluorescent dye, thereby allowing the emission of fluorescence. Fluorescence intensity is measured at end-point of the amplification process by the Biosearch Technologies Araya inline fluorescence detection system to qualitatively detect the 6'FAM signal representing N1 and/or N2 gene amplification as well as the Cal Fluor Orange 560 (CFO) signal representing RNase P amplification.

2. Materials

For pricing and availability of all the instruments, controls, primer-probe sets, reagents and consumables that comprise this test, please email Biosearch Technologies at genomics.americas@lgcgroup.com. Clinical laboratories potentially interested in this Biosearch Technologies test are advised *not* to reach out on an *ad hoc* basis to the various component manufacturers listed in the tables below.

2.1 Reagents and materials required for use of the BT-SCV2-UHTP-EP test

Component	Source	Storage conditions	Description
Collection device	XpressCollect™ (SteriPack, Cat: 60600) for HCP collection use, or HealthPulse@home Fusion conforming home-collection device for self-collection	Ambient	Standalone barcoded integrated sterile spun polyester swab-in-tube sample collection and transport device designed for high-throughput automation
Swabless tube assembly	XpressCollect™ SteriPack: Cat: ACR010002	Ambient	Swabless tube assembly that integrates with high throughput automation for use as NTC Tube
Tris(2-carboxyethyl) phosphine hydrochloride (TCEP)	Sigma, C4706-50G	2-8 °C	Component of direct lysis buffer
0.5M EGTA, pH 8.0	Boston BioProducts, Cat: BM-151 (Alternative: Sigma, Cat: 324626)	2-8 °C	Component of direct lysis buffer
10N NaOH	Sigma, 72068-100ML	Ambient	Component of direct lysis buffer
Molecular biology grade nuclease free water	Major laboratory suppliers	Ambient	Component of direct lysis buffer

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pH paper	Cytiva Whatman™ pH Indicator Papers, CF Strips PH Paper, 2614991	Ambient	Used to measure pH during preparation of direct lysis buffer
384-well sample plate	Biosearch Technologies, KBS-7001-139	Ambient	384-well plate, 100 µL well volume, 50 plates per box
Assay tubes	ThermoFisher – 4170	Ambient	0.75 mL blank matrix tubes
Assay tube rack	ThermoFisher – 4896	Ambient	Empty latch rack for 0.75 mL matrix tubes
Assay tube seal	ThermoFisher – 4463	Ambient	SepraSeal caps for assay tubes
Sample and Assay plate seal	Biosearch Technologies, KBS-0600-002	Ambient	Adhesive PCR foil seal, 135 mm × 80 mm, 100 sheets per box
Assay plates (alternative to ThermoFisher's 4170, 4896, 4463)	Biosearch Technologies, KBS-7001-131	Ambient	96-well V-bottom plate, 0.8 mL well volume
	Biosearch Technologies, KBS-7001-231	Ambient	96-well storage plate, 0.8 mL well volume, 50 plates per box
	Greiner Bio-one – 786201	Ambient	Masterblock®, 96-well V-bottom plate, 0.7 mL well volume
Adhesive barcode labels	Biosearch Technologies, KBS-0750-100	Ambient	Adhesive plate barcodes (roll of 5000).
	Various vendors	Ambient	Adhesive barcodes.
EpiScript RNase H-Reverse transcriptase	Biosearch Technologies, ERT12925K-ENZ	-20 °C	Component of assay: reverse transcriptase enzyme.
	Biosearch Technologies, ERT12925K-1.25ML	-20 °C	
	Biosearch Technologies, ERT12925K-12ML	-20 °C	
RapiDxFire qPCR 5X Master Mix GF, 1 mL, 10 mL, 100 mL	Biosearch Technologies, 30050-1	-20 °C	Component of assay: 5X qPCR Master Mix
	Biosearch Technologies, 30050-2	-20 °C	
	Biosearch Technologies 30050-100ML	-20 °C	
SuperROX Reference Dye, 15 µM 1 mL, 10 mL	Biosearch Technologies, SR-1000-1	2-8 °C	Component of assay: passive reference dye.
	Biosearch Technologies, SR-1000-10	2-8 °C	
UltraDx SARS-CoV-2 N1/N2/RnP Assay, 100X, 1010 µL	Biosearch Technologies, N1N2RNP-RES-1010	-20 °C	Component of assay: 100X primer and probe mix.
Positive Control Material for SARS-CoV-2	SeraCare AccuPlex™ 0505-0268	2-8 °C	AccuPlex SARS-CoV-2 POS Control Material (Full Genome) at 100,000 cp/mL 5 × 1 ML per kit.
Negative Control Material RNase P only	SeraCare AccuPlex 0505-0269	2-8 °C	AccuPlex RNaseP NEG Control at 82, 400 cp/mL 5 × 1 ML per kit.
No Template Control (NTC)	Manufactured per this IFU	Ambient	Prepared 1X direct lysis buffer
5 µL Array Tape PCR reaction consumable	Biosearch Technologies, AXAT384-13PP5ML-27C	Ambient	Nexar Array Tape, clean consumable, 384-well, 5 uL wells, 4 reels, 185 arrays per reel, 27 metre reel, 13" reel.

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Nexar Pipette 384 TipTray	Biosearch Technologies, AX225699	Ambient	Nexar Tips, 10 µL tips, 384 tips/tray, 1 tray of tips, for CyBio head.
Array Tape cover seal	Biosearch Technologies, AXCT17690CCM	Ambient	Nexar Cover Seal, Clean Consumable (DNase-, RNase-, Pyrogen-free), 3 rolls, 625 arrays per roll, 90 meters per roll.
70% Ethanol	Various	Ambient	Cleaning solution for surfaces and for use in PPE processes.
5% sodium hypochlorite	Clorox Germicidal Bleach (alternative: Various vendors, 5% sodium hypochlorite)	Ambient	Cleaning solution for decontamination of surfaces and for use in PPE processes and use as additive in Nexar pipette wash.
Sodium hypochlorite concentration test strips (both required)	Deardorff Fitzsimmons, Activate™ High-level Chlorine Test Strips – 77085	Ambient	Active high-level chlorine strips for measuring high bleach concentration in pipette wash.
	LaMotte, Insta-Test – 3002 Chlorine Dioxide Single Factor Test Strip	Ambient	Insta-Test Analytic High Range Chlorine Dioxide Test Strips for measuring Low bleach concentration in Pipette wash.

Table 1. List of required reagents and materials

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2.2 Instrumentation/equipment specified by the method

The Biosearch Technologies SARS-CoV-2 ultra-high-throughput End-Point RT-PCR Test utilizes the Biosearch Technologies [Nexar](#), [Hydrocycler²](#) and [Araya](#) instruments for PCR reaction setup, amplification and detection.

Component	Source	Description
Hybex microsample incubator	SciGene, PN: 1057-30	Isothermal incubator capable of holding sample temperature at 90 °C for a minimum of 10 minutes at a time.
Cap removal tool	Various vendors #3 Robertson screwdriver	Screwdriver with #3 Robertson square bit.
De-capping/re-capping unit	Biosearch Technologies, AX9921900	Modification A: Brooks Automation IntelliXcap de-capping instrument (46-8012 (Also known as: BBT-46-8012) modified to interface with SteriPack Sample Tube Cap removal and re-installation.
De-swabbing unit	Biosearch Technologies, AX9921905	Modification B: Brooks Automation IntelliXcap de-capping instrument (46-8012 (Also known as: BBT-46-8012) modified to interface with SteriPack Sample Swab removal.
Nexar	Biosearch Technologies, AXDS-0001-100 AXDS-0001-301	Nexar fully integrated system-unwind, dispense pipette with pipette wash, two expansion block modules with plate stacker and dehydrators Dispense Jet with Jet Wash, tape sealer and rewind.
Hydrocycler ²	Biosearch Technologies, KBS-0028-00/KBS-0028-001-PS	Hydrocycler ² PCR thermal cycler system.
Hydrocycler ² Array Tape spool holder	Biosearch Technologies KBS-0028-004	Hydrocycler ² 384-well Array Tape Basket (50 arrays).
Araya	Biosearch Technologies, AXDS-0004-100	Araya inline detection instrument.
Sample tube transfer tools and Hybex adaptor	Biosearch Technologies AX9921910	Kit of transfer tooling and custom interfacing parts for integration and use of the Hybex Incubator during the Lysis step.
1 Ton arbor press	Various	1 ton hand operated arbor press used to press transfer tools onto tube tops.
Arbor press plate	Biosearch Technologies, AX9922060	Plate used in conjunction with a 1 ton arbor press used to press the tube transfer tools onto the top of tubes.

Table 2. List of required instruments and equipment

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2.3 Required software

Component	Source	Description
Intellics™ for Nexar	Biosearch Technologies, AXDS-NI1804-001-SW Revision H or higher	Intellics for Nexar software. Protocol creation, Nexar pipette, Dispense Jet, and Araya Logfile correlation logic and results spot-checking software.
Nexar Instrumentation Control and User Interface Software	Biosearch Technologies, AX9917733-SW Revision J or higher	Control and user interface software for the Nexar system that includes system-unwind, CyBio Dispense Pipette and Pipette Wash, Two Expansion Block Modules with Plate Stacker in each, Dispense Jet 2.0 with Jet Wash, Tape Sealer and Rewind.
Hydrocycler ² control and user interface software	Biosearch Technologies, KBS-0028-001-SW Rev B (5.44.0) or higher	Control and User interface software for Hydrocycler ² system.
Araya	Biosearch Technologies, AX9917733-SW Revision J or higher	Control and user interface software for Araya inline detection instrument.
UgenTec FastFinder Analysis	UgenTec FastFinder Analysis v4.6.0 or higher	Cloud hosted data analysis software that utilizes an assay plugin object to execute clinical scoring.
UgenTec FastFinder Assay Plugin	UgenTec, Assay Plugin ID: BIOSEARCH-EUA-SARS-CoV-2-uHTP v2.0 or higher	Assay Decision Logic Plugin that implements specimen calling logic and clinical decision logic.

Table 3. List of required software

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3. Other equipment, software and materials required or recommended but not provided for the test

Optional equipment which can be used to increase throughput by automating certain operations of the workflow.

Component	Source	Description
Tube rack scanner	BioMicroLab Sample Scan ES, BML-SSES1D	Flatbed scanner capable of reading 2D barcodes off the bottom of the collection tubes and side mounted 1D barcode simultaneously to Tube Rack ID sample ID maps in 1 step.
Lysis Buffer dispenser	BioTek MicroFill, AF1000A (Or Equivalent)	BioTek microFill 96-/384-well microplate dispenser – bulk reagent dispenser – with waste pump modification.
96 tip sample re-array instrument	Biosearch Technologies, oKtopure™, KBS-0009-001 (or alternative 96 tip parallel pipette liquid handlers)	Parallel pipetting 96 tip head that can combine 4 source 96 well tube racks into a single 384-well plate as well as generating log files of source tube rack IDs into 384-well quadrants.
Handheld barcode scanner	Various	Handheld barcode scanners for scanning 1D barcodes on tubes and plates as needed.

Table 4. Other equipment, software and materials not specified by the method

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4. Warning and precautions

4.1 General warnings and precautions

This 'instructions for use' document must be read carefully prior to use. The assay, lysis buffer and control material preparation process instructions must be accurately followed. Reliability of results may be affected if deviations from the instructions in this document exist.

- For Use Under Emergency Use Authorization (EUA) Only.
- For *In Vitro* Diagnostic Use.
- For Prescription Use Only.
- This product has not been FDA cleared or approved but has been authorized for emergency use by FDA under an EUA for use by authorized laboratories.
- The product 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 product is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug, and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated or authorization is revoked sooner.
- Laboratories within the United States and its territories are required to report all SARS-CoV-2 test results to the appropriate public health authorities.
- All laboratory personnel using the test must be appropriately trained in PCR techniques, the specific processes and instruments used in the BT-SCV2-UHTP-EP test molecular test procedure, use appropriate laboratory and personal protective equipment when handling this kit, and use it in accordance with the authorized labeling.
- Never use reagents that appear damaged or are beyond their expiration date(s).
- After handling samples and reagents, remove gloves and wash hands thoroughly.
- Do not eat, drink, smoke or apply cosmetics in designated work areas.

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4.2 Sample collection, handling, transportation and storage

Please refer to Collection Device instructions for use. Inadequate or inappropriate sample collection, storage and transport may yield incorrect or invalid test results.

4.2.1 Handling precautions for specimen preparation in the laboratory

SAFETY WARNING

Handle all samples and controls as if they are capable of transmitting infectious agents. Refer to the [CDC Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Persons Under Investigation \(PUIs\) for Coronavirus Disease 2019 \(COVID-19\)](#).

- During preparation of samples, it is essential to minimize the risk of cross-contamination between samples and the inadvertent introduction of ribonucleases (RNases) into samples during and after the extraction procedure. Proper aseptic technique should always be used when working with RNA.
- It is recommended as best practice that the Hydrocycler² and Araya are located in a segregated location from where sample and assay materials are prepared in order to reduce the likelihood of amplicon contamination events that may occur with post PCR amplified arrays.
- All biological specimens should be handled as if infectious, using procedures as outlined in Biosafety in Microbiological and Biomedical Laboratories and in the CLSI Document M29-A4. Guidelines for specimen handling are available from the U.S. Centers for Disease Control and Prevention, Clinical and Laboratory Standards Institute and World Health Organization.
- Follow your institution's safety procedures for working with chemicals and handling biological samples.
- Only personnel proficient in handling infectious materials and the use of the SARS-CoV-2 ultra-high-throughput End-Point RT-PCR Test and associated instrumentation should perform this procedure.
- Wear laboratory gloves, laboratory coats and eye protection when handling samples and reagents. Gloves must be changed between handling samples, buffers and reagents to prevent contamination. Avoid contaminating gloves when handling samples and controls.
- Thoroughly clean and disinfect all laboratory work surfaces with a freshly prepared solution of 0.5% sodium hypochlorite in distilled or deionized water (dilute household bleach 1:10). Follow by wiping the surface with 70% ethanol.
- Wash hands thoroughly after handling samples and kit reagents, and after removing the gloves.

4.2.2 Precautions for instrument use

- Refer to the operations manuals for the equipment used in this procedure for instructions on safety precautions to be taken when working with each instrument.
- Review all Caution 'HOT Surface' identifications

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- The user should ensure the equipment is free of visible or known defects or issues before use.
- Consult the instrument operations manual for specifics on how to conduct operations using the system in conjunction with the test IFU documented here.
- Any problems, issues or errors encountered during instrument use should be logged through the appropriate issues management system to ensure they are addressed by the appropriate support team.
- Carefully inspect Array Tape before and after thermal cycling.
- Array Tape, post thermal cycling; must be handled with care to prevent array tape or seal damage. Damage to sealed Array Tape, post thermal cycling, may result in the release of amplicons and possible lab area contamination.

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5.1 Preparation of Direct Lysis Buffer

Prepare 100x Direct Lysis Buffer according to the following table as a stock solution. Volumes and quantities can be adjusted based on sample numbers. 25 mL of 100x Direct Lysis Buffer is sufficient for 5625 samples with 10% overage.

100x Direct Lysis Buffer can be stored at 2-8 °C and shall have an expiration date of 2 weeks from date of preparation.

25 mL of 100x Direct Lysis Buffer should be prepared according to the following table. Volumes and quantities can be adjusted based on sample numbers.

Reagent	Quantity	Notes
Tris (2-carboxyethyl) phosphine (TCEP), Hydrochloride	1790 mg	
0.5M EGTA pH 8.0	13 mL	
10N NaOH	Variable by batch (~1-2 mL)	Adjust pH to 7.0-7.5 using 10N NaOH and pH indicator paper (Cat: 2614-991)
Molecular Biology Grade Nuclease Free Water	Up to 25 mL	

Table 5. Components of the 100x Direct Lysis Buffer

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Prepare 1x Direct Lysis Buffer by diluting 100x Direct Lysis Buffer stock solution in molecular biology grade nuclease free water for active use. Adjust pH to 7.0-7.5 with 10N NaOH using pH indicator paper (Cat: 2614-991). 1x Direct Lysis Buffer should be prepared on the day of use and stored at 2-8 °C prior to use. It has an expiration of 24 hours from preparation. 400 µL of 1x Direct Lysis Buffer is required per sample, and a volume overage of 10% is recommended if preparing buffer for multiple specimens.

Component	Stock concentration	Volume per sample ¹ (µL)	Final concentration
100x Direct Lysis Buffer	100x	4	1x
Molecular biology grade nuclease free water	NA	396	NA
Final sample volume	NA	400	NA
Final pH	NA	NA	7.0-7.5

Table 6. Components of the 1x Direct Lysis Buffer

¹ Direct Lysis Buffer can be scaled as needed based on the number of specimens. Include 10% volume overage when making Direct Lysis Buffer for multiple specimens. 1x Direct Lysis Buffer should be prepared on the day of use. Prepare 400 µL 1x Direct Lysis Buffer per sample.

5.2 Sample registration, lysis/inactivation, replating and storage of lysed samples

- Once the specimens arrive at the test lab, unpack and scan the racks and tubes into the laboratory information system (LIMS) or equivalent data management system.
 - NOTE: Misalignment of results can come from situations including, but not limited to, moving the specimen location after entering it into the LIMS. It is recommended to avoid moving the specimen locations after entry into the LIMS.*
 - Specimens may be stored ambient for up to 72 hours or at a temperature setting between 2-8 °C until processing.
- Arrange the sample tubes into the racks according to Figure 1 below, reserving the last 3 locations in row H for control materials. Preparation instructions for the positive and negative controls can be found in the [Preparation and testing of control samples](#) section of this document.

NOTE: Biosearch Technologies recommends that control materials are located at positions H10-H12 in each tube rack even for partial racks.

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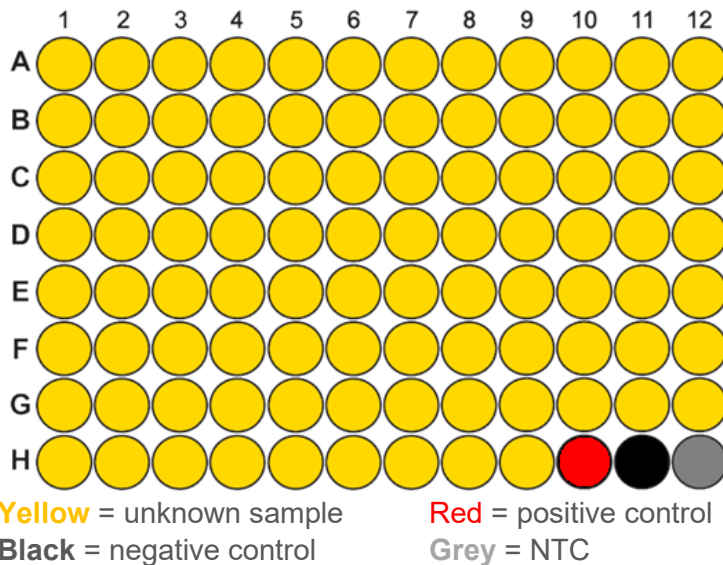


Figure 1. Layout of samples and controls in a 96-well rack

3. Remove filling plug from the specimen tube lids within a biosafety cabinet using automation or a #3 Robertson square bitscrewdriver (or equivalent). The swab should remain in place inside the specimen tube.

NOTE: Use of the automated de-capping/re-capping instrument Biosearch Technologies, AX9921900 is mandatory. It greatly improves the speed and reduces labor required to execute these specimen processing steps.

4. Pipette 400 μ L of 1x Direct Lysis Buffer to each specimen and control tube and recap specimen tubes.

NOTE: An automated reagent dispenser such as the BioTek MicroFill AF1000A instrument greatly improves the speed and reduces labor required to execute this step.

5. Spray the tube surfaces with 70% ethanol prior to removing the tubes from the biosafety cabinet.
6. Briefly vortex the rack of tubes.
7. Centrifuge tubes at 860-890 \times g for 2 minutes to collect liquid in the bottom of the tube.
8. Move the tubes from the tube racks to the SciGene Hybex Microsample Incubator

NOTE: The tube transfer tools AX9921910 and Arbor press with plate AX9922060 greatly improves the speed and reduces labor required to execute this step.

9. Heat samples at 90 $^{\circ}$ C \pm 1 $^{\circ}$ C for 10 \pm 1 minutes in the SciGene Hybex Microsample Incubator.
10. Remove specimens from heat block and centrifuge at 860-890 \times g for 2 minutes.
11. Twist to remove the lid/swab construct from the specimen collection tube and discard lid/swab construct in biohazard waste.
12. Construct a 384-well sample source plate containing lysed specimen material either manual or using any adequately programmed general purpose fluid handler. Transfer a minimum of

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- 50 µL of lysed specimen, including the positive, negative and NTC controls (1 from each 96-well rack), to the 384-well plate.
13. Attach a barcode label to the 384-well plate on the column 24 (i.e. right side of the plate skirt).
 14. Log and associate this barcode with the 4 source tube rack barcodes in the corresponding offsets of the 384-well plate for tracking in the LIMS.
 15. If not intending to be processed on the Nexar immediately after replating, seal the 96-well and 384-well plates with a peelable seal and store at 2-8 °C for up to 24 hours until ready for further processing. Otherwise, 384-well plates containing lysed specimen material should be sealed and stored at a temperature setting of 2-8 °C until results are reported.

5.3 Preparation, testing and storage of control materials

The following table summarizes the control materials validated for use in the method as well as their purpose and frequency.

Component	Description	Purpose	Frequency
Positive control swab (PC)	SeraCare AccuPlex SARS-CoV-2 POS Control Material	Full process positive control for SARS-CoV-2	One per 96-well tube rack (Well H10)
Negative control swab (NC)	SeraCare AccuPlex RNaseP NEG Control	Full process positive control for human RNase P	One per 96-well tube rack (Well H11)
No template control (NTC)	Swab-less tube to hold Lysis Buffer	Full process no template control	One per 96-well tube rack (Well H12)
Internal sample process control	Primer/probe set that detects human RNase P	Ensure that specimen was of good quality and integrity for RT-PCR	Two PCR replicates per sample on separate Array Tape dispenses

Table 7. Description of test controls

5.3.1 Positive control swab preparation

The positive control material is based on a targeted concentration of ~5x LOD determined for the control material. It is suggested that these materials are made in batches of 96 tubes to minimize waste of control materials.

1. Spot 42.5 µL of SeraCare AccuPlex SARS-CoV-2 POS Control Material (Cat: 0505-0268) onto each fresh sterile dry swab resulting in a positive control swab concentration of ~4250 copies/swab.
2. Place the swabs back in the tubes and allow the swabs to dry for at least 20 minutes in a biosafety cabinet.
3. Label and/or color code the positive control tubes so that they can be visually identified.
4. Prepared control swabs should be Quality Control (QC) verified by processing through the BT-SCV2-UHTP-EP test procedure prior to use as controls with patient specimens.
 - a. Process a minimum of two positives controls per lot preparation through the SARS-CoV-2 ultra-high-throughput End-Point RT-PCR test and confirm concordance with expected results.
 - b. Record QC results, documentation of pooling, dilution, lot number and preparation date.
5. Store prepared materials at 2-8 °C; or -20 °C; or -80 °C for up to 1 month. If stored for more than 1 month, QC should be repeated to verify expected performance.

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6. Positive control swabs should be processed like any other sample swab in the process workflow but should always be positioned in location H10 of the sample tube rack.

5.3.2 Negative control swab preparation

The negative control material is based on a targeted RNase P concentration equivalent to 5x LOD determined for the negative control material. It is suggested that materials are made in 96 tube batches to minimize waste of control materials. The batch size can be adjusted based on anticipated sample throughput.

1. Spot 42.5 µL of SeraCare AccuPlex RNaseP NEG Control Material (Cat: 0505-0269) onto each fresh sterile dry swab resulting in a negative control swab concentration of ~3502 copies/swab.
2. Place the swabs back in the tubes and allow the swabs to dry for at least 20 minutes in a biosafety cabinet.
3. Label and/or color code the Negative control tubes so that they can be visually identified.
4. Prepared control swabs should be Quality Control (QC) verified by processing through the BT-SCV2-UHTP-EP test procedure prior to use as controls with patient specimens.
 - a. Process a minimum of two negative control swabs per lot preparation through the SARS-CoV-2 ultra-high-throughput End-Point RT-PCR test and confirm concordance with expected results
 - b. Record QC results, documentation of pooling, dilution, lot number and preparation date.
5. Store prepared materials at 2-8 °C; or -20 °C; or -80 °C for up to 1 month. If stored for more than 1 month, QC should be repeated to verify expected performance.
6. Negative control swabs should be processed like any other sample swab in the process workflow but should always be positioned in location H11 of the sample tube rack.

5.3.3 No template control swab preparation

The no template control (NTC) is an empty tube or swabless tube that has not had any material previously dispensed into the tube. It is intended to contain an unaltered sample of the Direct Lysis Buffer material used for this batch of processing. NTC tubes should be processed like any other sample tube in the process workflow but should always be positioned in location H12 of the sample tube rack.

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5.4 Preparation of reagent and materials aliquots

Upon receipt, individual PCR reagent materials should be aliquoted in expected usage volumes to avoid freeze/thaw cycles of the master batch and stored in one time use aliquot volumes that are stored in accordance with manufacturer's guidelines. It is important to ensure reagents are thoroughly thawed and mixed before creating aliquots. On the day of use, assemble enough prepared master mix for the anticipated number of samples to be tested plus an overage. Master mix is prepared on the day of use with 2 aliquots each per 384-well sample plate. In the workflow each sample plate is dispensed with a volume of 3.85 μL into 2 replicate arrays followed by a full 384-well assay dispense of 1.15 μL after aspiration from separate assay plate source wells.

The PCR reagents should be prepared with the following target concentrations to generate a final reaction concentration according to the following table:

Reagent	Stock concentration	Target volume per reaction (μL)	Final target concentration in 5 μL reaction
RapiDxFire qPCR 5X Master Mix GF	5X	0.999	1X
EpiScript RNase H-Reverse transcriptase	200 U/ μL	0.072	2.87 U/ μL
UltraDx SARS-CoV-2 N1/N2/RP Assay	100X	0.05	1X
SuperROX, reference dye	15 μM	0.0288	0.086 μM
Prepared master mix volume	NA	1.15	NA

Table 8. Prepared Master Mix formulation

1. Remove aliquots of each of the following from the freezer, thaw and maintain on ice.
 - a. RapiDxFire qPCR 5X Master Mix
 - b. EpiScript RNase H-reverse transcriptase
 - c. UltraDx SARS-CoV-2 N1/N2/RP Assay
2. Remove aliquots of SuperROX passive reference dye from refrigerator.
3. Into the 1.5 mL tube containing RapiDxFire qPCR 5X Master Mix, combine reagents as described in the table below to make the prepared master mix.

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Reagent	Assay mix prepared for each sample plate/2 Array Tape arrays (μL)	% of total
RapiDxFire qPCR 5X Master Mix, GF	1016.8 μL	86.9%
EpiScript RNase H- Reverse Transcriptase, 200 U/μL	73.1 μL	6.2%
UltraDx SARS-CoV-2 N1/N2/RP Assay	50.9 μL	4.4%
SuperROX; concentration 15 μM	29.3 μL	2.5%
Total	1170.1 μL	100%

Table 9. Preparation of master mix for one 384-well sample plate (enough for two arrays)

IMPORTANT: Mix well reagents together by inverting or slow vortex.

4. Briefly centrifuge.
5. Each tube of prepared master mix is sufficient to aliquot into two assay plate wells. Each assay plate well corresponds to a single prepared 384-well array of Array Tape on the Nexar.
6. Add 560 μL to each well of the assay plate starting with A1 and A2.
 - a. For additional sample plates (four or more arrays), add additional 560 μL aliquots into the assay plate, in a top to bottom left to right order.

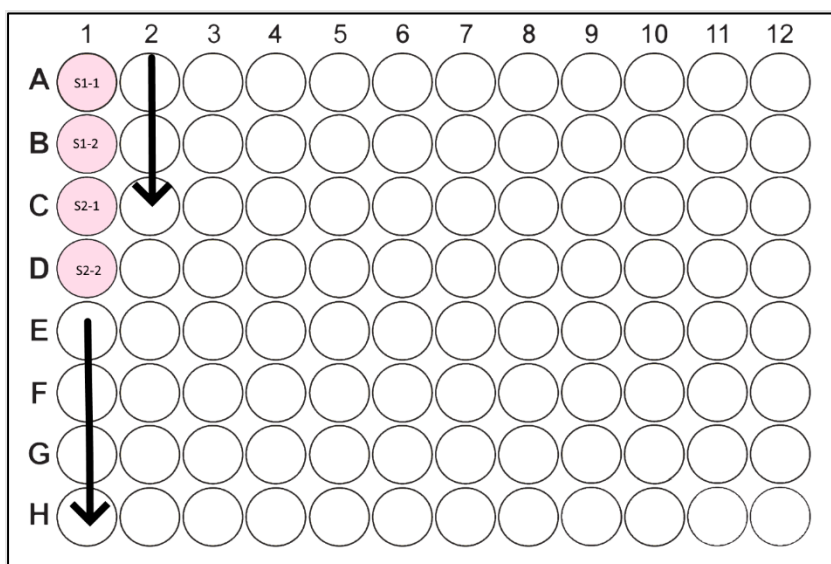


Figure 2. Assay plate layout

7. Prepared master mix should be stored at 4 °C, covered and protected from light. In this condition, the prepared master mix will be stable for up to 4 hours before use.
8. Details of the materials used for assay preparation should be documented in the LIMS and an

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assay plate barcode label should be applied to the plate on the right short edge next to column 12 for assay tracking in the Nexar dispense log file.

5.5 Prepare and execute PCR reaction setup on the Nexar

The Nexar is a modular inline liquid handling and assay processing system that transfers nucleic acid samples from a 384-well plate and prepared master mix from a 96-well plate/tube rack to create a final PCR reaction in an Array Tape well. It automatically executes Array Tape motion, barcode reading, sample and PCR reagent aspiration and dispense and application of the cover seal.

5.5.1 Precautions for instrument operations for sample and assay mixing

- Consult the instrument operations manual for specifics on how to conduct testing using the system. Only operators trained to use the instrument in a CLIA-certified high complexity lab should perform this testing.
- The user should ensure the equipment is free of visible or known defects or issues before use.
- Any problems, issues or errors encountered during instrument use should be logged through the appropriate issues management system to ensure they are addressed by the appropriate support team.
- Nexar tips are intended for use across multiple specimens following the defined wash procedure between specimen aspirations. As a precaution, Nexar tips, even with wash procedures in use, should be discarded daily or after each run as needed.

5.5.2 Instrument operations for Nexar sample and assay mixing processing

1. It is recommended that the first two arrays of each production run be considered 'Leading' arrays and be deliberately left empty to aid in the sealing processes. Leading arrays should be labeled with run identifier to aid the processing of the Nexar run. In addition, a single 'Trailing' array should be included to allow the user to have a region to remove and cut the cover seal without risk of improper sealing of the final array.
2. Prior to initiating the run on the Nexar liquid handler, the following system checks are recommended:
 - a. Ensure that there is enough Array Tape on the spool to complete all the source plates in the scheduled run. $\text{Number_Of_Arrays} = ((\text{Number of Source plates} \times 2) + 3)$
 - b. Ensure the sodium hypochlorite bottle has sufficient volume.
 - c. Ensure Nexar sodium hypochlorite concentration in the pipette wash routine is correct per the specified chlorine test strips.
 - d. Ensure the 20 L water carboy is full of type III water or better.

NOTE: One 20 L carboy should be able to complete a full run with washes of 26 384-well plates (52 arrays).

- e. Ensure there are no plates in the Nexar plate stackers or on the plate conveyors.

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- NOTE: Do not send to Nexar until the current/previous run is completed or it is possible that ancillary settings files may be overwritten for the protocol currently being executed on the system.*

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8. Acquire the 384-well plate containing lysed specimen material constructed in the sample registration, lysis/inactivation, replating and storage of lysed samples section.
9. Centrifuge sample plates for a minimum of 500 g for 1 min to collect condensation to the bottom of the plate.
10. Review the 96-well plate containing prepared master mix and ensure 560 µl of prepared master mix are in each required well of the 96-well plate (two per sample plate). For details on the creation of the prepared master mix, see the [Preparation of reagents materials and aliquots](#) section.

NOTE: Each well containing prepared master mix corresponds to one dispense of a full 384-well plate.

11. Remove adhesive seals from sample and assay plates.

IMPORTANT: Use caution when removing adhesive seals. Removal at a 45° angle will minimize the risk of sample transfer between wells.

12. After removal of seals place sample plates in the Dispense Pipette IN CHUTE location and Assay plates in the Dispense Jet IN CHUTE Location.
13. Login to the Nexar interface, add the protocol to the run list and select run playlist.
14. Sealed Array Tape samples should go from the Nexar directly to the Hydrocycler² after processing.
15. After the run, perform daily Dispense Jet maintenance process.
16. Used 384-well plates containing lysed specimen material should be sealed with a peelable seal and stored at a temperature setting of 4-8 °C until results are reported.
17. Used assay plates may be discarded.

5.6 Prepare and execute Hydrocycler² end-point thermal cycling protocol

The Hydrocycler² is a water bath thermal cycler that has been specifically modified to hold Nexar Array Tape as well as conventional microtiter plates.

5.6.1 Instrument operations for Hydrocycler² use

1. Prior to initiating the run on the Hydrocycler² system, the following system checks are recommended:
 - a. Ensure ballast water tank is filled to, but not over, the max limit.
 - b. Each water bath, 1, 2 and 3, must be set at 94 °C, 60 °C and 42 °C, respectively.
 - c. Select 'Pre-Heat Tanks'
 - d. Verify that the program settings are as follows:

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1. 42 °C for 15 minutes.
2. 94 °C for 3 minutes 10 seconds.
3. 45 cycles at temperature settings of 95 °C for 4 seconds and 60 °C for 30 seconds.
4. Recirculation set to OFF while tape is submerged and on when not submerged.
5. Mixing is turned OFF.

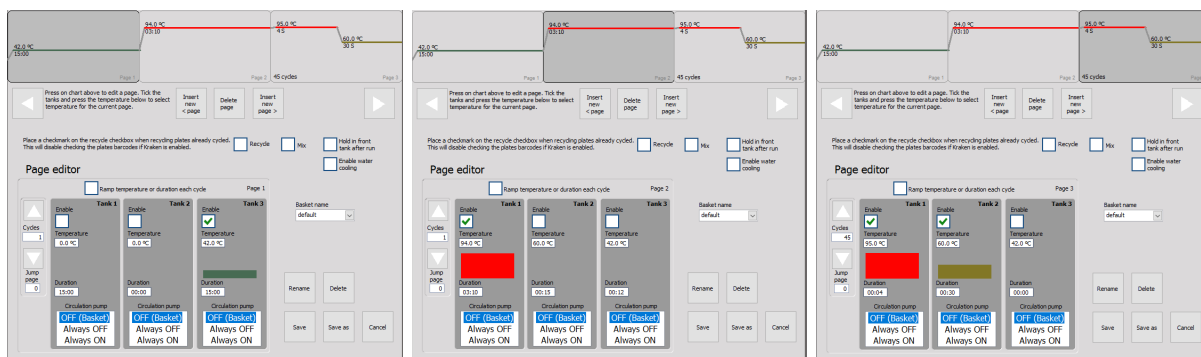


Figure 3. Hydrocycler² thermal cycling program

2. Initiate the run on the Hydrocycler².
3. After the run is complete, dry the array tape using large lint free cloth on a benchtop surface to remove any large water droplets that have accumulated on the surface of the tape.
4. Array Tape is stable for up to at 4 °C or ambient temperature in the dark for up to 1 week after thermal cycling.

5.7 Prepare and execute Araya end-point fluorescence detection

The Araya is used to scan and record end-point PCR fluorescence in Array Tape.

5.7.1 Prepare for Araya use

- Wipe up any water accumulated on the equipment surface immediately using a lint free paper towel.
- 70% ethanol should be used to wipe down the instrument as needed.
- Remove the hold down plate and use lint free paper towels soaked in 70% ethanol to clean under the plate as needed.

5.7.2 Instrument operations for Araya end-point detection system

1. Open the Araya HMI page
 - a. On the resulting instrument navigation page select 'Reset'.
 - b. Press 'Sync Tape Array' using the laptop before loading the array tape.
 - c. Load Array Tape onto the infeed spool so that the tape feeds into the unit with the wells pointing down.

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- d. Feed the tape into the scanner by holding the joystick to the left. Align the last column of the first array to be scanned with the yellow arrow closest to the optical reader module.
- e. Click on the middle OR Module of the instrument navigation page.
- f. If the entire spool is to be read then ensure the 'scan reel' box is checked, click on the 'Run Scan' button
- g. If only a portion of the tape is to be read, make sure 'scan reel' is not selected and the 'number of arrays' field displays the number in accordance with the number of arrays to be scanned click on the 'Run Scan' button

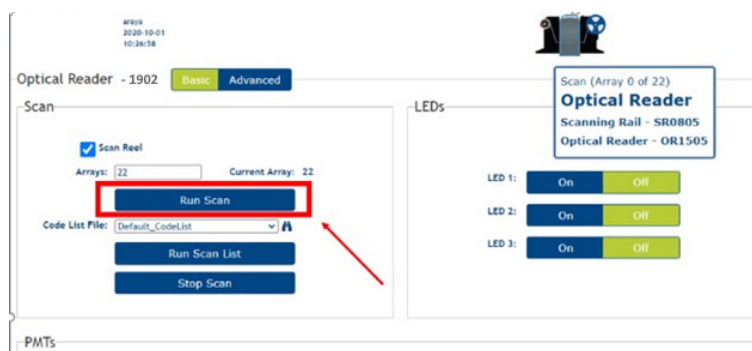


Figure 4. Araya instrument software interface

- h. Upon scanning the Araya readings files are stored in the instrument memory and are available for upload to the analysis software.
- i. Array Tape that has been read, can be stored in the dark at 4 °C or ambient temperature until disposal. If not enough 4 °C storage is available, tape may be stored at room temperature for up to 1 week in the case where a rescan of the tape is needed.

5.8 FastFinder analysis software review and authorization of results

The BT-SCV2-UHTP-EP test method utilizes the UgenTec FastFinder Analysis software for the [interpretation of results](#) as described below.

1. Login to the FastFinder software <yourdomain>.fastfinder.app/

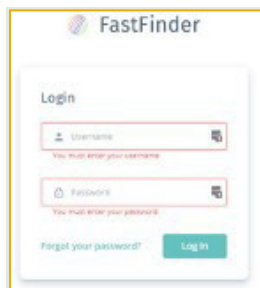
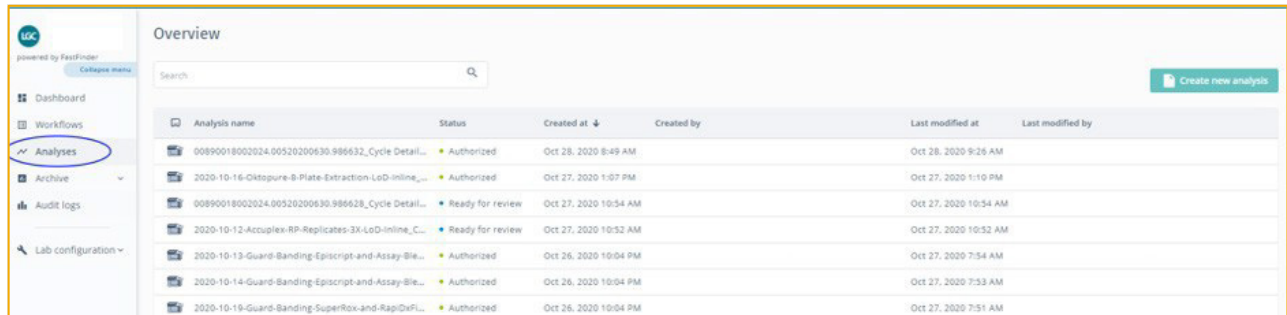


Figure 5. FastFinder login

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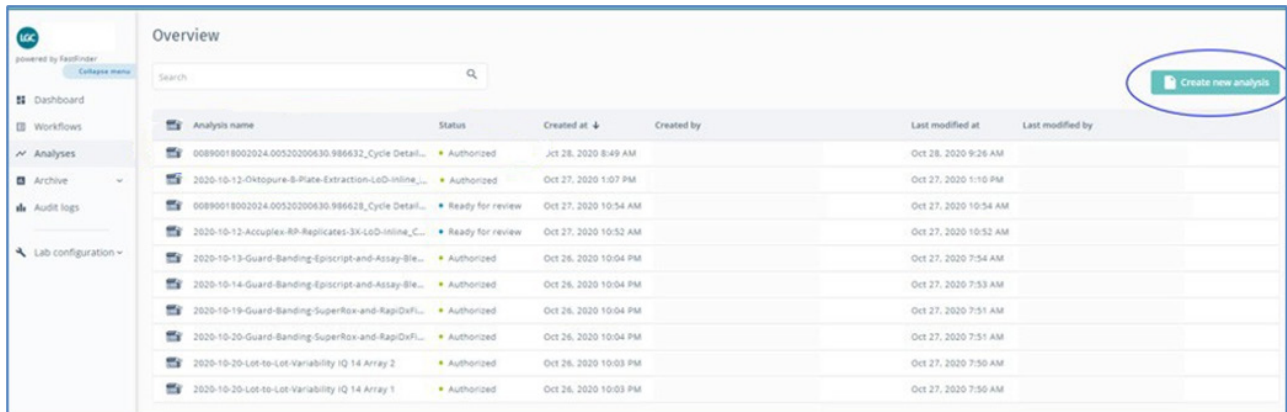
2. Navigate to the analyses section of the software



Analysis name	Status	Created at	Created by	Last modified at	Last modified by
00890018002024.00520200630.986632_Cycle Detail...	Authorized	Oct 28, 2020 8:49 AM		Oct 28, 2020 9:26 AM	
2020-10-16-Oktopure-8-Plate-Extraction-LoD-online...	Authorized	Oct 27, 2020 1:07 PM		Oct 27, 2020 1:10 PM	
00890018002024.00520200630.986628_Cycle Detail...	Ready for review	Oct 27, 2020 10:54 AM		Oct 27, 2020 10:54 AM	
2020-10-12-Accuplex-RP-Replicates-3X-LoD-online...	Ready for review	Oct 27, 2020 10:52 AM		Oct 27, 2020 10:52 AM	
2020-10-13-Guard-Banding-Episcrypt-and-Assay-Ble...	Authorized	Oct 26, 2020 10:04 PM		Oct 27, 2020 7:54 AM	
2020-10-14-Guard-Banding-Episcrypt-and-Assay-Ble...	Authorized	Oct 26, 2020 10:04 PM		Oct 27, 2020 7:53 AM	
2020-10-19-Guard-Banding-SuperBox-and-RapiDeFl...	Authorized	Oct 26, 2020 10:04 PM		Oct 27, 2020 7:51 AM	

Figure 6: Analyses view

3. Import results by selecting 'Create new analysis'



Analysis name	Status	Created at	Created by	Last modified at	Last modified by
00890018002024.00520200630.986632_Cycle Detail...	Authorized	Oct 28, 2020 8:49 AM		Oct 28, 2020 9:26 AM	
2020-10-12-Oktopure-8-Plate-Extraction-LoD-online...	Authorized	Oct 27, 2020 1:07 PM		Oct 27, 2020 1:10 PM	
00890018002024.00520200630.986628_Cycle Detail...	Ready for review	Oct 27, 2020 10:54 AM		Oct 27, 2020 10:54 AM	
2020-10-12-Accuplex-RP-Replicates-3X-LoD-online...	Ready for review	Oct 27, 2020 10:52 AM		Oct 27, 2020 10:52 AM	
2020-10-13-Guard-Banding-Episcrypt-and-Assay-Ble...	Authorized	Oct 26, 2020 10:04 PM		Oct 27, 2020 7:54 AM	
2020-10-14-Guard-Banding-Episcrypt-and-Assay-Ble...	Authorized	Oct 26, 2020 10:04 PM		Oct 27, 2020 7:53 AM	
2020-10-19-Guard-Banding-SuperBox-and-RapiDeFl...	Authorized	Oct 26, 2020 10:04 PM		Oct 27, 2020 7:51 AM	
2020-10-20-Guard-Banding-SuperBox-and-RapiDeFl...	Authorized	Oct 26, 2020 10:04 PM		Oct 27, 2020 7:51 AM	
2020-10-20-Lot-to-Lot-Variability IQ 14 Array 2	Authorized	Oct 26, 2020 10:03 PM		Oct 27, 2020 7:50 AM	
2020-10-20-Lot-to-Lot-Variability IQ 14 Array 1	Authorized	Oct 26, 2020 10:03 PM		Oct 27, 2020 7:50 AM	

Figure 7. Import results (create new analysis)

4. Navigate to the appropriate directory and select the pair of Araya readings files associated with a specified 384-well sample plate.

IMPORTANT: it is important to import only the two replicates reading files at one time and that the two files selected are associated with a single 384-well sample plate. To reduce this burden, the UgenTec FastFinder and Workflow solutions support automated uploads of paired data to their system from the customer's LIMS through automated scripts.

5. Adjust the sample and control well definitions in the PCR setup tab to define positive controls (O19-20, P19-20) negative controls (O21-22, P21-22) and no template controls (O23-24, P23-24) for Array 1 and Array 2. After well assignments have been populated, click Analyze or Re-analyze to execute the analysis.

IMPORTANT: ensure the Array 1 controls are used for Array 1 and Array 2 controls are used for Array 2.

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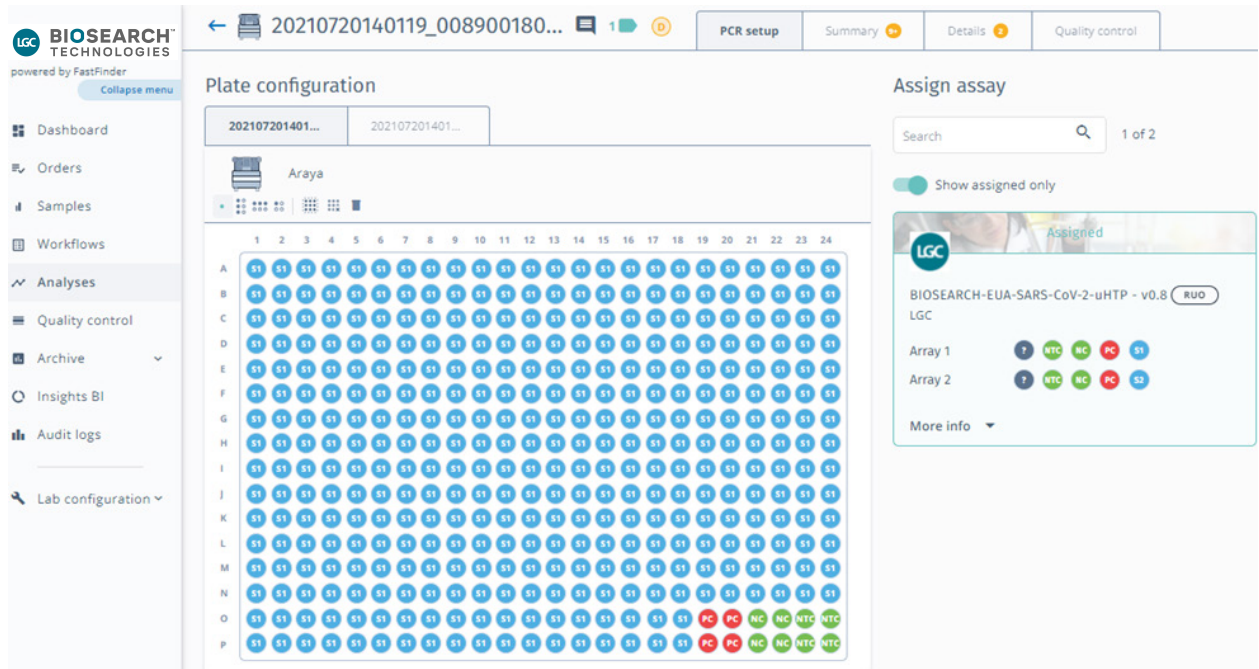


Figure 8. PCR setup view

6. A summary of the results can be reviewed from the 'Summary' tab.

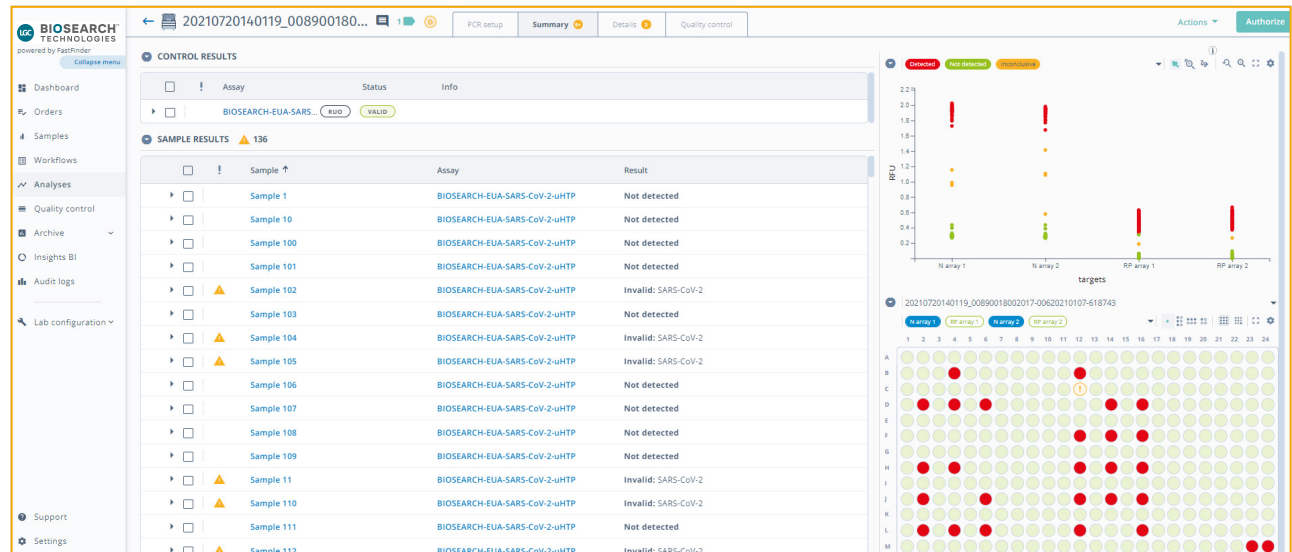


Figure 9. Summary of results view

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- After review and disposition of results click 'Authorize' and then export the results to CSV for incorporation into the LIMS.

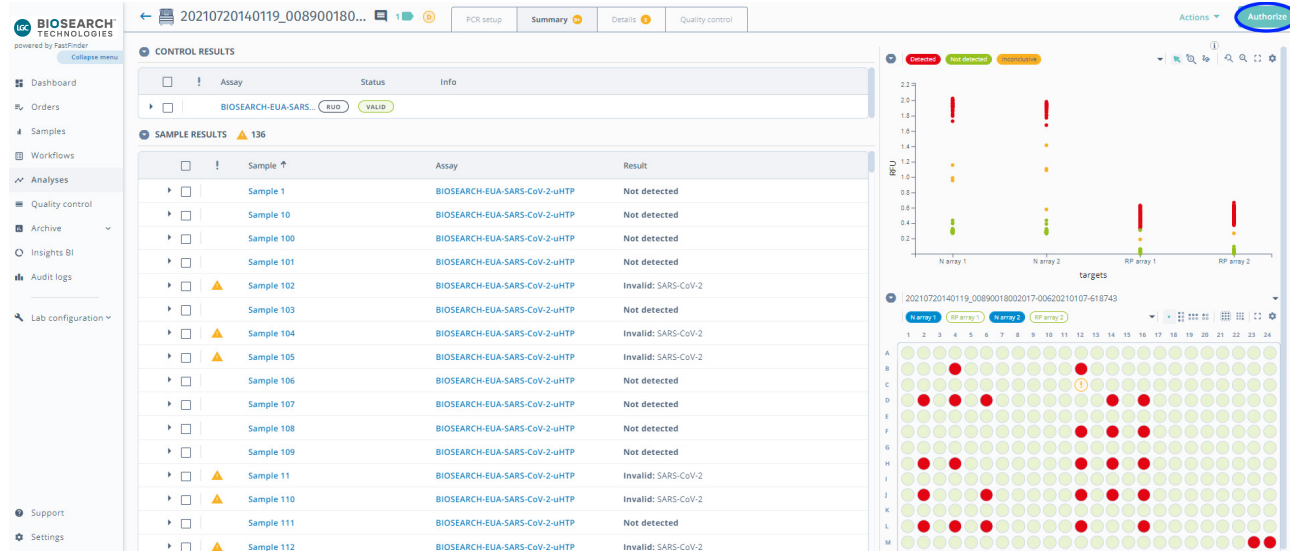


Figure 10. Authorize results

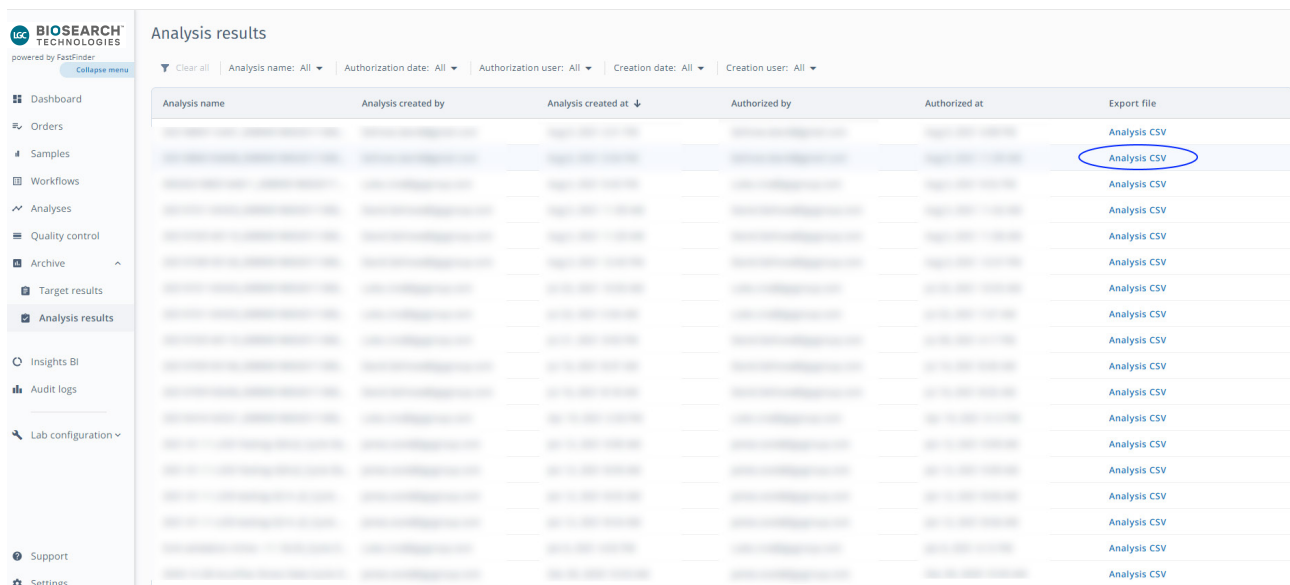


Figure 11. Export results in .csv format

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A	B	C	D	E	F	G	H	I	J
Sample ID	AssayResultTarget	AssayResultTargetCode	Outcome	IsInconclusive	IsInvalid	IsRetest	AssayResult	AssayResultNotifications	SampleType
Sample 1	SARS-CoV-2	SARS-CoV-2	Not detected	FALSE	FALSE	FALSE	Not detected		Regular
Sample 2	SARS-CoV-2	SARS-CoV-2	Not detected	FALSE	FALSE	FALSE	Not detected		Regular
Sample 3	SARS-CoV-2	SARS-CoV-2	Void	FALSE	TRUE	FALSE	Invalid	Warning: Extraction control(s) not detected.	Regular
Sample 4	SARS-CoV-2	SARS-CoV-2	Not detected	FALSE	FALSE	FALSE	Not detected		Regular
Sample 5	SARS-CoV-2	SARS-CoV-2	Not detected	FALSE	FALSE	FALSE	Not detected		Regular
Sample 6	SARS-CoV-2	SARS-CoV-2	Void	FALSE	TRUE	FALSE	Invalid	Warning: Extraction control(s) not detected.	Regular
Sample 7	SARS-CoV-2	SARS-CoV-2	Not detected	FALSE	FALSE	FALSE	Not detected		Regular
Sample 8	SARS-CoV-2	SARS-CoV-2	Void	FALSE	TRUE	FALSE	Invalid	Warning: Extraction control(s) not detected.	Regular
Sample 9	SARS-CoV-2	SARS-CoV-2	Void	FALSE	TRUE	FALSE	Invalid	Warning: Extraction control(s) not detected.	Regular
Sample 10	SARS-CoV-2	SARS-CoV-2	Not detected	FALSE	FALSE	FALSE	Not detected		Regular
Sample 11	SARS-CoV-2	SARS-CoV-2	Void	FALSE	TRUE	FALSE	Invalid	Warning: Extraction control(s) not detected.	Regular
Sample 12	SARS-CoV-2	SARS-CoV-2	Not detected	FALSE	FALSE	FALSE	Not detected		Regular
Sample 13	SARS-CoV-2	SARS-CoV-2	Not detected	FALSE	FALSE	FALSE	Not detected		Regular
Sample 14	SARS-CoV-2	SARS-CoV-2	Void	FALSE	TRUE	FALSE	Invalid	Warning: Extraction control(s) not detected.	Regular
Sample 15	SARS-CoV-2	SARS-CoV-2	Not detected	FALSE	FALSE	FALSE	Not detected		Regular
Sample 16	SARS-CoV-2	SARS-CoV-2	Void	FALSE	TRUE	FALSE	Invalid	Warning: Extraction control(s) not detected.	Regular
Sample 17	SARS-CoV-2	SARS-CoV-2	Void	FALSE	TRUE	FALSE	Invalid	Warning: Extraction control(s) not detected.	Regular
Sample 18	SARS-CoV-2	SARS-CoV-2	Not detected	FALSE	FALSE	FALSE	Not detected		Regular
Sample 19	SARS-CoV-2	SARS-CoV-2	Void	FALSE	TRUE	FALSE	Invalid	Warning: Extraction control(s) not detected.	Regular
Sample 20	SARS-CoV-2	SARS-CoV-2	Not detected	FALSE	FALSE	FALSE	Not detected		Regular
Sample 21	SARS-CoV-2	SARS-CoV-2	Not detected	FALSE	FALSE	FALSE	Not detected		Regular
Sample 22	SARS-CoV-2	SARS-CoV-2	Void	FALSE	TRUE	FALSE	Invalid	Warning: Extraction control(s) not detected.	Regular
Sample 23	SARS-CoV-2	SARS-CoV-2	Not detected	FALSE	FALSE	FALSE	Not detected		Regular
Sample 24	SARS-CoV-2	SARS-CoV-2	Void	FALSE	TRUE	FALSE	Invalid	Warning: Extraction control(s) not detected.	Regular
Sample 25	SARS-CoV-2	SARS-CoV-2	Not detected	FALSE	FALSE	FALSE	Not detected		Regular
Sample 26	SARS-CoV-2	SARS-CoV-2	Not detected	FALSE	FALSE	FALSE	Not detected		Regular
Sample 27	SARS-CoV-2	SARS-CoV-2	Not detected	FALSE	FALSE	FALSE	Not detected		Regular
Sample 28	SARS-CoV-2	SARS-CoV-2	Detected	FALSE	FALSE	FALSE	Detected		Regular
Sample 29	SARS-CoV-2	SARS-CoV-2	Not detected	FALSE	FALSE	FALSE	Not detected		Regular

Figure 12. Example of exported results file

- Final results should be correlated back to the original sample IDs using the LIMS or other methods to trace results from the results export to the original source tube.
 - Sample Tube ID
 - Tube rack + sample tube location
 - 384-well Source Plate + Tube Rack Offset
 - Array Tape Barcode + Source/Assay Plate Barcodes
 - Araya Readings Data/FastFinder Results Output
- Any results that have been flagged as 'Void', 'Invalid' or 'Retest' should be identified and retested as needed before publishing results back to the customer. A single retest from the source lysate tube is recommended if unexpected results are obtained. If this retest result is also 'Void' or 'Invalid', it is recommended to request an additional clinical sample from the patient.

6. Interpretation of results

Following acquisition of end-point fluorescence, categorization by the scoring thresholds and application of the decision logic rules table, as described, all laboratory and internal control performance should be examined prior to interpretation of patient results. If the laboratory or internal controls are not valid, the patient results cannot be interpreted, and specimens should be retested by reprocessing the 384-well sample plate, re-plating the prepared 96 lysate tube rack or by recollecting a new sample from the patient.

Determination of positive, negative or inconclusive SARS-CoV-2 patient results are made based on static thresholds for end-point signals that have been normalized using the ROX passive reference dye. In the scoring tables, the RP (RNase P) refers to the CAL Fluor™ Orange 560 (CFO) fluorescence marker which is included to interrogate the quality of the sample and the

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assay validity. The Ns in the table refer to the 6'FAM fluorescence marker interrogating the presence the N-gene target of the SARS-CoV-2 genome. Each patient sample is tested in duplicate (Array 1 and Array 2) during processing and both replicates are used to inform the result as 'Detected', 'Not detected', 'Invalid', 'Inconclusive' or 'Retest'. The results for each individual PCR reaction well are generated using the UgenTec FastFinder analysis software with the specific Assay Plugin using pre-defined static thresholds for the FAM, CFO and ROX signals.

6.1 Validity of positive and negative controls

To report results for an individual screening or patient sample, the positive, negative, no template controls must pass the controls decision logic. The Tables below present a tabular summary of control interpretation decision logic at the sample plate level and at the whole run level involving the 384-well array.

Positive control (PC): PC wells should yield a 'Detected' result for the N target (N1/N2) for 4 out of 4 wells on each array. For partial plates where fewer than 4 positive controls are present, all positive controls must yield a call of 'Detected' for the N target.

NOTE: AccuPlex Positive Control may contain trace amounts of RNase P and may show a 'Detected' results for this target and still be considered valid.

Scenario	N1/N2	RP	Ref	Control well result
Positive control (PC) – pass	+	N/A	+	Valid
PC negative or inconclusive	-, ?	N/A	+	Invalid
Missing or inconclusive reagent dispense	N/A	N/A	-, ?	Invalid
PC not present	N/A	N/A	N/A	Invalid

Table 10. Positive control decision logic (combined interpretation of both arrays)

Negative control (NC): NC wells should yield a 'Not detected' result for the N target and 'Detected' for RNase P internal control for 4 out of 4 wells on each array. For partial plates where less than 4 negative controls are present, all negative controls must yield calls of 'Not detected' for N targets and 'Detected' for RNase P targets.

Scenario	N1/N2	RP	Ref	Control well result
Negative control (NC) – pass	-	+	+	Valid
NC negative or inconclusive	-	-, ?	+	Invalid
NC positive or inconclusive for N target	+, ?	+	+	Invalid
Missing or inconclusive reagent dispense	N/A	N/A	-, ?	Invalid
NC not present	N/A	N/A	N/A	Invalid

Table 11. Negative control decision logic (combined interpretation of both arrays)

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No template control (NTC): The NTC should yield a 'Not detected' result for both the N and RP targets for 4 out of 4 wells on each array. For partial plates where fewer than 4 NTC wells are present, all NTC wells must yield a call of 'Not detected' for both the N and RP targets.

NOTE: For NTC wells, results that are discordant (inconclusive) for RP will be scored as valid and not count against the 4 out of 4 counts against the validity metric. However, any discordant (inconclusive) N target results will count against this validity metric. Additionally, any NTC sample well instances that show positive amplification for RP in both Array 1 and Array 2 replicates will be counted as an invalid instance.

Scenario	N1/N2	RP	Ref	Control well result
No template control (NTC) – pass	-	-/-, -/+ ¹ , +/- ¹	+	Valid
NTC duplicate RP positive	-	+/+ ²	+	Invalid
NTC positive or inconclusive for N target	+, ?	N/A	+	Invalid
Missing or inconclusive reagent dispense	N/A	N/A	- / ?	Invalid
No template control not present	N/A	N/A	N/A	Invalid

Table 12. No template control decision logic (combined interpretation of both arrays).

¹ For NTC results of the RP target only. Array 1 and Array 2 results are analyzed together to determine the validity of the NTC well. Only if the RP target is detected (invalid) in both PCR replicates for the same sample plate well location, will the NTC well location be considered invalid. If either one of the replicates the RP target is scored as negative, the control is considered valid.

² NOTE: For the purposes of NTC validity checks, discordant RP results between Array1 and Array2 are not considered invalid if at least one of the replicate wells scored as 'Negative' for the RP target. However, any discordant Array1 and Array2 results for the SARS-CoV-2 targets are considered invalid for the NTC wells.

In summary, Control results that meet the following conditions are considered valid. Therefore, clinical sample results may be interpreted as discussed in section 6.2 below.

Control type	Each full 384 array replicate (4 × 96 tube racks)	Each partial 384 array replicate (1-3 × 96 tube racks)
Positive Control (PC)	4 of 4 valid PC	100% valid PC
Negative Control (NC)	4 of 4 valid NC	100% valid NC
NTC	4 of 4 valid NTC	100% valid NTC

Table 13. Control summary logic

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6.2 Generation of clinical sample results

After the plate control well results are determined to be valid, the individual specimen results are then evaluated as follows:

Passive reference dye control (ROX): detection of passive reference dye that is included in the assay mixture is required for the reaction to be considered valid. Any result that returns a passive reference dye signal that is below the scoring threshold is considered 'Void' and 'Invalid' and must be retested.

Internal sample control (RP): detection of RP per scoring algorithms indicates that specimens of sufficient quantity and quality were present and tested. Detection of RP in a sample well is required to report a 'Not detected' i.e., negative SARS-CoV-2 result. Wells that return a 'negative' signal for N targets as well as 'negative' for RP will be scored as 'Void' and must be retested.

SARS-CoV-2 target (N): detection of N gene target per scoring algorithm indicates the presence of the SARS-CoV-2 virus. Detection of RP in the N positive wells is not required to score the sample as 'detected' as long as both replicate samples return the same result.

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Based on the result of control wells, passive reference dyes, and internal control (RP) and N targets of the individual specimen results, clinical results are generated as follows.

Scenario	Array 1			Array 2			Status	Warning/result	Action ¹
	N	RP	ROX	N	RP	ROX			
Pass ref. signal (ROX) invalid Array 1	N/A	N/A	-	N/A	N/A	N/A	Void-Invalid	Warn: pass ref. outside limits No reagent dispensed	Retest
Pass ref. signal (ROX) invalid Array 2	N/A	N/A	N/A	N/A	N/A	-	Void-Invalid	Warn: pass ref. outside limits No reagent dispensed	Retest
RP and N both not detected Array 1	-	-	+	N/A	N/A	+	Void-Inconclusive	Warn: RP extraction control not detected in reaction well	Retest
RP and N both not detected Array 2	N/A	N/A	+	-	-	+	Void-Inconclusive	Warn: RP extraction control not detected in reaction well	Retest
Results for N do not match: Rep01(-) and Rep02(+)	-	+/-	+	+	+/-	+	Void-Inconclusive	Warn: discordant array results for N	Retest
Results for N do not match: Rep01(+) and Rep 02(-)	+	+/-	+	-	+/-	+	Void-Inconclusive	Warn: discordant array results for N	Retest
N+RP	+	+	+	+	+	+	Detected	Result: SARS-CoV-2 detected	Report result
N Only	+	-	+	+	-	+	Detected	Result: SARS-CoV-2 detected	Report result
RP only	-	+	+	-	+	+	Not detected	Result: SARS-CoV-2 not detected	Report result

Table 14. Clinical sample decision logic

¹For any results that are flagged for retest, specimens from the failing quadrant should be retested in this sequence by either reprocessing the 384-well sample plate, by re-plating the prepared 96 lysate tube rack or by recollecting a new sample from the patient.

7. Performance evaluation summary

7.1 Analytical sensitivity (limit of detection)

Limit of detection (LoD) studies determine the lowest detectable concentration of SARS-CoV-2 at which greater than or equal to 95% of all (true positive) replicates test positive. The LoD of the

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BT-SCV2-UHTP-EP test was determined by Biosearch Technologies utilizing all required components of the test system from sample preparation to detection.

Contrived samples using a purified, chemically modified viral material (strain SARS-CoV-2, USA-WA1/2020, ZeptoMetrix® Corporation, catalog number [NATSARS\(COV2\)-ST](#), with starting concentration of 1.0×10^6 copies/mL as determined by RT-PCR) were created by dilution into negative clinical pooled matrix. The negative clinical matrix is NP swab material obtained from asymptomatic, non-exposed clinical lab staff volunteers tested and found negative for SARS-CoV-2; and then pooled for use in LoD studies.

The ZeptoMetrix modified SARS-CoV-2 virus was first qualified for use in the LoD studies by confirming the manufacturer's label value for viral copies per mL by determining viral RNA concentration with digital PCR. The manufacturer's label value was then used in setting up the contrived samples for preliminary and final LOD determinations.

7.1.1 Preliminary LoD

To determine the preliminary LoD, a series of contrived samples (see above) were spotted and dried onto reference swabs at concentrations of 4.0×10^3 , 2.0×10^3 , 1.0×10^3 , 7.5×10^2 , 5.0×10^2 , 2.5×10^2 , 1.25×10^2 , and 0 copies/swab and allowed to dry for at least 20 minutes. These swabs were then processed and scored in accordance with the IFU as normal specimen.

The lowest concentration which gave positive results 95% of the time during initial testing was defined as the preliminary LoD, which was found to be 250 copies/swab. The results are summarized below.

Sample	Concentration (copies/swab)	Results	Positive rate N Target (95%-CI)
ZeptoMetrix NATSARS (COV2)-ST	4000	20/20	100% (89.1-100%)
	2000	20/20	100% (89.1-100%)
	1000	20/20	100% (89.1-100%)
	750	20/20	100% (89.1-100%)
	500	20/20	100% (89.1-100%)
	250	19/20	95% (75.1-99.9%)
	125	18/20	90.0% (71.7%-98.2%)
	62.5	12/20	60.0% (39.4%-78.3%)
	31.25	10/20	50% (30.2%-69.8%)
	0 (neg matrix)	0/20	0% (0-13.9%)

Table 15. Preliminary LoD Testing

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7.1.2 Confirmatory LoD

The final LoD determination for the BT-SCV2-UHTP-EP test was confirmed to be 250 copies/swab. A summary of the results data is presented in the table below.

Sample	Concentration (copies/swab)	Results	Positive rate N Target (95%-CI)
ZeptoMetrix NATSARS (COV2)-ST	750	20/20	100% (89.1-100%)
	500	20/20	100% (89.1-100%)
	250	20/20	100% (89.1-100%)
	125 ¹	17/20	80% (56.3-94.3%)
	0 (neg matrix)	0/20	0% (0-13.9%)

Table 16. Biosearch Technologies limit of detection (LoD) determination

¹NOTE: No results at this concentration scored as “Not detected” per the decision logic. All results were “Inconclusive” due to failure of a R01 or R02 to amplify in one array copy that then succeeded in amplification in its replicate.

7.2 Inclusivity and cross-reactivity

The Biosearch Technologies SARS-CoV-2 ultra-high-throughput End-Point RT-PCR Test utilizes the same primers and probes sequences for the N1 and N2 regions of the nucleocapsid gene and for the RNase P target region as the [CDC 2019-Novel Coronavirus \(2019-nCoV\) Real-Time RT-PCR Diagnostic Panel](#). *In silico* analysis of primer and probe inclusivity and specificity was performed by CDC. The CDC has granted a right of reference to the performance data contained in the CDC's EUA request to any entity seeking an FDA EUA for a COVID-19 diagnostic device. Independent *in silico* inclusivity analysis performed in April 2022 predicted no significant impact from known SARS-CoV-2 mutations and/or variants of concern on the inclusivity of the primers and probes used in the BT-SCV2-UHTP-EP test.

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7.3 Interfering substances

Potential interfering substances from nasal specimens were tested for their ability to generate false negative results or false positive results using negative pooled samples that were spiked with the ZepetoMetrix SARS-CoV-2 control material (NATSARS(COV2)-ST) at $2.5 \times \text{LoD}$. Testing was performed with 3 replicates per substance. With one exception all replicates gave the expected results. For whole blood containing specimens, only 2 out of 3 replicates gave the expected result. A follow-on evaluation was performed with 6 separate blood spiked samples each tested in triplicate and all 18 replicates were detected for a combined total of 20/21 positive results.

Potentially interfering substance	Active ingredient concentration	Testing concentration	Positive specimen (2.5x LoD) concordance	Negative specimen concordance
Zicam Nasal Spray - Cold Remedy	Galphimia glauca 4x; Luffa operculata 4x; Sabadilla 4x	5% v/v	3 of 3 (100%)	3 of 3 (100%)
Zicam Nasal Spray - Allergy Relief	Galphimia glauca; Histaminum hydrochloricum; Luffa operculata; Sulphur	5% v/v	3 of 3 (100%)	3 of 3 (100%)
Zicam Nasal Spray - Congestion Relief	Oxymetazoline and Hydrochloride 0.05%	15% v/v	3 of 3 (100%)	3 of 3 (100%)
Neo-Synephrine	0.5% Phenylephrine hydrochloride	5% v/v	3 of 3 (100%)	3 of 3 (100%)
CVS Nasal Spray	0.05% Oxymetazoline hydrochloride	15% v/v	3 of 3 (100%)	3 of 3 (100%)
CVS No Drip Nasal Mist	0.05% Oxymetazoline hydrochloride	15% v/v	3 of 3 (100%)	3 of 3 (100%)
CVS Sinus Relief Nasal Mist	Sodium chloride 2.65%	15% v/v	3 of 3 (100%)	3 of 3 (100%)
CVS Fluticasone Propionate Nasal Spray, USP	50 mcg Fluticasone Propionate (Glucocorticoid)	5% v/v	3 of 3 (100%)	3 of 3 (100%)
CVS Nasal Allergy Spray	55 mcg Triamcinolone	5% v/v	3 of 3 (100%)	3 of 3 (100%)
CVS Sterile Saline Nasal Mist	3.0% sodium chloride	15% v/v	3 of 3 (100%)	3 of 3 (100%)
CVS Saline Nasal Spray	0.65% sodium chloride	15% v/v	3 of 3 (100%)	3 of 3 (100%)
Marie Originals, Natural nasal spray The Bioactive combo for Sinus congestion	Natural nasal spray: Melilotus officinalis 30C	5% v/v	3 of 3 (100%)	3 of 3 (100%)
Source Naturals Wellness Zinc Throat Spray	Zinc Glycinate 20 mg/1.77 mL	5% v/v	3 of 3 (100%)	3 of 3 (100%)

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EMD Millipore Mucin Bovine Submaxillary Gland, Lot 3716976	Mucin Bovine Submaxillary Gland,	2.5 mg/mL	3 of 3 (100%)	3 of 3 (100%)
Roche hgDNA, Ref: 1169112001 lot: 54105420	Human Genomic DNA	10 ng/uL	3 of 3 (100%)	3 of 3 (100%)
Mucinex Sinus-Max Nasal Spray	0.05% Oxymetazoline hydrochloride	15% v/v	3 of 3 (100%)	3 of 3 (100%)
Blood, human	Blood (EDTA)	5% v/v	20 of 21 (95.2%) ¹	3 of 3 (100%)
Flonase Allergy Relief: Fluticasone Propionate Nasal Spray	50 mcg Fluticasone Propionate (Glucocorticoid)	5% v/v	3 of 3 (100%)	3 of 3 (100%)
Nasacort Allergy Nasal Spray	55 mcg Triamcinolone acetonide	5% v/v	3 of 3 (100%)	3 of 3 (100%)
No Additive	No Additive	N/A	N/A	3 of 3 (100%)

Table 17. Interfering substances results summary.

¹During initial screening, one out of three replicates was inconclusive. Upon retest, it reported as negative for a final initial total of 2 of 3 (66.6%). A further evaluation of 6 samples tested in triplicates tests generated 18 additional specimen results (all positive) as expected for a grand total of 20 of 21 (95.2%). The 95% Confidence interval of this material was then 76.2%-99.9%. For other samples with 3/3 (100%) the 95% confidence interval is 36.8%-100%

7.4 Clinical evaluation

The BT-SCV2-UHTP-EP test clinical evaluation data demonstrate its capability of detecting SARS-CoV-2 in individuals suspected by their HCP and in asymptomatic individuals with or without reasons to suspect COVID-19 infection. Anterior nares nasal swab (ANS) specimens were prospectively obtained by HCP at a major hospital and clinic network located in the Northeastern U.S. The BT-SCV2-UHTP-EP test results were assessed for concordance with a high sensitivity molecular RT-PCR test for SARS-CoV-2 that had been previously authorized for emergency use.

All specimens (both cohorts, suspected and asymptomatic individuals) of the clinical concordance studies were collected by healthcare professionals as two paired anterior nares swabs. One swab was collected in the SteriPack XpressCollect tube (catalog# 60600) for testing with the investigational test (BT-SCV2-UHTP-EP test), and one was collected as a conventional nasal spun polyester swab in transport media for testing with the comparator. Paired sampling was performed with a standard cross-over collection procedure: #1- Left nares, #2- Right; #2- Left and #1- Right in order to maintain collection symmetry and to reduce the effect of sample bias. Paired specimens were transported to the laboratory that day and stored dry in their collection containers at 4 °C until ready to use. Each pair was run on the same day on their respective platform.

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7.4.1 Suspected individuals

A total of 52 clinical anterior nasal swab specimens collected from symptomatic in/out-patient individuals who had previously tested positive on the healthcare organization's standard-of-care (SoC) test (nasopharyngeal specimens, NPS), and 30 paired nasal swab specimens from at risk individuals (hospital-based HCP) who had previously tested negative on the SoC test (NPS) were consented and resampled within at most 4 days. Paired ANS were collected consecutively from all consenting individuals.

Results of the comparison between the investigational and the comparator device results on samples from were as follows:

9 (17.3%) of the positive samples in this cohort were low positive samples within 3 Cts of the comparators mean Ct at LoD of both targets (Orf 1a/b and E).

Biosearch Technologies SARS-CoV-2 End-Point RT-PCR Test	FDA-authorized comparator assay		
	Positive	Negative	Total
Positive	52	-	52
Inconclusive	-	1 ¹	1 ¹
Negative	-	29	29
Total	52	30	82
PPA	100.0% (52/52)		95% CI: 93.1%-100%
NPA ¹	96.7% (29/29 ¹)		95% CI: 88.3% - 100%

Table 18. Performance of the test with clinical specimens collected from symptomatic subjects

¹Inconclusive results with one positive and one negative target are considered neither concordant nor discordant and are excluded from the NPA calculation.

7.4.2 Individuals without reasons to suspect Covid-19

Individuals without reasons to suspect Covid-19 were recruited from a volunteer testing event at the healthcare organization and consisted of asymptomatic hospital employees who did not provide direct patient care and were therefore not at risk. Subjects were interviewed to confirm the absence of Covid-19 relevant symptoms and/or known exposure to an infected person in the prior two weeks and samples were collected consecutively as subjects consented. HCPs (defined by CDC as 'individuals at risk'), providing direct care to potentially positive patients, were not included. Due to low prevalence, this cohort did not include any positive samples. Therefore, positive samples of the asymptomatic screening cohort were enriched with consecutive positive samples collected from asymptomatic individuals without risk factors or exposure who were identified as positive based on a previous test result. These individuals were re-sampled within 4 days.

Individuals without symptoms or exposure or other reasons to suspect COVID-19 were tested with an EUA authorized highly sensitive comparator test and a Biosearch Technologies test.

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8 (30.0%) of the positive samples in this cohort were low positive samples within 3 Cts of the comparators mean Ct at LoD of both targets (Orf 1a/b and E); this includes the one false negative sample generated by the investigational device.

Biosearch Technologies SARS-CoV-2 End-Point RT-PCR Test	FDA-authorized comparator assay		
	Positive	Negative	Total
Positive	26	1	27
Inconclusive	0	0	0
Negative	1 ¹	147	148
Total	27	148	175
PPA ¹	96.3% (26/27 ¹) 95% CI: 81.7% - 99.3%		
NPA	99.3% (147/148) 95% CI: 96.3% - 99.9%		

Table 19. Performance of the test with clinical specimens collected from a screening population

¹Comparator target 1 Ct was 37.43 vs 38.0 cutoff; target 2 Ct was negative. Per IFU, the result of this sample is 'positive'.

8. Limitations

- The performance of this test was established based on the evaluation of a limited number of clinical specimens. Clinical performance has not been established with all circulating variants but is anticipated to be reflective of the prevalent variants in circulation at the time and location of the clinical evaluation. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of SARS-CoV-2 and their prevalence, which change over time
- As with any molecular test, mutations within the target regions of the BT-SCV2-UHTP-EP test could affect primer and/or probe binding resulting in failure to detect the presence of virus, or the virus being detected less predictably.
- The performance of this test was validated using the procedures provided in this package insert only. Modifications to these procedures may alter the performance of the test.
- The test cannot rule out diseases caused by other bacterial or viral pathogens. If the SARS-CoV-2 virus mutates in the RT-PCR amplicon region being tested, COVID-19 may not be detected or may be detected less predictably.
- 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.
- Results from the D BT-SCV2-UHTP-EP test should be correlated with the clinical history, epidemiological data, and other data available to the clinician evaluating the patient.
- The performance of the test has not been established for monitoring treatment of COVID-19 infection.
- The performance of the test has not been established for screening of blood or blood products for the presence of COVID-19.

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8.1 Conditions of authorization for the laboratory

The molecular test Letter of Authorization, along with the authorized fact sheet for HCPs, the authorized fact sheet for patients and authorized labeling are available on the U.S.FDA website: <https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/in-vitro-diagnostics-euas>.

However, to assist clinical laboratories using the test, the relevant conditions of authorization are listed below:

- Authorized laboratories¹ using this product must include with test result reports, all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these fact sheets may be used, which may include mass media.
- Authorized laboratories using this product must use the 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 use your product are not permitted.
- Authorized laboratories when testing anterior nasal swab specimens collected using a collection kit that conforms to the HealthPulse@home Fusion EUA authorized for use with this product must follow any specimen accessioning protocol provided with and/or developed for the collection kit when accepting specimens for testing.
- Authorized laboratories that receive this product must notify the relevant public health authorities of their intent to run this product prior to initiating testing.
- Authorized laboratories using this product must have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.
- Authorized laboratories must collect information on the performance of this product and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and to Biosearch Technologies (via email: genomics.americas@lgcgroup.com) any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of this product of which they become aware.
- All laboratory personnel using this product must be appropriately trained in RT-PCR techniques and use appropriate laboratory and personal protective equipment when handling this kit, and use this product in accordance with the authorized labelling.
- Authorized distributors and authorized laboratories using this product must ensure that any records associated with this EUA are maintained until otherwise notified by FDA. Such records must be made available to FDA for inspection upon request.

¹The letter of authorization refers 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.”

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