Clear Labs

Instructions for Use

Rx only

For use under Emergency Use Authorization (EUA) Only

For *In Vitro* Diagnostic Use

REF

Clear Dx™ SARS-CoV-2 Test

For use with Clear Dx™ System Only

IVD

	Clear Labs 1559 Industrial Rd, San Carlos, CA 94070 USA

Table of Contents

Contents

General Information

Intended Use

Clear Dx™ SARS-CoV-2 Test is a multiplexed RT-PCR and next-generation DNA sequencing (NGS) *in vitro* diagnostic test on the Oxford Nanopore GridION Sequencer intended for the qualitative detection of nucleic acid from SARS-CoV-2 in nasopharyngeal swab, oropharyngeal swab, anterior nasal swab, mid-turbinate nasal swab, nasopharyngeal wash/aspirate, nasal aspirate, and bronchoalveolar lavage specimens from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to laboratories that are 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 nasopharyngeal swab, oropharyngeal swab, anterior nasal swab, mid-turbinate nasal swab, nasopharyngeal wash/aspirate, nasal aspirate, and bronchoalveolar lavage 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 Clear Dx™ SARS-CoV-2 Test is intended for use by qualified clinical laboratory personnel specifically instructed and trained on the Clear Dx™ system, in the techniques of real-time PCR and NGS, and in vitro diagnostic procedures. The Clear Dx™ SARS-CoV-2 Test is only for use under the Food and Drug Administration's Emergency Use Authorization.

Summary and Explanation of Test

Coronavirus disease 2019 (COVID-19) is a severe respiratory disease that was initially identified in Wuhan, China but has become a global pandemic since then. The virus that causes COVID-19 is designated as Severe Respiratory Syndrome Coronavirus 2 (SARS CoV-2); previously, it was referred to as 2019 Novel Coronavirus (2019 nCoV). SARS-CoV-2 belongs to the same family of betacoronaviruses as that of SARS CoV and MERS CoV that have caused epidemics in humans in 2003 and 2012 respectively. All these betacoronaviruses are enveloped, positive-sense, single-stranded RNA viruses

with relatively large genome sizes that bind to specific receptors on the host cell and cause respiratory illnesses.

The Clear Dx™ SARS-CoV-2 Test is a targeted next-generation sequencing test intended for detection of SARS-CoV-2 virus RNA under FDA Emergency Use Authorization. This test is fully automated to run on the Clear Dx™ system and can process up to 192 samples in a run including positive and negative run controls. The automated test goes from extracted RNA from clinical respiratory specimens to a full report with no human intervention.

Principles of the Procedure

The entire workflow for the detection of SARS-CoV-2 RNA includes multiple steps - specimen collection from patients, extraction of RNA from the clinical specimens and subsequently testing for the presence of SARS CoV-2. Clear Dx™ SARS-CoV-2 Test, which includes the targeted NGS-based assay using the extracted RNA, liquid handling steps, bioinformatic analysis and reporting, is fully automated on the Clear Dx™ system.

The Clear Dx™ system (illustrated in Figure 1) uses a Hamilton STAR robotic platform for automation of liquid handling and includes all the required ancillary equipment, such as thermocyclers, barcode reader, and magnet block, needed for the test. The system also houses the GridION nanopore sequencer developed by Oxford Nanopore Technologies (ONT).

Figure 1. Clear Dx™, a fully automated platform from RNA to results

An overview of the different steps involved in the test is illustrated in the figure below.

Automated workflow of the Clear Dx™ SARS-CoV-2 Test

RNA Extraction:

The Clear Dx™ SARS-CoV-2 Test starts with RNA (in elution buffer), extracted from respiratory specimens, either using MagMAX Viral RNA Isolation Kit (catalog # AM1939) through the manual workflow or using MagMAX Viral/Pathogen Nucleic Acid Isolation Kit (Catalog # A42352 from Thermo Fisher Scientific) through the automated workflow on KingFisher Flex Purification system with 96-Deep well head (Catalog # 5400630 from Thermo Fisher Scientific), as recommended by the manufacturer. These kits and extraction apparatuses are not provided with the Clear Dx SARS-CoV-2 test kit.

Assay & Bioinformatics workflow:

The automated Clear Dx™ SARS-CoV-2 Test starts with synthesizing cDNA from the extracted RNA for each of the samples loaded into the well plates in independent reverse transcription reactions. Then the viral target amplicons are captured from the synthesized cDNA through a multiplex PCR process using a panel of barcoded target capture primers. After this "Target-capture" PCR step, there is a Solid Phase Reversible Immobilization (SPRI) bead-based cleanup during which all the excess primers and any short amplification products are removed. Following this, the amplicons are subjected to another round of PCR, termed as "Barcoding PCR", where the second set of barcodes are added to the amplicons using the rapid library primers from ONT. Following this step, the dual barcoded amplicons from all the samples are pooled together and cleaned up through another SPRI bead process. After this step, the ONT sequencing adapters are ligated to all the barcoded amplicons and then sequenced on the GridION sequencer.

Our bioinformatics pipeline, Clear Dx™ BIP (version Dv9.0) performs a series of steps including demultiplexing, error correction and alignments on the sequencing reads of the amplicons to make the detection calls. The SARS-CoV-2 detection algorithm takes the relative ratios of the sequencing signal for SARS-CoV-2 primers, the internal PCR control, and the human housekeeping gene into account to make an invalid/positive/negative call. Samples with insufficient total read coverage are classified as invalid. The remaining samples have their SARS-CoV-2 signal compared to empirically derived thresholds. These thresholds distinguish true SARS-CoV-2 signal from noise. Each primer has its own threshold and the pipeline leverages the redundancy of SARS-CoV-2 specific primers to make a call.

Warnings and Precautions

- ✓ For *in vitro* diagnostic use
- ✓ For prescription use only
- ✓ For use under Emergency Use Authorization (EUA) 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;
- ✓ This product has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens; and
- ✓ 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.
- ✓ Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments (CLIA) of 1988, 42 U.S.C. §263a, that meet requirements to perform high complexity tests.
- ✓ Carefully read the entire Instructions for Use for the Clear Dx™ system.
- ✓ Only personnel adequately trained on the use of this assay and in handling potentially infectious materials should perform these procedures.
- ✓ The Clear Dx™ system method includes sample preparation procedures that handle concentrated viral RNA. Because viruses can cause human illness, appropriate safety precautions must be taken when handling samples, media, reagents, and other supplies and equipment that could be contaminated with potentially pathogenic virus/bacteria.
- ✓ Reagents used with the system should pose no hazards when used as directed. Before using the reagents, please review the Safety Data Sheets (SDS) included with your Clear Dx™ purchase.
- ✓ Use only supplied or specified disposable laboratory ware.
- ✓ Use appropriate personal protective equipment when collecting and handling specimens from individuals suspected of being infected with SARS-CoV-2 as outlined in CDC Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with 2019 Novel Coronavirus (2019-nCoV).
- ✓ Do not attempt to place or adjust or remove something from inside the robot once it has started. An accidental collision with the robotic liquid handling arm can result in erroneous results.
- ✓ Wear disposable, powderless gloves, protective eye wear, and laboratory coats when handling specimens and reagents. Wash hands thoroughly after handling specimens and reagents.
- ✓ Dispose of all material that have come into contact with specimens and reagents in accordance with applicable national, international, and regional regulations.

- ✓ Maintain proper storage conditions during specimen shipping to ensure the integrity of the specimen. Specimen stability under shipping conditions other than those recommended has not been evaluated.
- ✓ Avoid cross-contamination during the specimen handling steps. Specimens can contain extremely high levels of virus or other organisms. Ensure that specimen containers do not come in contact with one another, and discard used materials without passing them over any open containers. Change gloves if they come in contact with specimens.
- ✓ Do not use the reagents and controls after the expiration date.
- ✓ Store assay components at the recommended storage condition. See Equipment and Materials for reagent storage and handling requirements (page 8-10), and Clear Dx™ Test Procedure (page 12-32) for more information. Do not freeze Clear DX Reagent Kit 3 (CLDX-K300). Doing so will adversely impact assay performance.
- ✓ Detection of SARS-CoV-2 RNA on the Clear Dx™ SARS-CoV-2 Test may be affected by sample collection methods, patient factors (ex. Presence of symptoms) and/or stage of infection.
- ✓ Quality control requirements must be performed in conformance with local, state, and/or federal regulations or accreditation requirements and your laboratory's standard quality control procedures.

Equipment and Materials

Storage and Handling Requirements for Reagent Kits Provided

Pre-packaged reagents & kits for Clear Dx™ SARS-CoV-2 Test:

Clear Dx™ SARS-CoV-2 Test comes with 5 reagent kits, labeled CLDX-Kxxx, as shown in the table below. These kits contain different pre-packaged and metered reagents such as primers, enzyme mixes, buffers, SPRI beads and sequencing-specific reagents from ONT.

SKU #	Kit Name	Description	# of tubes OR 96-well plates	Storage conditions	Max. # of samples / Kit
CLDX-K100	Clear Dx™ Reagent Kit 1	Library Prep Reagents	22	-20 °C	192
CLDX-K200	Clear Dx™ Reagent Kit 2	Enzyme mixes for RT and PCR	8	-20 °C	192
CLDX-K300	Clear Dx™ Reagent Kit 3	SPRI beads	4	2-8 °C	192
CLDX-K400	Clear Dx™ SARS-CoV-2 Index 1 Kit	Barcoded primers set 1	1	-20 °C	96
CLDX-K500	Clear Dx™ SARS-CoV-2 Index 2 Kit	Barcoded primers set 2	1	-20 °C	96

Reagent & kit configuration for Clear Dx™ SARS-CoV-2 test

All the reagents will be sealed with pierceable foil to avoid evaporation and contamination. During the automated run, the foil seal will be pierced by the pipette tips. All the critical reagents and the primer plates (CLDX-K400 & CLDX-K500) will be barcoded with 2D barcodes to track the reagent usage for the run and also to track against their expiration dates. All the kit boxes and reagents will be clearly labeled. The reagent tubes will have colored lids to avoid operator error. The details of the components of the kits are listed in the table below.

Item #	Item Description	Components	Vol, ul	Conc	Qty	UOM
CLDX-K100	**Clear DX Reagent Kit 1**				**1**	**Kit**
CLDX-K100-101	LP1: EDTA/Waste Tube	EDTA	1,400	200 mM	1	Tube
CLDX-K100-102	LP2: Pool Tube	Empty Tube	N/A	N/A	1	Tube
CLDX-K100-103	LP3: Internal Control Tube	G-block internal control DNA	1,500	0.015 fg/uL	1	Tube
CLDX-K100-104	LP4: Water Tube	Molecular Grade Water	1,800	N/A	1	Tube
CLDX-K100-105	LP5: Water Tube	Molecular Grade Water	1,800	N/A	1	Tube
CLDX-K100-106	LP6: Sequence Library Control Tube	Tris-HCl buffer with g-block control primers	15	10 mM	1	Tube
CLDX-K100-107	LP7: CL Elution Buffer Tube	Tris-HCl buffer	50	10 mM	1	Tube
CLDX-K100-108	LP8: Rapid Adapter (RAP 8x) Tube	ONT Rapid Adaptor	8	Proprietary	1	Tube
CLDX-K100-109	LP9: Library Loading Beads (LLB) Tube	ONT Library Loading Beads	45	Proprietary	1	Tube
CLDX-K100-110	LP10: Sequencing Buffer (SQB) Tube	ONT Sequencing Buffer	50	Proprietary	1	Tube
CLDX-K100-111	LP11: Flush Tether (FLT) Tube	ONT Flush Tether	50	Proprietary	1	Tube
CLDX-K100-112	LP12: Flush Buffer (FLB) Tube	ONT Flush Buffer	1,170	Proprietary	1	Tube
CLDX-K100-113	LP13: Ethanol Tube	Clear Labs, Inc.	Empty	N/A	1	Tube
CLDX-K100-114	LP14: Ethanol Tube	Clear Labs, Inc.	Empty	N/A	1	Tube
C-10074	Empty Tube	Empty Tube	N/A	N/A	8	Tube

Item #	Item Description	Components	Vol, ul	Conc	Qty	UOM
CLDX-K200	**Clear DX Reagent Kit 2**				**1**	**Kit**
CLDX-K200-101	RT-MM Tube	NEB Reverse Transcript Enzyme Master Mix	800	2X	4	Tube
CLDX-K200-102	Enzyme PCR1 Tube	TFS Platinum II HS Master Mix	650	2X	4	Tube

Item #	Item Description	Components	Vol, ul	Conc	Qty	UOM
CLDX-K300	**Clear DX Reagent Kit 3**				**1**	**Kit**
CLDX-K300-101	SPRI Beads Tube	Beckman Coulter AMPure XP	500	N/A	4	Tube

Item #	Item Description	Components	Vol, ul	Conc	Qty	UOM
CLDX-K400	**Clear DX SARS-CoV-2 Index 1 Kit**				**1**	**Kit**
CLDX-K400-101-001	PCR1 primers-Row 1	Clear Labs SARS-CoV-2 Primer Set - Row 1	40	125 - 750 nM	1	Well/Plate
CLDX-K400-101-002	PCR1 primers-Row 2	Clear Labs SARS-CoV-2 Primer Set - Row 2	40	125 - 750 nM	1	Well/Plate
CLDX-K400-101-003	PCR1 primers-Row 3	Clear Labs SARS-CoV-2 Primer Set - Row 3	40	125 - 750 nM	1	Well/Plate
CLDX-K400-101-004	PCR1 primers-Row 4	Clear Labs SARS-CoV-2 Primer Set - Row 4	40	125 - 750 nM	1	Well/Plate
CLDX-K400-101-005	PCR1 primers-Row 5	Clear Labs SARS-CoV-2 Primer Set - Row 5	40	125 - 750 nM	1	Well/Plate
CLDX-K400-101-006	PCR1 primers-Row 6	Clear Labs SARS-CoV-2 Primer Set - Row 6	40	125 - 750 nM	1	Well/Plate
CLDX-K400-101-007	PCR1 primers-Row 7	Clear Labs SARS-CoV-2 Primer Set - Row 7	40	125 - 750 nM	1	Well/Plate
CLDX-K400-101-008	PCR1 primers-Row 8	Clear Labs SARS-CoV-2 Primer Set - Row 8	40	125 - 750 nM	1	Well/Plate
CLDX-K400-101-101	MM PCR2-ONT 1	TFS Platinum II HS MM + ONT Barcode 1	18	Proprietary	1	Well/Plate
CLDX-K400-101-102	MM PCR2-ONT 2	TFS Platinum II HS MM + ONT Barcode 2	18	Proprietary	1	Well/Plate
CLDX-K400-101-103	MM PCR2-ONT 3	TFS Platinum II HS MM + ONT Barcode 3	18	Proprietary	1	Well/Plate
CLDX-K400-101-104	MM PCR2-ONT 4	TFS Platinum II HS MM + ONT Barcode 4	18	Proprietary	1	Well/Plate
CLDX-K400-101-105	MM PCR2-ONT 5	TFS Platinum II HS MM + ONT Barcode 5	18	Proprietary	1	Well/Plate
CLDX-K400-101-106	MM PCR2-ONT 6	TFS Platinum II HS MM + ONT Barcode 6	18	Proprietary	1	Well/Plate
CLDX-K400-101-107	MM PCR2-ONT 7	TFS Platinum II HS MM + ONT Barcode 7	18	Proprietary	1	Well/Plate
CLDX-K400-101-108	MM PCR2-ONT 8	TFS Platinum II HS MM + ONT Barcode 8	18	Proprietary	1	Well/Plate
CLDX-K400-101-109	MM PCR2-ONT 9	TFS Platinum II HS MM + ONT Barcode 9	18	Proprietary	1	Well/Plate
CLDX-K400-101-110	MM PCR2-ONT 10	TFS Platinum II HS MM + ONT Barcode 10	18	Proprietary	1	Well/Plate
CLDX-K400-101-111	MM PCR2-ONT 11	TFS Platinum II HS MM + ONT Barcode 11	18	Proprietary	1	Well/Plate
CLDX-K400-101-112	MM PCR2-ONT 12	TFS Platinum II HS MM + ONT Barcode 12	18	Proprietary	1	Well/Plate

Item #	Item Description	Components	Vol, ul	Conc	Qty	UOM
CLDX-K500	**Clear DX SARS-CoV-2 Index 2 Kit**				**1**	**Kit**
CLDX-K500-101-001	PCR1 primers-Row 9	Clear Labs SARS-CoV-2 Primer Set - Row 9	40	125 - 750 nM	1	Well/Plate
CLDX-K500-101-002	PCR1 primers-Row 10	Clear Labs SARS-CoV-2 Primer Set - Row 10	40	125 - 750 nM	1	Well/Plate
CLDX-K500-101-003	PCR1 primers-Row 11	Clear Labs SARS-CoV-2 Primer Set - Row 11	40	125 - 750 nM	1	Well/Plate
CLDX-K500-101-004	PCR1 primers-Row 12	Clear Labs SARS-CoV-2 Primer Set - Row 12	40	125 - 750 nM	1	Well/Plate
CLDX-K500-101-005	PCR1 primers-Row 13	Clear Labs SARS-CoV-2 Primer Set - Row 13	40	125 - 750 nM	1	Well/Plate
CLDX-K500-101-006	PCR1 primers-Row 14	Clear Labs SARS-CoV-2 Primer Set - Row 14	40	125 - 750 nM	1	Well/Plate
CLDX-K500-101-007	PCR1 primers-Row 15	Clear Labs SARS-CoV-2 Primer Set - Row 15	40	125 - 750 nM	1	Well/Plate
CLDX-K500-101-008	PCR1 primers-Row 16	Clear Labs SARS-CoV-2 Primer Set - Row 16	40	125 - 750 nM	1	Well/Plate
CLDX-K400-101-101	MM PCR2-ONT 1	TFS Platinum II HS MM + ONT Barcode 1	18	Proprietary	1	Well/Plate
CLDX-K400-101-102	MM PCR2-ONT 2	TFS Platinum II HS MM + ONT Barcode 2	18	Proprietary	1	Well/Plate
CLDX-K400-101-103	MM PCR2-ONT 3	TFS Platinum II HS MM + ONT Barcode 3	18	Proprietary	1	Well/Plate
CLDX-K400-101-104	MM PCR2-ONT 4	TFS Platinum II HS MM + ONT Barcode 4	18	Proprietary	1	Well/Plate
CLDX-K400-101-105	MM PCR2-ONT 5	TFS Platinum II HS MM + ONT Barcode 5	18	Proprietary	1	Well/Plate
CLDX-K400-101-106	MM PCR2-ONT 6	TFS Platinum II HS MM + ONT Barcode 6	18	Proprietary	1	Well/Plate
CLDX-K400-101-107	MM PCR2-ONT 7	TFS Platinum II HS MM + ONT Barcode 7	18	Proprietary	1	Well/Plate
CLDX-K400-101-108	MM PCR2-ONT 8	TFS Platinum II HS MM + ONT Barcode 8	18	Proprietary	1	Well/Plate
CLDX-K400-101-109	MM PCR2-ONT 9	TFS Platinum II HS MM + ONT Barcode 9	18	Proprietary	1	Well/Plate
CLDX-K400-101-110	MM PCR2-ONT 10	TFS Platinum II HS MM + ONT Barcode 10	18	Proprietary	1	Well/Plate
CLDX-K400-101-111	MM PCR2-ONT 11	TFS Platinum II HS MM + ONT Barcode 11	18	Proprietary	1	Well/Plate
CLDX-K400-101-112	MM PCR2-ONT 12	TFS Platinum II HS MM + ONT Barcode 12	18	Proprietary	1	Well/Plate

Additional Materials Required and Provided

Item	Manufacturer	Storage conditions	Clear Labs Part Number	Quantity
MinION Flow Cell	Oxford Nanopore Technologies	2 – 8 °C	R-10023	1
RNA Sample Plate, White Barcoded	4titude	Room temp.	C-10080	1
Hamilton lid cover	Hamilton Robotics	Room temp.	C-10012	Case of 50 plates
Clear plate, Non-Barcoded	4titude	Room temp.	C-10010	Case of 50 plates
Clear plate, barcoded	4titude	Room temp.	C-10009	Case of 50 plates
50 µL CO-RE Filter Tips	Hamilton Robotics	Room temp.	C-10028	Case of 5760 tips
300 µL CO-RE Filter Tips	Hamilton Robotics	Room temp.	C-10013	Case of 5760 tips

Special Instrumentation Required (but not Provided)

The Clear Dx™ SARS-CoV-2 Test is to be used with the Clear Dx™ system (# 51160-01) with the Venus 4 version software.

The system includes:

1. Hamilton STAR robotic workstation
2. Hamilton On-Deck Thermal Cyclers (2)
3. Oxford Nanopore GridION Sequencer - MinKNOW 18.12.4
4. ALPAQUA Magnum FLX on deck magnet

This instrumentation is not included with the test kit. Listed below are the equipment and hardware specifications for the Clear Dx™ System and other hardware accessories that are part of the system.

Clear Dx™ Hardware Specifications:

Specification	Required Configuration
Delivered Hardware System	Clear Dx™ documentation package, Hamilton STAR with instrument bench, ONT GridION, MicroTik Ethernet router, Microcentrifuge

Hamilton STAR Hardware Specifications:

Technical

Dimensions of Hamilton STAR (W x H x D)	65.5 in. x 35.5 in. x 31.2 in.
Dimensions of Hamilton Star Bench (W x H x D)	65.5 in. x 28.7 in. (32.2 in. with casters) x 31.2 in.
Weight of Hamilton Star	145kg (319 lbs.)
Weight of Hamilton Star Bench	68kg (150 lbs.)
Hamilton Consumables Included for Installation	
Tips for Star	Part No: 235903 - 300µL Filter tips - CORE FILTER TIPS 300µL, 5760/CS - Quantity 1 Part No: 235948 - 50µL Filter tips - CORE FILTER TIPS 50µL COND PK/5760 - Quantity 1
PCR Consumables	Part No: 814300 - Hamilton PCR Comfort Lids - 50 plates per case - Quantity 1
Optional Hardware - APC Smart-UPS SRT 2200VA	
Brand	APC

Manufacturer	APC
Model	2200VA
Product Line	APC Smart-UPS SRT

Additional Materials and Reagents Required (but not Provided)

- Cole-Parmer C1008-B Microcentrifuge, Catalog # UX-17701-11 or equivalent
- Vortex Genie-2, from Stellar Scientific, Catalog # SI-0236 or equivalent
- 100% absolute ethanol, from VWR, Catalog # EM-4455S or equivalent
- Twist Synthetic SARS-CoV-2 RNA Control 2 (Accession # MN908947.3; Catalog # 102024)
- Molecular grade PBS buffer (Gibco DPBS, no Ca^{2+} no Mg^{2+}, Catalog # 14-190-144 from Fisher Scientific)
- MagMAX Viral RNA Isolation Kit (Catalog # AM1939 from Thermo Fisher Scientific)
- DynaMag-2 Magnet for manual RNA extraction using MagMAX kits (Catalog # 12321D from Thermo Fisher Scientific)
- KingFisher Flex Purification system with 96-Deep well head (Catalog # 5400630 from Thermo Fisher Scientific)
- MagMAX Viral/Pathogen nucleic acid isolation kit (Catalog # A42352 from Thermo Fisher Scientific)

Procedures

Clear Dx™ System Setup and Initialization

Prior to setting up the Clear Dx™ system for running the test, the frozen reagents are thawed to room temperature. Clear Dx™ automation software includes a user- friendly interface. The interface guides the operator step by step during sample and reagent loading with multiple dialogues and interactive images. Different reagent tubes of the kit are numbered and color-coded with the same numbers and colors on the automated system as well as on the user interface. Once all the reagents are loaded and before the automated workflow starts, the system runs a verification step to make sure that all reagents and consumables are loaded correctly. If the operator had loaded a wrong reagent or a reagent in a wrong position, the system would give an alert and provide the user with the instructions to correct the error.

Specimen Collection, Transport and Storage

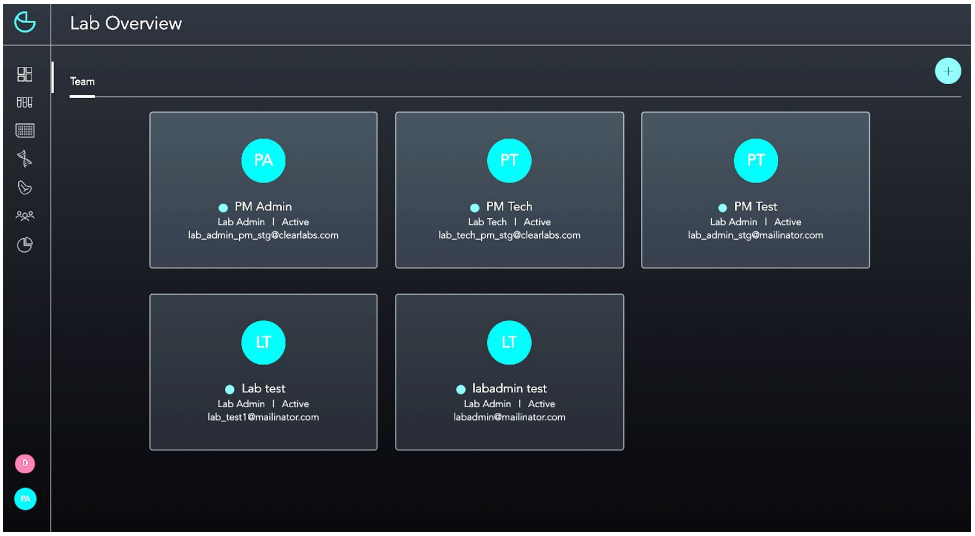
- ✓ The clinical specimens can be collected either in Universal Transport Medium (UTM), Viral Transport Medium (VTM), PBS buffer or normal saline or equivalent. The collection media are not included in the test kit.

- ✓ Swab specimens from patients are collected in 1-3 mL tubes containing any of the aforesaid transport media at the collection site and are immediately refrigerated upon collection (2 to 8°C). Nasopharyngeal wash/aspirate, nasal aspirate, and bronchoalveolar lavage specimens similarly should be immediately refrigerated upon collection (2 to 8°C). Note: All specimens must be shipped in accordance with applicable national, international, and regional transportation regulations

- ✓ If the specimens cannot be tested within 72 hours of collection, they should be frozen at -80°C until tested. **NOTE:** An increased rate of invalid results was observed for clinical specimens that are subjected to one or more freeze thaw cycles prior to testing with the Clear Dx™ SARS-CoV-2 assay.

Sample Registration in the Clear View Application

The Clear View interface for Laboratories:



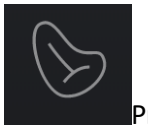
Navigate the icons on the left side of the application to access the various features.


Lab Overview - View and manage the users and their privileges within your laboratory.


Sample View - Create samples, and view a list of registered samples.


Rack View - View and create virtual racks of samples that will be processed in the Clear Dx™ System.


Test Run View - View completed test runs and results.


Product View - Create products and assign test specificities to facilitate the sample registration process.


Clients View - View and manage Laboratory Clients.


Result Analytics View - View subtypes and groupings.

Notifications - View notifications related to test runs and result availability

User Avatar - View account info, created user accounts, resources, and logout

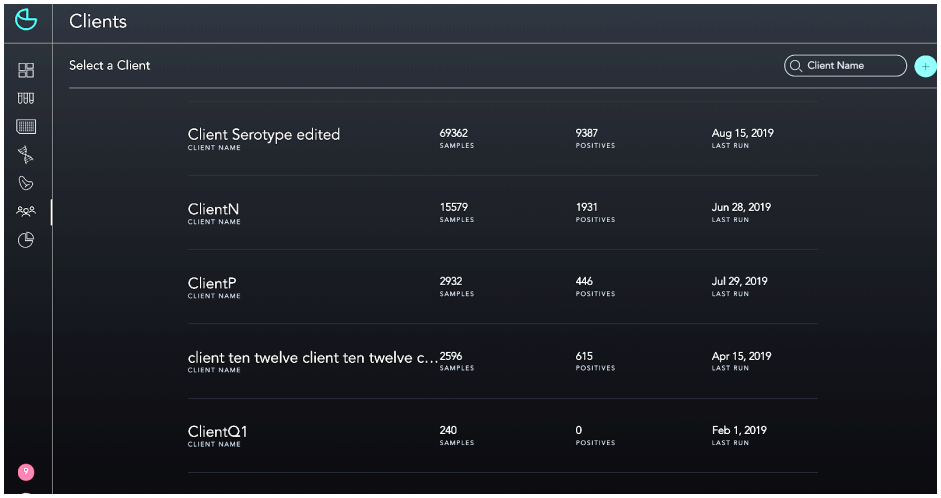
Company, Client, and User Creation and Management

Company Creation

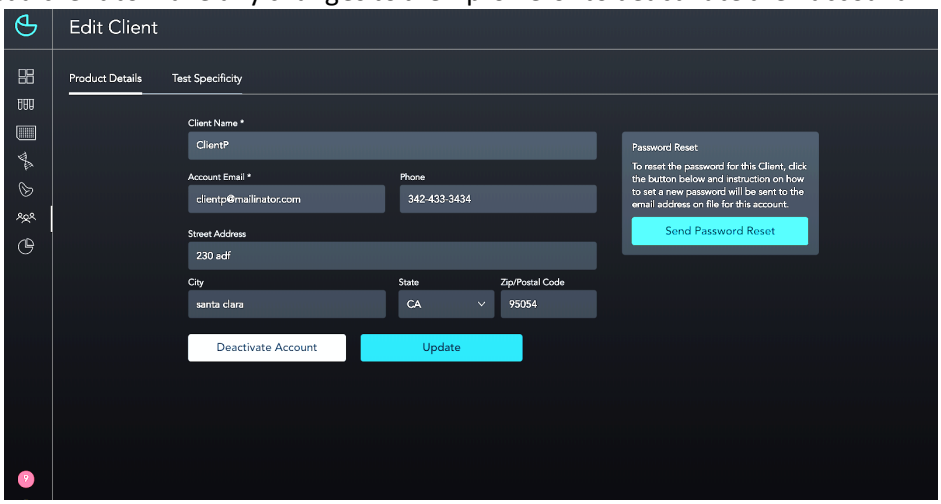
Your Clear Labs Field Application Scientist will assist you with the Company and Client (Laboratory Client) creation process.

To Manage Existing Clients:

1. Click the “Clients” icon in the left bar. This will display the list of Clients the laboratory has created.



2. Select a client to make any changes to their profile or to deactivate their account.

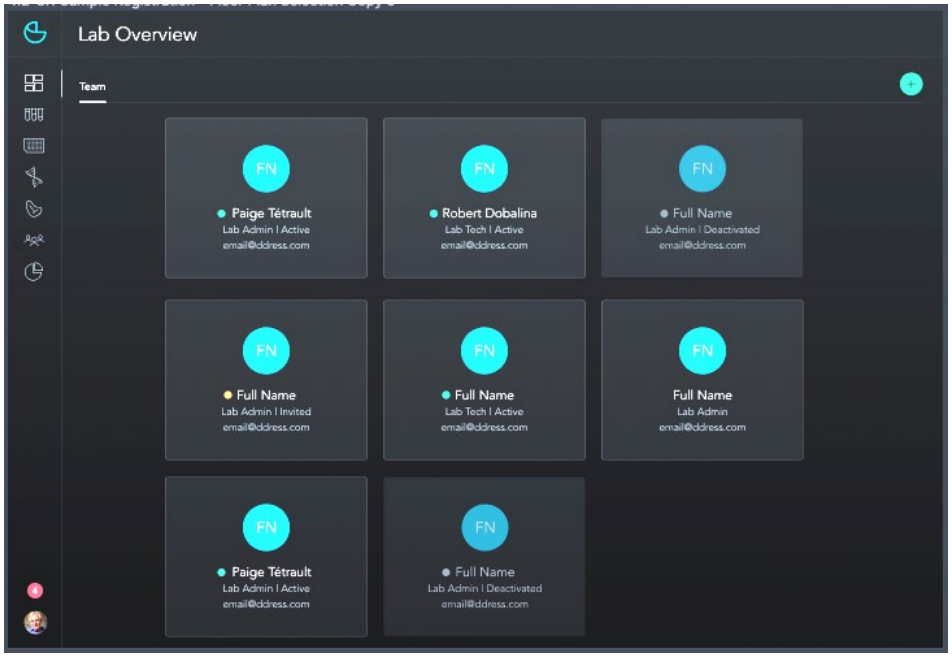


To Add a Client:

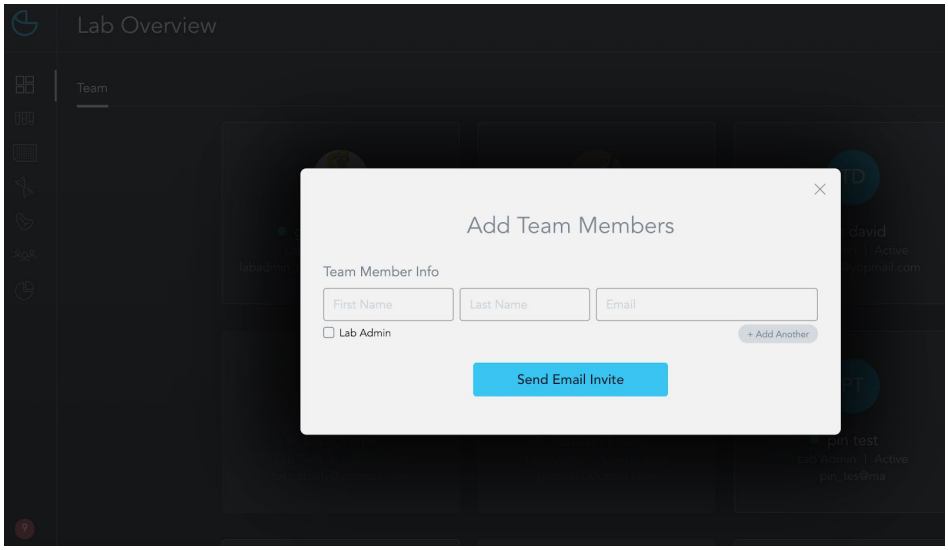
1. Click the "+" icon on the top right of the Clients page.
2. Enter the required information in the Product Details.
3. Select the default tests that will populate when products are created for this Client.

User Creation (Clear Labs Admin level only)

Click on the Lab Overview icon to view existing users within the laboratory.



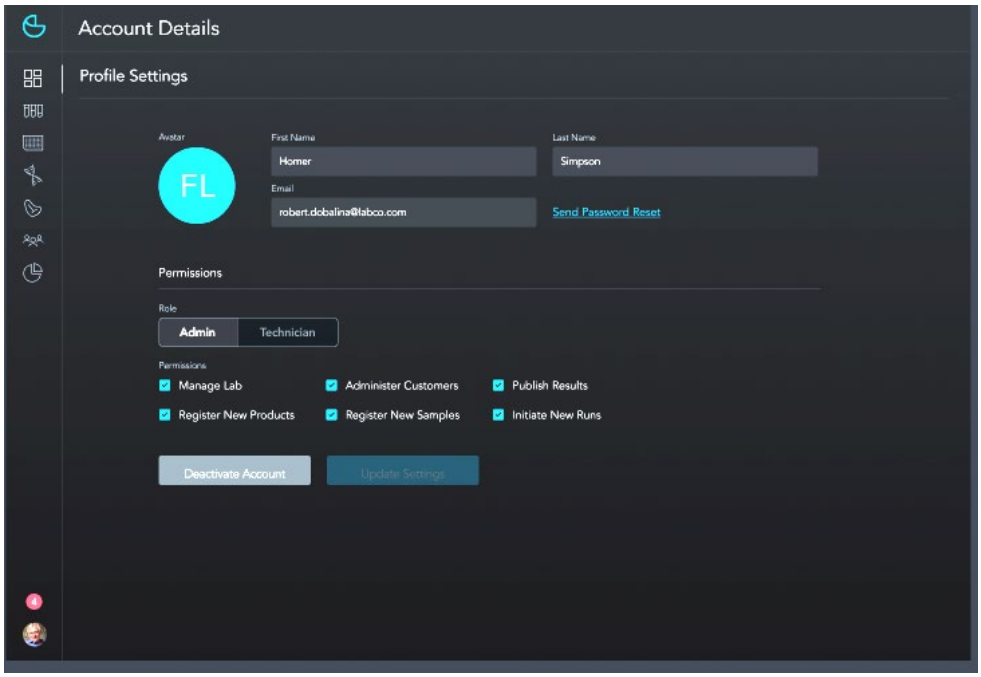
Begin the user creation process by clicking “Add” near the top of the screen.
Complete the fields for name and email address. The added user will be notified via email to complete the registration process.



Privilege Management

Administrators are able to manage permissions for each individual within the company. You can do so within the Users page that was used to create users. Click your user avatar icon near the bottom left of the screen, select the user, and modify their privileges accordingly (if you have permission to do so.) You can

choose to alter the Role of a user, which will enable or disable a predefined set of actions or choose specific permissions you would like that user to have. Be wary of changing permissions on your own account, as authorization conflicts may lock you out of your account.



Logging In

Once an account is created for a user, the created user will receive an e-mail supporting them through a final creation of and logging into the account.

Product (Specimen Type) Creation

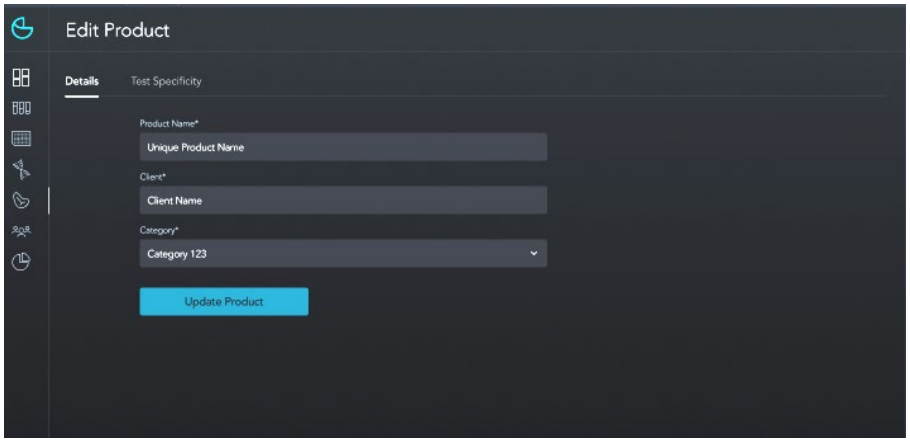
Registering products for Laboratory Clients is a one-time process that allows the laboratory to capture sample metadata so that results associated with a particular product (i.e. specimen) types (e.g. nasopharyngeal swab, oropharyngeal swab, anterior nasal swab, mid-turbinate nasal swab, nasopharyngeal wash/aspirate, nasal aspirate, and bronchoalveolar lavage specimens) can be tracked over time. Additionally, the product creation process is an opportunity to assign the test settings to particular products. The entire process facilitates the sample creation and registration process.

Creating a product (specimen type)

Lab Administrators and Techs can create products. In order to register a new product into Clear View,

1. Select the Products page from the navigation bar. This page lists all products created within the system, and the client they are associated with.

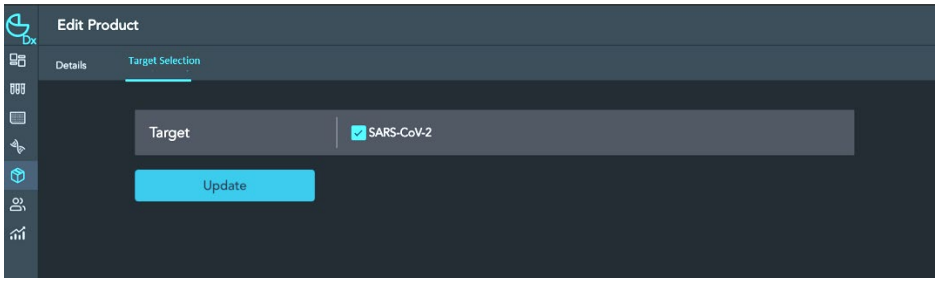
2. Click the green plus icon to begin the product creation process.



3. Name the product (specimen type).
4. Select the Client.
5. Assign a product category.

Target Selection

You will then be guided to Target Selection, where you are able to define what analyses the particular product will undergo. Clear Dx™ currently accommodates SARS-CoV-2 screening and subtyping by default.

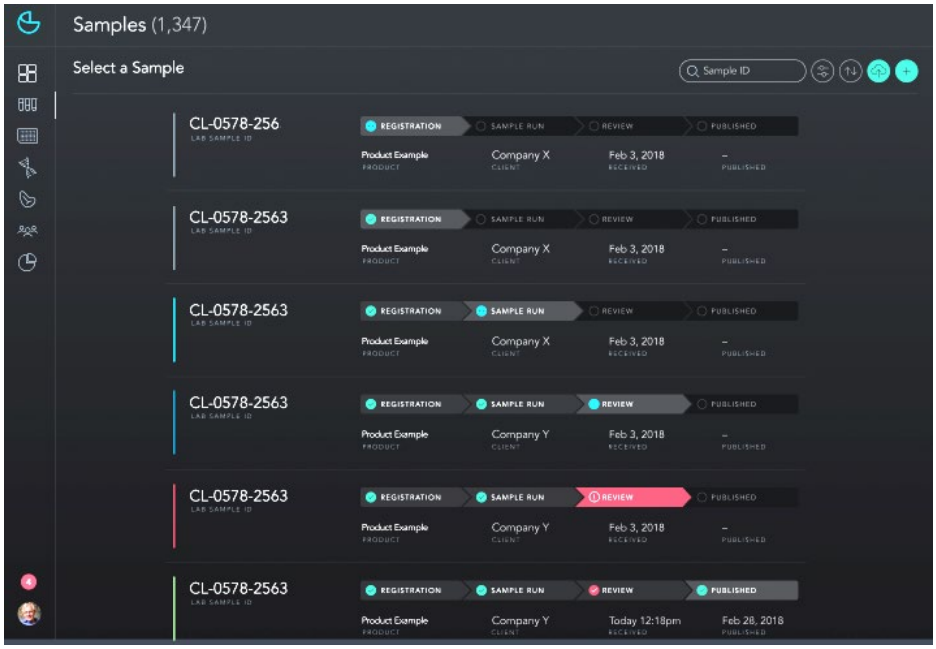


Sample Creation

Samples can be created by the laboratory for Laboratory Clients.

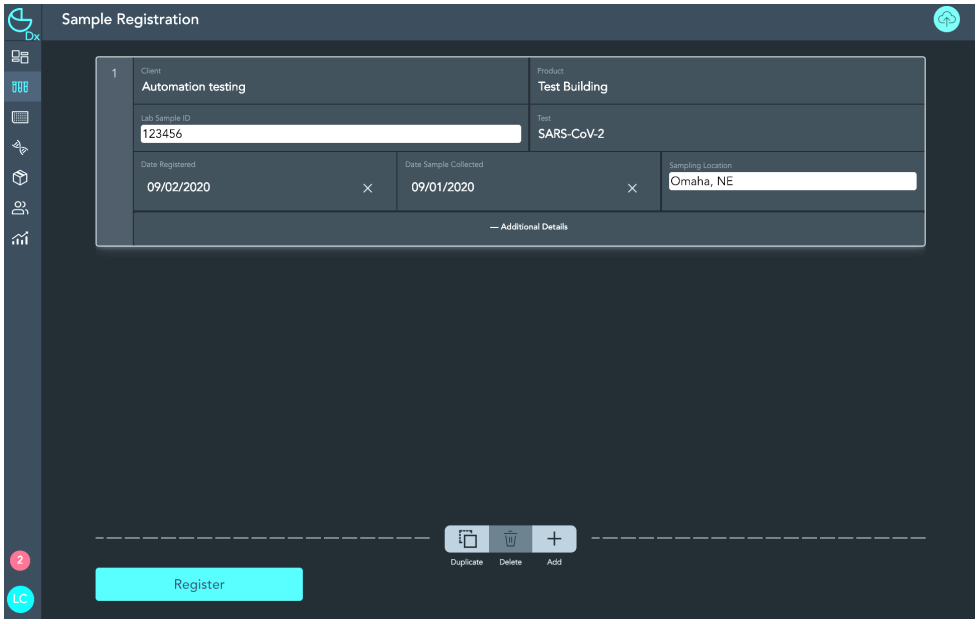
Sample Intake

1. Select the Samples page on the navigation bar. Within this page, you are able to view all samples created in the past and create new ones for upcoming test runs.



2. Click the green plus icon on the top right to begin the sample creation process.

Manual sample creation



1. Enter the Laboratory Client ID
2. Enter the Product associated with that client.
3. Enter the Lab Sample ID

4. Enter additional details as desired.
5. Click the "Add" button to add more samples. Alternatively, click the "Duplicate" button to duplicate the sample. This is helpful and efficient if the additional samples share many of the same properties. Be sure to update the dissimilar sample information (i.e., Lab Sample ID) accordingly.

Importing Multiple Samples

In the Sample Registration Screen,

1. Select "Import Samples" at the top right of the screen and select the "Import Samples" option to import a list of samples originating from an external LIMS system.
2. Upload a .csv file containing a list of samples you wish to be imported into Clear View.

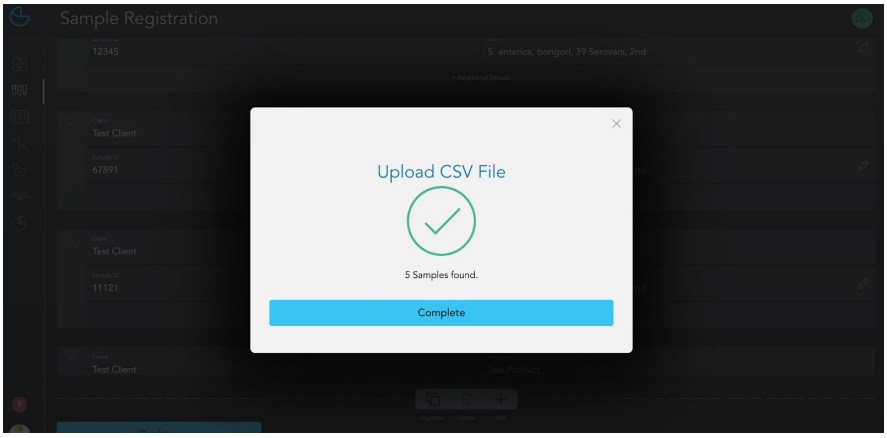
Example:

	A	B	C	D	E	F
1	Client Name	Lab Sample ID	Product Name	Date Registered	Date Sample Collected	Sampling Location
2						
3						
4						
5						
6						

The .csv file must contain the following headers:

i. Client Name
ii. Lab Sample ID
iii. Product Name
iv. Date Registered
v. Date Sample Collected
vi. Sampling Location

3. Clear View will then ask you to match the headers found within the .csv file with those known to the software. Minimally, the .csv should contain identifying metadata such as Client Name, Lab Sample ID, and Product Name (i.e. Specimen Type).

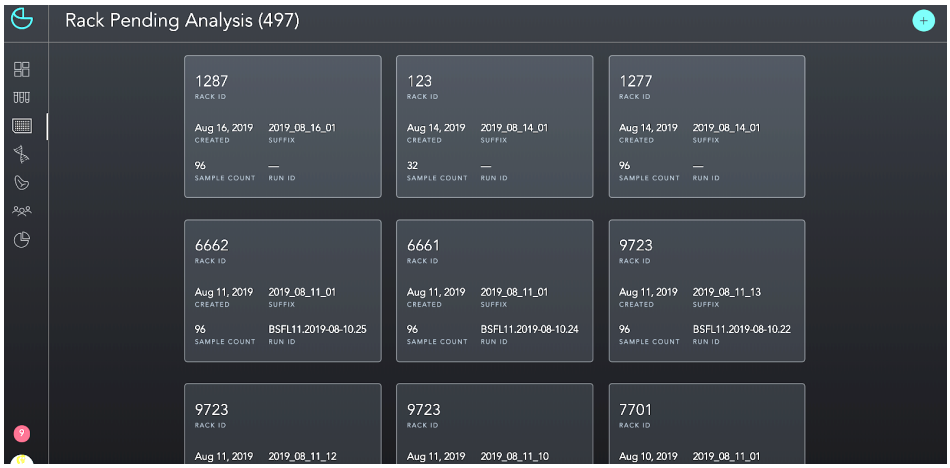


4. Click “Complete” to finish importing the samples list. Once successful, the samples that have been imported may be registered.

Sample Rack Creation

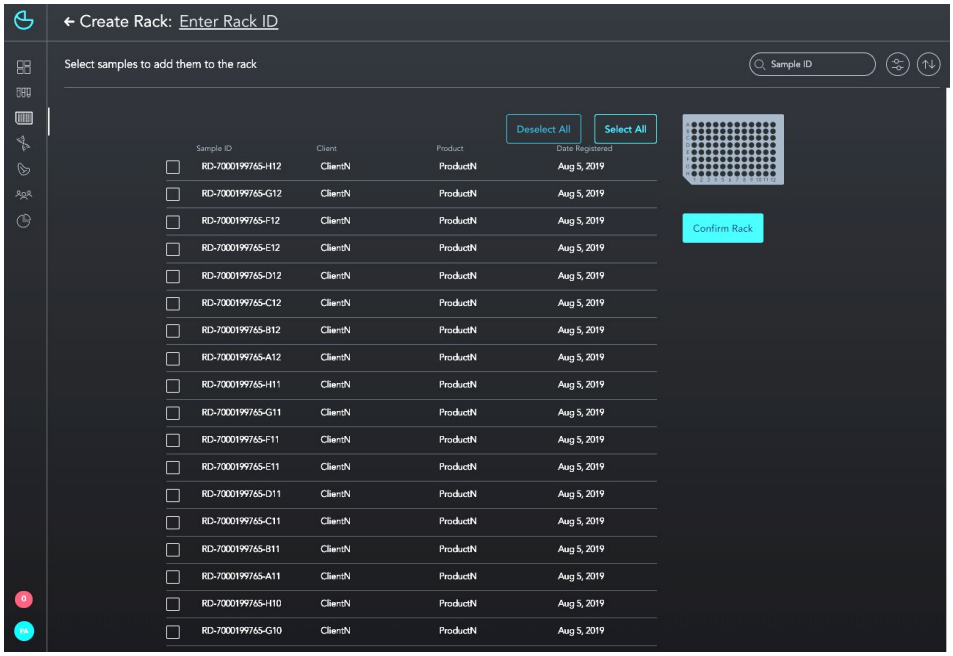
In order for the Clear Dx™ System to accept samples, samples must first be assigned to a “rack” in Clear View. A rack in Clear View captures the physical 96-well 1-D barcoded RNA sample tray (part number C-10080) and the order and arrangement of samples in the tray that will be loaded into the Clear Dx™ System. A Laboratory Technician can create Sample Racks:

1. Select the Racks page on the navigation bar. The screen will display a list of racks that are pending analysis.

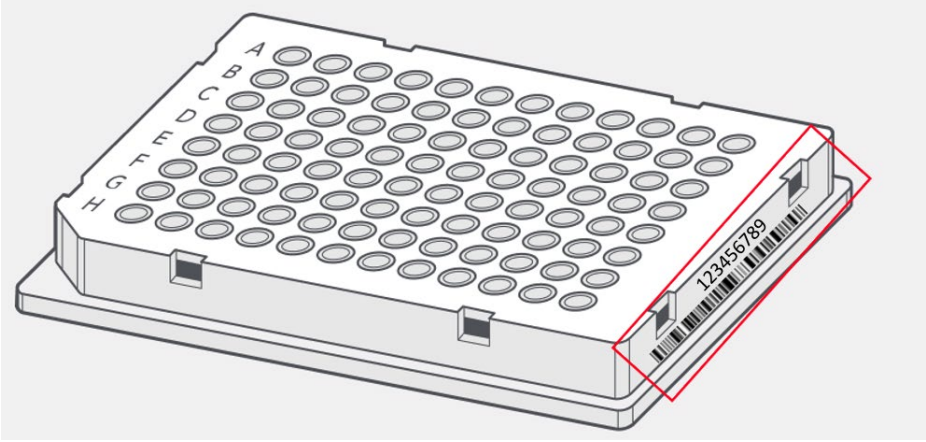


2. Click the green plus icon on the top right to begin the Sample Rack creation process.

3. A list of registered samples for the selected target genus are displayed. Add samples to the rack in a manner that represents the physical layout of the 96-well RNA sample plate, which is manually prepared following extraction (see Sample Preparation below).

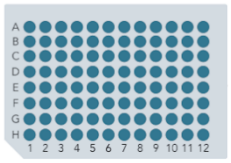


4. Enter Rack ID. The rack ID can be found on the right hand side of the RNA sample tray (see red box below).



5. Once all samples have been selected, click "Confirm Rack," and you will be given a confirmation of the samples that will be included on this Sample Rack. You also have the ability to print the layout of the sample rack for reference by clicking the green download icon.

Rack ID: 7000199807
Suffix: 2019_08_23_01
Customer: CL-PM
Lab: PM Lab1
Printed Date: Aug 23, 2019, 3:08 PM



A	1 1096	9 1088	17 1080	25 1072	33 1064	41 1056	49 1048	57 1040	65 1032	73 1024	81 1016	89 1008
B	2 1095	10 1087	18 1079	26 1071	34 1063	42 1055	50 1047	58 1039	66 1031	74 1023	82 1015	90 1007
C	3 1094	11 1086	19 1078	27 1070	35 1062	43 1054	51 1046	59 1038	67 1030	75 1022	83 1014	91 1006
D	4 1093	12 1085	20 1077	28 1069	36 1061	44 1053	52 1045	60 1037	68 1029	76 1021	84 1013	92 1005
E	5 1092	13 1084	21 1076	29 1068	37 1060	45 1052	53 1044	61 1036	69 1028	77 1020	85 1012	93 1004
F	6 1091	14 1083	22 1075	30 1067	38 1059	46 1051	54 1043	62 1035	70 1027	78 1019	86 1011	94 1003
G	7 1090	15 1082	23 1074	31 1066	39 1058	47 1050	55 1042	63 1034	71 1026	79 1018	87 1010	95 1002
H	8 1089	16 1081	24 1073	32 1065	40 1057	48 1049	56 1041	64 1033	72 1025	80 1017	88 1009	96 1001
	1	2	3	4	5	6	7	8	9	10	11	12

Publishing, Reporting and Result Analytics

A notification will be sent to the laboratory users when results are available.

Sample Preparation

For manual extraction of RNA, 400µL of each sample should be processed using the MagMAX Viral RNA Isolation Kit (Catalog # AM1939). For automated extraction, 200µL of each sample should be processed using the MagMAX Viral/Pathogen Nucleic Acid Isolation Kit (Catalog # A42352 from Thermo Fisher Scientific) on KingFisher Flex Purification system with 96-Deep well head (Catalog # 5400630 from Thermo Fisher Scientific). The elution volume is 50µL for both the manual and automated extraction protocols. The user is instructed to refer to the instructions for use for the extraction kits and the instrument mentioned above, provided in the manufacturer's (Thermo Fisher Scientific) website. The total viral RNA from the virus particles of SARS-CoV-2 present in the nasopharyngeal (NP) swab, oropharyngeal (OP) swab, anterior nasal swab, mid-turbinate nasal swab, nasopharyngeal wash/aspirate, nasal aspirate, or bronchoalveolar lavage (BAL) specimens is co-extracted alongside any human RNA present in the clinical specimen. The final expected elution volume for the extracted RNA is 50µL. Once the Clear Dx™ system is setup and initialized to perform the test, 20µL (only 6 µL total is used for the reaction) of extracted RNA in elution buffer of each of the registered samples is manually loaded into individual wells of the 96-well skirted PCR plates (RNA sample plate, #C10080) provided with the test kit. The plates are then placed in their respective positions in the Clear Dx™ system. Each Clear Dx™ SARS-CoV-2 Test run can process up to two 96-well plates; 192 samples, including controls.

Instrument Maintenance Check

Perform the steps in this section prior to using the Clear Dx™ System.

1. If not already on, turn on all hardware: The Clear Dx™ System, GridION sequencer, On-Deck Thermocycler controllers (ODTC), Plate/tube scanner, desktop computer and monitor.
2. Open the Microlab STAR Maintenance icon on your computer.
3. Check the box for either daily or weekly maintenance. *Note: The Weekly Check overrides the Daily Check.*
4. Click the Run Process icon (green arrow) near the top.
5. At the prompt to perform deck and tip waste maintenance, click Yes
6. At the prompt for carrier maintenance, click OK. (This prompt only appears during the Weekly Maintenance process.)
7. Clean the deck with sanitizing wipes, then click OK.
8. Empty and clean the tip waste, then click OK. *Note: This step is only necessary when the tip waste is full (or close to full) and can be done at any time between runs.*
9. At the prompt to check the channel tightness, click Yes. This verifies the integrity of the O-rings, and takes about two minutes. Note: If a failure message occurs, discontinue usage and contact Clear Labs for service.
10. At the prompt to check Capacitive Liquid Level Detection, click Yes (This tests the machine's ability to detect liquids) Note: If a failure message occurs, discontinue usage and contact Clear Labs for service.
11. Once the last check concludes, click OK, then exit the program. You can now proceed to loading the Clear Dx™ System.

Instrument Setup and Loading

The Clear Dx™ System is designed to accommodate and process as many as 190 clinical specimens in a single run, in addition to a single positive and a single negative control per run (192 samples total per run). Up to two 96-well PCR barcoded RNA sample plates can be loaded onto the instrument at a time, and each sample plate will be loaded with the corresponding number of consumables illustrated in the matrix below:

Item	Description	Plates Needed for up to 96 samples	Plates Needed for >96 and up to 192 samples
RNA Sample Plate	Plate used to load extracted RNA into Clear Dx™ instrument. White in color.	1	2
PCR 1 Plate	Plate used for primary PCR. Clear in color.	1	2

Item	Description	Plates Needed for up to 96 samples	Plates Needed for >96 and up to 192 samples
RT Plate	Plate used to carry out RT PCR. Clear in color.	1	2
Hamilton Lid Cover	Lid to contain reactions. Clear in color.	1	2
Cover Plate	Plate used to provide contamination-free lid parking. Clear in color.	1	2
PCR 2 Plate	Plate used for secondary PCR. Clear in color.	1	1
Reagent Plate	Plate used to mix reagents. Clear in color.	1	1
50 µL CO-RE Filter Tips	Pipette tips to be used with Hamilton Star platform.	Stock completely	Stock completely
300 µL CO- RE Filter Tips	Pipette tips to be used with Hamilton Star platform.	Stock completely	Stock completely
MinION Flow Cell	Flow cell to be used for sequencing	1	1
Clear Dx™ Reagent Kit 1	Library preparation kit	1	1
Clear Dx™ Reagent Kit 2	Sample Processing kit	1/2	1
Clear Dx™ Reagent Kit 3	Library clean up kit	1	1
Clear Dx™ SARS-CoV-2 Index 1 Kit and 2 kit	Index plates	One index plate	Two index plates

For efficiency purposes, it is ideal to populate a sample plate with as many samples as possible before including additional plates. The following instructions assume that one 96-well sample plate is being processed.

Loading the Clear Dx™ System is an easy-to-follow process guided by a series of prompts. Simply follow the prompts on the Clear Dx™ system interface screen to complete the process.

Note: The current configuration of the Clear Dx™ system cannot accept fewer than 48 samples per run. If fewer than 48 samples are being loaded into the system, it is required that “blank” samples be added to

both the virtual sample carrier and the physical sample carrier to make up the difference. The Clear Dx™ system will report an error if fewer than 48 samples are detected in the sample carrier.

Preparation

1. Before each run, remove the required tubes, as requested by the software user interface, from Reagent Kits I, II, III, and a Flow Cell from appropriate storage conditions and bring them to room temperature. (Remove the Flow Cell from the bag and place it on a level surface). Thirty minutes at room temperature is recommended.
2. Remove Tube #13 and #14 from Reagent Kit I and fill each tube with 2 mL of 100% absolute ethanol (not supplied).
3. If present, remove all the used consumables from the previous run to be ready for loading fresh consumables. Clean up any liquid spill and disinfect the robot surface by wiping down with Isopropyl alcohol wipes.
4. Open the "Clear Dx™" application on Clear Dx™ System interface.
5. A "Get Ready Screen will be displayed. Follow the on-screen instructions to complete the required steps and confirm that each step was completed successfully when prompted. Continue the process for each screen that follows.
   Note: Open the door to load the Clear Dx™ System.

Load Samples and Controls

Follow the on-screen prompts to load the RNA sample plates. The number of plates and samples onboard will inform the Clear Dx™ System how many reagent sets need to be loaded in subsequent steps. Proceed to the next step when complete.

Load Flow Cell

1. When prompted, retrieve the now room temperature flow cell and load it into slot X1 on the GridION sequencer.
   a. Remove Flow Cell from packaging, retain the packaging.
   b. Orient the Flow Cell so the yellow window faces the rear.
   c. Insert it at an angle until you feel the rear end catch, then set down the front end.
   d. When secure and connected, the "X1" light will turn green.
      Note 1: Keep the Flow Cell as level as possible to avoid introduction of air bubbles
      Note 2: Be careful not to bend the bottom pins of the flow cell.
2. Gently rotate the priming gate 90° clockwise, using the thumb as the fulcrum.
   Note: Be careful not to over-rotate.
3. Remove the Flow cell port's cover and retain.
4. Clean the stainless steel plug with lint-free wipes.
5. Install plug into the Flow cell port. Gently push down the plug until secure, making sure there's no free movement.

6. Click “Flow Cell QC” to begin the quality check (a 7-minute process). Follow the prompts as they are displayed. While the Flow Cell is undergoing QC check, the remaining loading steps are completed.
   Note: If the Flow Cell fails, do the following:
   1. Remove it from the GridION sequencer and close the priming gate fully by rotating clockwise
   2. From the waste port located at the end of the waste reservoir, fully aspirate the contents within the Flow Cell
   3. Place the included sticker on the top face of the Flow Cell and place back in the plastic housing
   4. Insert into the original sealing bag and seal securely. Contact Clear Labs service to coordinate return and replacement of the Flow Cell.

Load Plates

Follow the prompts to load the two Index plates, Lid and Cover into their designated locations.
Note: All plates are loaded with well A1 to the top left, barcodes on the right side.

Load Tips

Follow the prompts to load the Tips into the Tip Carriers until both are fully stocked.

1. Fill the left side with 50 µL Tips (the racks will have pink barcodes).
2. Fill the right side with 300 µL Tips (the racks will have yellow barcodes).

Note: Unused tips from previous runs can be loaded. Make sure to use aseptic technique when performing a transfer and take care not to mix different sized tips with a rack. Clear Dx™ System does not track tip inventory. Tips are consumed from the back rack first, moving forward.

Load Reagent Tubes

For convenience, reagents from Kit I, Kit II, and Kit III are numbered for their respective locations on the sliding racks on the instrument deck. Clear Dx™ System will calculate the required reagents according to the number of samples.

1. Using a microcentrifuge, give all tubes with reagents and index plate(s) a 1-2 sec spin.
2. Vortex the “SP” tubes for 20 seconds before loading.
3. Follow the prompts to scan the reagent kits into the system.

Complete Flow Cell QC Check

The Flow Cell QC check started in the “Load Flow Cell” section should be complete. Follow the onscreen prompts to continue.

Pre-Run Check

Begin the Pre-Run Check when prompted and follow the onscreen instructions.
Note: Close door before beginning Pre-Run Check

Once the Pre-Run Check finishes, click “Confirm”.

Secure Index Plate(s)

Follow the prompts to secure the Index Plate by installing its Down Holder. Click “Continue” to proceed.

1. Open door. Make sure the corner pins of the holder are facing upward.
2. Click “Continue”.

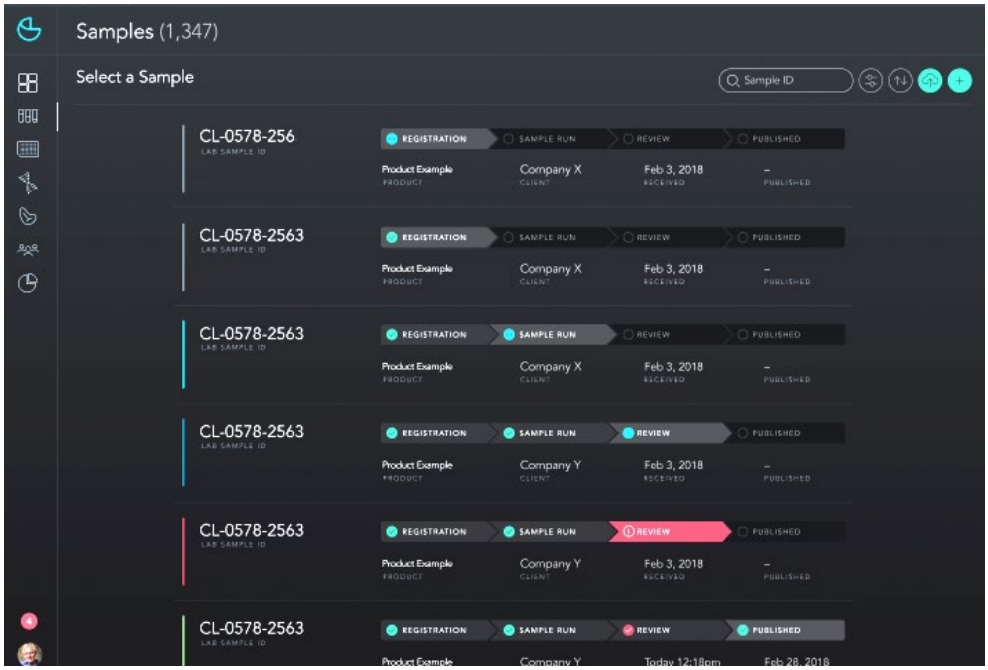
Start Test Run

1. Close the door.
2. Click “Start Test Run” to begin the automated testing process, which should take 8-9 hours
3. Once the run concludes, click “OK” at the final screen.

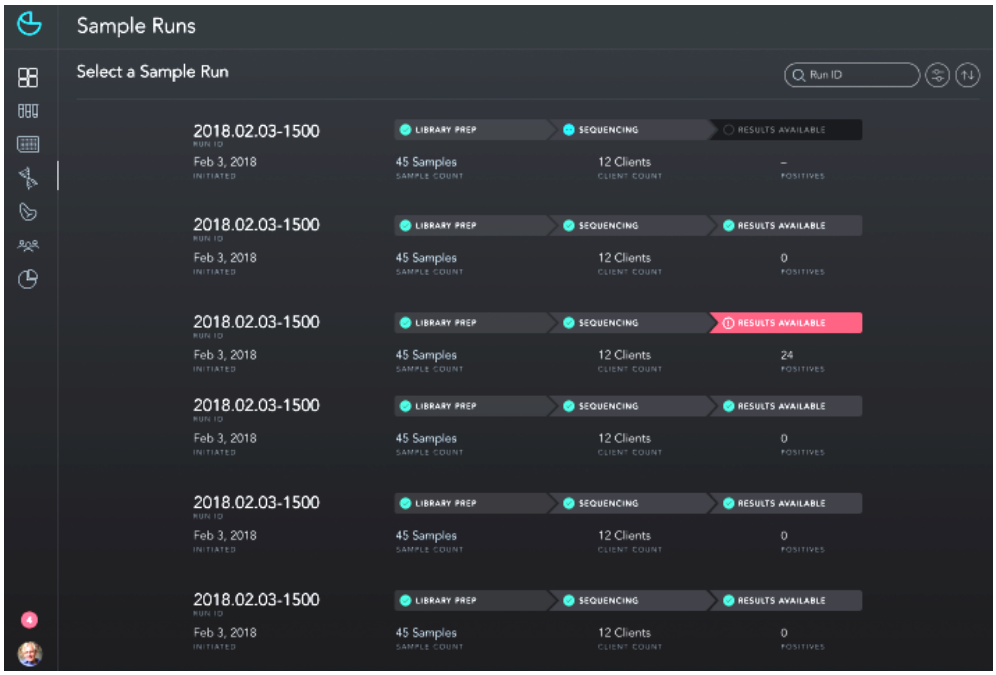
Data Analysis

Results Access

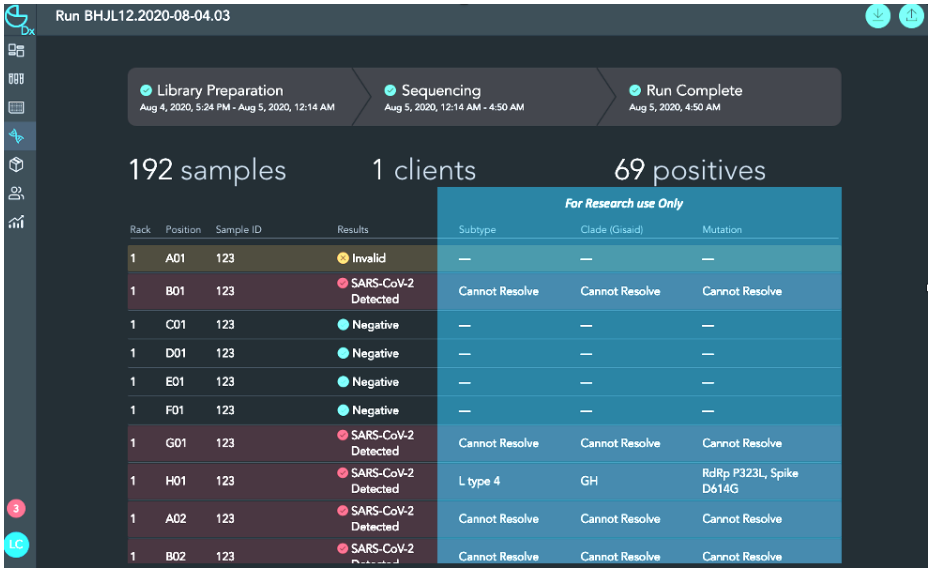
Samples View - click an individual sample to see the result details.



Runs View - View a list of test runs.

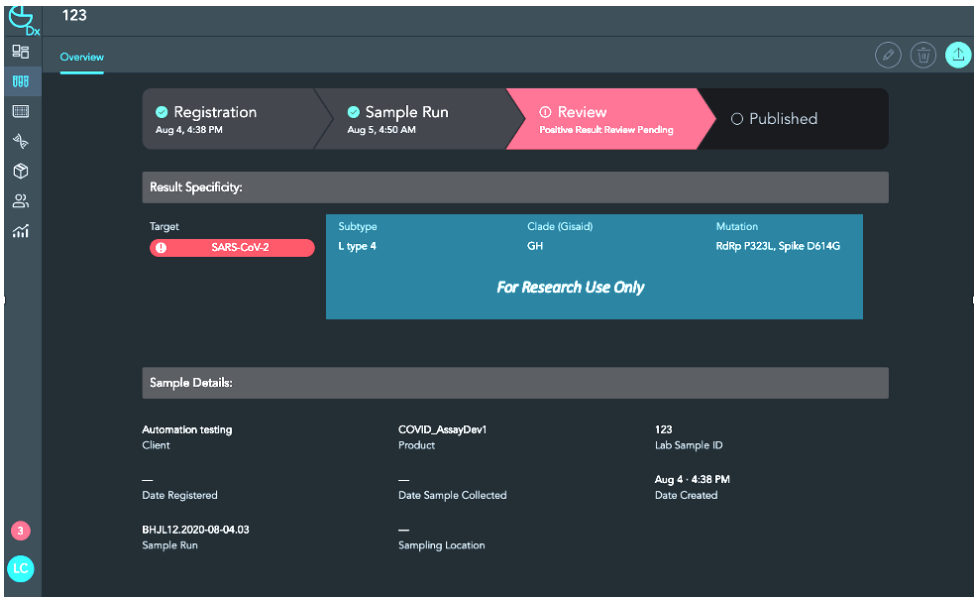


Select a run to view the samples and higher level results.



A “Negative” result is displayed when the targeted analyte is not detected in the sample.
A “Positive” result is displayed when the target is detected in the sample.
An “Invalid” result is generated when the built-in internal control technologies detect an unsuccessful reaction. Contact Clear Labs for further support.

Select an individual sample to see additional details.



Reporting Clear Dx™ Results

Results for a Clear Dx™ run can be exported from the Run Details page in the Clear View application as a .csv file by clicking on the Download icon near the top right side of the browser window. Use this feature if results need to be uploaded into a laboratory information management system (LIMS) for communication to other parties. An example report is pasted below.

Rack	Well	Lab Sample ID	Clear ID	Client Name	Product Name	Target Result	Target Virus	Date Sample Collected	Run Complete	Sampling Location
1	A01	12345-1	CL-A0-0006-1222	Lab A	NP Swab	POSITIVE	SARS-CoV-2	8/9/2020	8/11/2020 7:33	Omaha, NE
1	B01	12345-2	CL-A0-0006-1221	Lab A	NP Swab	POSITIVE	SARS-CoV-2	8/9/2020	8/11/2020 7:33	Omaha, NE
1	C01	12345-1	CL-A0-0006-1220	Lab A	NP Swab	POSITIVE	SARS-CoV-2	8/9/2020	8/11/2020 7:33	Omaha, NE
1	D01	12345-2	CL-A0-0006-1219	Lab A	NP Swab	POSITIVE	SARS-CoV-2	8/9/2020	8/11/2020 7:33	Omaha, NE
1	E01	12345-1	CL-A0-0006-1218	Lab A	NP Swab	NEGATIVE	SARS-CoV-2	8/9/2020	8/11/2020 7:33	Omaha, NE
1	F01	12345-2	CL-A0-0006-1217	Lab A	NP Swab	NEGATIVE	SARS-CoV-2	8/9/2020	8/11/2020 7:33	Omaha, NE
1	G01	12345-1	CL-A0-0006-1216	Lab A	NP Swab	NEGATIVE	SARS-CoV-2	8/9/2020	8/11/2020 7:33	Omaha, NE
1	H01	12345-2	CL-A0-0006-1215	Lab A	NP Swab	NEGATIVE	SARS-CoV-2	8/9/2020	8/11/2020 7:33	Omaha, NE
1	A02	12345-1	CL-A0-0006-1214	Lab A	NP Swab	POSITIVE	SARS-CoV-2	8/9/2020	8/11/2020 7:33	Omaha, NE
1	B02	12345-2	CL-A0-0006-1213	Lab A	NP Swab	POSITIVE	SARS-CoV-2	8/9/2020	8/11/2020 7:33	Omaha, NE
1	C02	12345-1	CL-A0-0006-1212	Lab A	NP Swab	POSITIVE	SARS-CoV-2	8/9/2020	8/11/2020 7:33	Omaha, NE
1	D02	12345-2	CL-A0-0006-1211	Lab A	NP Swab	POSITIVE	SARS-CoV-2	8/9/2020	8/11/2020 7:33	Omaha, NE

The report includes the following information:

Title – The Run ID associated with the report.

Rack – The sample plate number. The Clear Dx™ system can accept up to two plates of samples.

Well – The sample position in the sample plate. Sample plates are 8x12 arrays with letters indicating rows, and numbers indicating columns.

Lab Sample ID – The unique identifier assigned by a laboratory to a sample.

Clear ID – The unique identifier assigned by the Clear Dx™ system to a sample.

Client Name – Laboratory-designated description that can be used to differentiate sample owners.

Product Name – Laboratory-designated description that can be used to differentiate sample types.

Target Result – Positive, Negative, or Invalid.

Target Virus – The target analyte.

Date Sample Collected – optional field for capturing the date sample was collected.

Run Complete – Date/time stamp for run completion.

Sampling Location – Optional field for capturing the geographical origin of the sample.

Post Processing Procedures

Emptying the Clear Dx™ System

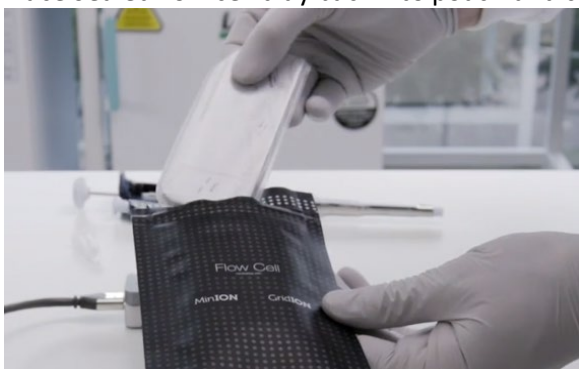
Flush Flow Cell

1. Remove Flow Cell and prepare to flush.
    1. Replace the retained plastic SpotOn port cover.
    2. Hold Flow Cell over a receptacle containing a solution of 10% bleach.
    3. Dispense 4 mL of DI water into the priming port.

    4. Close priming port by turning gate counter clockwise.
    5. Remove excess liquid from waste channel.

    6. Return flow cell to tray. Apply the original plastic snap to close the spotON port.

7. Place sealed flow cell tray back into pouch and close zippered pouch.



8. Store sealed, flushed Flow Cells at room temperature. Contact Clear Labs service to arrange return shipping.

Remove & Dispose Consumables

Empty tip racks can be removed at this time and partial racks can be consolidated. Be sure to use aseptic technique when handling unused pipette tips and take care not to mix different sized tips within a rack.

The following items should be treated as biohazards:

- RNA sample plates (white)
- RT PCR plates (Clear)
- PCR plates (Clear)
- Reagent tubes
- Used sample plates
- Tip waste bag
- Reagent Plate (clear)

The following items potentially contain nucleic acids and need to be neutralized upon removal. Some of them also have ethanol in them. The suggested method is to submerge the listed items in 10% bleach or a DNAse for 30 minutes before disposal (not autoclaved) as hazardous waste.

- Kit 1, tubes 1, 13 and 14 (neutralize with a DNAse - not bleach)
- Waste tubes (1-8) on Rack 2 (neutralize with a DNAse - not bleach)
- PCR covers
- PCR Plates (clear)
- Cover plate (clear)

Technical Support

For questions or comments, please contact your Clear Labs Field Application Scientist. You can also email service@clearlabs.com

Quality Control

The following controls are used in Clear Dx™ SARS-CoV-2 Test for ascertaining the validity of the sample, assay and run. The validity of these controls are factored into making the final calls for the samples.

- A ***"No template" (negative) control (NTC)*** is needed to verify the absence of viral template contamination in all reagents of all steps of the workflow to monitor false positive signals. This will be accomplished by the use of **molecular grade PBS buffer (Gibco DPBS, no Ca^{2+} no Mg^{2+}, Catalog # 14-190-144 from Fisher Scientific)** or equivalent as a negative specimen, alongside other clinical specimens input in the extraction step. This control will be in all assay runs, representing one negative sample irrespective of the total number of clinical specimens run (minimum 48 – maximum 190 clinical specimens/run). This is not provided with the kit.

- A ***positive assay control*** is needed to verify that the assay run from extracted RNA to result is performing as intended. This is not subjected through any extraction and is included in the test run only after the extraction step. This provides certainty of reverse transcription reaction, robotic liquid handling operations, stability of assay reagents, proper operation of other ancillary instrumentation such as thermocycler, magnets, etc. on the automation platform and the bioinformatics pipeline and reporting software. To accomplish this, Clear Dx™ uses **Twist Synthetic SARS-CoV-2 RNA Control 2 (Accession # MN908947.3; Catalog # 102024)** diluted by 20,000x to 50,000 copy equivalents/mL (roughly 5x the LoD from extracted RNA). This control is a non-encapsulated product consisting of 6 non-overlapping 5 kb synthetic RNA fragments

spanning near the entire viral genome (>99.9%). This control will be in all runs, representing one negative sample irrespective of the total number of clinical specimens run (minimum 48 – maximum 190 clinical specimens/run). This is not provided with the kit.

- An ***extraction control or sample quality control*** validating that human RNA is present, is needed for clinical samples and is used to determine if the RNA extraction process occurred successfully and the sampling procedure is valid. For this reason, Clear Dx™ SARS-CoV-2 kit includes **barcoded primers for human RNase P** added into each of the barcoded primer set to be used for individual sample. These primers will amplify the human RNase P in all samples and hence will serve as positive extraction control as well as a control for sample quality.

- An ***internal control*** is needed to verify successful PCR amplification within each sample well. Clear Dx™ SARS-CoV-2 Test uses a synthetic DNA template of 367bp, added in the PCR mix, along with corresponding indexed primers to that target, to conclusively identify that PCR occurred in each well, delineating invalid wells. Internal control is present in all sample wells, including the assay controls. Along with the positive assay control, this also verifies the entire assay in every single well. The internal control template and primers are provided with the test kit in reagent kits CLDX-K100 and CLDX- K400 & K500 respectively.

- In addition to internal control, Clear Dx™ SARS-CoV-2 kit also includes a ***sequencing library control***, a synthetic DNA sequence of 600bp length. This is added just prior to sequencing in the assay workflow and is needed to verify the complete processing of the pooled sequencing library. Note that there are no library normalization steps part of the assay. This allows for the identification of errors in sequencing or library preparation. The sequencing library control is added once per sequencing run, present in all runs regardless of the number of clinical specimens completed (minimum 48 – maximum 190 clinical specimens/run).

Interpretation of Results

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

a) Negative control: If a positive result is identified in the NTC of the assay, all positive results in clinical samples must be re-extracted and retested with a new reagent kit. If the NTC well results in a negative viral signal, it can be concluded that no viral template contamination is present.

b) Positive control: If the positive control well with the viral RNA control does not result in a positive result in a run, then all negative samples in the run cannot be conclusively ruled out for viral presence and they need to be re-tested from their extracted RNA. However, the samples that were called positive, can be declared as positive.

c) Extraction control (or) Sample quality control: In clinical samples where human RNA is expected to be present, if the extraction control human RNA target does not amplify and does not produce any sequencing reads, and no viral reads are detected, then the sample must be re-extracted and retested or resampled if failed multiple times. A minimum threshold of 10 sequencing reads for RNAse P marker is established to call this control "positive". This threshold has been established based on training data from Nasopharyngeal clinical swab specimens.

d) Internal control: If any sample well fails to produce less than 20 sequencing reads at the final step of the BIP, including either reads of viral amplicons or internal control but excluding RNAse P, then that individual sample and well must be ruled "invalid", and retested. This threshold of 20 reads was determined based on a large set of training data comprising several runs.

e) Sequencing library control: If sequencing results have no sequencing library control reads in the run, and no reads for internal control or any of the viral amplicons, it can be concluded that the library preparation or sequencing portion of the Clear Dx™ SARS-CoV-2 assay did not complete. In this instance the user is reported of a sequencing run failure and is prompted to reload all reagents and samples and retest all wells.

f) Run-level performance control: Along with the Sequencing library control having non-zero reads, the total number of reads in a run that survive past the quality filters should be greater than 200,000 for a run to be considered valid. This threshold has been established based on a large training dataset from our development runs. If a run has < 200,000 reads, the user is reported of a sequencing run failure and is prompted to repeat the run with a new test kit, starting from the extracted RNA.

The acceptance criteria for a valid result for a clinical sample based on all the above controls is shown in the Table below.

SARS-CoV-2 Detected in Sample*	Internal Control in Sample	Extraction control in Sample	Sample Result	Interpretation	Action
+	Pass	Pass	SARS-CoV-2 Detected	SARS-CoV-2 RNA Detected	Report results to sender and appropriate public health authorities

SARS-CoV-2 Detected in Sample*	Internal Control in Sample	Extraction control in Sample	Sample Result	Interpretation	Action
+	Pass	Fail	SARS-CoV-2 Detected	SARS-CoV-2 RNA Detected	Report results to sender and appropriate public health authorities
+	Fail	Pass	SARS-CoV-2 Detected	SARS-CoV-2 RNA Detected	Report results to sender and appropriate public health authorities
+	Fail	Fail	SARS-CoV-2 Detected	SARS-CoV-2 RNA Detected	Report results to sender and appropriate public health authorities
-	Pass	Pass	SARS-CoV-2 Not Detected	SARS-CoV-2 RNA Not Detected	Report results to sender and appropriate public health authorities
-	Fail	Pass	SARS-CoV-2 Not Detected	SARS-CoV-2 RNA Not Detected	Report results to sender and appropriate public health authorities
-	Pass	Fail	Invalid	Invalid Result	Repeat test from extracted RNA. If a second test yields 'Invalid', report results to sender and request a second sample if clinically indicated
-	Fail	Fail	Invalid	Invalid Result	Repeat test from extracted RNA. If a second test yields 'Invalid', report results to sender and request a second sample if clinically indicated

Limitations of the test

- ✓ If Clear Dx™ automation fails for unforeseen reasons like a power failure or ethernet cable malfunction, the entire run fails, and all the RNA used for the test get compromised. For this reason, we always recommend saving the RNA extracts for all the samples in 2-8°C until the results for all the samples have been successfully obtained without being required to repeat.
- ✓ Automated liquid handling requires precise positioning of the robotic arm and for some reasons if the robotic platform gets dislodged from its stable position because of a lab accident or an earthquake, the system must be realigned to its original position. If such an incident happened but went unnoticed, then the specific run may produce erroneous results.
- ✓ False-negative results may arise from degradation of the viral RNA during shipping and storage.

- ✓ An increased rate of invalid results was observed for clinical specimens that are subjected to one or more freeze thaw cycles prior to testing with the Clear Dx™ SARS-CoV-2 assay.
- ✓ As with any molecular test, mutations within the target regions of the Clear Dx™ SARS-CoV-2 Test could affect the primer binding resulting in failure to detect the presence of the virus.
- ✓ Negative results do not preclude infection with the SARS-CoV-2 virus and should not be the sole basis of a patient treatment and management or public health decision. Follow up testing should be performed according to the current CDC recommendations.
- ✓ Typically, RNA may persist even after the virus is no longer viable in the patients. A positive result in Clear Dx™ test indicates just the detection of the SARS-CoV-2 RNA and no claim is made on the viability of the actual virus. However, unlike most assays, Clear Dx™ test looks for several longer regions of the viral genome.
- ✓ The performance of the Clear Dx™ SARS-CoV-2 test was established using nasopharyngeal swab specimens only. Oropharyngeal swab, anterior nasal swabs, mid-turbinate nasal swabs, nasopharyngeal wash/aspirate, nasal aspirates and bronchoalveolar lavage specimens are considered acceptable specimen types for use with the Clear Dx™ SARS-CoV-2 test, but performance has not been established.
- ✓ 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.

Conditions of Authorization for Labs

The Clear Dx™ SARS-CoV-2 test Letter of Authorization, along with the along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Patients, and authorized labeling are available on the FDA website: https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/in-vitro-diagnostics-euas.

However, to assist clinical laboratories using the Clear Dx™ SARS-CoV-2 test, the relevant Conditions of Authorization are listed below:

- ✓ Authorized laboratories* using the Clear Dx™ SARS-CoV-2 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.
- ✓ Authorized laboratories using the Clear Dx™ SARS-CoV-2 test must use the Clear Dx™ SARS-CoV-2 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 the Clear Dx™ SARS-CoV-2 test are not permitted.
- ✓ Authorized laboratories that receive the Clear Dx™ SARS-CoV-2 test must notify the relevant public health authorities of their intent to run the Clear Dx™ SARS-CoV-2 test prior to initiating testing.
- ✓ Authorized laboratories using the Clear Dx™ SARS-CoV-2 test 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 the Clear Dx™ SARS-CoV-2 test and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and Clear Labs Technical Support (via email: service@clearlabs.com) any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of Clear Dx™ SARS-CoV-2 test of which they become aware.
- ✓ All laboratory personnel using the Clear Dx™ SARS-CoV-2 test must be appropriately trained on the Clear Dx™ system, in the techniques of real-time PCR and NGS and use appropriate laboratory and personal protective equipment when handling this kit, and use the Clear Dx™ SARS-CoV-2 test in accordance with the authorized labeling.
- ✓ Clear Labs, authorized distributors, and authorized laboratories using the Clear Dx™ SARS-CoV-2 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.

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

Clear Dx™ SARS-CoV-2 Test Performance

Limit of Detection (LoD) - Analytical Sensitivity:

The analytical sensitivity (LoD) of the Clear Dx™ SARS-CoV-2 Test was determined by making serial dilution of Twist Synthetic SARS-CoV-2 RNA Control 2 (Accession # MN908947.3; Catalog # 102024) in pooled negative nasopharyngeal clinical specimens at four different concentrations – 10000 copies/mL, 5000 copies/mL, 2000 copies/mL and 1000 copies/mL, along with 100pg/µL of carrier RNA and testing them through the entire workflow. All the contrived samples at different RNA concentrations were extracted independently using the MagMAX Viral RNA Isolation Kit (Catalog # AM1939) manual workflow as recommended by the manufacturer with 400 µL as the input sample volume and 50 µL as the elution volume. The extracted RNA from all the contrived samples were run together in four replicate runs by four different operators through Clear Dx™ SARS-CoV-2 test.

The results are summarized in the table below. 97% of the detection calls were accurate for the input concentration of 2000 copies/mL RNA in the negative contrived samples. So, the **LoD of Clear Dx™ SARS-CoV-2 is claimed to be 2000 copies/mL in the clinical sample**.

SARS-CoV-2 Twist RNA (copies/mL)	No. of replicates	Detected as Positive in Clear Dx™ SARS-CoV-2 test	Percent accuracy
10000	3	3	100 %
5000	27	27	100%
2000	33	32	97%
1000	33	27	82%

Results from the experiment to determine analytical sensitivity of the Clear Dx™ SARS-CoV-2 test

LoD Bridging Study for automated extraction with KingFisher Flex Purification System and MagMax Viral/Pathogen nucleic acid isolation kit

A bridging study was conducted to validate the use of automated extraction of SARS-CoV-2 RNA using MagMax Viral/Pathogen nucleic acid isolation kit (Catalog # A42352) on KingFisher Flex Purification system with 96-Deep well head (Catalog # 5400630) for the Clear Dx SARS-CoV-2 test. Inactivated intact virus particles cultured using USA/WA1/2020 SARS-COV-2 isolate from Zeptometrix (Catalog # NATSARS(COV)-ST), available at a stock concentration of 1,000,000 copies/ml, were diluted in negative clinical NP swab specimen matrix to prepare contrived SARS-CoV-2 samples with different virus concentrations. For the automated workflow, the specimen volume processed was 200µl and the elution volume was 50µl. The comparator manual extraction method using MagMax Viral isolation kit used 400µl specimen volume and 50µl elution volume.

The results are summarized in the table below. As observed in the table, the LoD of the Clear Dx™ SARS-CoV-2 test with the automated extraction method is 5400 copies/mL, which is equivalent to or lower than the LoD of the manual extraction method.

Concentration of SARS-CoV-2 in the contrived samples	Factor of LoD	Detection rate for manual extraction using MagMax Viral Isolation Kit	Detection rate for automated extraction using KingFisher Flex and MagMax Viral/Pathogen Nucleic Acid Isolation Kit
1800 copies/ml	1/3x LoD	11/12 (91.7%)	12/12 (100%)
5400 copies/ml	LoD	24/24 (100%)	24/24 (100%)
10800 copies/ml	2x LoD	12/12 (100%)	12/12 (100%)
16200 copies/ml	3x LoD	24/24 (100%)	24/24 (100%)
50000 copies/ml	~ 10x LoD	10/10 (100%)	10/10 (100%)
150000 copies/ml	~ 50x LoD	10/10 (100%)	10/10 (100%)

Original EUA-authorized method: Manual extraction using MagMax Viral RNA Isolation kit
New extraction method: Automated extraction using MagMax Viral Pathogen kit on KingFisher Flex Purification system with 96-Deep well head

Inclusivity:

The inclusivity test was evaluated using *in silico* analysis. In total, 582,249 SARS-CoV-2 genomes were downloaded from GISAID database (https://www.gisaid.org, as of June 28, 2021). A preliminary curation was conducted to remove genomes with 1) lengths <= 29,000 bp; 2) more than 500 bps of ambiguity characters (gaps or N). There were 417,424 genomes left for inclusivity check. A BLASTn (NCBI) analysis was performed to quantify the level of primer homology by querying each of our 21 SARS-CoV-2 primer sequences against the downloaded SARS-CoV-2 genomes. The table below summarizes the homology analysis for all 417,424 SARS-CoV-2 genomes.

Percent of Primer Pairs Homology	Mean	Median	5th Percentile	95th Percentile
100% homology	94.80%	98.70%	82.40%	99.20%
>80% homology	99.80%	99.90%	99.60%	100.00%

Summary of inclusivity analysis for the Clear Dx SARS-CoV-2 primers

To further assess risk for false negative results and ensure that any risk has been adequately mitigated, an additional *in silico* analysis was performed to determine the potential PCR amplification of SARS-CoV-2 target regions in the curated genomes using the Clear Dx™ SARS-CoV-2 primer pairs. The table below shows the distribution of perfect primer pairs (where both forward and reverse primers have 100% identity with the target) in the 417,424 genomes. Even in the worst

case, Clear Dx™ SARS-CoV-2 assay has 13 primer pairs with 100% alignment, indicating that 100% of sequences evaluated in this analysis are predicted to be detected. .

Number for 100% perfect aligned primer pairs	Number of genomes
21	153460
20	135922
19	76841
18	42274
17	8159
16	720
15	42
14	2
13	4
Total genome number	**417424**

***In silico* primer amplification profile for the curated genome set**

Cross-reactivity (Exclusivity):

An *in silico* cross-reactivity analysis was performed to assess if the 21 viral primer pairs in the Clear Dx™ SARS-CoV-2 Test have any cross reactivity with the genomes of the microorganisms commonly present in the clinical specimens collected for SARS-CoV-2 tests. A cohort of 62,916 non SARS-CoV-2 genome sequences, compiled from GenBank on 04/20/2020, was considered for this analysis. A BLASTn (NCBI) analysis was then performed to quantify the number of primer pairs with more than 80% homology with each of the genomes in the cohort. The results of the analysis are shown in the table below.

Organism	Number of genomes (or genome segments) included in analysis	Number of genomes with ≥80% homology to any SARS-CoV-2 viral primers
Human adenovirus	615	0
Bordetella pertussis	1257	0
Candida albicans	1	0
Chlamydia pneumoniae	4	0
Human coronavirus 229E	25	0
Human coronavirus HKU1	39	0

Organism	Number of genomes (or genome segments) included in analysis	Number of genomes with ≥80% homology to any SARS-CoV-2 viral primers
Human coronavirus NL63	59	0
Human coronavirus OC43	170	0
Human enterovirus	322	0
Haemophilus influenzae	109	0
Homo sapiens	1	1
Influenza A	55584	0
Influenza B	1939	0
Legionella pneumophila	168	0
MERS	520	0
Human metapneumovirus	156	0
Mycobacterium tuberculosis	354	0
Mycoplasma pneumoniae	153	0
Human parainfluenza virus	125	0
Pneumocystis jirovecii	15	0
Pseudomonas aeruginosa	381	0
Human rhinovirus	223	0
RSV	9	0
SARS-CoV-1	268	268
Staphylococcus epidermidis	43	0
Streptococcus pneumoniae	86	0
Streptococcus pyogenes	276	0
Streptococcus salivarius	24	0

Summary of *in silico* cross-reactivity analysis results

Of the organisms listed above, only SARS-CoV-1 showed ≥ 80% homology to any of the CoV-2 primers. Those primers are shown in the table below along with the number of SARS genomes (out of a total of 268) for which there was evidence of homology (≥ 80%).

Primer	Number of SARS-CoV-1 genomes with ≥ 80% homology to primer
10_F	7
13_F	260
14_R	268
20_F	266
21_F	268
21_R	268

Primers in Clear Dx™ SARS-CoV-2 Test that show homology to SARS CoV-1

Though many SARS-CoV-2 primers show ≥ 80% homology to SARS CoV-1 genomes, the **assay is not expected to produce a false positive** even in the unlikely case of encountering SARS CoV-1. That is because, unlike methods such as qPCR, sequencing allows for the direct comparison of amplicon sequences to both SARS-CoV-1 and SARS-CoV-2 alleles in order to make the proper taxonomic assignment. Primer 21 is designed to include SARS CoV-1 and that is why all the SARS CoV-1 genomes tested have > 80% homology for that primer set. But the amplicon targeted by these primers has almost 10% difference in their alleles and our BIP can correctly distinguish that. A more detailed study on SARS CoV-1 is presented in the next sub- section.

In addition to the prokaryotic genomes listed above, primer sequences were also aligned against eukaryotic genomes *Candida albicans* and *Homo sapiens* (GRCh38). In some cases, shown in the table below, some primers showed ≥ 80% homology to human chromosomes.

Primer	Human chromosomes with ≥ 80% homology to primer
2_R	1,11,20
3_F	X
3_R	1,12,14,16,20
6_F	11
8_F	14
11_R	17
14_R	3,7,8,9,11,22

16_R	3
18_R	20

Primers in the Clear Dx™ SARS-CoV-2 Test that show homology to human chromosomes

In 10 of the 20 cases of homology with human chromosomes, there are mismatches at or near the 3' end of the primer that would likely be disruptive to primer extension. Note that any human reads that happen to be present in the sequencing output will be identified as not belonging to SARS-CoV-2 on the basis of sequence composition and will be discarded.

We also performed a wet testing experiment for evaluating cross-reactivity, where 25 microorganisms commonly found in the upper respiratory tract of the humans are tested through the Clear Dx™ SARS-CoV-2 Test starting with their extracted RNA or DNA. RNA or DNA of these microorganisms at appropriate concentrations were tested in duplicates along with positive Twist synthetic RNA controls and negative (NTC) controls in a single 96-plate. The results are reported in the table below. None of these pathogens show any cross-reactivity to the SARS-CoV-2 primers in Clear Dx™ assay.

Microorganism name	Material used	Source	Catalog #	Conc. tested (Genome equivalents/µL)	SARS-CoV-2 detected?
Bordetella pertussis	DNA	ATCC	9797D-5	10^6	No
Candida albicans	DNA	ATCC	10231 (#3147)	10^6	No
Chlamydia pneumoniae	DNA extract	ATCC	53592	10^6	No
Coronavirus 229 E	RNA	ATCC	VR-740D	10^5	No
Coronavirus HKU1	Synthetic RNA	ATCC	VR-3262SD	10^5	No
Coronavirus NL63	Synthetic RNA	ATCC	VR-3263SD	10^5	No
Coronavirus OC43	RNA	ATCC	VR-1558D	10^5	No
Enterovirus	RNA	ATCC	VR-1825D	10^5	No
Haemophilus influenzae	DNA	ATCC	51907D-5	10^6	No
Influenza A H1N1	RNA	ATCC	VR-1736D	10^5	No
Influenza B H3N2	RNA	ATCC	VR-1535D	10^5	No
Legionella pneumophila	DNA	ATCC	33152DQ	10^6	No
MERS Coronavirus	RNA	ATCC	3248SD	10^5	No
Metapneumovirus 8	RNA	ATCC	VR-3250SD	10^5	No
Mycobacterium tuberculosis	DNA	ATCC	25177DQ	10^6	No

Microorganism name	Material used	Source	Catalog #	Conc. tested (Genome equivalents/µL)	SARS-CoV-2 detected?
Mycoplasma pneumoniae	DNA	ATCC	25177DQ	10^6	No
Parainfluenza 1	RNA extract	ATCC	VR-94D	10^5	No
Parainfluenza 2	RNA extract	ATCC	VR-92D	10^5	No
Parainfluenza 3	RNA extract	ATCC	VR-93D	10^5	No
Pseudomonas aeruginosa	DNA	ATCC	47085DQ	10^6	No
Rhinovirus 1A	RNA	ATCC	VR-283DQ	10^5	No
Respiratory Syncytial Virus A	RNA	ATCC	VR-1580DQ	10^5	No
SARS Coronavirus	Synthetic RNA	Twist	SARS 2003	10^5	No
Staphylococcus epidermidis	DNA	ATCC	12228DQ	10^6	No
Streptococcus pneumoniae	DNA	ATCC	700669DQ	10^6	No
Streptococcus pyogenes	DNA	ATCC	BAA-572D-5	10^6	No

Summary of the wet testing results of the cross-reactivity study

Microbial Interference Studies:

As explained in the section above, only SARS-CoV-1 has homology to primers/probes of the SARS-CoV-2 in the Clear Dx™ test. But the bioinformatic analysis of Clear Dx™ platform can clearly differentiate the reads based on the allelic difference of the sequences if they are one or the other.

We performed a microbial interference study by adding synthetic SARS CoV-1 RNA (10^7 genome equivalents/mL) from Twist Bioscience to SARS-CoV-2 synthetic RNA (Accession # MN908947.3; Catalog # 102024) at 10,000 (~5x LoD) & 100,000 genome (~50x LoD) equivalents/mL. We did not observe any degradation of detection calls of SARS-CoV-2 because of the addition of SARS CoV-1. The results are summarized in the table below.

Sample	# of replicates	SARS-CoV-2 detected?
SARS-CoV-2 RNA (100,000 copies/mL)	3	Yes
SARS-CoV-2 RNA (10,000 copies/mL)	3	Yes
SARS CoV-1 RNA (10^7 copies/mL)	3	No
SARS-CoV-2 RNA (100,000 copies/mL) + SARS CoV-1 RNA (10^7 copies/mL)	3	Yes

SARS-CoV-2 RNA (10,000 copies/mL) + SARS CoV-1 RNA (10^7 copies/mL)	3	Yes

Summary of the microbial interference study with SARS CoV-1

Endogenous Interference Substances Studies:

Clear Dx™ SARS-CoV-2 Test uses RNA extracted from standard, well-established extraction methods as discussed in the earlier sections. The assay is not expected to experience any impact from any potential interfering substances.

Clinical Performance Evaluation:

Clinical evaluation was performed by directly comparing the results of Clear Dx™ SARS-CoV-2 Test against a Real-Time Reverse Transcriptase (RT) PCR Diagnostic authorized by the FDA for Emergency Use Authorization on the samples provided by the same lab as described below.

50 positive and 32 negative clinical nasopharyngeal (NP) patient specimens either in viral transport media (VTM) or sterile saline buffer were procured. RNA from these clinical samples were extracted using the manual workflow with MagMAX Viral RNA Isolation kit (Catalog #AM1939). The specimen volume processed was 400 µL for all the samples and the elution volume was 50µl. The extracted RNA from these clinical samples were run alongside 14 negative controls. The summary of the results is shown in the table below. The samples ranged in Ct value from 15-38 (7-log range).

		Clear Dx™ SARS-CoV-2 Test		
		Positive	**Negative**	**Total**
EUA Comparator assay	**Positive**	50	0	50
	Negative	0	32	32
Positive Percent Agreement (PPA)		**50/50 (100%) 95%CI 92.9-100%**		
Negative Percent Agreement (NPA)		**32/32 (100%) 95%CI 89.3 – 100%**		

Summary of Clinical evaluation results

FDA SARS-CoV-2 Reference Panel Testing

The analytical sensitivity and MERS-CoV cross-reactivity were further evaluated at the instruction of the FDA using reference material (T1), blinded samples and a standard protocol provided by the FDA. The study included a range finding study and a confirmatory study for LoD. Blinded sample testing was used to establish specificity and to confirm the LoD. The extraction of RNA was performed manually using the MagMax Viral RNA Isolation Kit. The results are summarized in the table below. Based on the results from this testing, the LoD of the product was subsequently ascertained to be 5.4 x 10^3 NDU/mL and reported in the FDA website containing the reference panel results for the EUA-authorized molecular diagnostic results.

Reference Materials Provided by FDA	Specimen Type	Product LoD	Cross-Reactivity
SARS-CoV-2	NP swabs	5.4 x 10^3 NDU/mL	N/A
MERS-CoV		N/A	ND

NDU/mL = RNA NAAT detectable units/mL
N/A: Not applicable
ND: Not detected at the highest concentration supplied

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

Appendix A: Clear Dx™ System Installation and Operations Qualification Protocols

To verify Clear Dx SARS-CoV-2 runs that can be loaded in the web base application, the instrument can be properly run by the instrument PC software, the instrument can successfully execute the protocol on Clear Dx™ validation plates, installation and operations Qualification Protocol testing that is conducted using the System Operational Qualification Protocols

- VAL-0007, Clear Dx™ System Installation Qualification Protocol
- VAL-0008, Clear Dx™ System Operational Qualification Protocol

Operational Qualification testing is performed by Clear Lab's qualified Field Applications personnel along with the qualification personnel in the laboratory where the instrument is installed following the protocols that will be provided by Clear Labs to each laboratory.

Test Scripts

Test Script 1.2.1: Operational Qualification Runs

Test Requirements:

The Executor must have the Clear Dx™ Validation plates.

The Executor must be logged in with Admin privileges.

All test scripts must be executed by trained laboratory personnel.

The Executor should follow and reference the procedures in the Clear Dx™ Instructions for Use as well as the 1.2.1 Test Scripts

Test Inputs:

Clear Dx™ Validation Plates

Clear Dx™ Reagent kits as necessary

Any prepared associated .csv files

Acceptance Criteria for each Passing Run: (all the following criteria are built-in our Clear DxTM SARS-CoV-2 test report and no subjective interpretation is involved)

< 2 invalid wells

< 1 false positive

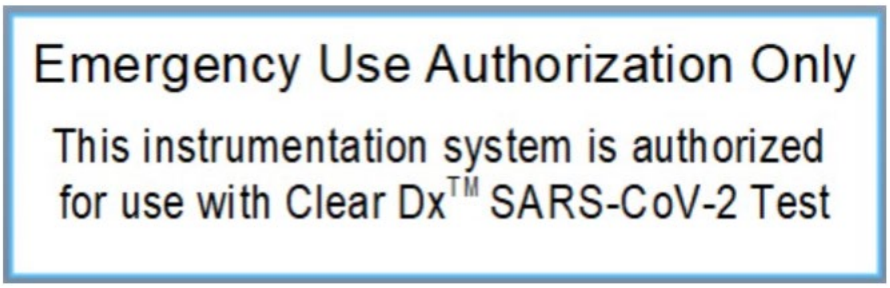
< 1 false negative

Acceptance Criteria for OQ Completion: 3 passing runs total, with at least 2 consecutive passing runs

Appendix B: Clear Labs Dx Instrument System Label

For EUA Clear Labs Dx™ Instrument System

Please print and place this label (Item Number: 71-0004, rev. A) on the front panel of the instrument. If the instrument includes labeling indicating "For Research Use Only", please cover with the below "Emergency Use Authorization Only" labeling. The instrument should retain this labeling throughout the EUA use of the Clear Labs Clear Dx™SARS-CoV-2 Assay Kit.



Appendix C: Symbols Legend

Symbol	Meaning
	Catalog Number
	In Vitro Diagnostic Medical Device
	Batch Number
	Manufacturer
	Expiration Date YYYY-MM-DD
	Temperature Limitation
	For Prescription Use Only

Clear Labs Technical Support

Phone: 650-257-3304

Email service@clearlabs.com

For technical assistance, contact your local technical support office or click the Contact us link at:
https://www.clearlabs.com/contact-us/

END OF DOCUMENT