Instructions for use

Biosearch Technologies SARS-CoV-2 Real-Time and End-Point RT-PCR Test

For Emergency Use Authorization. | For Rx Only. | For *In Vitro* Diagnostic Use. |
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BIOSEARCH™ TECHNOLOGIES
GENOMIC ANALYSIS BY LGC

Instructions for use

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Intended use

The Biosearch Technologies SAR-CoV-2 Real-Time and End-Point RT-PCR Test is a reverse transcriptase (RT)-polymerase chain reaction (PCR) test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in anterior or mid-turbinate nasal swabs, nasopharyngeal swabs, oropharyngeal swabs, and nasopharyngeal washes/aspirates or nasal aspirates collected from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a 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 respiratory 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 test results to the appropriate public health authorities.

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

The Biosearch Technologies SARS-CoV-2 Real-Time and End-Point RT-PCR Test is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the techniques of real-time and/or end-point PCR and *in vitro* diagnostic procedures. The Biosearch Technologies SARS-CoV-2 Real-Time and End-Point RT-PCR Test is only for use under the Food and Drug Administration's Emergency Use Authorization (EUA).

Summary and explanation of test

Special instrument requirements

The Biosearch Technologies SARS-CoV-2 Real-Time and End-Point RT-PCR test is to be used with the Biosearch Technologies oKtopure™ , IntelliQube™ and Hydrocycler²™ instruments. These instruments will be subject to an on-site qualification process performed by LGC technicians after installation to verify critical instrument parameters, prior to reporting of patient results.

The Biosearch Technologies SARS-CoV-2 Real-Time and End-Point RT-PCR Test is a molecular, reverse transcription polymerase chain reaction (RT-PCR), *in vitro* diagnostic test that is based on the widely used nucleic acid amplification technology. The Biosearch Technologies SARS-CoV-2 Real-Time and 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 in anterior or mid-turbinate nasal swabs, nasopharyngeal swabs, oropharyngeal swabs and nasopharyngeal washes/aspirates or nasal aspirates.

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Principles of the procedure

Nucleic acids are isolated and purified from upper respiratory specimens using the Biosearch Technologies oKtopure in combination with sbeadex™ viral RNA purification kits. The sbeadex viral RNA purification chemistry uses a magnetic bead-based approach to purify the viral RNA from 200 µL of swab specimen with a final elution volume of 50 µL. Using a one-step RT-PCR approach, the viral RNA template is converted to cDNA and subsequently amplified in either the IntelliQube PCR System (real-time workflow) or in the Hydrocycler² (end-point workflow) following dispense of 0.8 µL of assay mix containing RapiDxFire™ qPCR Master Mix, EpiScript™ RNase H- Reverse Transcriptase, SuperROX™, and BHQ™ Probes and primers. In the process, the probe anneals to a specific target sequence located between the forward and reverse primers. During the extension phase of the PCR cycle, the 5' nuclease activity of Taq polymerase degrades the probe, causing the reporter dye to separate from the quencher dye,generating a fluorescent signal. With each cycle, additional reporter dye molecules are cleaved from their respective probes, increasing the fluorescence intensity. Fluorescence intensity is monitored either at each PCR cycle or at end-point by the IntelliQube PCR System.

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Components manufactured by Biosearch Technologies and specified in the Test

Material and reagent consumables:

Component	Source	Storage condition	Description
oKtopure tips	KBS-0010-003	Ambient	96-tips per tray, 200 µL, box of 10 trays
oKtopure filters	KBS-09-MS027	Ambient	oKtopure mandrel filters, 100 filters/pack
Waste collection plate	KBS-7001-044	Ambient	96-well deep-well plate, 2 mL well volume, 50 plates per box
	KBS-7001-031	Ambient	96-well deep-well plate, 2 mL well volume, 50 plates per box
Lysis and MagMix plate	KBS-7001-130	Ambient	96-well deep-well plate, 1.2 mL well volume, 50 plates per box
Elution and PCR sample plate	KBS-7001-139	Ambient	384-well plate, 120 µl well volume, 100 plates per box
	KBS-7001-132	Ambient	384-well plate, 100 µl well volume, 50 plates per box
	KBS-7001-133	Ambient	384-well plate, 200 µl well volume, 50 plates per box
Lysis plate seal	KBS-0606-002	Ambient	Adhesive PCR film, 135 mm × 80 mm, 100 sheets per box
Elution and assay plate seal	KBS-0600-002	Ambient	Adhesive PCR foil seal, 135 mm × 80 mm, 100 sheets per box
RNA purification kit	NAP40-028-04	sbeadex Suspension: 2-8 °C All Others: Ambient	sbeadex viral RNA kit, 200 µl sample input, 10,000 purifications
Protease solution	NAP30-002-02	2-8 °C	Protease solution, 20 mg/mL, 10 mL
	NAP30-002-03	2-8 °C	Protease solution, 20 mg/mL, 100 mL
Positive control	0505-0211	2-8 °C	AccuPlex™ SARS-CoV2 full genome with RNaseP reference material, 6 × 1.76 mL tubes per box
IntelliQube sample pipette tips	AX843799	Ambient	384-tips per tray, 10 µL
	AX840999	Ambient	384-tips per tray, 40 µL
Array Tape™	AXIT384-13WP050CC	Ambient	IntelliQube Array Tape, Clean Consumable (DNAse-, RNAse-, Pyrogen-free), 384-well, 2 reels, 50 arrays per reel
	AXIT768-13WP050CC	Ambient	IntelliQube Array Tape, Clean Consumable (DNAse-, RNAse-, Pyrogen-free), 768-well, 2 reels, 50 arrays per reel
Cover seal for Array Tape	AX8591CVRTCC	Ambient	IntelliQube Cover Seal, Clean Consumable (DNAse-, RNAse-, Pyrogen-free) Pressure Sensitive, 30 meter roll with 350 seals

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PCR assay – oligo blends	N1RNP-1010	-20 °C Storage 2-8 °C Daily Use	2019 nCoV N1/RnP Blend -100X Stock, 1,010 µL
	N2RNP-1010	-20 °C Storage 2-8 °C Daily Use	2019 nCoV N2/RnP Blend -100X Stock, 1,010 µL
5X Master Mix	30050-1	-20 °C Storage 2-8 °C Daily Use	RapiDxFire qPCR 5X Master Mix GF, 1 mL
	30050-2	-20 °C Storage 2-8 °C Daily Use	RapiDxFire qPCR 5X Master Mix GF, 10 mL
	30050-100ML	-20 °C Storage 2-8 °C Daily Use	RapiDxFire qPCR 5X Master Mix GF, 100 mL
Reverse Transcriptase	ERT12925K-ENZ	-20 °C Storage 2-8 °C Daily Use	Episcript RNase H- Reverse Transcriptase, 200 U/µL,0.125 mL
	ERT12925K-1.25ML	-20 °C Storage 2-8 °C Daily Use	Episcript RNase H- Reverse Transcriptase, 200 U/µL, 1.25 mL
	ERT12925K-12ML	-20 °C Storage 2-8 °C Daily Use	Episcript RNase H- Reverse Transcriptase, 200 U/µL, 12 mL
Passive reference dye	SR-1000-1	2 - 8 °C	SuperROX, 15 µM, 1 mL
	SR-1000-10	2 - 8 °C	SuperROX, 15 µM, 10 mL

Table 1. Biosearch Technologies SARS-CoV-2 Real-Time and End-Point RT-PCR Test – Consumables included with the Test.

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Equipment, software and materials:

Component	Source	Description
Automation for RNA purification	KBS-0009-001	oKtopure – high-throughput nucleic acid purification robot
RNA purification reagent reservoir	KBS-09-050	Maxi buffer reservoir
	KBS-0009-006	Standard buffer reservoir
oKtopure tip blocks	KBS-09-127	192-hole tip blocks for the oKtopure
Handheld barcode scanner	KBS-0025-003	Handheld, USB linear barcode scanner
	Various vendors	
PCR automation	AXDS-0002-100	IntelliQube integrated inline liquid handling, assay processing and analytical system
Bulk thermal cycling (end-point only)	KBS-0028-001	Hydrocycler[2]
Heat source for sample lysis	KBS-0099-101	75 L oven
	KBS-0099-102	40 L oven
	Various vendors	Forced convection laboratory oven capable of reaching 95 ºC
Real-Time Data Analysis Software	UgenTec FastFinder Analysis V4.0.1 and Assay Plugin "LGC-EUA-SARS-CoV-2-RT v1.1"	UgenTec Software FastFinder Analysis 4.0.1 with Real Time Assay Plugin 1.1
End-Point Data Analysis Software	UgenTec FastFinder Analysis V4.0.1 and Assay Plugin "LGC- EUA-SARS-CoV-2-EP v1.1"	UgenTec Software FastFinder Analysis 4.0.1 with End-Point Assay Plugin 1.1
Sanosil Super 25	Sanosil	Highly concentrated water disinfectant
HaloMist	Halosil International	Water disinfectant
Sanostrips 200	Sanosil	Measuring Strips MS200 for determination of H_2O_2 concentration

Table 2. Biosearch Technologies SARS-CoV-2 Real-Time and End-Point RT-PCR Test – equipment, software and materials specified in the Test.

Components manufactured by Biosearch Technologies and other suppliers and specified in the Test

Component	Source	Description
PCR reagent diluent and NTC	Various vendors	Molecular or PCR grade water
Assay tubes	ThermoFisher – 4170	0.75 mL blank matrix tubes
Assay tube rack	ThermoFisher – 4896	Empty latch rack for 0.75 mL matrix tubes

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Assay tube seal	ThermoFisher – 4463	SepraSeal caps for assay tubes – recommend purchase in two colors
Assay plate (alternative to ThermoFisher's 4170, 4896, 4463)	KBS-7001-131	96-well V-bottom plate, 0.8 mL well volume
	KBS-7001-231	96-well storage plates, 0.8 mL (case 50 plates)
	Greiner Bio-one – 786201	Masterblock®, 96-well V-bottom plate, 0.7 mL well volume
Adhesive barcode labels	KBS-0750-100	Adhesive plate barcodes (roll of 5000)
	Various vendors	Adhesive barcodes
Sterilizing basin	Various vendors	~20 L volume, polypropylene sterilizing basins for oKtopure tip blocks
5% Sodium hypochlorite	Various vendors	Sodium hypochlorite for tip and tip block decontamination
Sodium hypochlorite concentration test strips (both required)	LaMotte – 3002	Insta-Test Analytic High Range Chlorine Dioxide Test Strips
	Deardoff Fitzsimmons – 77085	Active High-Level Chlorine Strips
Pipettors	Various vendors	Single and multichannel adjustable pipettors (2 µL to 1,000 µL)
Pipette tips	Various vendors	Filtered, disposable pipette tips
PCR reagent preparation tubes	Various vendors	1.5 mL centrifuge tubes, 15 mL and 50 mL conical tubes
Tube racks	Various vendors	Racks for 1.5 mL, 15 mL and 50 mL tubes
Cold storage	Various vendors	Laboratory fridges and freezers (4 ºC, -20 ºC)
Ice or cold block	Various vendors	
Flammable cabinet	Various vendors	
Compressor	Various vendors	
RO water supply	Various vendors	
Disposable, powder free gloves	Various vendors	
Centrifuge	Various vendors	Centrifuge with rotors compatible with standard and deep-well plates
Vortex	Various vendors	Vortex – including plate adapter
Ethanol	Various vendors	70-80% ethanol, molecular biology grade or equivalent
Isopropanol	Various vendors	70% isopropanol, molecular biology grade or equivalent
Microcentrifuge	Various vendors	

Table 3. Biosearch Technologies SARS-CoV-2 Real-Time and End-Point RT-PCR Test – other equipment and materials required.

Control material(s) to be used with Biosearch Technologies SARS-CoV-2 Real-Time and End-Point RT-PCR Test

Component	Source: ref #	Description	Purpose	Frequency
Positive Control	0505–0211	AccuPlex SARS-CoV-2 RNaseP and viral template at 15,000 copies/mL of N1/N2 Target and 30,000 copies/mL of RP target, with polyA carrier RNA.	Full Process Positive Control (PC) material	One per 96-well source plate
Negative Control	Major laboratory suppliers	Molecular or PCR grade water.	Negative Template Control (NTC)	One per 96-well source plate
Internal Control	N/A	The human RNase P gene should be present in a properly collected and extracted sample.	Extraction control for each individual patient sample	Analyzed in every patient sample well

Table 4. Biosearch Technologies SARS-CoV-2 Real-Time and End-Point RT-PCR Test – control materials.

Controls that are provided with the test include:

a. A "no template" (negative) control is needed to ensure there are no unexpected amplification events that may indicate a failure in the assay, a contamination event in the lab, or other mishandling of samples as part of the extraction, purification and amplification process. This control is included as an input into one well of each 96-well source plate that contains the raw clinical samples prior to lysis or purification and should be identified in the plate map file as "NTC."

b. The positive template control material is a SARS-CoV-2 Full Genome and human RNase P with spiked carrier RNA. The control material contains the whole genome of the 2019-Coronavirus strain (SARS-CoV-2) corresponding to GenBank sequence NC_045512.2., using LGC SeraCare Life Sciences proprietary AccuPlex Technology. The concentration of this control has been set to a viral load of 15,000 ± 2,000 cp/mL SARS-CoV-2; and 30,000 ± 4,000 cp/mL RNase P verified through digital PCR with an additional inclusion of carrier RNA at a concentration of ~5 µg/mL. This concentration represents a target concentration for N1, N2, and RP of between 2X and 3X the LoD of this method.

   This control is needed to provide assurance that the extraction and purification process was executed as expected and generated an acceptable purified sample concentration as well as a verification that the RT-PCR process is functioning as expected. This control is included as an input into one well of each 96-well source plate that contains the raw clinical samples prior to lysis or purification and should be identified in the plate map file as "PC."

   Human RNase P serves as an internal/extraction control and is detected using the 2019-nCoV N1/RnP and 2019-nCoV N2/RnP assay blends. Detection of this target indicates that human nucleic acid is present and implies that human biological material was collected and successfully extracted and amplified. It does not necessarily indicate that the specimen is of appropriate

quality to enable detection of SARS-CoV-2. This RNase P control can be used to flag samples that have a low concentration of purified nucleic acid for re-extraction and retesting as needed.

Warnings and precautions

As with any test procedure, good laboratory practice is essential to the proper performance of this assay. Due to the high sensitivity of this test, care should be taken to keep reagents and amplification mixtures free of contamination. The Biosearch Technologies SARS-CoV-2 Real-Time and End-Point RT-PCR Test workflow should be performed by qualified and trained staff to avoid the risk of erroneous results.

- For *in vitro* diagnostic use only.
- For prescription use only.
- For Emergency Use Authorization (EUA) Only.
- The Biosearch Technologies SARS-CoV-2 Real-Time and End-Point RT-PCR Test has not been FDA cleared or approved; the test has been authorized by FDA under an Emergency Use Authorization (EUA) for use by laboratories certified under the Clinical Laboratory Improvement Amendments (CLIA) of 1988, 42 U.S.C. §263a, that meet requirements to perform high complexity tests.
- This test has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.
- 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 authorization is terminated or revoked sooner.
- Specimens should always be treated as if infectious and/or biohazardous in accordance with safe laboratory procedures. Refer to Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with SARS-CoV-2.
- Follow necessary precautions when handling specimens. Use personal protective equipment (PPE) consistent with current guidelines for the handling of potentially infectious samples. Refer to Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition – CDC.
- Do not eat, drink, smoke, apply cosmetics or handle contact lenses in areas where reagents and human specimens are handled.
- Modifications to assay reagents, assay protocol or instrumentation are in violation of the product Emergency Use Authorization.

- Do not use the kit after the indicated expiry date. Please see Table 1, material and reagent consumables specified in the Test, for storage conditions.
- Avoid freeze/thaw of the PCR reagents.
- Dispose of waste in compliance with local, state, and federal regulations.
- Safety Data Sheets (SDS) are available upon request.
- Positive results are indicative of the presence of SARS-CoV-2 RNA.
- Handle all samples and controls as if they are capable of transmitting infectious agents.

Sample collection, handling and storage

Proper specimen collection, storage and transport are critical to the performance of this test. Inadequate specimen collection, improper specimen handling and/or transport may yield a false result. Sample handling and storage should be consistent with CDC guidelines. The Biosearch Technologies SARS-CoV-2 Real-Time and End-Point RT-PCR Test has been validated for use with anterior and mid-turbinate nasal swabs, nasopharyngeal swabs, oropharyngeal swabs and nasopharyngeal washes/aspirates or nasal aspirates. The collected samples should be handled and stored according to the CDC's recommendations (2-8°C for 72 hours, ≤-70°C for >72 hours).

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).

Instructions for use

Minimize the risk of contamination

- Use appropriate biosafety environmental containment for sample and reagent handling.
- Always use caution when transferring specimens from primary containers to secondary tube(s).
- Precautions must be taken to prevent cross contamination of samples. This entails only re-using consumables were appropriate and using aseptic pipetting techniques.

Biosearch Technologies SARS-CoV-2 detection workflow

RNA purification

oKtopure preparation:

1) Load new mandrel filters (KBS-09-MS027) into the oKtopure head. If new filters are already present, skip this step.

   IMPORTANT: Mandrel filters should be replaced prior to every run to prevent cross contamination and ensure proper dispense volumes if previous filters encountered fluids.

2) Load oKtopure tip blocks (KBS-09-127) onto the instrument.
3) Load new oKtopure tips (KBS-0010-003) into the tip blocks. This may be performed through the oKtopure software user interface.
   a. For the first run of the day, load tips into tip positions 1-12. For subsequent runs, only the buffer tips should be reused, new tips should be loaded into tip positions 5-12.

      IMPORTANT: Buffer only tips should be reused for no more than 3 runs.

   b. When performing tip transfer using the oKtopure software user interface:
      i. Prior to loading tips to positions 1-12 verify "Skip wash buffer tips" box is not checked Figure 1.
      ii. If only loading tips to positions 5-12, verify "Skip wash buffer tips" is checked Figure 1.

Figure 1. Load oKtopure Tip Racks.

Extraction reagents preparation:

Lysis Buffer Mixture

Prepare the required amount of Lysis Buffer Mix within 4 hours of intended use.

1) Prepare the Lysis BufferMix.
   a. For the number of required 96-well plate extractions, prepare the Lysis Buffer Mix containing Lysis Buffer SB found in the sbeadex viral RNA purification kit (NAP40-028-04) and Protease solution (NAP30-002-02 or NAP30-002-03) according to Table5.

Component	Volume per 96-well plate[1]
Lysis Buffer SB	19.2 mL
Protease Solution (20 mg/mL)	0.48 mL
Total volume per plate	19.68 mL

Table 5. Lysis Buffer SB and Protease Solution volumes for 1 plate.

[1] Include percent overage required for liquid handling.

b. Using the oKtopure to prefill 8 sample plates requires 180 mL of Lysis Buffer Mix using a standard buffer reservoir (KBS-0009-006). Table 6 below references how to prepare Lysis Buffer Mix for 8 sample plates.

Component	Volume
Lysis Buffer SB	175.61 mL
Protease Solution (20 mg/mL)	4.39 mL
Total volume	180 mL

Table 6. Lysis Buffer SB and Protease Solution volumes for 8 plates.

2) Mix well by inversion. The solution will remain stable at room temperature for up to 4 hours.

Binding Bead Mix

1) Thoroughly mix sbeadex particle suspension by vortexing until solution is homogenous.
2) Combine and mix the Binding Buffer SB and sbeadex particle suspension following the table below.
   a. For the number of required 96-well plate extractions, prepare the Binding Bead Mix according to Table 7.

Component	Volume per 96-well plate[1]
Binding Buffer SB	30.72 mL
sbeadex particle suspension	1.92 mL
Total volume per plate	32.64 mL

Table 7. Binding Buffer SB and sbeadex particle suspension volumes for 1 plate.

[1] Include percent overage required for liquid handling.

b. Using the oKtopure to prefill 8 sample plates requires 306 mL of Binding Bead Mix using a standard buffer reservoir (KBS-0009-006). Prepare the Binding Bead Mix according to Table 8.

IMPORTANT: The standard buffer reservoir (KBS-0009-006) is required for this action to keep Binding Bead Mix homogenous. Failure to do so may cause bead settling and inconsistent bead transfer.

Component	Volume
Binding Buffer SB	288 mL
sbeadex particle suspension	18 mL
Total volume	306 mL

Table 8. Binding Buffer SB and sbeadex particle suspension for 8 plates.

3) Mix well by inversion, then store at room temperature until use.

Prepare sample plate:

1) Combine the following into each reaction well of a 96-well MagMix plate (KBS-7001-130).
   a. Add 205 µL of Lysis Buffer Mix prepared previously.
      i. Optional: Pre-fill plate function on the oKtopure may be used to add Lysis Buffer Mix. Be sure to designate the dispense volume to 205 µL and select the appropriate number of plates to be filled. Figure 2 may be used as a reference.

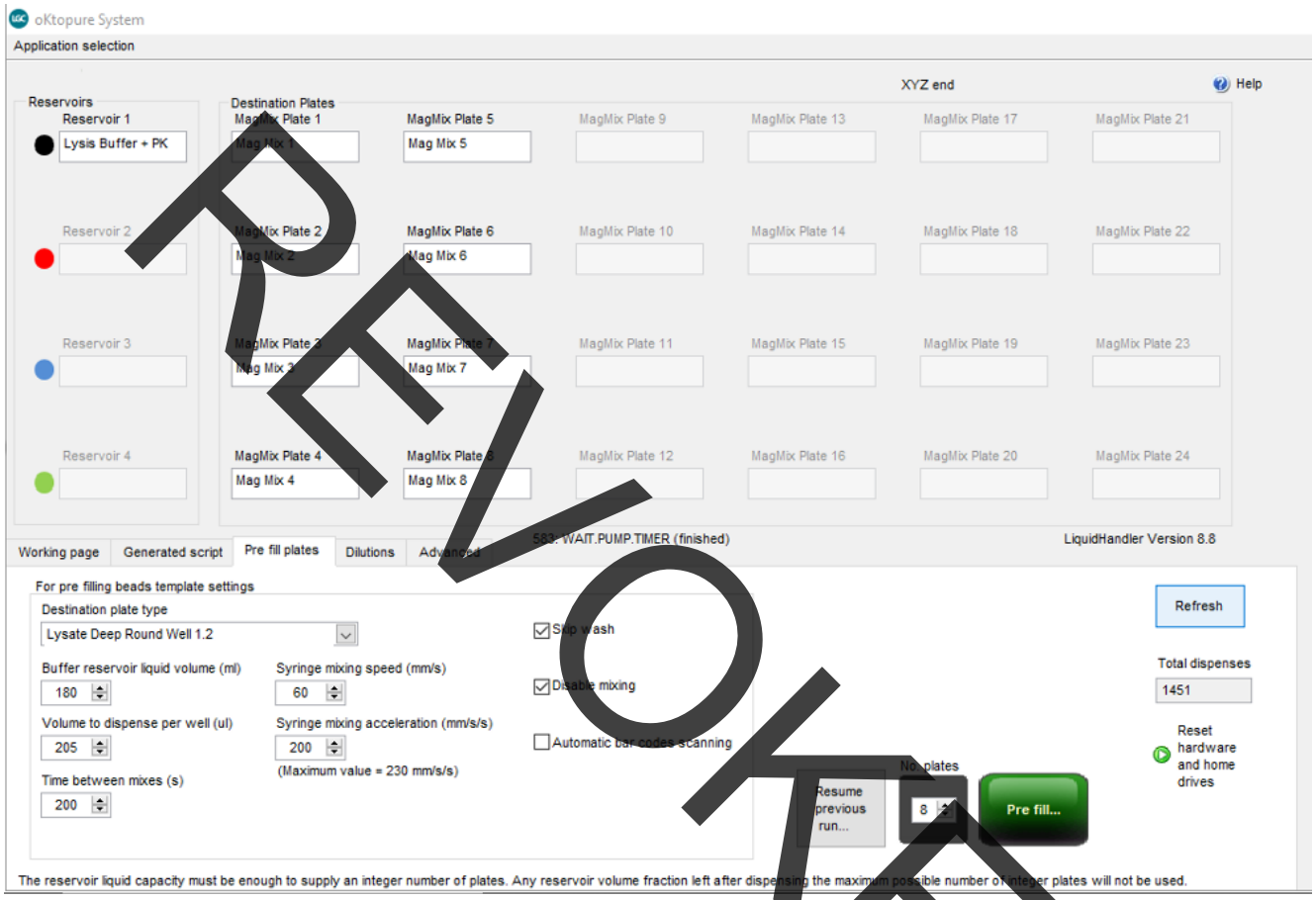

Figure 2. oKtopure pre-fill plates layout for dispensing Lysis Buffer Mix.

   ii. Replace used tips after completion of Pre-fill routine.

b. Add 200 µL of sample to each well.
   i. Record sample position within the plate.

c. Add extraction controls to each 96-well MagMix plate (KBS-7001-130):
   i. Add 200 µL of the positive control material (0505-0211). This positive control must be named "PC" in the plate map files used to reference sample locations.
   ii. Add 200 µL of nuclease free water. This negative control must be named "NTC" in the plate-map files used to reference sample locations.
   iii. The user may determine the location of the controls, but they must be labeled as "PC" and "NTC" in the sample plate file.

2) Seal the 96-well MagMix plates with an adhesive PCR film (KBS-0606-002). Firmly press seal to the 96-well MagMix plates using a roller or equivalent method.

IMPORTANT: The adhesive PCR film (KBS-0606-002) must be used to seal the 96-well MagMix plate (KBS-7001-130) at this step. Alternative seals may not tolerate the 95 °C incubation, resulting in cross-contamination risk.

3) Vortex the 96-well MagMix plates for 30 seconds. Avoid splashing liquid onto the seal to prevent potential contamination when unsealing the plate after incubation.
4) Centrifuge the 96-well MagMix plates at 2,500 × g for 1 minute.
5) Incubate the 96-well MagMix plates at 95 °C for 30 minutes in an oven. Use caution when removing the plates as they may be very warm.
6) Allow 96-well MagMix plates to cool at ambient temperature (20 ± 5 °C) for a minimum of 10 minutes.
7) Centrifuge the 96-well MagMix plates at 2,500 × g for 1 minute.
8) Leave the plates sealed and set the 96-well MagMix plates aside until ready to add Binding Bead Mix.
9) Carefully unseal plate.
10) Add 340 µL of Binding Bead Mix.
    a. Optional: Pre-fill plate function on the oKtopure may be used to add Binding Bead Mix. Designate the dispense volume to 340 µL and select the appropriate number of plates to be filled. Figure 3 may be used as a reference.

    IMPORTANT: Buffer reservoir (KBS-0009-006) is required for this action to keep Binding Bead mix homogenous. Failure to do so may cause bead settling and inconsistent bead transfer.

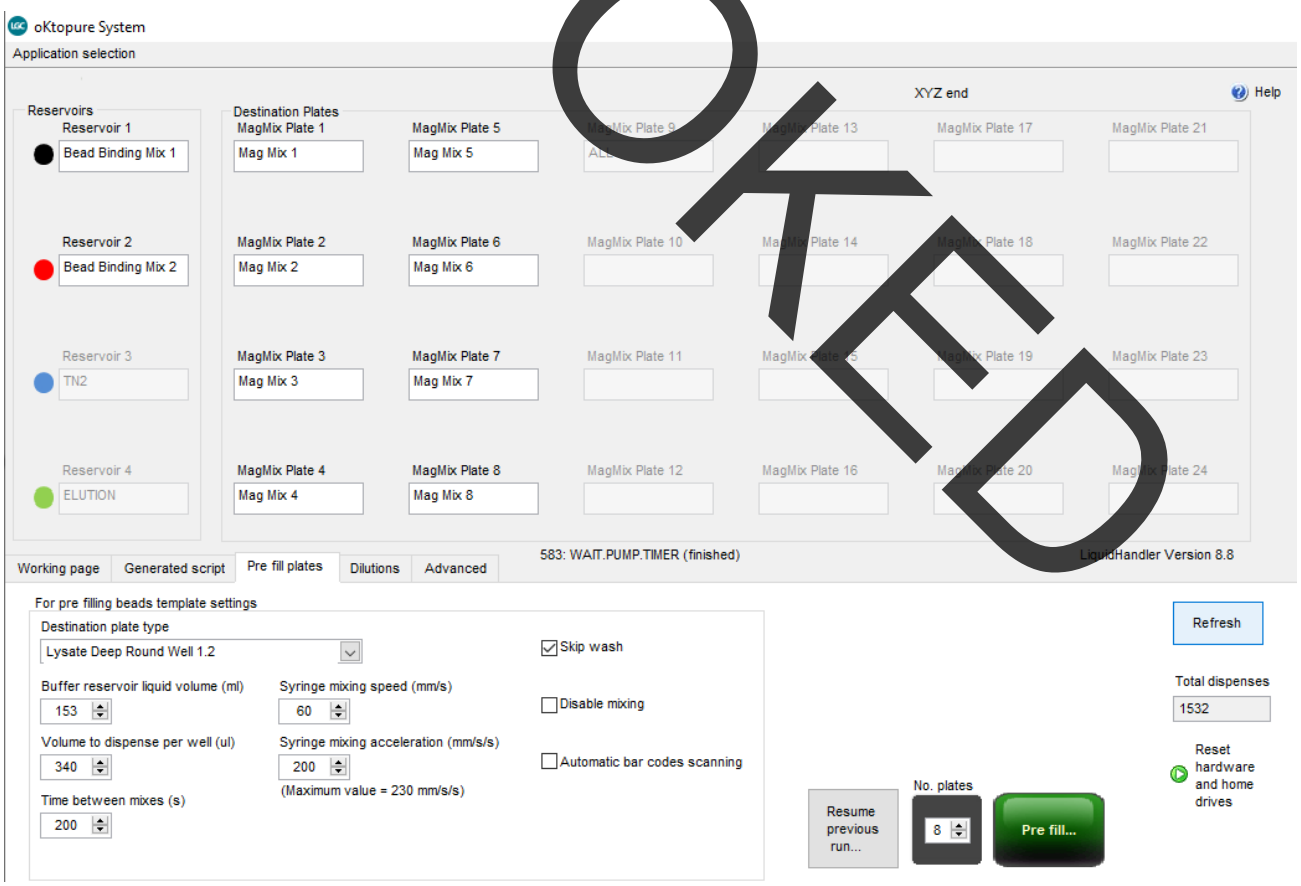

Figure 3. oKtopure pre-fill plates layout for dispensing Binding Bead Mix.

    b. Replace used tips after completion of Pre-fill routine.

Performing the oKtopure run:

1) Place the 96-well MagMix plates (KBS-7001-130) now containing sample, Lysis Buffer Mix, and Binding Bead Mix onto magnet locations 9-12 and 21-24. If less than 8 plates are being extracted, refer to oKtopure software for designated plate locations.
2) Apply an adhesive foil seal (KBS-0600-002) to two 384-well destination plates (KBS-7001-139, KBS-7001-132, or KBS-7001-133) and add plates to locations 13 and 25.
3) Add and fill the wash buffer reservoirs (KBS-09-050) with the appropriate volumes of each wash buffer based on the equations below. The standard buffer reservoir (KBS-0009-006) is required for the Elution AMP. Table 9 can be used to reference volumes required for an 8 plate extraction.

NOTE: Graduated cylinders should be used to measure the appropriate buffer volumes and carefully pour into the reservoirs. Alternatively, a pre-determined minimum fill line can be marked on the side of the reservoir instead of measuring with a graduated cylinder.

IMPORTANT: Buffer reservoir (KBS-0009-006) is required for the Elution AMP. Heating the maxi buffer troughs (KBS-09-050) will cause troughs to warp and no longer seat properly into the oKtopure deck positions.

a. BN1 volume (mL) = # of plates × 96 wells/plate × 0.3 mL/well + 45 mL overage
b. TN1 volume (mL) = # of plates × 96 wells/plate × 0.24 mL/well + 45 mL overage
c. TN2 volume (mL) = # of plates × 96 wells/plate × 0.34 mL/well + 45 mL overage
d. Elution AMP volume (mL) = # of plates × 96 wells/plate × 0.05 mL/well + 45 mL overage

Component	oKtopure deck position	Volume for 8 plate extraction
Wash Buffer BN1	1	275 mL
Wash Buffer TN1	2	230 mL
Wash Buffer TN2	3	306 mL
Elution AMP	4	83 mL

Table 9. Wash and Elution Buffer volumes for 8 plates.

4) Seal the buffer trough containing Elution AMP with an adhesive foil seal (KBS-0600-002) and place in a 65 °C oven. The instrument will prompt the user to add the elution reservoir trough at the elution step.
5) Add the waste collection plates (KBS-7001-031 or KBS-7001-044 or KBS-7001-244) onto the deck locations directly left of the MAG/MIX plate locations containing a 96-well MagMix plate (KBS-7001-130) for extraction.
6) Using a handheld barcode scanner, scan the plate barcodes in the MAG/MIX locations and Destination Plate locations as shown in Figure 4 below. If previous barcodes are present, the "Clear purification plates" button can be selected to clear the barcodes.

IMPORTANT: Always scan barcodes in the order they are loaded onto the instrument deck and verify proper plate orientation. Failure to do so will result in incorrect plate mapping.

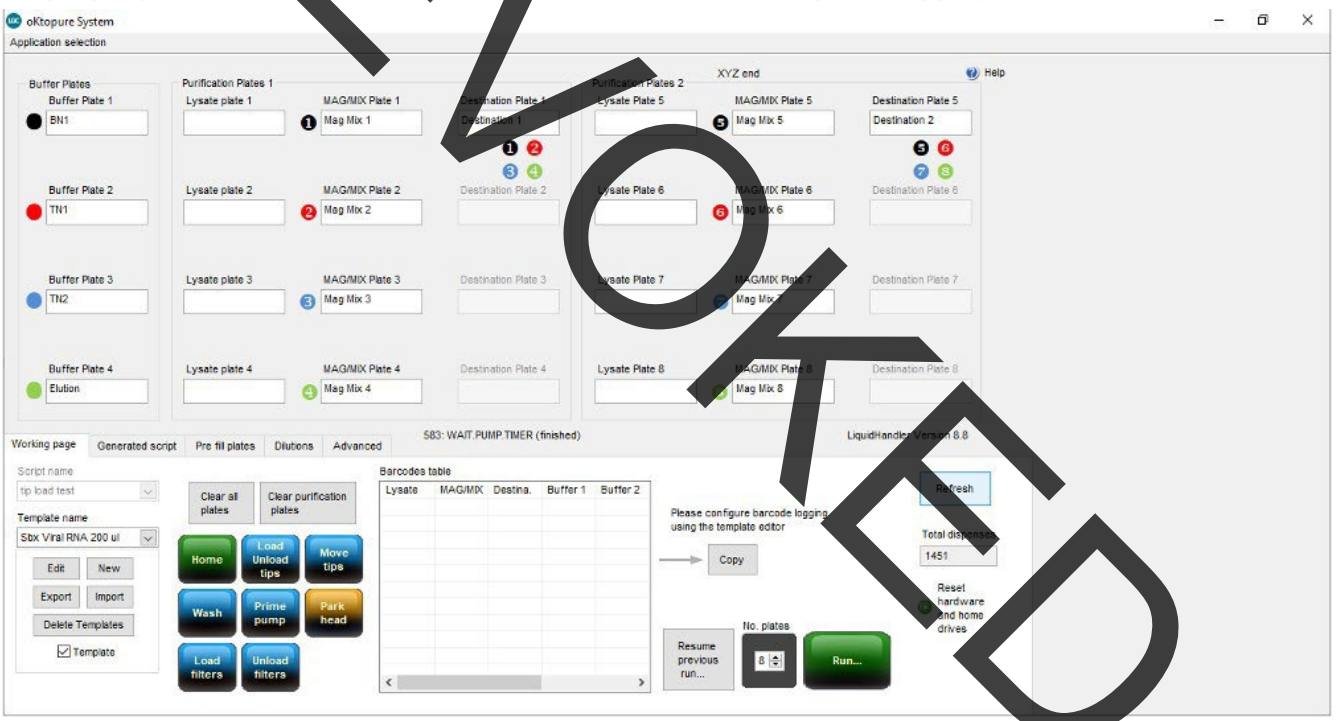

Figure 4. oKtopure plate barcode entry.

7) From the working page, select the "Sbx Viral RNA 200 ul 384 – 8.8" protocol. Confirm the selected number of plates is correct and start the run.
8) Confirm the appropriate barcodes have been scanned and continue.
9) When prompted, transfer the heated elution buffer reservoir from the 65 °C oven to oKtopure deck position 4 and remove seal.
10) Remove the seals from the 384-well destination plates.
11) Select "Resume."

12) Upon completion of the run, seal the plates with the adhesive foil seal (KBS-0600-002) and keep on ice or at 4 °C until use.

IMPORTANT: Use caution when applying adhesive seals to ensure pressure is applied evenly across the plate for proper sealing across all wells. Failure to do so can lead to potential well-to-well contamination during plate handling.

13) Remove the used tip blocks from the deck and dispose of the used sample tips. The buffer only tips can be reused in subsequent runs throughout the day.

IMPORTANT: Buffer only tips should be reused for no more than 3 runs.

IMPORTANT: oKtopure tip blocks used to hold the samples tips must be decontaminated according to the oKtopure user manual after every run to avoid risk of cross-contamination.

14) Remove the mandrel filters from the dispense head.
15) If this is the first run of the day, then proceed to the instrument daily startup routine section. If the daily startup routines were already completed, then proceed to the respective real-time and end-point RT-PCR sections depending on the desired workflow.

IntelliQube real-time RT-PCR detection of SARS-CoV-2

NOTE: This section describes the real-time RT-PCR modality of operation. For end-point operation, see p. 40.

Instrument daily startup routine:

IntelliQube

1) Check Carboys.
   a. Empty Waste Carboy.
   b. Fill Source Water Carboy.
   c. Check Bleach Carboy; refill if low.
2) Purge Dispense Jet.
   a. Manual Control > Maintenance > Jet Purge
3) Wash Dispense Jet and re-pressurize.
   a. Manual Control > Jet > "EUA Jet Configuration" > Cycles: 200 > Wash Tips
   b. Manual Control > Jet > Pressurize to a target pressure of 1.6 psi
4) Clean Dispense Jet with Ethanol.
   a. Prepare 96-well deep-well plate or matrix tube rack with 70-80% ethanol.
      i. Fill wells A1, B1, C1, and D1 with 700µL of 70-80% ethanol.
      ii. Place on deck position 1.
   b. Manual Control > Jet > Select All Tips > Select Plate Deck 1 > Select Appropriate Plateware > Select Full Dispense Pattern > Aspirate. Refer to Figure 5
   c. Wait 5-10 minutes.

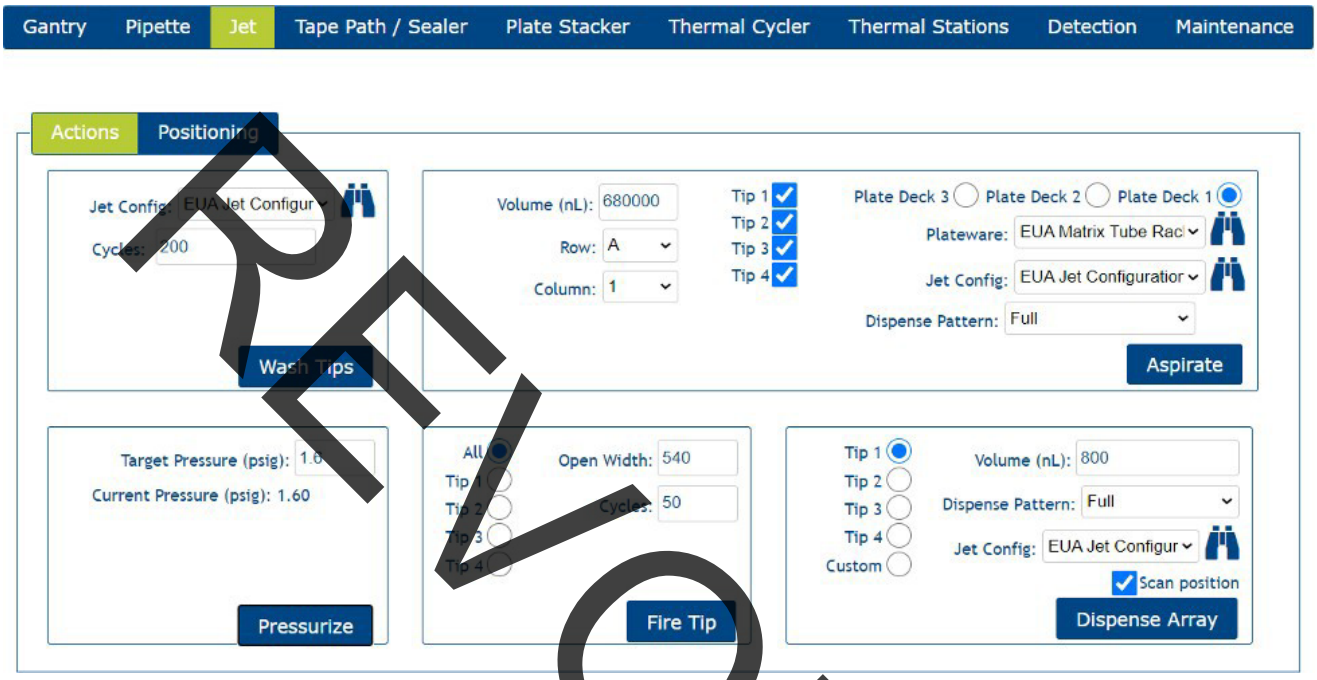

Figure 5. IntelliQube Dispense Jet manual control.

5) Flush the DispenseJet.
   a. Manual Control > Jet > Cycles: 200 > Wash Tips
6) Check the sodium hypochlorite concentration in the pipette wash protocol
   a. Under the Pipette tab in manual control, select the EUA Pipette Wash configuration and EUA 384 Tip 10 µL.
   b. Select Wash Tips.
   c. Once the basin has filled and the Dispense Pipette begins to move down to start the aspiration process, fault the instrument by opening the guard door.
   d. Test the bleach concentration by moving the test strip across the wash fluid in the basin.
   e. Verify the bleach strip measures 2500ppm.
   f. Close the guard door and recover the instrument.
   g. Select Water Only Pipette Wash configuration and perform 2 washes.
   h. Repeat steps 6a and 6b.
   i. Allow the wash to continue to the last flush cycle.
   j. Once the basin has filled and the Dispense Pipette begins to move down to start the aspiration process, fault the instrument by opening the guard door.
   k. Test the bleach concentration by moving the test strip across the wash fluid in the basin.
   l. Verify the bleach strip measures 0 ppm.
   m. Close the guard door and recover the instrument.
   n. Make any adjustments needed to the wash pattern and repeat to verify adjusted settings are correct.

2X PCR reagent preparation for a two 384-well sample plate run:

***Note: If preparing less than two full 384-well plates, consult Table 10 below to determine the appropriate volumes of reagents to be used based on sample number.**

1) Thaw all reagents and keep on ice or at 4 °C until use.
2) Gently vortex reagents and briefly centrifuge before use.
3) Calculate amount of assay to prepare.
   a. For less than 384 samples, take the (# of samples x 0.8 µL + 98 µL) x 1.05 = total volume of assay required.
      - Example: (96 samples x 0.8 µL + 98 µL) x 1.05 = 183.5 µL
   b. For a single 384-well sample plate, 405 µL × 1.05 = total volume of assay required.
   c. For multiple 384-well sample plates, 405 µL × # of plates × 1.05 = total volume of assay required.
4) Prepare the 2X mixture of PCR reagents using a 1.5-2 mL tube referencing Table 10. Table 11 and Table 12 give examples assuming 2 tubes required for each assay.
5) Example calculation for the preparation of enough reagent to test a single 384-well plate, the total volume of reagent needed would be:

   405 µL x 1 X 1.05 = **425.25 µL** of each N1 or N2 reagent total.

   Using Table 10 below the individual component volumes would be:

   425.25 / 2.5 = **170.1 µL - RapiDxFire qPCR 5X Master Mix GF**

   425.25 / 33.3 = **12.8 µL - EpiScript RNase H- Reverse Transcriptase**

   425.25 / 50 = **8.5 µL - 2019-nCoV N1/RnP or N2/RnP blend**

   425.25 / 100 = **4.25 µL - SuperROX Reference Dye**

   425.25 – 170.1 – 12.8 – 8.5 – 4.25 = **229.6 µL - Molecular grade water**

	Stock concentration	Working 2X concentration	Volume (µL)
RapiDxFire qPCR 5X Master Mix GF[1]	5X	2X	=Total/2.5
EpiScript RNase H- Reverse Transcriptase[2]	200 U/µL	6 U/µL	=Total/33.3
2019-nCoV N1[3] or N2[4]/RnP blend	100X	2X	=Total/50
SuperROX Reference Dye[5]	15 µM	150 nM	=Total/100
Molecular grade water	-	-	=Total – sum of all components
		Total	xxxx

Table 10. Variable reagent volume calculations.

[1] Part numbers: 30050-1, 30050-2, or 30050-100ML
[2] Part numbers: ERT12925K-ENZ, ERT12925K-1.25ML or ERT12925K-12ML
[3] Part number: N1RNP-1010 – 2019-nCoV N1/RnP Blend
[4] Part number: N2RNP-1010 – 2019-nCoV N2/RnP Blend
[5] Part numbers: SR-1000-1 or SR-1000-10

	Stock concentration	Working 2X concentration	Volume (µL)
RapiDxFire qPCR 5X Master Mix GF	5X	2X	340.2
EpiScript RNase H- Reverse Transcriptase	200 U/µL	6 U/µL	25.5
2019-nCoV N1/RnP blend	100X	2X	17.0
SuperROX Reference Dye	15 µM	150 nM	8.5
Molecular grade water	-	-	459.3
		Total	850.5

Table 11. N1/RnP reagent volumes for 2 x 384-well sample plate run.

	Stock concentration	Working 2X concentration	Volume (µL)
RapiDxFire qPCR 5X Master Mix GF	5X	2X	340.2
EpiScript RNase H- Reverse Transcriptase	200 U/µL	6 U/µL	25.5
2019-nCoV N2/RnP blend	100X	2X	17.0
SuperROX Reference Dye	15 µM	150 nM	8.5
Molecular grade water	-	-	459.3
		Total	850.5

Table 12. N2/RnP reagent volumes for 2 x 384-well sample plate run.

6) Vortex to mix, briefly centrifuge, transfer 405 µL of the assay mixture into 0.75 mL matrix tubes (ThermoFisher – 4170) and seal with a SepraSeal cap (ThermoFisher – 4463). If running less than 384 samples, the assay mixture volume should be adjusted accordingly as described above. If assay mixture will not be immediately used, store on ice or at 4 °C until ready for use. The assay mixture is stable at 2-8°C for 24 hours.
7) When ready to begin the IntelliQube run, place the matrix tubes containing the 2X PCR reagent mixture into the matrix rack (ThermoFisher – 4896) into the positions indicated by the IntelliQube protocol. The assay plate layout can be viewed in the Intellics software by clicking on the protocol in the protocols list and selecting "Quick Review" → "PlateSummary."
   a. For example, when running two 384-well plates, place the matrix tubes containing the 2019-nCoV N1/RnP blend mixture into positions A1 and C1 of the matrix rack (ThermoFisher – 4896). Place the matrix tubes containing the 2019-nCoV N2/RnP blend into positions B1 and D1 of the same matrix rack.

IMPORTANT: Always confirm proper placement of assay tubes based on the protocol prior to initiating a run. Improperly positioned reagents can lead to misclassification of sample and control results.

NOTE: As an alternative to matrix tubes and matrix rack, a 96-well plate (KBS-7001-131, KBS-7001-231 or Greiner Bio-one 786201) may be used. The assay mixtures must be pipetted into the same well locations designated above – A1 and C1 for 2019-nCoV N1/RnP; B1 and D1 for 2019-nCoV N2/RnP. Plates should be sealed with an adhesive foil seal (KBS-0600-002) and stored on ice or at 4 °C until ready for use.

Creating an IntelliQube protocol from template:

1) Start by selecting protocol "EUA COVID TEMPLATE – INLINE" and then select "Use as Template."
2) Check all the boxes shown in Figure 6 below and select "Create."

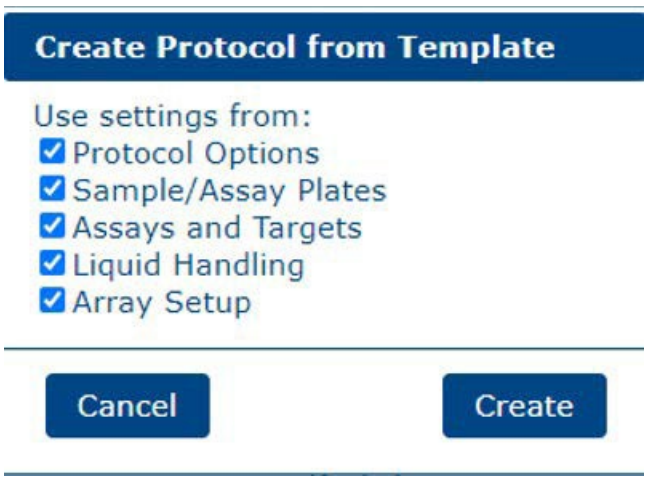

Figure 6. Create protocol from template.

3) Edit the protocol name to reflect the run being performed and verify all settings match Figure 7 below. The Dye Calibration Set selected will be specific to each instrument and preset as a default.

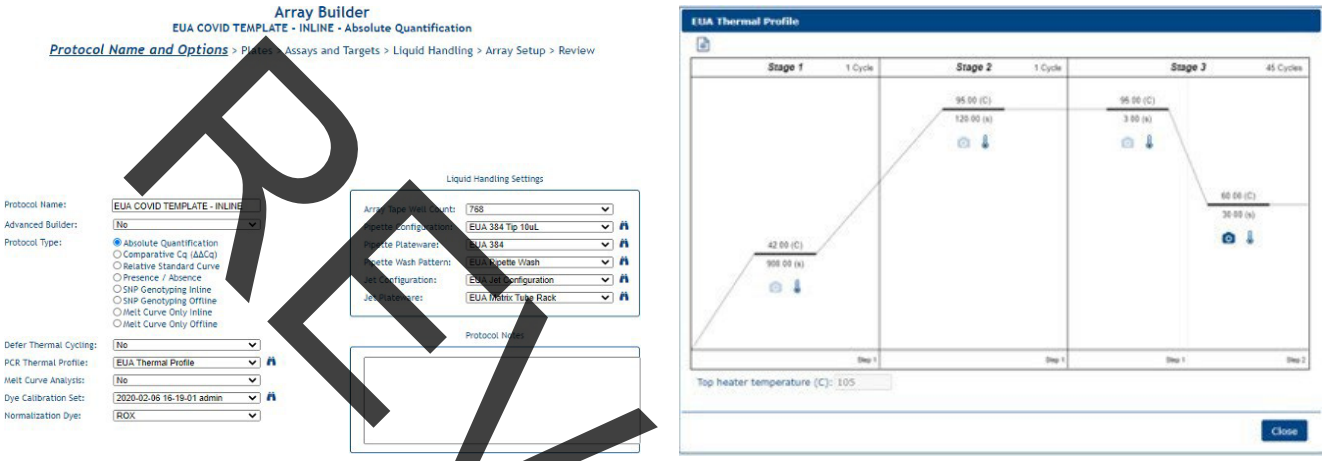

Figure 7. IntelliQube real-time protocol settings summary.

4) Referencing Figure 8, import the sample plate information for the protocol. This can be done by selecting “Browse” and directing the software to the correct plateware .csv files.

Figure 8. IntelliQube real-time sample plate import.

5) Verify assays 2019-nCoV N1/RnP and 2019-nCoV N2/RnP are included as shown in Figures 9a and 9b.

Figure 9a. IntelliQube sssay selection screen.

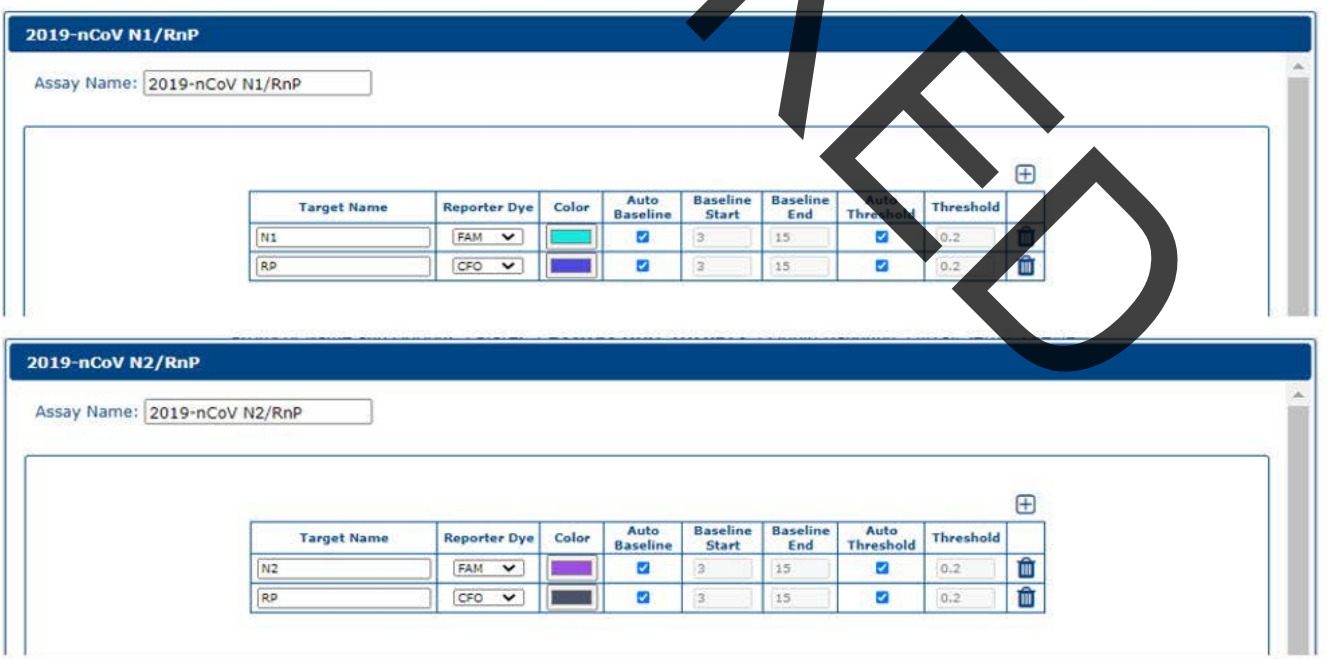

Figure 9b. IntelliQube assay information details.

6) Using Figure 10 as a reference, select all sample plates by both assays and click the right arrow to create the combinations.

Figure 10. IntelliQube – real-time sample/assay liquid handling.

7) Assign the assay plate barcode by typing or scanning the barcode into the provided space and selecting "Apply Barcode" Figure 11.

Figure 11. IntelliQube assay plate barcode definition.

8) Review protocol plate layouts in the "Array Setup" page and select "Next" or "Skip to Review" to progress to the "Review" page.
9) Review the "Protocol Summary" to ensure protocol was created correctly. Figure 12 can be used as a reference.

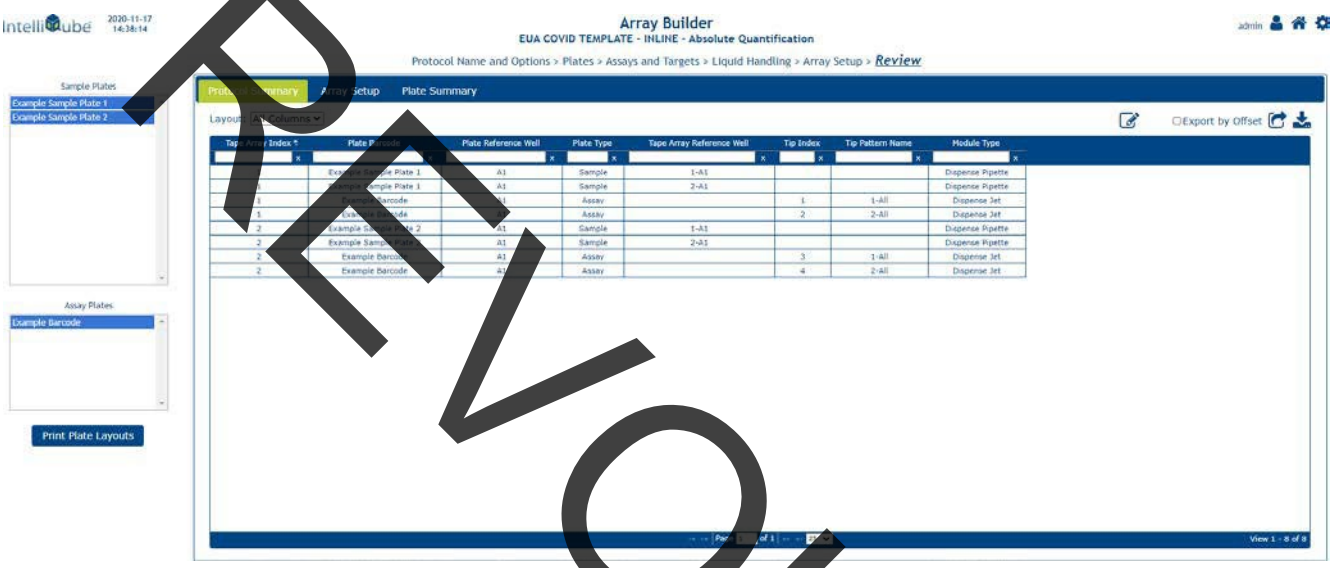

Figure 12. IntelliQube real-time protocol summary.

10) A layout of the sample and assay plates including required volumes can be viewed and printed by selecting the "Print Plate Layouts."
11) Select "Finish" to complete the protocol setup.

Loading the IntelliQube to begin a run:

1) If this is the first run of the day, perform all daily startup routine procedures.
2) Before each run, confirm there is an adequate supply of Array Tape (AXIT768-13WP050CC), cover seal (AX8591CVRTCC), sodium hypochlorite, and RO source water. If the waste line is not directly plumbed to a drain, confirm the waste carboy is empty.
3) It is recommended to replace the Dispense Pipette tips (AX843799 or AX840999) on a daily basis at a minimum.
4) The two 384-well extracted RNA sample plates should be centrifuged at 2,500 × g for 1 min.
5) Each sample plate should be barcoded to match the barcodes specified in the IntelliQube protocol. The required barcodes for a given protocol are visible on the instrument HMI.
6) After carefully removing the plate seals, the sample plates can be placed in any location within the sample plate stacker.

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

7) The matrix tube rack containing the prepared 2X PCR reagent mixtures must also be barcoded to match the barcode specified in the IntelliQube protocol.
8) Place the matrix tube rack in the Plate Deck 1 position and remove the matrix tube caps using a decapper tool.
9) From the IntelliQube HMI, select the appropriate protocol from the list and hit "Run."
10) Upon completion of the run, the dye cycle details data will be automatically exported and uploaded to FastFinder Analysis software.
11) Proceed to the next section for the interpretation of results using the real-time RT-PCR workflow.

Interpretation of results using the real-time RT-PCR workflow

This test utilizes the UgenTec FastFinder Analysis software to implement the threshold and decision logic described below.

Figure 13. FastFinder login.

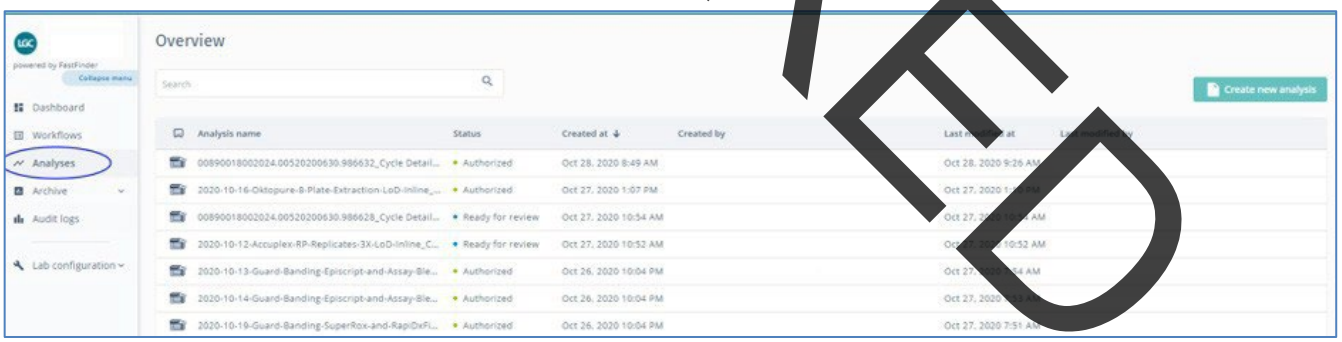

Figure 14. FastFinder Analyses module.

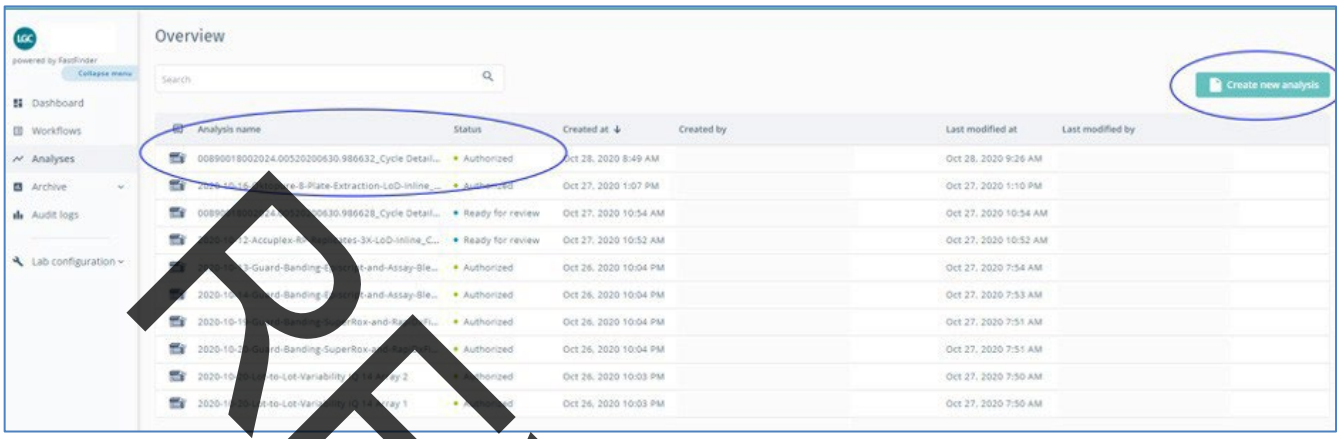

Figure 15. FastFinder import and view results.

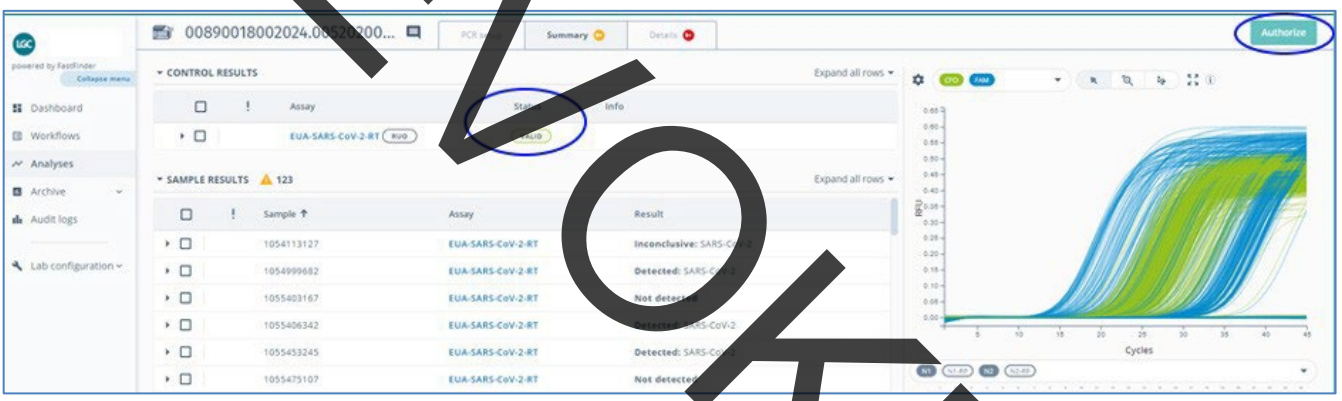

Figure 16. FastFinder review and authorize results.

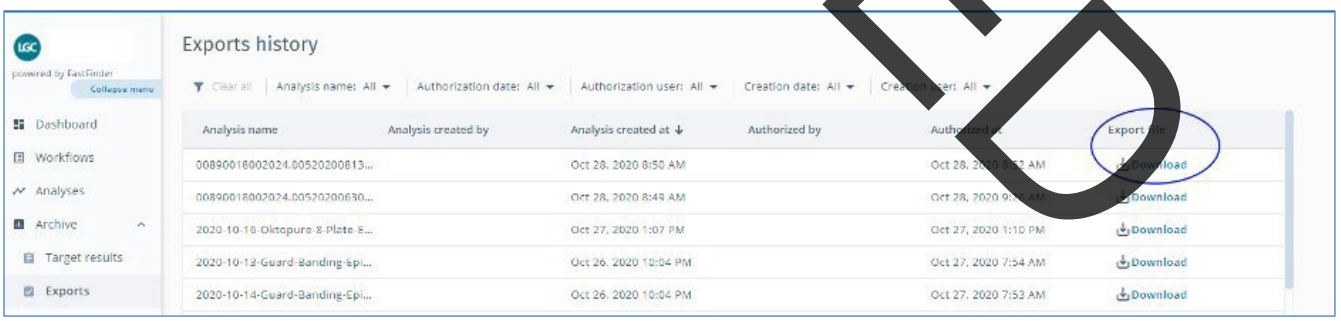

Figure 17. FastFinder export results.

	A	B	C	D	E	F	G	H	I	J	K	L	M
1	Sample ID	AssayResultTarget	AssayResultTargetCode	Outcome	IsInconclu	IsInvalid	IsRetest	AssayResu	SampleTy	Filename(	Barcode(s	Instrumer	Analysis
2	1055385714	SARS-CoV-2	SARS-CoV-2	Not detected	FALSE	FALSE	FALSE		Regular	00890018(	00890018(	"IQ520014	2020-10-2
3	1055406342	SARS-CoV-2	SARS-CoV-2	Not detected	FALSE	FALSE	FALSE		Regular	00890018(	00890018(	"IQ520014	2020-10-2
4	1055412246	SARS-CoV-2	SARS-CoV-2	Not detected	FALSE	FALSE	FALSE		Regular	00890018(	00890018(	"IQ520014	2020-10-2
5	1055475312	SARS-CoV-2	SARS-CoV-2	Not detected	FALSE	FALSE	FALSE		Regular	00890018(	00890018(	"IQ520014	2020-10-2
6	1055476049	SARS-CoV-2	SARS-CoV-2	Not detected	FALSE	FALSE	FALSE		Regular	00890018(	00890018(	"IQ520014	2020-10-2
7	1055477048	SARS-CoV-2	SARS-CoV-2	Not detected	FALSE	FALSE	FALSE		Regular	00890018(	00890018(	"IQ520014	2020-10-2
8	1055479328	SARS-CoV-2	SARS-CoV-2	Not detected	FALSE	FALSE	FALSE		Regular	00890018(	00890018(	"IQ520014	2020-10-2
9	1055486077	SARS-CoV-2	SARS-CoV-2	Not detected	FALSE	FALSE	FALSE		Regular	00890018(	00890018(	"IQ520014	2020-10-2
10	1055662067	SARS-CoV-2	SARS-CoV-2	Not detected	FALSE	FALSE	FALSE		Regular	00890018(	00890018(	"IQ520014	2020-10-2
11	1055670422	SARS-CoV-2	SARS-CoV-2	Not detected	FALSE	FALSE	FALSE		Regular	00890018(	00890018(	"IQ520014	2020-10-2
12	1055750465	SARS-CoV-2	SARS-CoV-2	Not detected	FALSE	FALSE	FALSE		Regular	00890018(	00890018(	"IQ520014	2020-10-2
13	1055772600	SARS-CoV-2	SARS-CoV-2	Not detected	FALSE	FALSE	FALSE		Regular	00890018(	00890018(	"IQ520014	2020-10-2
14	1055780482	SARS-CoV-2	SARS-CoV-2	Void	TRUE	FALSE	FALSE		Regular	00890018(	00890018(	"IQ520014	2020-10-2
15	1055790729	SARS-CoV-2	SARS-CoV-2	Not detected	FALSE	FALSE	FALSE		Regular	00890018(	00890018(	"IQ520014	2020-10-2
16	1056532107	SARS-CoV-2	SARS-CoV-2	Detected	FALSE	FALSE	FALSE		Regular	00890018(	00890018(	"IQ520014	2020-10-2
17	1056724448	SARS-CoV-2	SARS-CoV-2	Detected	FALSE	FALSE	FALSE		Regular	00890018(	00890018(	"IQ520014	2020-10-2
18	1056794001	SARS-CoV-2	SARS-CoV-2	Detected	FALSE	FALSE	FALSE		Regular	00890018(	00890018(	"IQ520014	2020-10-2
19	1056852849	SARS-CoV-2	SARS-CoV-2	Detected	FALSE	FALSE	FALSE		Regular	00890018(	00890018(	"IQ520014	2020-10-2
20	1057124563	SARS-CoV-2	SARS-CoV-2	Void	TRUE	FALSE	FALSE		Regular	00890018(	00890018(	"IQ520014	2020-10-2
21	1057548346	SARS-CoV-2	SARS-CoV-2	Detected	FALSE	FALSE	FALSE		Regular	00890018(	00890018(	"IQ520014	2020-10-2

Figure 18. FastFinder example csv results export file.

The test utilizes a control scheme that requires the addition of one positive and one negative control into each 96-well sample source plate. The plates are processed on the oKtopure as four separate 96-well sample plates that are combined into a single 384-well elution plate.The IntelliQube real-time RT-PCR process generates fluorescence data from the amplification of the targets of interest. The fluorescence values are exported to the FastFinder Analysis software. Validation of the results is performed automatically by the FastFinder Analysis software based on performance of the Positive and Negative Controls, the passive reference dye, presence of the RNase P target, and concordance of the N1 and N2 targets. The outcomes generated can take the values of “Detected,” “Not Detected” or “Void” in the case where one or more of the target results were invalid or inconclusive.

Real-time: Control well individual target interpretation

Target	+ Present	- Not present
Passive Reference Dye	RFU >= 0.2	RFU <0.2
N1	Cq <= 36	None or Cq >36
N2	Cq <= 36	None or Cq >36
N1-RP and N2-RP	Cq <= 37	None or Cq >37

Table 13. Interpretation of individual target results for the PC and NTC controls.

Positive and Negative controls are examined by the FastFinder Analysis software and are evaluated against the following control scenarios shown in Table 14 prior to interpretation of clinical results. Failed control wells are automatically flagged in the FastFinder analysis software. If any of the included controls are not marked as Valid, the corresponding patient results from the same source plate cannot be interpreted and will be marked as "Void" by default with a warning message related to the type of failure. Positive and Negative Control wells must be run for each 96-well sample extraction plate that is represented in analysis. Each control well corresponding to a 96-well source plate must pass for the samples analyzed with that plate to be considered valid. If there are failed control wells, the corresponding source plates can be removed as described below in order to complete the analysis for samples with valid controls. Cq value interpretation is defined by the UgenTec FastFinder software scoring algorithms.

Control	Scenario	N1	N1-RP	N2	N2-RP	Pass Ref [1]	Control results	Outcome for real-time or end-point clinical samples	Suggested user action
Positive Control (PC)	All N gene and RNase P targets detected	+	+	+	+	+	Valid	As scored by clinical sample decision logic	Report result. Option to mark any sample for re-test as needed.
	Any N gene or RNase P target not detected	?	?	?	?	?	Invalid	All wells marked as "Invalid Assay Controls"	Repeat test by repeating RT-PCR or re-extracting the original samples associated with the failed source plates and repeating RT-PCR[2].
No Template Control (NTC)	All N gene and RNase P targets not detected	-	-	-	-	+	Valid	As scored by clinical sample decision logic	Report result. Option to mark any sample for re-test as needed.
	Any N gene target or RNase P target detected	?	?	?	?	?	Invalid	All wells marked as "Invalid Assay Controls"	Repeat test by repeating RT-PCR or re-extracting the original samples associated with the failed source plates and repeating RT-PCR[2].

Table 14. Interpretation of PC and NTC control results.

[1] If the passive reference fails, the test can be repeated from the RT-PCR stage without re-extracting the samples.

[2] A single control failure may be resolved by repeating RT-PCR, otherwise re-extract the original samples and repeat RT-PCR

For any 96-well source plate that contains a control failure, it is possible in the FastFinder application to manually omit this source plate from analysis through the PCR setup tab inside the Analysis module such that only valid source plates are used for analysis and generation of results. This allows for valid results to still be analyzed while invalid results are omitted from analysis. The procedure is as follows:

1) Identify the source plate where the control failure is present. (Figure 19,20)
2) Remove the selected assignment of wells from the PCR setup tab. (Figure 21)
3) Reanalyze the results. (Figure 22)
4) Review and authorize the final result containing only the source plates with valid controls (Figure 23)

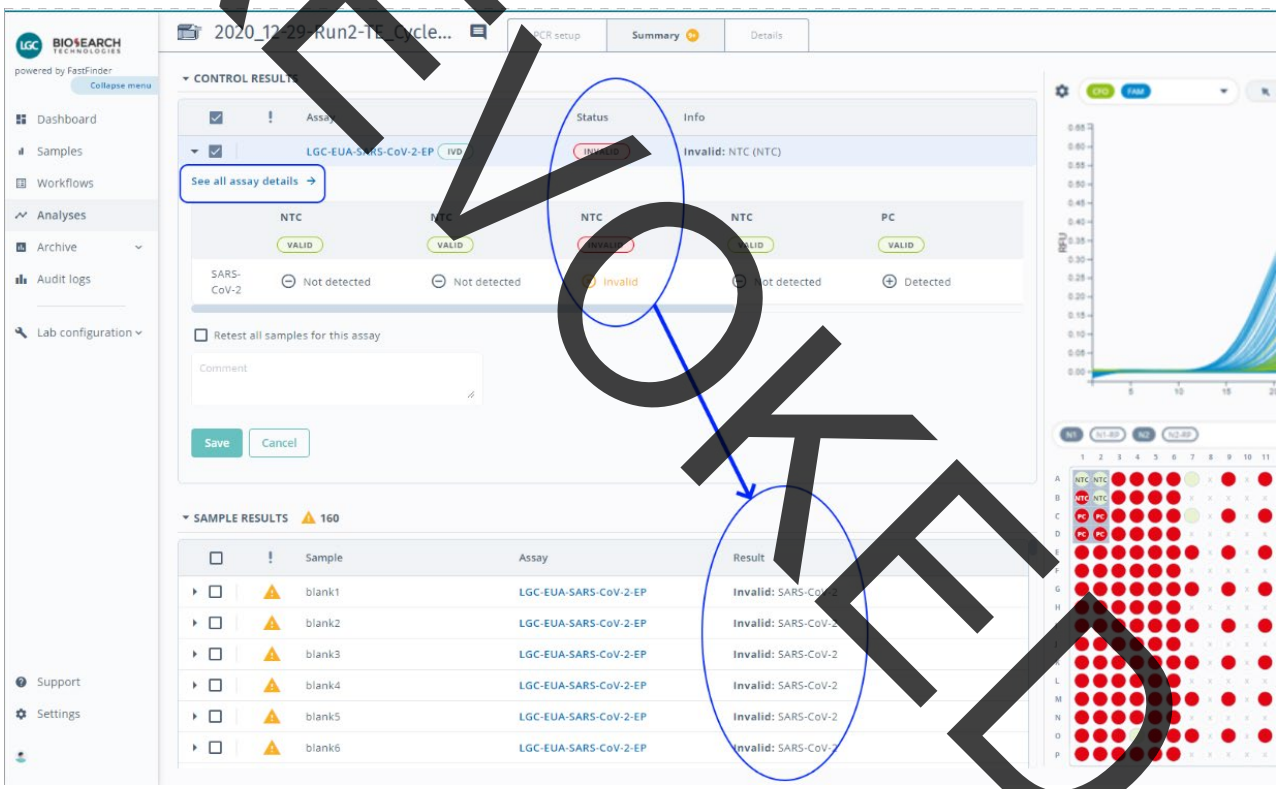

Figure 19. Identify control failures and select assay details in the analysis summary tab.

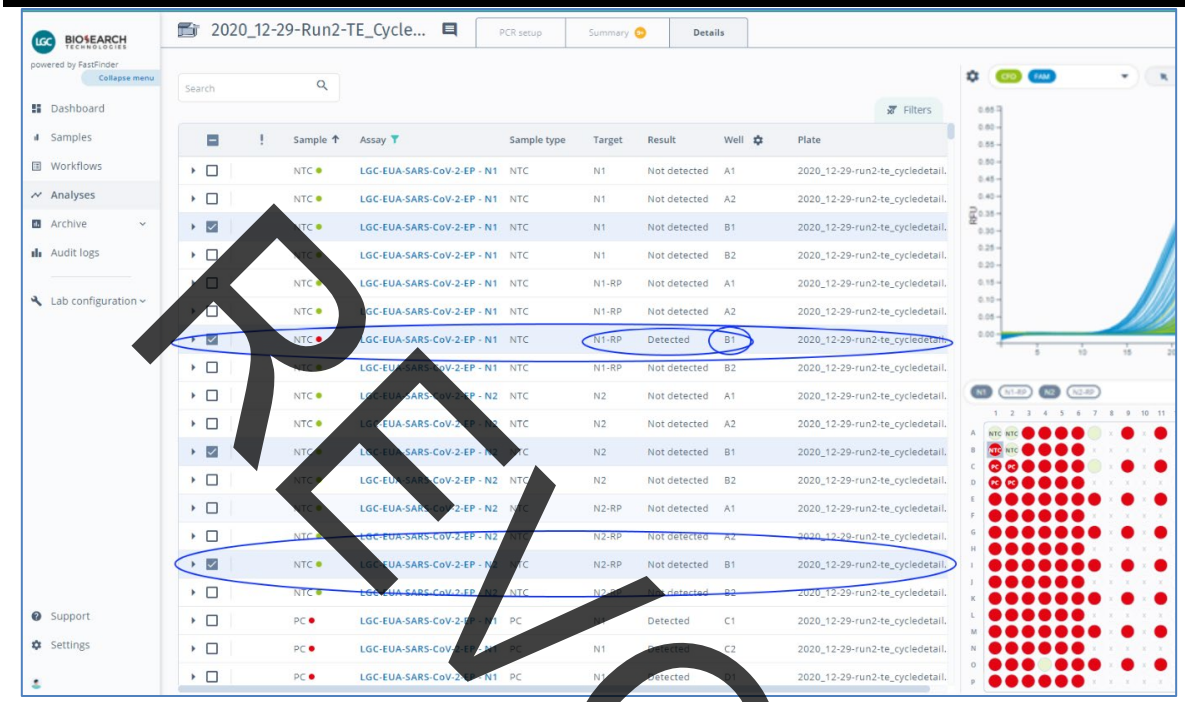

Figure 20. Identify the control failure well location in the details tab.

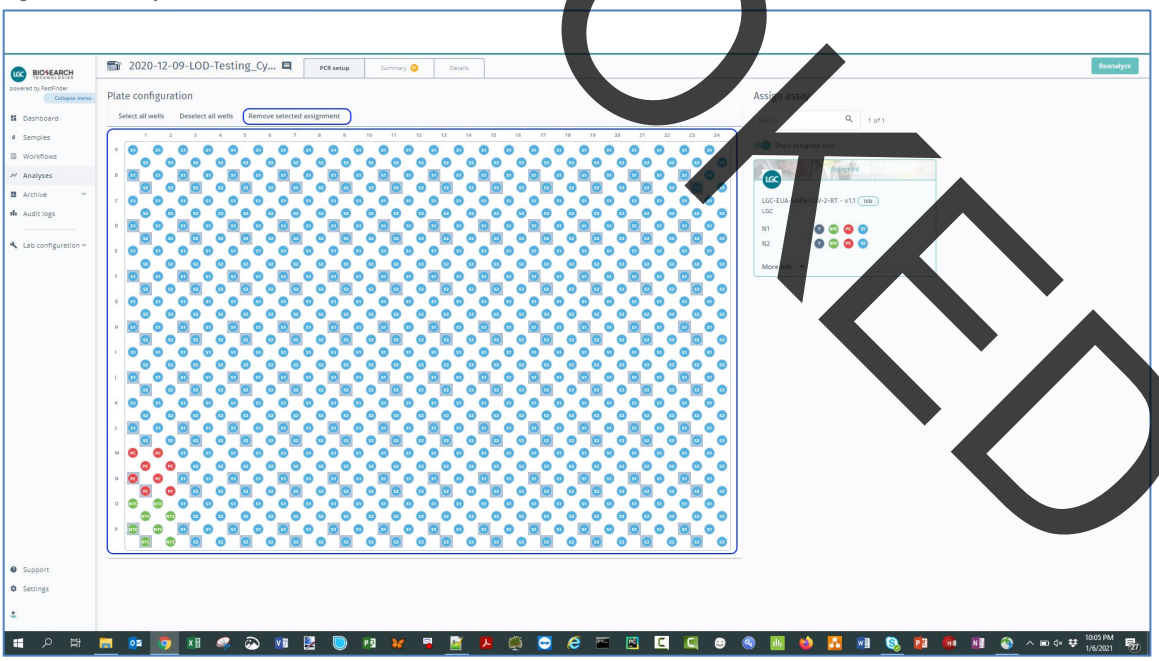

Figure 21. Select and remove assignment for all N1 and N2 wells for the associated 96-well source plate in PCR setup tab.

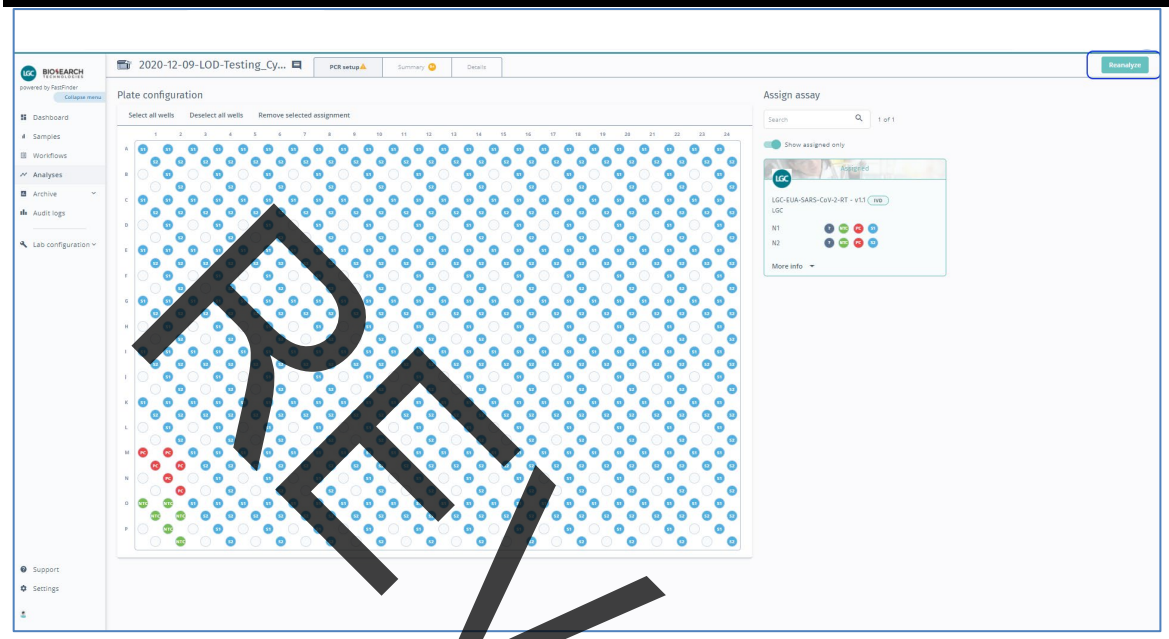

Figure 22. Reanalyze after removal of all wells associated with the specific source plate.

After all controls included in analysis have been identified as Valid, the analysis pipeline evaluates the results of the individual patient samples and assigns an overall call of "Detected," "Not Detected" or "Void" to each sample set per the Positive/Negative calling threshold and decision logic.

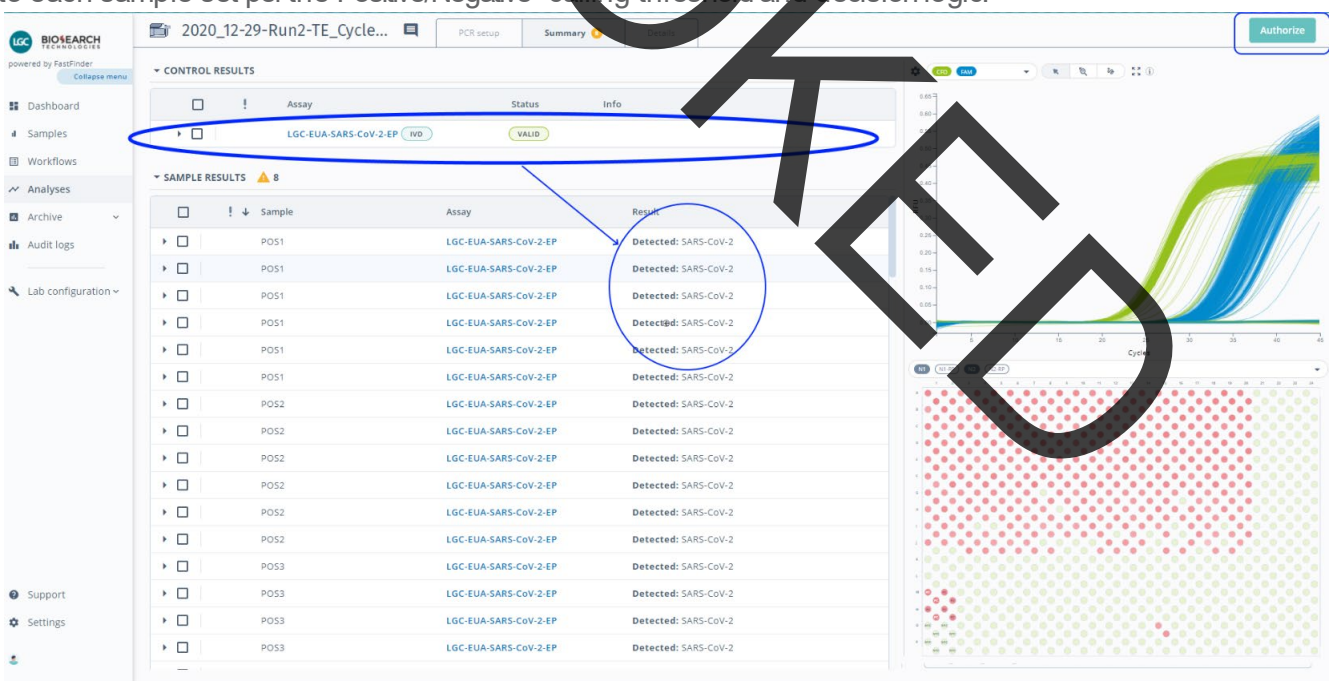

Figure 23. Review and authorize sample results.

Real-time: Clinical sample individual target interpretation

Target	+ "Positive"	- "Negative"
Passive Reference	RFU >= 0.2	RFU <0.2
N1	Cq Value <= 37.9	None or Cq Value >37.9
N2	Cq Value <= 37.5	None or Cq Value >37.5
N1-RP and N2-RP	Cq Value <= 31.7	None or Cq Value >31.7

Table 15. Interpretation of clinical sample individual target results

Clinical samples are examined by the FastFinder analysis software and results from Passive Reference Dyes and combinations of N1, N1_RP, N2, and N2_RP individual target results are automatically combined and evaluated against the following decision logic shown in Table 16 to define the clinical result. If any result other than "Detected" or "Not Detected" is determined for a given sample, the overall call for the clinical sample will be "Void" and it is recommended to repeat the test by re-extracting the original sample and repeating the RT-PCR.

Scenario	Passive Ref N1	N1	N1_RP	Passive Ref N2	N2	N2_RP	Outcome	Assay Result (User Interface)	Message	User action
Negative sample or concentration below LOD	+	-	+	+	-	+	Not detected	SARS-CoV-2 not detected	N/A	Report results
Positive sample above LOD	+	+	+	+	+	+	Detected	SARS-CoV-2 detected	N/A	Report results
RNase P amplification absent, but both N1 and N2 are present.	+	+		+	+	-	Detected	SARS-CoV-2 detected	Extraction control not detected	Report results
N1 or N2 are present, but not both, or, any other scenario not represented above	?	?	?	?	?	?	Void	Inconclusive	Various	Repeat test by re-extracting the original samples and repeating the RT-PCR[1]

Table 16. Clinical sample Real-Time decision logic.

[1] A single inconclusive result may be evaluated by re-extracting the original sample and repeating RT-PCR. If a second inconclusive result is obtained, the result should be reported as "Inconclusive" and a request to recollect sample should be made.

IntelliQube end-point RT-PCR detection of SARS-CoV-2

NOTE: This section describes the end-point RT-PCR modality of operation. For real-time operation, see p. 22.

Instrument daily startup routine:

IntelliQube

1) Check Carboys.
   a. Empty Waste Carboy.
   b. Fill Source Water Carboy.
   c. Check Bleach Carboy; refill if low.
2) Purge Dispense Jet.
   a. Manual Control > Maintenance > Jet Purge
3) Wash Dispense Jet andre-pressurize.
   a. Manual Control > Jet > "EUA Jet Configuration" > Cycles: 200 > Wash Tips
   b. Manual Control > Jet > Pressurize to a target pressure of 1.6 psi

4) Clean Dispense Jet with Ethanol.
   a. Prepare 96-well deep-well plate or matrix tube rack with 70-80% ethanol.
      i. Fill wells A1, B1, C1, and D1 with 700µL of 70-80% ethanol.
      ii. Place on deck position 1.
   b. Manual Control > Jet > Select All Tips > Select Plate Deck 1 > Select Appropriate Plateware > Select Full Dispense Pattern > Aspirate. Refer to Figure 24.
   c. Wait 5-10 minutes.

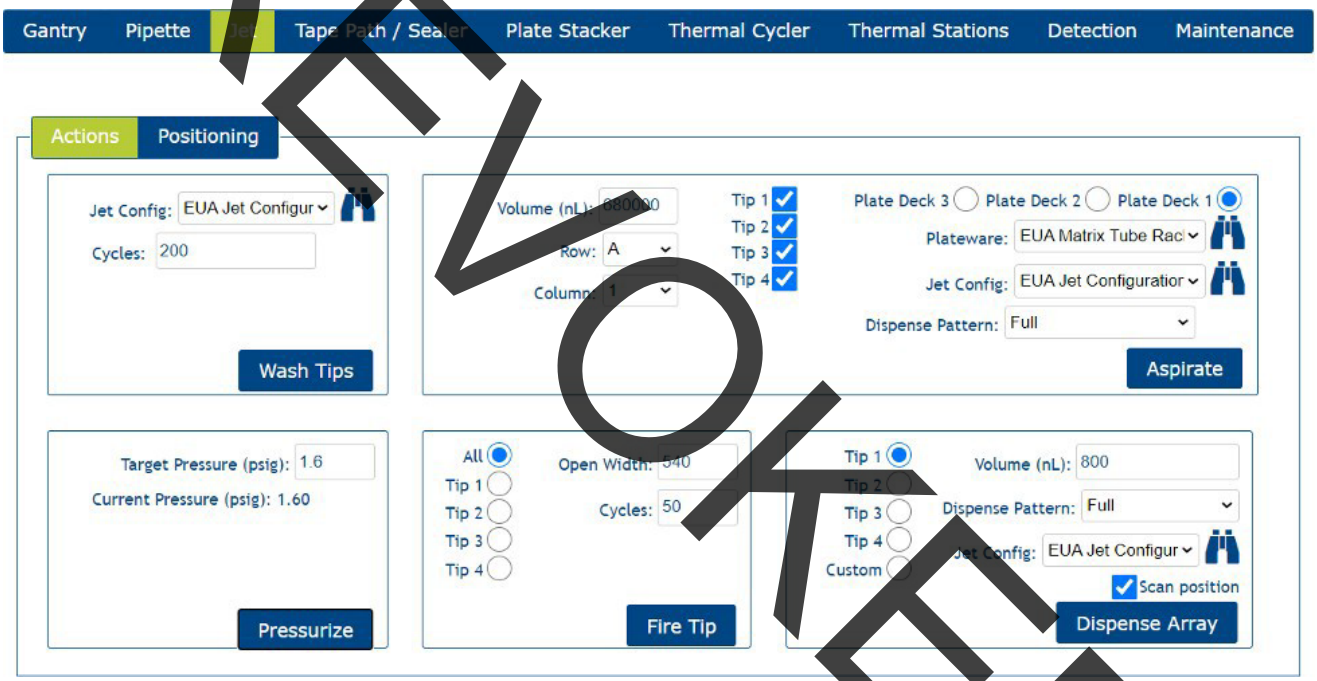

Figure 24. IntelliQube daily startup up routine operations control screen.

5) Flush the Dispense Jet.
   a. Manual Control > Jet > Cycles: 200 > Wash Tips

6) Check the sodium hypochlorite concentration in the pipette wash protocol.
   a. Under the Pipette tab in manual control, select the EUA Pipette Wash configuration and EUA 384 Tip 10 µL.
   b. Select Wash Tips.
   c. Once the basin has filled and the Dispense Pipette begins to move down to start the aspiration process, fault the instrument by opening the guard door.
   d. Test the bleach concentration by moving the test strip across the wash fluid in the basin.
   e. Verify the bleach strip measures 2500 ppm.
   f. Close the guard door and recover the instrument.
   g. Select Water Only Pipette Wash configuration and perform 2 washes.
   h. Repeat steps 6a and 6b.
   i. Allow the wash to continue to the last flush cycle.
   j. Once the basin has filled and the Dispense Pipette begins to move down to start the aspiration process, fault the instrument by opening the guard door.
   k. Test the bleach concentration by moving the test strip across the wash fluid in the basin.
   l. Verify the bleach strip measures 0 ppm.
   m. Close the guard door and recover the instrument.
   n. Make any adjustments needed to the wash pattern and repeat to verify adjusted settings are correct.

Hydrocycler[2] preparation

1) Check water level.
   a. Add water to Ballast tank if required.
2) Pre-heat tanks.
   a. Locate the EUA Thermal Profile and verify setting match Figure 25.
   b. Select "Pre-heat Tanks."

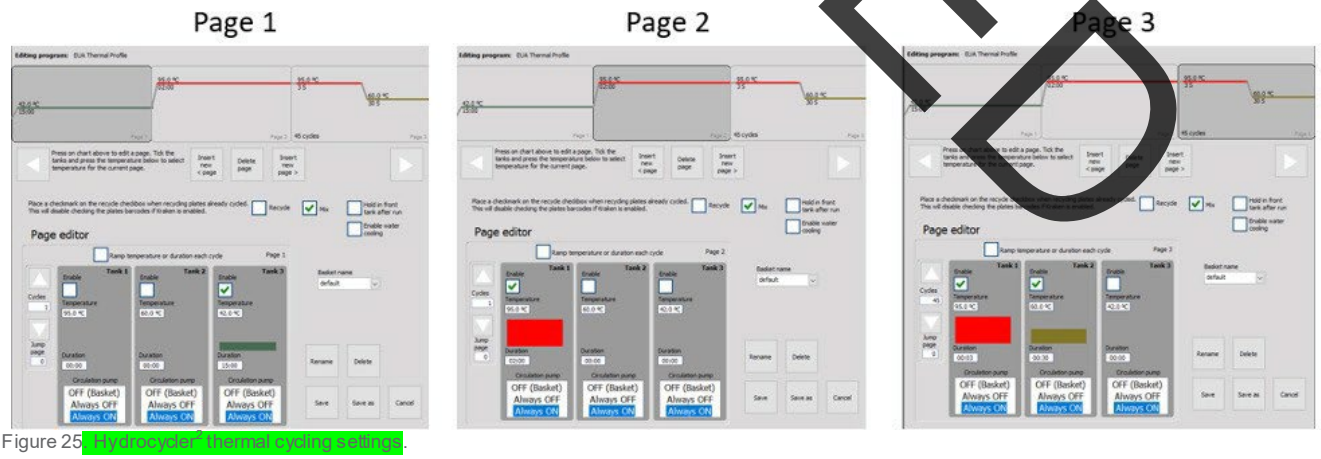

Figure 25: Hydrocycler[2] thermal cycling settings.

2X PCR reagent preparation 1x to 10x 384-well sample plate run:

1) Thaw all reagents and keep on ice or at 4 °C until use.
2) Gently vortex reagents and briefly centrifuge before use.
3) Calculate amount of 2X assay to prepare.
   a. For less than 384 samples, take the (# of samples x 0.8 µL + 98 µL) x 1.05 = total volume of assay required.
      - Example: (96 samples x 0.8 µL + 98 µL) x 1.05 = 183.5 µL
   b. For a single 384-well sample plate, 405 µL × 1.05 = total volume of assay required.
   c. For multiple 384-well sample plates, 405 µL × # of plates × 1.05 = total volume of assay required.
4) Prepare the 2X mixture of PCR reagents using a 5-15 mL tube referencing Table 17. Table 18 and Table 19 give examples assuming 10 tubes required for each assay.
5) Example calculation for the preparation of enough reagent to test a single 384-well plate, the total volume of reagent needed would be:

   405 µL x 1 X 1.05 = **425.25 µL** of each N1 or N2 reagent total.

   Using Table 10 below the individual component volumes would be:

   425.25 / 2.5 = **170.1 µL - RapiDxFire qPCR 5X Master Mix GF**

   425.25 / 33.3 = **12.8 µL - EpiScript RNase H- Reverse Transcriptase**

   425.25 / 50 = **8.5 µL - 2019-nCoV N1/RnP or N2/RnP blend**

   425.25 / 100 = **4.25 µL - SuperROX Reference Dye**

   425.25 – 170.1 – 12.8 – 8.5 – 4.25 = **229.6 µL - Molecular grade water**

	Stock concentration	Working 2X concentration	Volume (µL)
RapiDxFire qPCR 5X Master Mix GF[1]	5X	2X	=Total/2.5
EpiScript RNase H- Reverse Transcriptase[2]	200 U/µL	6 U/µL	=Total/33.3
2019-nCoV N1[3] or N2[4]/RnP blend	100X	2X	=Total/50
SuperROX Reference Dye[5]	15 µM	150 nM	=Total/100
Molecular grade water	-	-	=Total – sum of all components
		Total	xxxx

Table 17. Variable reagent volume calculations.

[1] Part numbers: 30050-1, 30050-2, or 30050-100ML
[2] Part numbers: ERT12925K-ENZ, ERT12925K-1.25ML or ERT12925K-12ML
[3] Part number: N1RNP-1010 – 2019-nCoV N1/RnP Blend
[4] Part number: N2RNP-1010 – 2019-nCoV N2/RnP Blend
[5] Part numbers: SR-1000-1 or SR-1000-10

	Stock concentration	Working 2X concentration	Volume (µL)
RapiDxFire qPCR 5X Master Mix GF	5X	2X	1701
EpiScript RNase H- Reverse Transcriptase	200 U/µL	6 U/µL	127.7
2019-nCoV N1/RnP blend	100X	2X	85.1
SuperROX Reference Dye	15 µM	150 nM	42.5
Molecular grade water	-	-	2296.2
		Total	4252.5

Table 18. N1/RnP reagent volumes for 10 x 384-well plate sample run.

	Stock concentration	Working 2X concentration	Volume (µL)
RapiDxFire qPCR 5X Master Mix GF	5X	2X	1701
EpiScript RNase H- Reverse Transcriptase	200 U/µL	6 U/µL	127.7
2019-nCoV N2/RnP blend	100X	2X	85.1
SuperROX Reference Dye	15 µM	150 nM	42.5
Molecular grade water	-	-	2296.2
		Total	4252.5

Table 19. N2/RnP reagent volumes for 10 x 384-well plate sample run.

6) Vortex to mix, briefly centrifuge, transfer 405 µL of the assay mixture into 0.75 mL matrix tubes (ThermoFisher – 4170) and seal with a SepraSeal cap (ThermoFisher – 4463). If running less than 384 samples, the assay mixture volume should be adjusted accordingly as described above. If assay mixture will not be immediately used, store on ice or at 4 °C until ready for use.
7) When ready to begin the IntelliQube run, place the matrix tubes containing the 2X PCR reagent mixture into the matrix rack (ThermoFisher – 4896) into the positions indicated by the IntelliQube protocol. The assay plate layout can be viewed in the Intellics software by clicking on the protocol in the protocols list and selecting "Quick Review" → "PlateSummary."

IMPORTANT: Always confirm proper placement of assay tubes based on the protocol prior to initiating a run. Improperly positioned reagents can lead to misclassification of sample and control results.

NOTE: As an alternative to matrix tubes and matrix rack, a 96-well plate (KBS-7001-131 or Greiner Bio-one 786201) may be used. The assay mixtures must be pipetted into the wells designated in the IntelliQube protocol. Plates should be sealed with an adhesive foil seal (KBS-0600-002) and stored on ice or at 4 °C until ready for use.

Creating an IntelliQube protocol from template:

1) Start by selecting protocol "EUA COVID TEMPLATE– OFFLINE" and selecting "Use as Template."
2) Check all the boxes shown in Figure 26 below and select "Create."

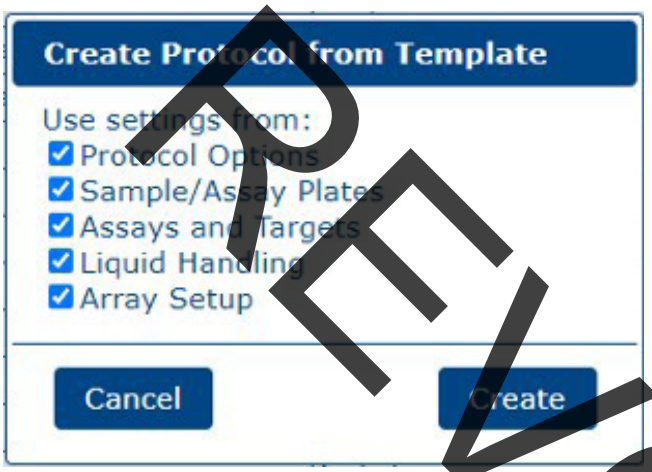

Figure 26. IntelliQube create protocol from template.

3) Edit the protocol name to reflect the run being performed and verify all settings match Figure 27 below. The Dye Calibration Set selected will be specific to each instrument and preset as a default.

Figure 27. IntelliQube end-point protocol setup screen.

4) Referencing Figure 28, import the sample plate information for the protocol. This can be done by selecting “Browse” and directing the software to the correct plateware .csv files.

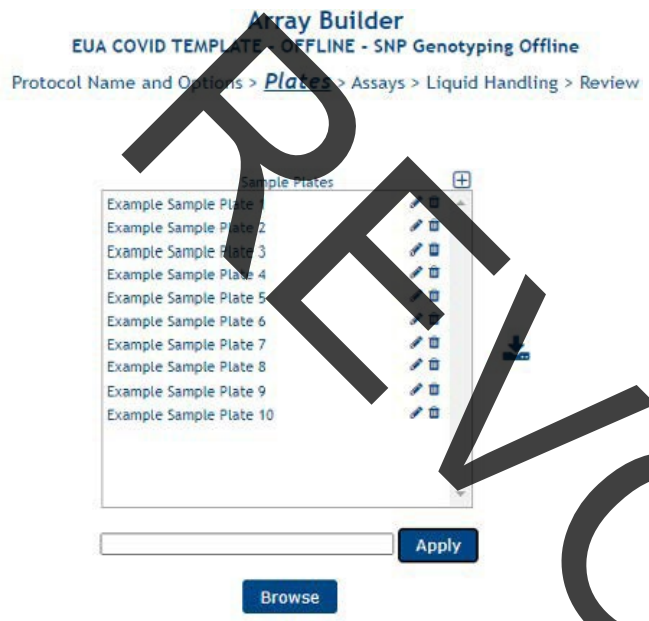

Figure 28. IntelliQube end-point sample plate import.

5) Verify assays 2019-nCoV N1/RnP and 2019-nCoV N2/RnP are included as shown in Figure 29.

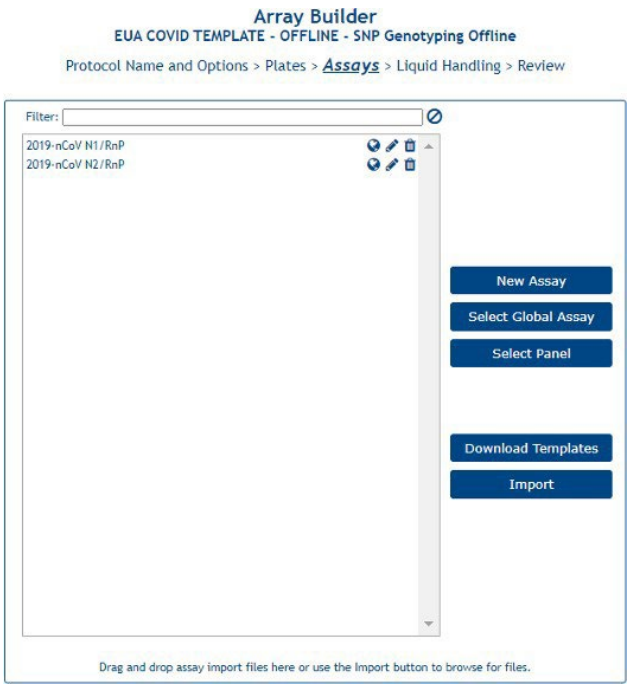

Figure 29. IntelliQube assay selection screen.

6) Using Figure 30 as a reference, select all sample plates by both assays and click the right arrow to create the combinations.

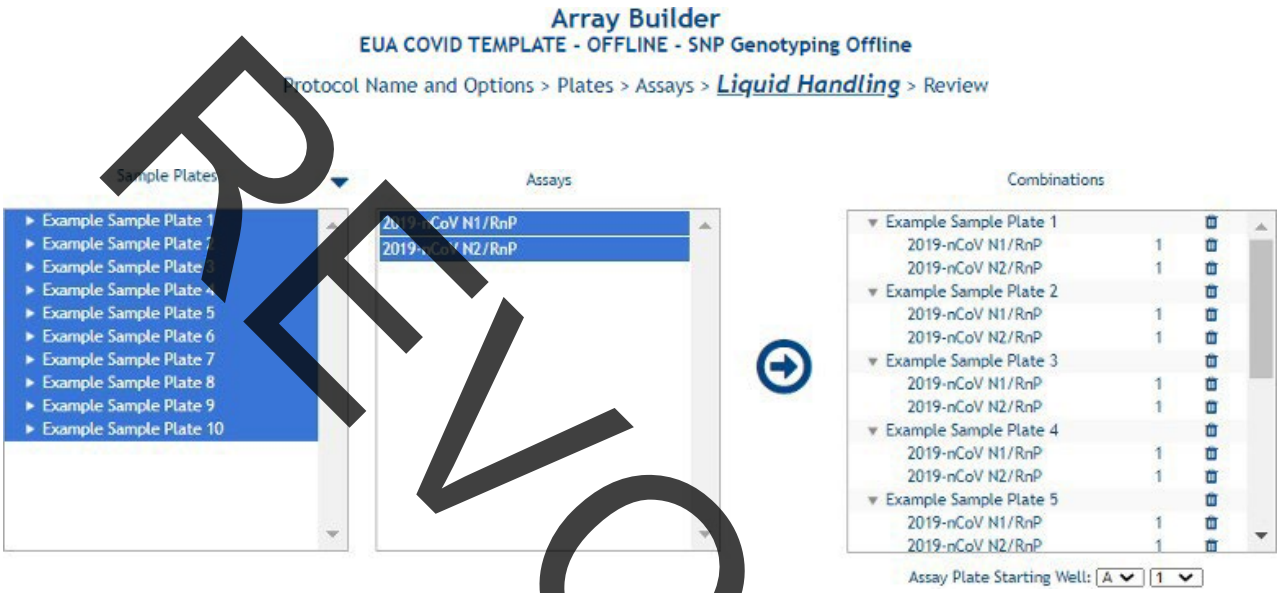

Figure 30. IntelliQube end-point sample/assay liquid handling.

7) Assign the assay plate barcode by typing or scanning the barcode into the provided space and selecting "Apply Barcode" as shown in Figure 31.

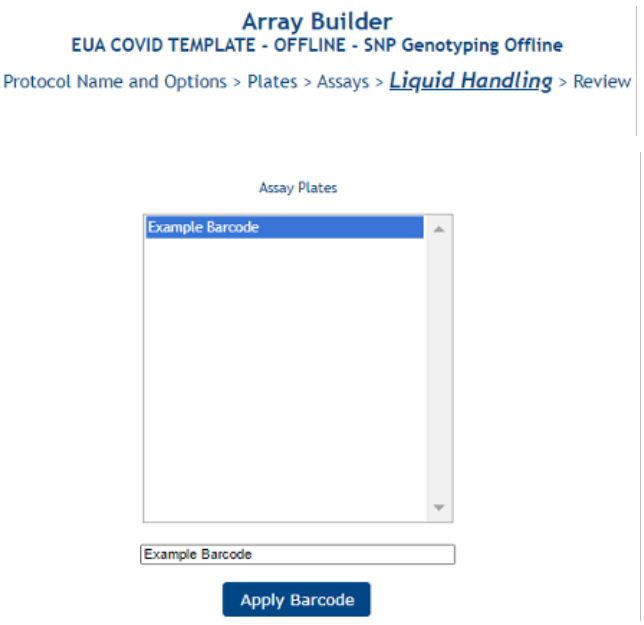

Figure 31. IntelliQube assay plate barcode definition.

8) Review the "Protocol Summary" to ensure protocol was created correctly. Figure 32 can be used as a reference.

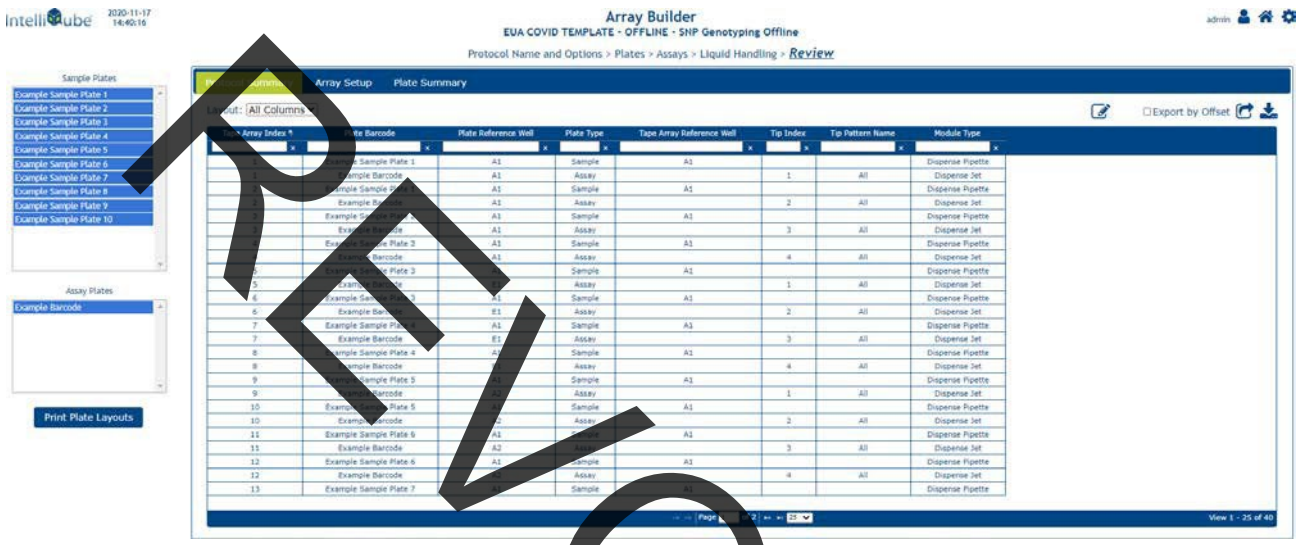

Figure 32. End-point protocol summary.

9) A layout of the sample and assay plates including required volumes can be viewed and printed by selecting the "Print Plate Layouts."
10) Select "Finish" to complete the protocol setup.

Loading the IntelliQube to begin the run:

1) If this is the first run of the day, perform all daily startup routine procedures.
2) Before each run, confirm there is an adequate supply of Array Tape (AXIT384-13WP050CC), cover seal (AX8591CVRTCC), sodium hypochlorite, and RO source water. If the waste line is not directly plumbed to a drain, confirm the waste carboy is empty.
3) It is recommended to replace the Dispense Pipette tips (AX843799 or AX840999) on a daily basis at a minimum.
4) The extracted 384-well RNA sample plates should be centrifuged at 2,500 × g for 1 min.
5) Each sample plate should be barcoded to match the barcodes specified in the IntelliQube protocol. The required barcodes for a given protocol are visible on the instrument HMI.
6) After carefully removing the plate seals, the sample plates can be placed in any location within the sample plate stacker.

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

7) The matrix tube rack containing the prepared 2X PCR reagent mixtures must also be barcoded to match the barcode specified in the IntelliQube protocol.
8) Place the matrix tube rack in the Plate Deck 1 position and remove the matrix tube caps using a decapper tool.
9) From the IntelliQube HMI, select the appropriate protocol from the list and hit "Run." A window will pop up informing that this is the dispensing portion of the two part protocol.
10) As the tape is being dispensed and beginning to exit the IntelliQube, attach the Array Tape to the rewind spool using a piece of tape.
11) After the IntelliQube has completed the dispensing portion of the protocol, detach the rewind spool from the IntelliQube.
12) Using a spare piece of empty Array Tape containing four arrays, wrap the outside of the dispensed arrays containing samples with the spare piece of tape.
13) Use two clips to secure the spare piece of tape holding the sample containing arrays in place on the spool.
14) Attach the spool containing the samples to the Hydrocycler^2 basket.
15) Insert the basket into the Hydrocycler^2.
16) Verify the EUA Thermal Profile is selected (Figure 33) in the Hydrocycler^2 software and select "Next."

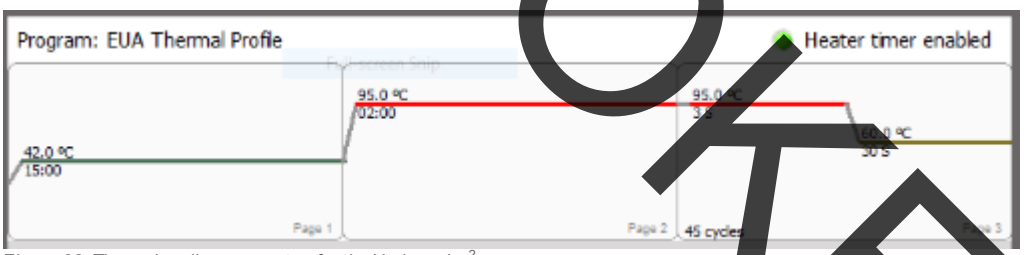

Figure 33. Thermal cycling parameters for the Hydrocycler^2.

17) Select "Run."
18) After completion of the thermal cycle protocol, remove the basket and detach the spool.
19) Unwind the arrays from the spool and dry with a paper towel.
20) Re-spool the dried Array Tape onto an empty Array Tape spool.
21) Insert the spool back into the IntelliQube.
22) From the IntelliQube HMI, select the appropriate protocol from the list and hit "Run." A window will pop up informing that this is the detection portion of the two part protocol.
23) Upon completion of the run, the dye cycle details data will be automatically exported and uploaded to FastFinder Analysis software.
24) Proceed to the next section for the interpretation of results using the end-point RT-PCR workflow.

Interpretation of results using the end-point RT-PCR workflow

This test utilizes the UgenTec FastFinder Analysis software to implement the threshold and decision logic described below.

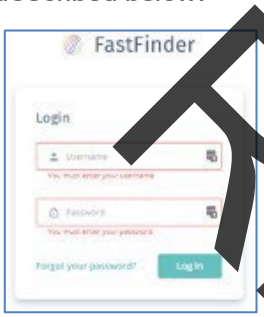

Figure 34. FastFinder login.

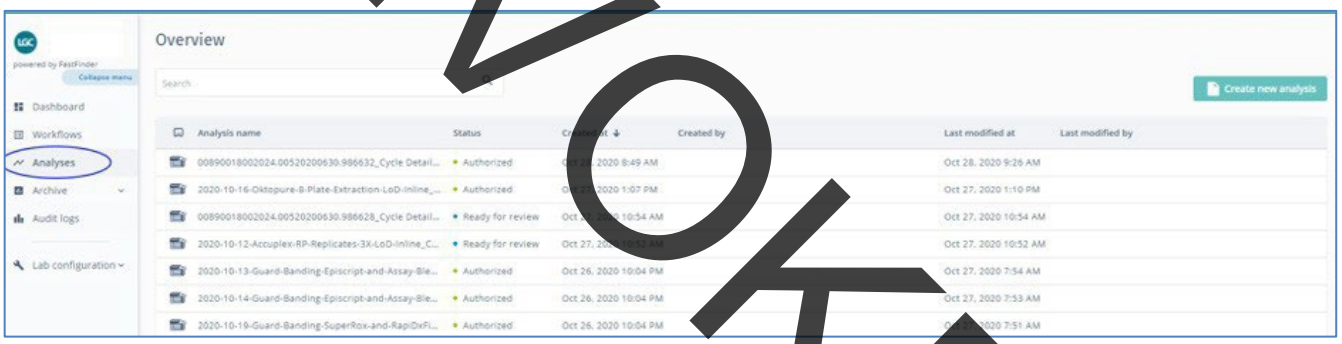

Figure 35. Analysis module.

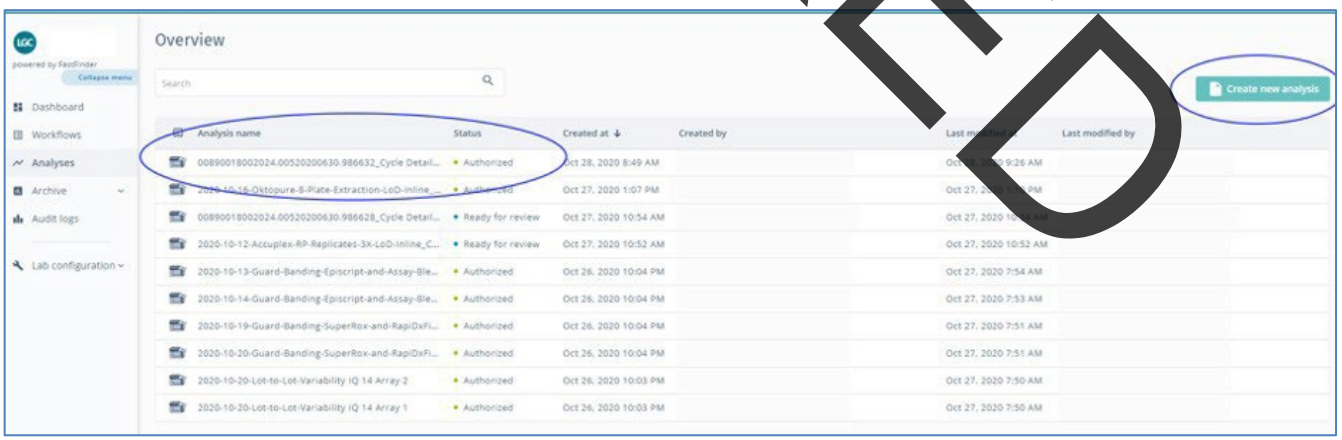

Figure 36. Import and view results.

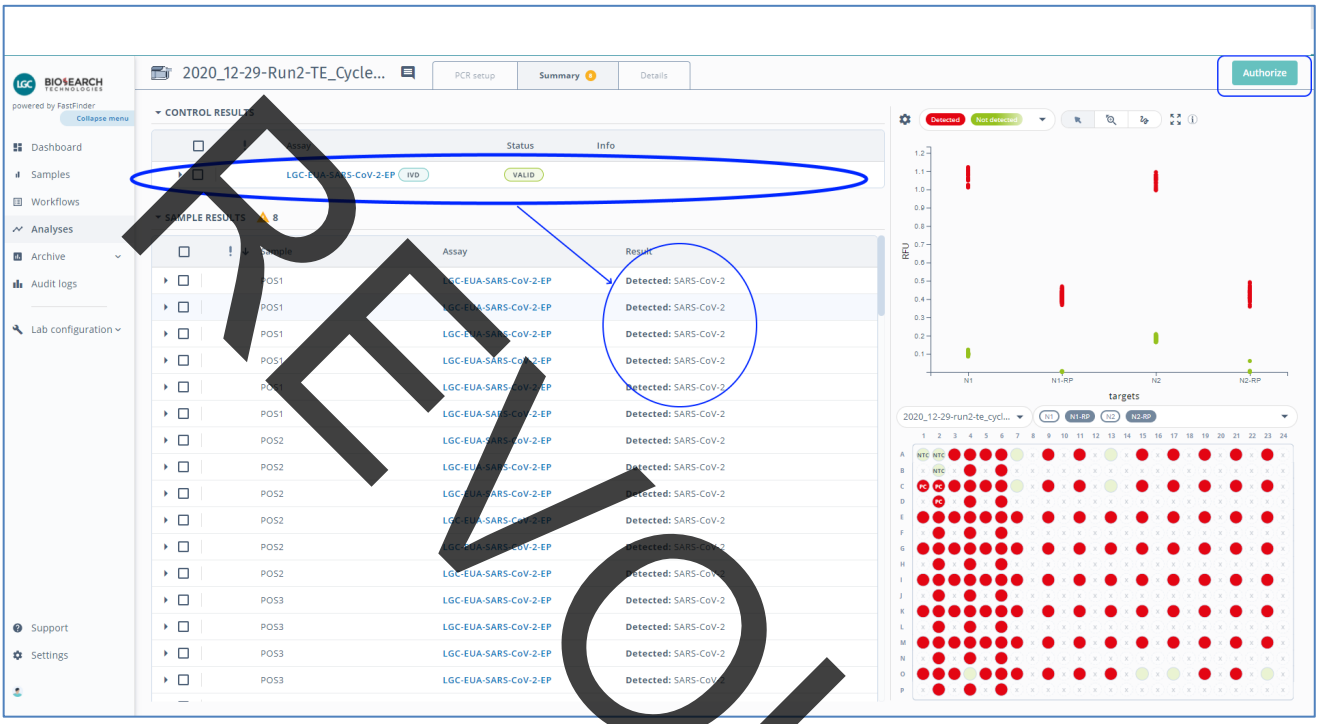

Figure 37. Review and authorize results.

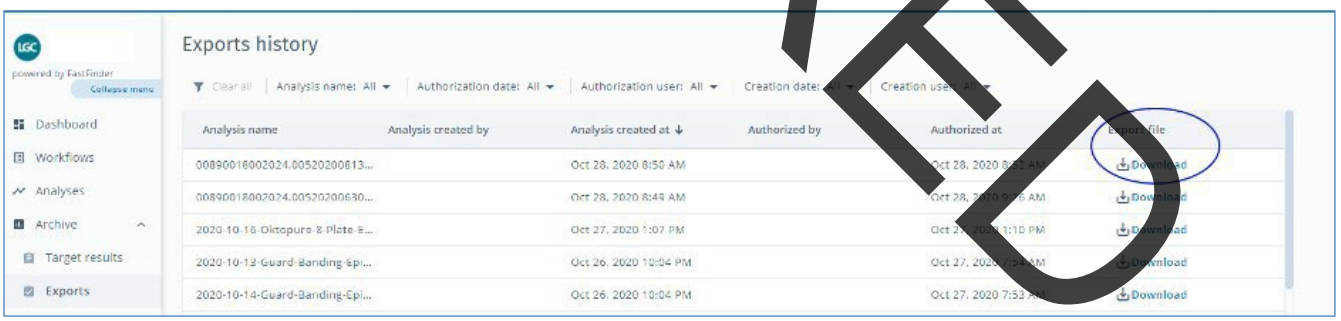

Figure 38. Export results.

	A	B	C	D	E	F	G	H	I	J	K	L	M
1	Sample ID	AssayResultTarget	AssayResultTargetCode	Outcome	IsInconclu	IsInvalid	IsRetest	AssayResu	SampleTy	Filename(	Barcode(s	Instrumer	Analysis
2	1055385714	SARS-CoV-2	SARS-CoV-2	Not detected	FALSE	FALSE	FALSE		Regular	00890018(	00890018(	"IQ520014	2020-10-2
3	1055406342	SARS-CoV-2	SARS-CoV-2	Not detected	FALSE	FALSE	FALSE		Regular	00890018(	00890018(	"IQ520014	2020-10-2
4	1055412246	SARS-CoV-2	SARS-CoV-2	Not detected	FALSE	FALSE	FALSE		Regular	00890018(	00890018(	"IQ520014	2020-10-2
5	1055475312	SARS-CoV-2	SARS-CoV-2	Not detected	FALSE	FALSE	FALSE		Regular	00890018(	00890018(	"IQ520014	2020-10-2
6	1055476049	SARS-CoV-2	SARS-CoV-2	Not detected	FALSE	FALSE	FALSE		Regular	00890018(	00890018(	"IQ520014	2020-10-2
7	1055477048	SARS-CoV-2	SARS-CoV-2	Not detected	FALSE	FALSE	FALSE		Regular	00890018(	00890018(	"IQ520014	2020-10-2
8	1055479328	SARS-CoV-2	SARS-CoV-2	Not detected	FALSE	FALSE	FALSE		Regular	00890018(	00890018(	"IQ520014	2020-10-2
9	1055486077	SARS-CoV-2	SARS-CoV-2	Not detected	FALSE	FALSE	FALSE		Regular	00890018(	00890018(	"IQ520014	2020-10-2
10	1055662067	SARS-CoV-2	SARS-CoV-2	Not detected	FALSE	FALSE	FALSE		Regular	00890018(	00890018(	"IQ520014	2020-10-2
11	1055670422	SARS-CoV-2	SARS-CoV-2	Not detected	FALSE	FALSE	FALSE		Regular	00890018(	00890018(	"IQ520014	2020-10-2
12	1055750465	SARS-CoV-2	SARS-CoV-2	Not detected	FALSE	FALSE	FALSE		Regular	00890018(	00890018(	"IQ520014	2020-10-2
13	1055772600	SARS-CoV-2	SARS-CoV-2	Not detected	FALSE	FALSE	FALSE		Regular	00890018(	00890018(	"IQ520014	2020-10-2
14	1055780482	SARS-CoV-2	SARS-CoV-2	Void	TRUE	FALSE	FALSE		Regular	00890018(	00890018(	"IQ520014	2020-10-2
15	1055790729	SARS-CoV-2	SARS-CoV-2	Not detected	FALSE	FALSE	FALSE		Regular	00890018(	00890018(	"IQ520014	2020-10-2
16	1056532107	SARS-CoV-2	SARS-CoV-2	Detected	FALSE	FALSE	FALSE		Regular	00890018(	00890018(	"IQ520014	2020-10-2
17	1056724448	SARS-CoV-2	SARS-CoV-2	Detected	FALSE	FALSE	FALSE		Regular	00890018(	00890018(	"IQ520014	2020-10-2
18	1056794001	SARS-CoV-2	SARS-CoV-2	Detected	FALSE	FALSE	FALSE		Regular	00890018(	00890018(	"IQ520014	2020-10-2
19	1056852849	SARS-CoV-2	SARS-CoV-2	Detected	FALSE	FALSE	FALSE		Regular	00890018(	00890018(	"IQ520014	2020-10-2
20	1057124563	SARS-CoV-2	SARS-CoV-2	Void	TRUE	FALSE	FALSE		Regular	00890018(	00890018(	"IQ520014	2020-10-2
21	1057548346	SARS-CoV-2	SARS-CoV-2	Detected	FALSE	FALSE	FALSE		Regular	00890018(	00890018(	"IQ520014	2020-10-2

Figure 39. Example csv results export file.

The test utilizes a control scheme that requires the addition of one positive and one negative control into each 96-well sample source plate. The plates are processed on the oKtopure as four separate 96-well sample plates that are combined into a single 384-well elution plate. The IntelliQube end-point RT-PCR process generates fluorescence data from the amplification of the targets of interest. The fluorescence values are exported to the FastFinder Analysis software. Validation of the results is performed automatically by the FastFinder Analysis software based on performance of the Positive and Negative Controls, the passive reference dye, presence of the RNase P target and concordance of the N1 and N2 targets. The outcomes generated can take the values of "Detected," "Not Detected" or "Void" in the case where one or more of the target results were invalid or inconclusive.

End-point: Control well individual target interpretation

Target	+ Present	- Not present	? Inconclusive
Passive Reference Dye	RFU >= 0.2	RFU <0.2	N/A
N1	Part of positive cluster	Part of negative cluster	Not part of either cluster
N2	Part of positive cluster	Part of negative cluster	
N1-RP and N2-RP	Part of positive cluster	Part of negative cluster	

Table 20. Interpretation of individual target results for the PC and NTC controls.

Positive and Negative controls are examined by the FastFinder analysis software and are evaluated against the following control scenarios shown in Table 21 prior to interpretation of unknown clinical results. Failed control wells are automatically flagged in the FastFinder analysis software. If any of the included controls are not marked as Valid, the corresponding patient results from the same source plate cannot be interpreted and will be marked as "Void" by default with a warning message related to the type of failure. Positive and Negative Control wells must be run for each 96-well sample extraction plate that is represented in analysis. Each control well corresponding to a 96-well source plate must pass for the samples analyzed with that plate to be considered valid. If there are failed control wells, the corresponding source plates can be removed as described below in order to complete the analysis for samples with valid controls. End-point cluster calling is defined by the UgenTec FastFinder software scoring algorithms.

Control	Scenario	N1	N1-RP	N2	N2-RP	Pass Ref [1]	Control results	Outcome for real-time or end-point clinical sample	Suggested user action
Positive Control (PC)	All N gene and RNase P targets detected	+	+	+	+	+	Valid	As scored by clinical sample decision logic	Report result. Option to mark any sample for re-test as needed.
	Any N gene or RNase P target not detected	?	?	?	?	?	Invalid	All wells marked as "Invalid Assay Controls"	Repeat test by re-extracting the original samples and repeating the RT-PCR
No Template Control (NTC)	All N gene and RNase P targets not detected	-	-	-	-	+	Valid	As scored by clinical sample decision logic	Report result. Option to mark any sample for re-test as needed.
	Any N gene or RNase P target detected,	?	?	?	?	?	Invalid	All wells marked as "Invalid Assay Controls"	Repeat test by re-extracting the original samples and repeating the RT-PCR

Table 21. Interpretation of PC and NTC control results.
[1] If the passive reference fails, the test can be repeated from the RT-PCR stage without re-extracting the samples.

For any 96-well source plate that contains a control failure, it is possible in the FastFinder application to manually omit this source plate from analysis through the PCR setup tab inside the Analysis module such that only valid source plates are used for analysis and generation of results. This allows for valid results to still be analyzed while invalid results are omitted from analysis. The procedure is as follows:

1) Identify the source plate where the control failure is present. (Figure 40,41)
2) Remove the selected assignment of wells from the PCR setup tab. (Figure 42,43,44)
3) Reanalyze the results. (Figure 45,46)
4) Review and authorize the final result containing only the source plates with valid controls. (Figure 47)

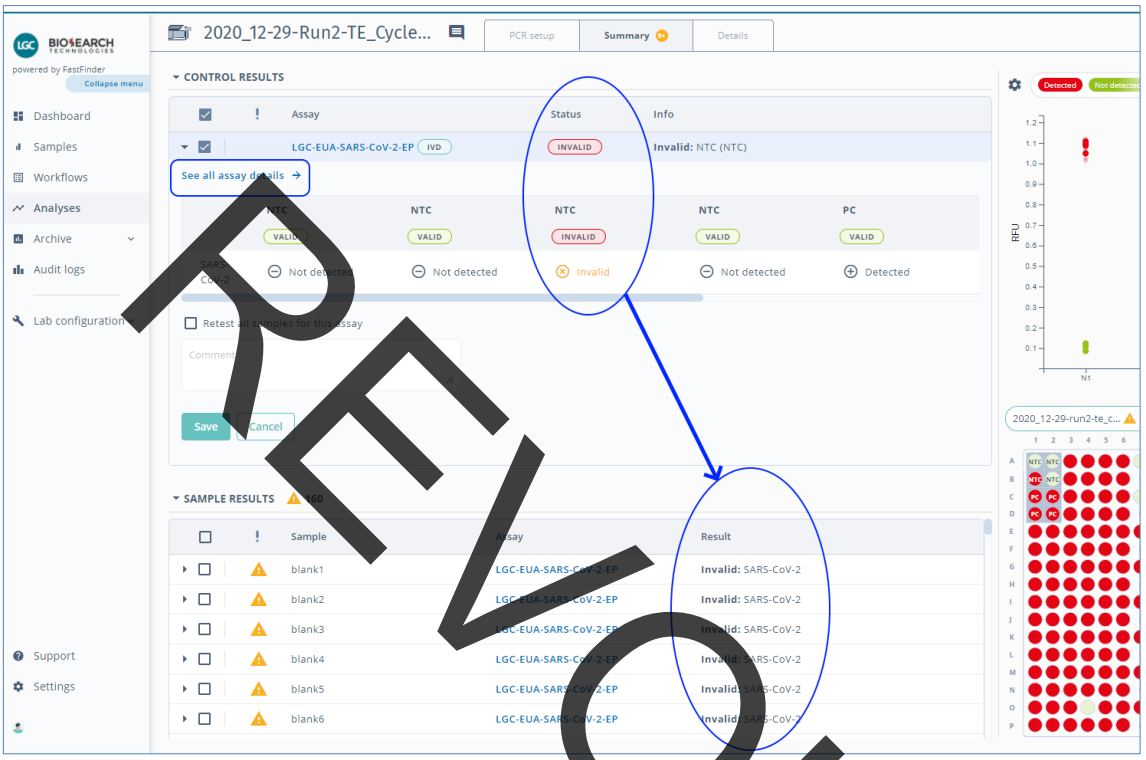

Figure 40. Identify control failures and select assay details in the analysis summary tab.

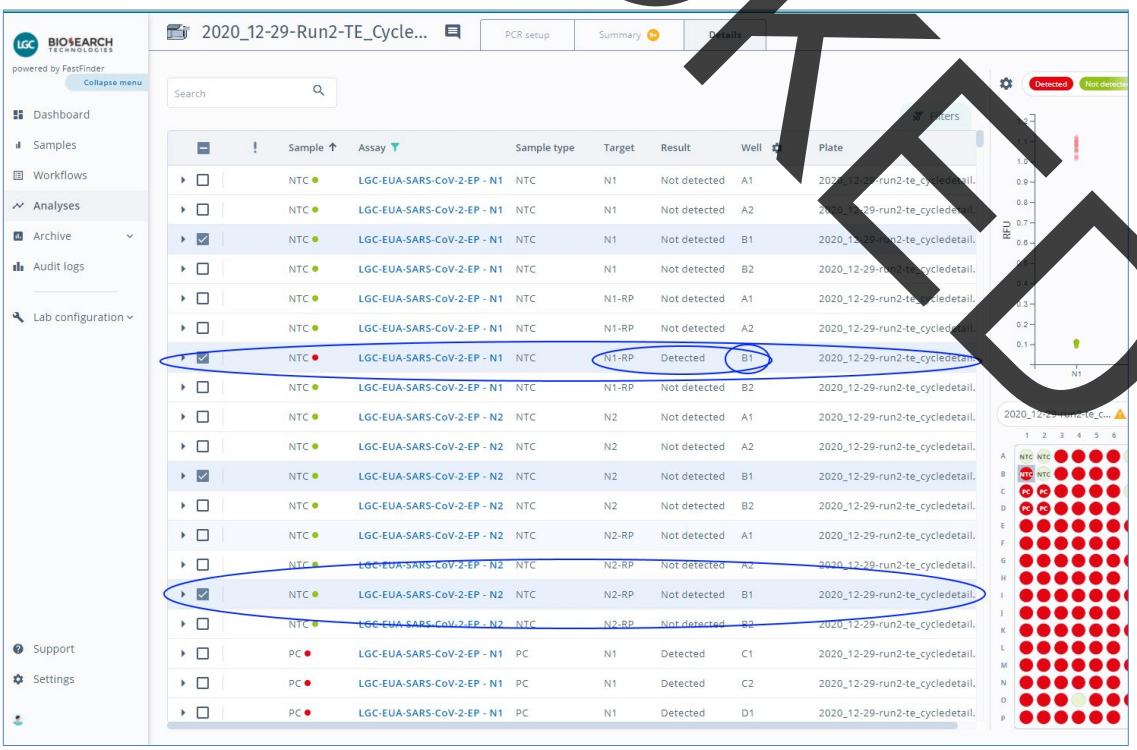

Figure 41. Identify the control failure well location in the details tab.

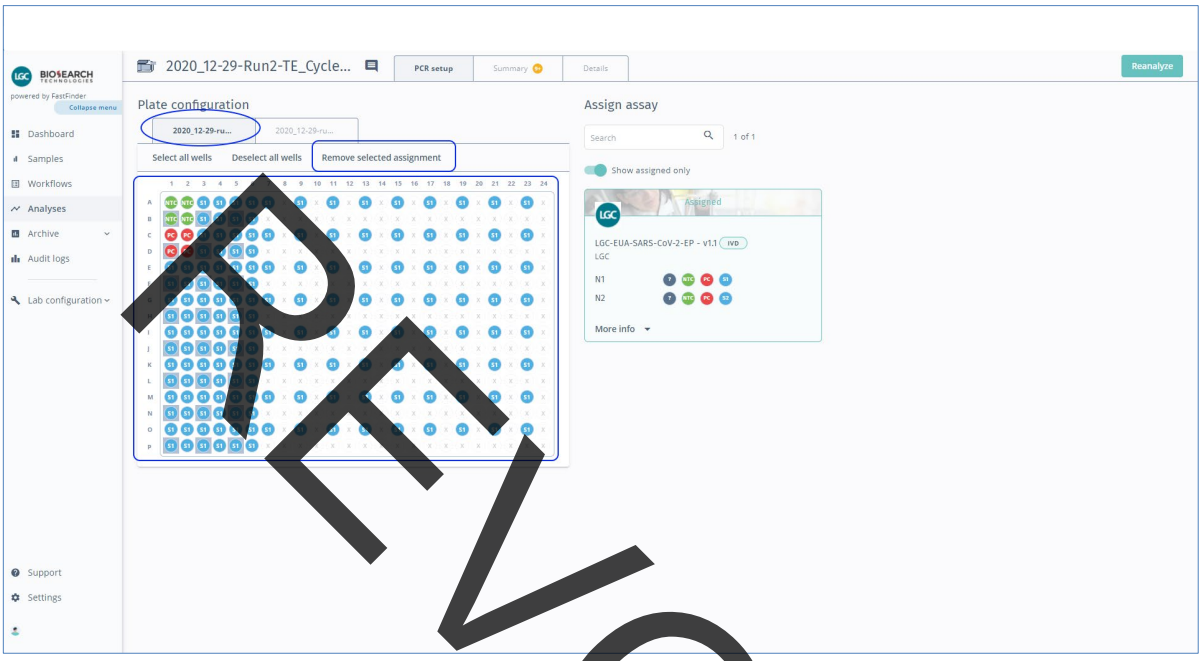

Figure 42. Select and remove assignment for N1 wells in PCR setup tab.

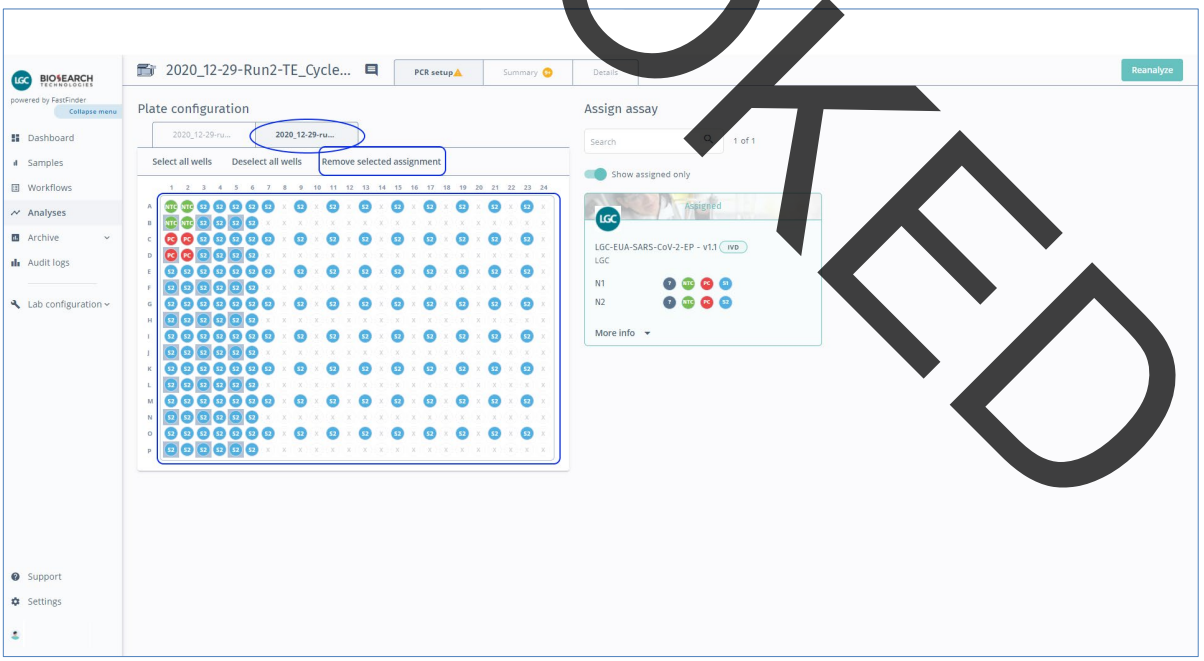

Figure 43. Select and remove assignment for N2 wells in PCR setup tab.

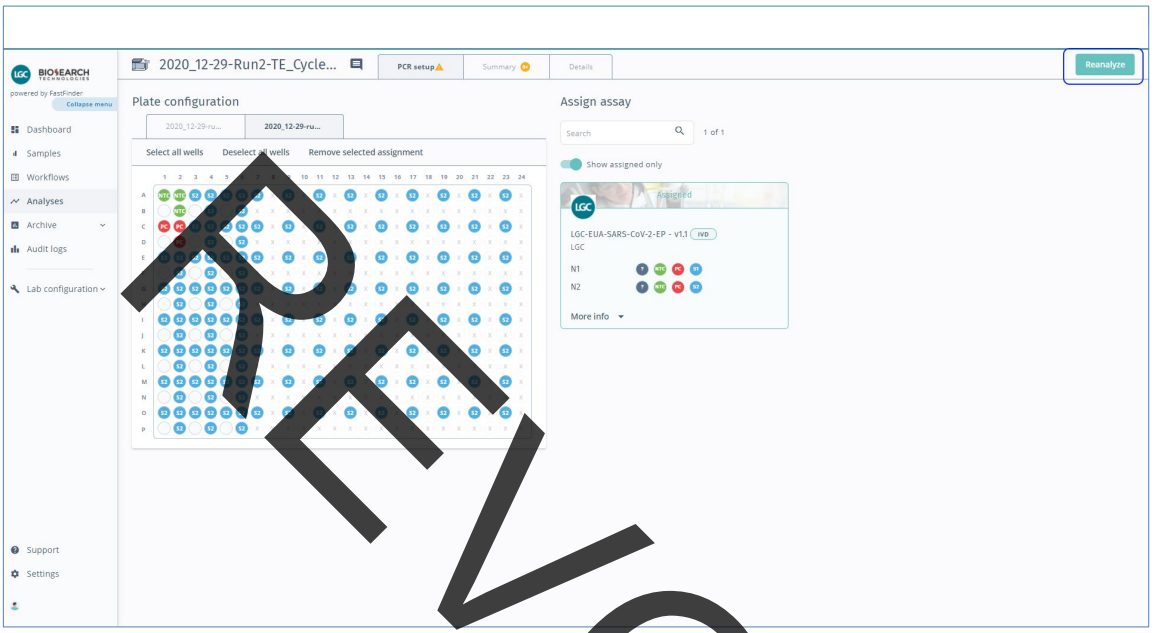

Figure 44. Reanalyze after removal of all wells associated with the specific source plate.

After all controls included in analysis have been identified as Valid, the analysis pipeline evaluates the results of the individual patient samples and assigns an overall call of "Detected," "Not Detected" or "Void" to each sample set per the Positive/Negative calling threshold and decision logic.

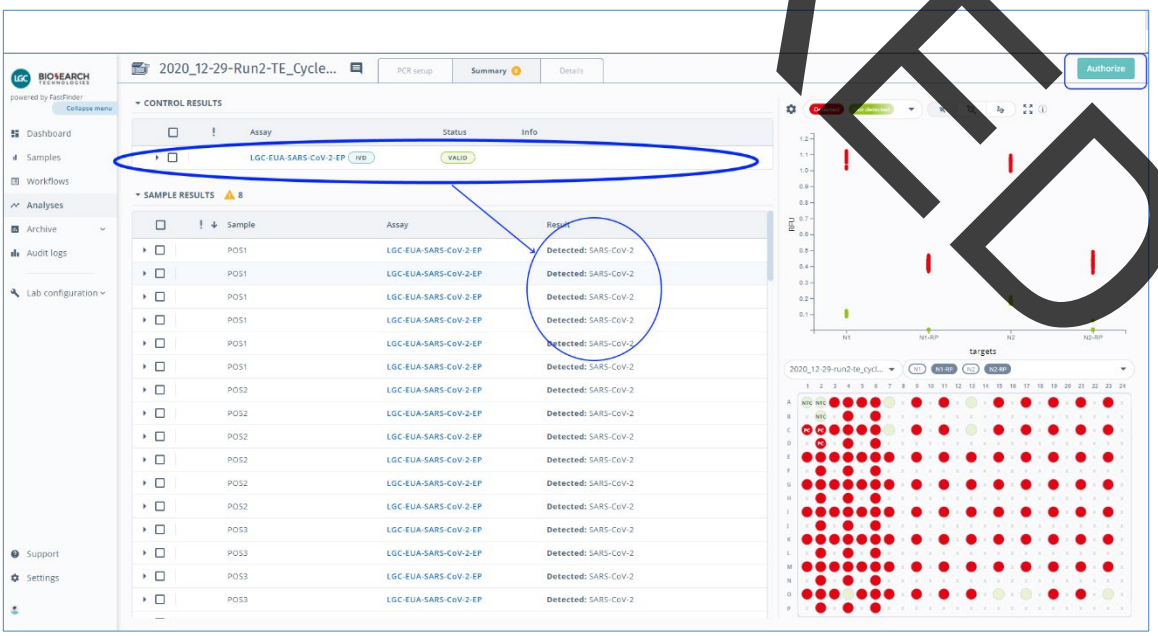

Figure 45. Review and authorize sample results.

End-point: Clinical sample individual target interpretation			
Target	**+ "Positive"**	**- "Negative"**	**? Inconclusive**
Passive Reference	RFU >= 0.2	RFU <0.2	N/A
N1	Part of positive cluster	Part of negative cluster	Not in positive or negative cluster
N2			
N1-RP and N2-RP			

Table 22. Interpretation of clinical sample individual target results.

Clinical samples are examined by the FastFinder Analysis software and results from Passive Reference Dyes and combinations of N1, N1_RP, N2, and N2_RP individual target results are automatically combined and evaluated against the following decision logic shown in Table 23 to define the clinical result. If any result other than "Detected" or "Not Detected" is determined for a given sample, the overall call for the clinical sample will be "Void" and it is recommended to repeat the test by re-extracting the original sample and repeating the RT-PCR. End-point cluster calling is defined by the UgenTec FastFinder software scoring algorithms.

Scenario	Passive Ref N1	N1	N1_RP	Passive Ref N2	N2	N2_RP	Outcome	Assay result (User Interface)	Message	User action
Negative sample or concentration below LoD	+	-	+	+	-	+	Not detected	SARS-CoV-2 not detected	N/A	Report results
Positive sample above LoD	+	+	+	+	+	+	Detected	SARS-CoV-2 detected	N/A	Report results
RNase P amplification absent, but both N1 and N2 are present.	+	+	-	+	+	-	Detected	SARS-CoV-2 detected	Extraction control not detected	Report results
N1 or N2 are present, but not both, or, any other scenario not represented above	?	?	?	?	?	?	Void	Inconclusive	Various	Repeat test by re-extracting the original samples and repeating the RT-PCR[1]

Table 23. Clinical sample end-point decision logic.

[1] A single inconclusive result may be evaluated by re-extracting the original sample and repeating RT-PCR. If a second inconclusive result is obtained, the result should be reported as "Inconclusive" and a request to recollect sample should be made.

Limitations

- The use of this assay as an *in vitro* diagnostic under the FDA Emergency Use Authorization (EUA) is limited to laboratories that are certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. § 263a, to perform high complexity tests.
- The SARS-CoV-2 Real-Time and End-Point RT-PCR Test may only be performed using the oKtopure automated extraction instrument, as well as the IntelliQube and/or Hydrocycler^2 qPCR instruments using clinical specimens that have been collected as per testing lab procedures or following vendor instructions.
- The performance of the SARS-CoV-2 Real-Time and End-Point RT-PCR Test was established using archived nasopharyngeal swab specimens. Anterior or mid-turbinate nasal swabs, nasopharyngeal swabs, oropharyngeal swabs and nasopharyngeal wash/aspirates or nasal aspirates are also considered acceptable specimen types for use with the SARS-CoV-2 Real-Time and End-Point RT-PCR Test.

- 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.

- Validation studies for non-saliva respiratory specimens were performed using BD Universal Viral Transport media (www.bd.com, phone number 201-847-6800) and UTM (Copan Universal Transport Medium, www.copanusa.com, phone number: (800)-216-4016). Compatibility with other specimen collection media and/or transport media has not been evaluated. Use of this assay is limited to personnel who have been trained in the procedure. Failure to follow the instructions provided in this package insert may cause erroneous results.

- Reliable results are dependent on adequate specimen collection. Because the collection and transport system does not allow for microscopic assessment of specimen adequacy, training of clinicians in proper specimen collection techniques is necessary.

- Careful compliance with the instructions in this package insert is necessary to avoid erroneous results.

Conditions of Authorization for the Laboratory

To assist clinical laboratories running the SARS-CoV-2 Real-Time and End-Point RT-PCR Test, the relevant Conditions of Authorization are listed below:

A. Authorized laboratories[1] using the SARS-CoV-2 Real-Time and End-Point RT-PCR Test 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.

B. Authorized laboratories using the SARS-CoV-2 Real-Time and End-Point RT-PCR Test must use the SARS-CoV-2 Real-Time and End-Point RT-PCR 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 SARS-CoV-2 Real-Time and End-Point RT-PCR Test are not permitted.

C. Authorized laboratories that receive the SARS-CoV-2 Real-Time and End-Point RT-PCR Test must notify the relevant public health authorities of their intent to run the test prior to initiating testing.

D. Authorized laboratories using the SARS-CoV-2 Real-Time and End-Point RT-PCR 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 SARS-CoV-2 Real-Time and End-Point RT-PCR Test and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and LGC, Biosearch Technologies (techsupport@lgcgroup.com) 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 test must be appropriately trained in PCR techniques and use appropriate laboratory and personal protective equipment when handling this kit and use the test in accordance with the authorized labeling.

G. LGC, Biosearch Technologies, its authorized distributor(s) and authorized laboratories using the SARS-CoV-2 Real-Time and End-Point RT-PCR 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.

[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."

Performance evaluation

Analytical sensitivity

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.

To determine the LoD, recombinant virus containing a full length SARS-CoV-2 RNA (LGC SeraCare, AccuPlex SARS-CoV-2, Cat: 0505-0168, 100,000 copies/mL stock as determined by reverse transcription digital PCR) was diluted in pooled nasopharyngeal swab specimen matrix previously confirmed to be negative by another FDA-authorized assay. The final LoD was confirmed by testing 5 panel members with target concentrations at 10,000, 7,500, 5,000, 2,500 and 1,250 copies/mL, determined prior to extraction, tested in replicates of 40. The results are summarized in Table 24 and Table 25. The lowest concentration level with observed positive rates ≥ 95% for both the N1 and N2 targets was 5,000 viral genome copies/mL for the real-time workflow and 5,000 viral genome copies/mL for the end-point workflow.

Sample	Concentration (copies/mL)	Total replicates	Positive rate (%)			Mean Cq	
			N1	N2	N1 and N2	N1	N2
AccuPlex SARS-CoV-2	10,000	40	100%	100%	100%	29.36	29.06
	7,500	40	100%	100%	100%	29.60	29.33
	5,000	40	100%	100%	100%	29.99	29.71
	2,500	40	95%	97.5%	92.5%	31.13	30.92
	1,250	40	72.5%	75%	55%	32.11	31.62
	0 (blank)	40	0%	0%	0%	-	-

Table 24. LoD determination with the real-time workflow.

Sample	Concentration (copies/mL)	Total replicates	Positive rate (%)		
			N1	N2	N1 and N2
AccuPlex SARS-CoV-2	10,000	40	100%	100%	100%
	7,500	40	100%	100%	100%
	5,000	40	100%	100%	100%
	2,500	40	82.5%	100%	82.5%
	1,250	40	77.5%	90%	72.5%
	0 (blank)	40	0%	0%	0%

Table 25: LoD determination with the end-point workflow.

Inclusivity (analytical sensitivity) and Cross-reactivity (analytical specificity)

The Biosearch Technologies SARS-CoV-2 Real-Time and End-Point RT-PCR Test uses the same primers and probes for the N1 and N2 regions of the nucleocapsid gene as the CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel, manufactured by Biosearch Technologies. *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. No additional laboratory testing to evaluate inclusivity and analytical specificity was performed with the Biosearch Technologies SARS-CoV-2 Real-Time and End-Point RT-PCR Test.

Clinical evaluation

A clinical evaluation study was performed to evaluate performance of the Biosearch Technologies SARS-CoV-2 Real-Time and End-Point RT-PCR Test using upper respiratory specimens including nasopharyngeal, mid-turbinate, and anterior nares swabs (banked and acquired from a clinical laboratory). A total of 128 individual clinical specimens (91 were nasopharyngeal and 47 were mid-turbinate and anterior nares samples) were analyzed using the Biosearch Technologies SARS-CoV-2 Real-Time and End-Point RT-PCR Test and an FDA-authorized comparator assay. Positive Percent Agreement (PPA) was 100.0% for both specimen types for both real-time and end-point workflows, and the Negative Percent Agreement (NPA) was 100.0% for both real-time and end-point workflows. Real-time results are summarized in Tables 26 and 27, and end-point results are summarized in Tables 28 and 29.

Biosearch Technologies SARS-CoV-2 End-Point RT-PCR Test	FDA-authorized Comparator Assay		
	Positive	Negative	Total
Positive	39	0	39
Inconclusive	1[1]	0	1
Negative	0	51	51
Total	40	51	91
PPA	100.0% (91.0% - 100.0%)		
NPA	100.0% (93.0% - 100.0%)		
Inconclusive Rate	1.1% (1/91)		

Table 26. Real-Time Clinical Performance – Nasopharyngeal (NP) Swab Samples.
[1] Samples with repeat inconclusive results were excluded from the calculation of PPA and NPA.

Biosearch Technologies SARS-CoV-2 Real-Time RT-PCR Test	FDA-authorized Comparator Assay		
	Positive	Negative	Total
Positive	24	0	24
Inconclusive	0	0	0
Negative	0	13	13
Total	24	13	37
PPA	100.0% (86.2% - 100.0%)		
NPA	100.0% (77.2% - 100.0%)		

Table 27. Real-Time Clinical Performance – Mid-turbinate and Anterior Nasal Swab Specimens

Biosearch Technologies SARS-CoV-2 End-Point RT-PCR Test	FDA-authorized Comparator Assay		
	Positive	Negative	Total
Positive	40	0	40
Inconclusive	0	0	0
Negative	0	51	51
Total	40	51	91
PPA	100.0% (91.2% - 100.0%)		
NPA	100.0% (93.0% - 100.0%)		

Table 28. End-point Clinical Performance - Nasopharyngeal (NP) Swab Samples

Biosearch Technologies SARS-CoV-2 End-Point RT-PCR Test	FDA-authorized Comparator Assay		
	Positive	Negative	Total
Positive	24	0	24
Inconclusive	0	0	0
Negative	0	13	13
Total	24	13	37
PPA	100.0% (86.2% - 100.0%)		
NPA	100.0% (77.2% - 100.0%)		

Table 29. End-point Clinical Performance – Mid-turbinate and Anterior Nasal Swab Specimens

Summary of changes

Revision Doc ID number	Description of change	Date
GEN/861/SW/1120	Initial release. Notification to FDA of completion of clinical validation.	November 2020
GEN/861/SW/1120/v2/02082021	Version 2 release. FDA review.	Feb 8, 2021
GEN/861/SW/1120/v3/03242021	Version 3 release. FDA authorization.	March 24, 2021
GEN/861/SW/1120/v4/05032022	Version 4 release. Update limitations section and FDA review.	May 3, 2022

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