EMERGENCY USE AUTHORIZATION (EUA) SUMMARY
Assurance SARS-CoV-2 Panel DTC
(Assurance Scientific Laboratories)

For *In vitro* Diagnostic Use
For use under Emergency Use Authorization (EUA) only

(Direct to consumer product for testing of anterior nasal swab specimens self-collected by individuals using either the Simplicity COVID-19 Home Collection Kit or the Everlywell COVID-19 Test Home Collection Kit DTC using the Assurance SARS-CoV-2 Panel DTC will be performed at Assurance Scientific Laboratories, or other laboratories designated by Assurance Laboratories that are also certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, and meet the requirements to perform high complexity tests, as described in the laboratory procedures that were reviewed by the FDA under this EUA.)

INTENDED USE

The Assurance SARS-CoV-2 Panel DTC is a direct to consumer product for testing of anterior nasal swab specimens self-collected at home using either: (1) the Simplicity COVID-19 Home Collection Kit; or (2) the Everlywell COVID-19 Test Home Collection Kit DTC by any individuals, 18 years and older, including individuals without symptoms or other reasons to suspect COVID-19. Testing of self-collected anterior nasal swab specimens is limited to laboratories designated by Assurance Scientific Laboratories, that are certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C.§263, and meet the requirements to perform high-complexity tests.

All test results from specimens collected with the Simplicity COVID-19 Home Collection Kit are delivered to the user via an online portal. Individuals with positive and invalid/indeterminate results additionally will be contacted by a healthcare provider. The direct to consumer home collection system is intended to enable users to access information about their COVID-19 infection status that could aid with determining if self-isolation or quarantine is appropriate and to assist with healthcare decisions after discussion with a healthcare provider.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in anterior nasal swab specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with medical history and other diagnostic information is necessary to determine 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.

The Assurance SARS-CoV-2 Panel DTC is not a substitute for visits to a healthcare provider. The information provided by this kit should not be used to start, stop, or change any course of treatment unless advised by your healthcare provider.

Testing with the Assurance SARS-CoV-2 Panel DTC is intended for use by trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures. The COVID-19 RT-PCR is only for use under the Food and Drug Administration's Emergency Use Authorization.

DEVICE DESCRIPTION AND TEST PRINCIPLE

The Assurance SARS-CoV-2 Panel DTC is a direct to consumer product for testing of anterior nasal swab specimens self-collected at home using either the Simplicity COVID-19 Home Collection Kit or the Everlywell COVID-19 Test Home Collection Kit DTC by individuals, 18 years and older, without symptoms or other reasons to suspect COVID-19.

The Simplicity COVID-19 Home Collection Kit will be available direct to consumer (DTC) without a prescription at a physical retail location and online direct to consumer for any individual 18 years and older. When ordering a kit online individual must verify they are 18 years or older. Individuals are recommended to complete a screening questionnaire when registering their kit after receiving it, but it is not a requirement. After sending the self-collected (unsupervised) anterior nasal swab back to Assurance Scientific Laboratories, or other laboratories designated by Assurance Laboratories, testing is performed using the Assurance SARS-CoV-2 Panel DTC. All test results from specimens collected with the Simplicity COVID-19 Home Collection Kit are delivered to the user via an online portal. Additionally, individuals with positive and invalid results are contacted by a healthcare provider. Healthcare provider will be a contracted Telehealth company (i.e., PWN Health) or employee of Assurance Scientific Labs. Specifically, all individuals with positive and invalid results will receive a text stating the results of the test, providing an option to speak with a HCP if desired and requesting acknowledgement of the text. Individuals that do not acknowledge receipt of the text, will be called by a HCP. For purposes of this EUA, a healthcare provider includes any healthcare professional with prescribing abilities including, but not limited to, physicians, nurses, pharmacists, technologists, laboratory directors, and epidemiologists. The healthcare provider contacting individuals with test results will have prescribing privileges for that individual, should medication be indicated for treatment.

The Simplicity COVID-19 Home Collection Kit is composed of a swab, collection tube containing saline, label for tube if not already on the tube, return shipping box/envelope, biohazard bag with absorbent for specimen, instructions for collection and shipment, alcohol prep pad, return shipping label, and the Fact Sheet for Individuals for the Assurance SARS-CoV-2 Panel DTC. Instructions are included in the kit to direct the home users on how to appropriately collect the nasal swab specimen and place it in the saline transport tube, how to properly package the specimen and how to mail the specimen back to the laboratory using the pre-paid shipping label.

The Everlywell COVID-19 Test Home Collection Kit DTC is a direct to consumer product for self-collecting an individual anterior nasal swab specimen at home (unsupervised) and sending that specimen for testing to laboratories designated by Everlywell for testing with a real-time reverse transcription polymerase chain reaction (rRT-PCR) test for the qualitative detection of nucleic acid from SARS-CoV-2 that is authorized for testing individuals without symptoms or other reasons to suspect COVID-19 and has been issued an EUA for use with

Home Collection Kits, that includes the Everlywell COVID-19 Test Home Collection Kit DTC. Assurance Scientific Laboratories using the Assurance SARS-CoV-2 Panel DTC has been designated by Everlywell. Test results for the anterior nasal swab specimens collected with the Everlywell COVID-19 Test Home Collection Kit DTC are reported back to Everlywell and delivered to the user via the process outlined in the Everlywell COVID-19 Test Home Collection Kit DTC EUA summary (found at https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/vitro-diagnostics-euas).

The Simplicity COVID-19 Home Collection Kit and Everlywell COVID-19 Test Home Collection Kit DTC are direct to consumer products for self-collecting an individual nasal swab specimen at home and sending that specimen for testing with the Assurance SARS-CoV-2 Panel DTC. The Assurance SARS-CoV-2 Panel DTC is a real-time reverse transcription polymerase chain reaction (rRT-PCR) test. The SARS-CoV-2 primer and probe set(s) is designed to detect RNA from the SARS-CoV-2 in respiratory specimens from individuals. It is authorized for testing individuals suspected of COVID-19 by their HCP and individuals without symptoms or other reasons to suspect COVID-19.

Sample Preparation

Two extraction chemistries are validated for COVID-19 PCR testing with the Assurance SARS-CoV-2 Panel DTC: Abnova Precipitor32 or Indical Indimag 48 (using the Zymo Quick-RNA Viral Kit RNA Extraction Kit). The underlying workflow involves adding a lysis buffer that will disrupt cellular material and release nucleic acids. The lysis buffer inactivates nucleases present in the specimen. Magnetic silica is added to the lysed specimen and under high salt concentrations, the nucleic acids bind to the magnetic silica. Following two washes, the nucleic acids are eluted from the magnetic silica into the elution buffer.

Amplification

Detection of SARS-CoV-2 RNA uses reverse transcriptase PCR (RT-PCR) to detect the viral nucleoprotein (N) gene. This portion of the genome is conserved in other bat-derived betacoronaviruses and not conserved among other coronaviruses. RT-PCR amplifies RNA targets by first producing cDNA from the RNA target. The cDNA is then amplified by PCR. The TaqPath 1-Step RT-qPCR Master Mix allows this process to proceed without the addition of reagents between the RT and PCR steps.

The addition of a TaqMan probe serves to eliminate detection of nonspecific amplification in the reaction. The probe consists of an oligonucleotide with a 5’-reporter dye (FAM) and a 3’-quencher dye (BHQ1). If the target is present, the probe will anneal between the forward and reverse primer sites. In this setting, the proximity of the reporter dye to the quencher dye results in suppression of the reporter fluorescence. The 3’ end of the probe is blocked so that the probe cannot be extended during PCR. DNA polymerase exonuclease activity cleaves the TaqMan probe during PCR. This separates the reporter dye from the quencher dye, resulting in increased fluorescence of the reporter. This allows detection of the accumulation of PCR products.

Detection

The BioRad CFX96 or CFX384 is used for qualitative and quantitative detection with

fluorescent-based PCR chemistries. During PCR, light from a lamp is focused on each well of the microplate. The light excites the fluorescent dye in each well and emission between 500 nm and 600 nm is detected. The system allows data analysis and reporting in a variety of formats.

INSTRUMENTS USED WITH TEST

Instruments

The Assurance Scientific Laboratories - Assurance SARS-CoV-2 Panel DTC, is a real-time RT-PCR test is to be used with the Abnova Precipitor32 or Indical Indimag 48 (using the Zymo Quick-RNA Viral Kit RNA Extraction Kit) and the BioRad CFX96 and BioRad CFX384 with the BioRad CFX Maestro software.

Designated laboratories will receive an FDA accepted instrument qualification protocol included as part of the laboratory SOP and will be directed to execute the protocol prior to testing clinical samples. Designated laboratories must follow the authorized SOP, which includes the instrument qualification protocol, as per the letter of authorization.

Collection Kits

- The Assurance SARS-CoV-2 Panel DTC can be used with the Everlywell COVID-19 Test Home Collection Kit DTC to self-collect anterior nasal swab specimens at home. Everlywell has granted Assurance Scientific Laboratories a right of reference to the data supporting the use of this authorized home collection kit. The IFU for the Everlywell COVID-19 test home collection kit DTC is available at https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/vitro-diagnostics-euas.

- The Assurance SARS-CoV-2 Panel DTC is also for use with the Simplicity COVID-19 Home Collection Kit to self-collect anterior nasal swab specimens at home.

Reagents

The primary reagents used in this assay, including primer and probe designs, are adapted from the "CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel" document effective March 30, 2020.

Kits and Reagents	Manufacturer	Catalog #
Abova Precipitor32: Viral Total Nucleic Acid Purification Kit	Abnova	U0382
Zymo Quick-RNA Viral Kit RNA Extraction Kit	Zymo	R2140 or R2141
TaqPath 1-Step RT-qPCR Master Mix, CG	ThermoFisher	A15299
Primer: COVID-19_N1-F	IDT or Biosearch	Custom

Primer: COVID-19_N1-R	IDT or Biosearch	Custom
Probe: COVID-19_N1-P	IDT or Biosearch	Custom
Primer: RP-F	IDT or Biosearch	Custom
Primer: RP-R	IDT or Biosearch	Custom
Probe: RP-P	IDT or Biosearch	Custom
Template: 2019-nCoV_N_Positive Control	IDT or Biosearch	
Template: Hs_RPP30 Positive Control	IDT or Biosearch	

Simplicity COVID-19 At-Home Kit Components:

The test kit sent to users includes the following components:

Name	Description	Quantity	Material Supplier
Assurance SARS-CoV-2 Panel DTC Fact Sheet for Individuals	Assurance SARS-CoV-2 Panel DTC Fact Sheet for Individuals	1	Assurance Scientific Laboratories
Flocked Nasal Swab	Flocked Nasal Swab	1	Miraclean or Puritan
Collection Tube	Saline 0.85%, 3 mL	1	Edge Biologicals (T-0627) or equivalent
Tube Label	Label for individual to label tube with name, date of birth and collection date	1	various
Alcohol Prep Pad	Pad with alcohol on it	1	various
Biohazard bag with Absorbent Sheet	Biohazard bag with zipper closure that contains and absorbent sheet	1	various
Poly mailer	Poly mailer with UN3373 label and return label	1	various
Specimen shipping box	Specimen shipping box with UN3373 label	1	various
Instructions for Use	Document with instruction for use, help, and contact information	1	various

CONTROLS TO BE USED WITH THE ASSURANCE SARS-COV-2 PANEL DTC

1. A “no template” control (NTC) serves as a negative control and is included in every assay plate to identify specimen contamination. Molecular grade, nuclease free water is used as the NTC.

2. A positive template control is included in each assay plate to ensure the reagents and instruments are performing optimally. The positive control is a synthetic RNA (ultramers) containing the target sequence of gene N of the COVID-19 virus. Two markers in gene N, as defined by the “CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel” document effective March 30, 2020, will be targeted and detected by the primer and probe sets, COVID-19_N1 and COVID-19_N2.

3. An internal control (Hs_RPP30 Positive Control) targeting human RNase P mRNA (RP)

is used to verify optimal RNA extraction, amplification, and the presence of nucleic acid in the samples.

INTERPRETATION OF RESULTS

These controls will be analyzed on each plate.

- Positive control assays using ultramers for each N gene assay will be analyzed on each plate. Lung RNA will be used for the RNase P assay. These will be analyzed in the 30 Ct range to prevent issues due to template degradation.
- The extraction control with be the RNase P assay.

External Control results are interpreted as defined by the CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel Instructions for Use.

Control Type	External Control Name	Used to Monitor	2019-nCoV_N1	RP	Expected Ct Values
Positive	nCoV PC	Substantial reagent failure including primer and probe integrity	+	+	<40.00 Ct
Negative	NTC	Reagent and/or environmental contamination	-	-	None detected

- If controls do not amplify as expected, then the extracted sample analysis will be repeated on another plate.

The table below lists the expected results for the Assurance SARS-CoV-2 Panel DTC.

SARS-CoV-2 N1	RP	Result Interpretation	Ct	Report	Actions
+	±	SARS-CoV-2 detected	<40	Positive SARS-CoV-2	Report results to appropriate public health authorities and individual*.
-	+	2019-nCoV not detected	<40	Not Detected	Report results to appropriate public health authorities and individual*.
-	-	Invalid Result	≥40	Invalid	Repeat extraction and rRT-PCR. If the repeated result remains invalid, request a new specimen from the individual.

* For at home collection using the Everlywell COVID-19 Test Home Collection Kit DTC, reporting will be done through an Application Program Interface to Physicians Wellness Network (PWN). For details on this process, please refer to Everlywell's - Everlywell COVID-19 Test Home Collection Kit DTC EUA summary that can be found at https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/vitro-diagnostics-euas. Please see the Device Description and test Principle section above for the reporting process used for the Simplicity COVID-19 Home Collection Kit.

SAMPLE INTAKE - INSPECTION OF SAMPLES

Specimen Accessioning:
A laboratory protocol was submitted and reviewed by FDA, but a summary is provided below. Specimens received at the clinical laboratory for testing with the Simplicity COVID-19 Home Collection Kit undergo the following accessioning prior to acceptance for testing

1. The laboratory receives the sample
2. The laboratory retrieves the sample information by entering individual's information in the LIS and retrieving an electronic record.
3. The sample is processed if it has sufficient volume and fully labeled without errors

The following errors require rejection of the sample or resolution by the ordering provider:

1. Sample was not registered by the individual
2. Insufficient volume or leakage
3. The label does not contain, the individual name, date of birth and collection date
4. The information on the label does not match the electronic record

For the Everlywell COVID-19 Test Home Collection Kit DTC specimen accessioning will be preformed via the Everlywell SOP "Receiving and Processing Everlywell Samples"

Overview of Manufacturing and Distribution:
The saline tubes will be manufactured by Edge Biologicals or equivalent manufacturer. The swabs will be manufactured by Miraclean, Puritan, or equivalent product manufacturer. These products will be manufactured by Edge Biologicals, Miraclean, Puritan, or equivalent personnel consistent with practices for the production of home collection kits based on cGMP. Material manufactured by Edge Biologicals, Miraclean, Puritan, or equivalent may be bottled and kitted by GBF Medical Group (1055358) or equivalent.

The current manufacturing capabilities include the ability to manufacture approximately 600,000 products per week; however, in the event of a surge in demand, this could be increased to 1 million products per week within a few weeks.

PERFORMANCE EVALUATION

Note: The Assurance SARS-CoV-2 Panel DTC represent the same real-time RT-PCR test used for different indications of use. In addition, Everlywell granted Assurance Scientific Laboratories a right of reference to the data supporting the use of the Everlywell COVID-19 test home collection kit DTC for testing by Assurance Scientific Laboratories.

1) Limit of Detection (LoD) -Analytical Sensitivity:

The LoD study was performed using viral genomic RNA from BEI using the Abnova Total Nucleic Acid Purification Kit and Zymo Research Quick DNA/RNA Viral MagBead kit for RNA extraction and the CFX96 thermocycler. 10-fold serial dilutions of genomic RNA were

spiked into pooled respiratory matrix (NP and OP swabs collected in liquid Amies) to obtain the preliminary LoD. The LoD was confirmed by testing approximately 2 or 3-fold dilutions of genomic RNA spiked into matrix. The concentrations of genomic RNA in the tables below show the amount of genomic RNA spiked into the matrix; the LoD was determined assuming 100% extraction efficiency. The claimed LoD is 9 genomic copies/µL for all methods. It should be noted that an LoD of 5 copies/ul was found for the assay used with the Abnova. However, because this is within 2X of the LoD found with Zymo (indicating comparable sensitivity), a more conservative LoD estimation of 9 copies/ul is claimed for the assay.

Table 1. Limit of Detection Confirmation of the Assurance SARS-CoV-2 Panel with Abnova

Targets	**2019-nCoV_N1**	
Concentration (genomic copies/µL)	9	5
Positives/Total	20/20	20/20
Mean Ct[1]	30.74	32.48
Standard Deviation (Ct)	0.29	0.36

[1]Mean Ct reported for dilutions that are ≥ 95% positive. Calculations only include positive results.

Table 2. Limit of Detection Confirmation of the Assurance SARS-CoV-2 Panel with Zymo

Targets	**2019-nCoV_N1**	
Concentration (genomic copies/µL)	29	9
Positives/Total	20/20	20/20
Mean Ct[1]	30.29	31.57
Standard Deviation (Ct)	0.33	0.35

[1] Mean Ct reported for dilutions that are ≥ 95% positive. Calculations only include positive results.

The assay LoD was also confirmed on the CFX384 thermocycler. Because assay performance is comparable between Zymo and Abnova extraction methods (i.e., within 2X), only Zymo was used to confirm the assay LoD of 9 copies/ul on the CFX384. See results in table below.

Table 3. Confirmation of the LOD with the CFX384

Extraction Method	**Zymo**	
Targets	**2019-nCoV_N1**	
Concentration (genomic copies/µL)	29	9
Positives/Total	19/19	20/20
Mean Ct[1]	26.86	27.84
Standard Deviation (Ct)	0.36	0.32

[1]Mean Ct reported for dilutions that are ≥ 95% positive. Calculations only include positive results.

The LoD of the assay was also confirmed in normal saline by conducting a side-by-side bridging study comparing the two transport media. The two media are considered equivalent if the lowest concentrations that yield 100% positivity in each medium are within 3-fold of each other.

The bridging study was performed by testing 3-fold serial dilutions using heat inactivated virus from BEI spiked into pooled respiratory matrix from samples collected in saline and liquid Amies. The samples were extracted using the Abnova Precipitor Total Nucleic Acid kit and then tested on the BioRad CFX96 thermocycler. Dilutions were tested in triplicate until a <100% hit rate was observed. The lowest concentration at which all three replicates were positive in saline and Amies was 6.31E-03 and 1.89E-02 $TCDI_{50}$/reaction, respectively. These concentrations are within 3-fold of one another therefore the media are considered to be comparable.

Table 4. Bridging Study Demonstrating Equivalent Assay Performance in Amies and saline

Virus	Saline - N1				Amies - N1			
$TCID_{50}$/reaction	Ct	Ct	Ct	Mean Ct	Ct	Ct	Ct	Mean Ct
5.11E -01	30.14	27.05	29.94	29.04	31.41	30.7	31.21	31.11
1.70E -01	31.85	28.69	31.47	30.67	33.45	32.39	33.71	33.18
5.68E -02	32.7	33.04	33.13	32.96	34.37	33.96	34.59	34.31
1.89E -02	34.86	34.22	35.67	34.92	36	35.86	38.23	36.70
6.31E -03	37.92	37.96	36.63	37.50	38.18	38.09	-	38.14
2.10E -03	-	-	38.1	38.10	38.27	38.11	-	38.19
7.01E -04	37.08	37.03	-	37.06	-	-	-	-

2) Reactivity (Inclusivity):

The Assurance SARS-CoV-2 Panel utilizes the identical oligonucleotide sequences as those used in the FDA authorized CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel authorized February 4, 2020. An alignment was performed with the N1 and N2 oligonucleotide primer and probe sequences designed by the CDC with all publicly available in the Global Initiative on Sharing All Influenza Data (GISAID, https://www.gisaid.org) database as of June 20, 2020 (31,623 sequences), to demonstrate the predicted inclusivity of the 2019- nCoV Real-Time RT-PCR Diagnostic Panel. With the exception of one nucleotide mismatch with frequency > 1% (2.00%) at the third position of the N1 probe, the frequency of all mismatches was < 1%, indicating that prevalence of the mismatches was sporadic. Only one sequence (0.0032%) had two nucleotide mismatches in the N1 probe, and one other sequence from a different isolate (0.0032%) had two nucleotide mismatches in the N1 reverse primer. No sequences were found to have more than one mismatch in any N2 primer/probe region. The risk of these mismatches resulting in a significant loss in reactivity causing a false negative result is extremely low due to the design of the primers and probes, with melting temperatures > 60°C and with annealing temperature at 55°C that can tolerate up to two mismatches at any positions in the primer.

3) Cross-reactivity (Analytical Specificity):

The sequences for the N1 and N2 primers/probes used in this assay are identical to the primer/probe sequences used in the FDA emergency use authorized CDC 2019-Novel Coronavirus (2019-nCoV) Diagnostic Panel authorized February 4, 2020. CDC has provided a

universal right of reference to their Cross-Reactivity Study data, which is available at https://www.fda.gov/media/134922/download

4) Clinical Evaluation:

Clinical Performance of the Assurance SARS-CoV-2 Panel

Everlywell performed the study summarized below and provided a right of reference to Assurance Scientific Laboratories. To evaluate the performance of the Assurance SARS-CoV-2 Panel, 286 consecutively received nasal swabs collected with the Everlywell COVID-19 test home collection kit were tested with the Assurance SARS-Cov-2 Panel at Assurance Scientific Laboratories. Samples were then deidentified, frozen at -80℃ and shipped to another laboratory CLIA certified to perform high complexity tests where they were tested with the comparator, a highly sensitive EUA-authorized RT-PCR SARS-CoV-2 Assay. Of the 286 samples, one had insufficient sample volume to permit testing by both assays and six had indeterminate results when tested with the comparator assay. The remaining sample were used to evaluate the performance of the Assurance SARS-CoV-2 Panel. Study results are in the table below.

Table 5. Clinical Performance of the Assurance SARS-CoV-2 Panel

		FDA EUA- Authorized Comparator		
		Positive	**Negative**	**Total**
Assurance SARS-CoV-2 Panel	**Positive**	59	5	64
	Negative	3	212	215
	Total	62	217	279

PPA (95% CI) = 95.16% (86.50% - 98.99%)
NPA (95% CI) = 97.70% (94.71% - 99.25%)

Performance Among Individuals Without Symptoms or Other Reasons to Suspect COVID-19

Of the 286 samples tested above 242 were from individuals without symptoms or other reasons to suspect COVID-19. Results are in the table below.

Table 6. Clinical Performance of the Assurance SARS-CoV-2 Panel Among Asymptomatic Individuals

		FDA EUA- Authorized Comparator		
		Positive	**Negative**	**Total**
Assurance SARS-CoV-2 Panel	**Positive**	41	5	46
	Negative	2	194	196
	Total	43	199	242

PPA (95% CI) = 95.35% (84.19% - 99.43%)
NPA (95% CI) = 97.49% (94.23% - 99.18%)

5) Sample Shipping and Stability Study

Shipping stability under simulated summer conditions of foam or spun polyester nasal swabs shipped dry (in an empty tube) or in saline (0.9%), has been demonstrated by Quantigen Biosciences, Inc. with support from The Gates Foundation and UnitedHealth Group. Quantigen Biosciences, Inc. has granted right to reference to any sponsor wishing to pursue an EUA to leverage their COVID-19 swab stability data as part of that sponsor's EUA request. Because there are no known clinically impactful differences between virus absorption onto and release from spun polyester versus flocked nylon nasal swabs into saline, FDA has determined that the Quantigen data for spun polyester can be leveraged to support use of flocked nylon swabs in saline (0.9%).

Assurance conducted the winter stability to confirm that low temperatures do not cause sample degradation. Replicates for the study were made by diluting clinical samples into negative clinical matrix. See winter conditions in table below.

Table 7. Simulated Winter Shipping Conditions

Storage Temperature	Time at Storage Temp (hours)	Total Time (hours)
N/A	0	0
-10°C	8	8
18°C	4	12
-10°C	2	14
10°C	36	50
-10°C	6	56

All samples provided the expected results, meeting the stated study acceptance criteria of ≥95% agreement for low positive samples (2x LoD) and 100% agreement for high positive samples (10x LoD). Study results are summarized in the table below. This data supports stability for 48-hour shipping.

Table 8. Winter Sample Shipping and Stability Study

			Mean Ct Values			
			N1		RNase P	
	N	Positives	Initial	Post	Initial	Post
Negative	10	0/10	N/D	N/D	26.07	25.71
2x LoD	28	28/28	34.68	33.04	26.08	25.85
10x LoD	10	10/10	29.75	29.03	26.73	26.87

N/D – Not Detected

6) Home Collection Kit Stability

Saline Tube (Reagent) Stability

The tubes used in the Simplicity COVID-19 Home Collection Kit are manufactured at Edge Biologicals. Edge Biologicals manufactures saline and fills the tubes in-house. Filled tubes are then sent to Sterigenic for sterilization via gamma irradiation. For each lot, a tube is retained and

tested on its expiration date to affirm that sterility, pH and product integrity have been maintained. Edge Biologicals assures the product integrity for sterile saline a year from the manufacture date, which has been quality tested throughout its shelf life to ensure the sterility and pH has not been affected. The expiration date of the Simplicity COVID-19 Home Collection Kits will correspond with the kit component that has the earliest expiration date, which will likely be the saline.

7) Self-Collection Validation

Simplicity COVID-19 Home Collection Kit:

A usability study was conducted using the Simplicity COVID-19 Home Collection Kit user instructions. Each participant received a notification via email that they will receive the Assurance Home Collection Kit. The email provided the following: kit instructions that are also provided in the kit, survey that is included in the kit, and the link for video instructions for the Simplicity COVID-19 Home Collection Kits. Each participant received a kit and followed the instructions in the kit to register the sample, collect the anterior nasal swab specimens, fill out the survey and ship the sample to the laboratory. Once the sample was received in the lab, it was inspected for packing and shipping appropriateness according to the criteria outlined for acceptance and rejection criteria. If the sample met the criteria it was tested in the lab. If a sample was rejected, it was noted in the results. The samples collected during the study were tested for specimen adequacy using the EUA authorized Assurance SARS CoV-2 Panel that includes a human sample control, RNase P, to determine if sufficient sample was collected by the user.

30 participants meeting the following inclusion and exclusion criteria were included in the study.

Inclusion criteria:

- age 18-75
- willingness to receive and perform test
- willingness to fill out survey

Exclusion criteria:

- No access to internet
- < 18 years old
- Previous or current at home collection experience
- Previous or current Healthcare experience
- Previous or current Lab experience
- Unwillingness to perform test

The study evaluated 30 participants for collecting and shipping an anterior nasal swab specimen. Of the 30 participants, 50% were 60 and older, 30% were between 40 and 59, and 20% were less than 39 years of age. All samples that were received by the laboratory met acceptance criteria for testing. All participants were able to collect their sample without assistance and 29/30 were confident that they collected a good sample. Overall, the survey results demonstrate that lay users can adequately perform the sample self-collection at home following the instructions provided in the Simplicity COVID-19 Home Collection Kit.

Question #	Question	"Yes" Responses	"No" Responses	No Response
1	Did you read the entire instructions prior to collecting the sample?	29	1	0
2	Was it difficult to register the kit?	2	25	3
3	Did you know what to do if you had any questions during the sample collection?	27	2	1
4	Did you watch the instructional video?	21	9	0
4B	If you watched the video, were the instructions helpful?	21	0	9*
5	Was it difficult to understand what information you needed to write down on the Kit ID Label?	1	28	1
6	Did you wash your hands prior to collecting the sample?	30	0	0
7	Did you need help while collecting your sample?	0	30	0
8	Are you confident that you collected the sample properly?	29	1	0
9	Was collecting the sample uncomfortable?	5	25	0
10	Instructions clearly explained how to collect the sample	27	1	2
11	Instructions clearly explained how to properly close the collection tube and pack the sample for shipping	28	1	1
12	I knew when to collect the sample	30	0	0
13	I knew when to ship the sample	30	0	0
14	Was it difficult to pack the sample for shipping?	0	28	2
15	I understood that I should place the absorbent pad in the biohazard bag.	28	2	0
16	I understood that if I did not follow the procedure exactly, I might get a false result.	29	1	0
17	Was it difficult to remove the swab from the packaging without touching the swab tip or touching any surface with the swab?	2	28	0
18	Was it difficult to open the collection tube while holding the swab?	3	27	0
19	Was it difficult to break the swab at the collection tube	6	24	0
20	Was it difficult to open the collection tube without spilling or coming into contact with the liquid?	1	29	0
21	I did not spill or come into contact with the liquid in the collection tube.	19	11	0
22	I knew what to do if the liquid from the collection tube spilled or leaked.	25	5	0
	I understood that if any liquid is spilled or leaks from the collection tube, I cannot			

23	return the test.	29	1	0
24	Did you touch any surface with the swab?	2	28	0
25	I knew what to do if I touched any surface with the swab.	28	2	0
26	I understood from the directions how deep I should insert the swab into my nose.	30	0	0
27	I understood how many times I should rotate the swab in the nostril	30	0	0
28	I collected the sample from both nostrils before placing it in the collection tube.	29	0	0
29	I put the swab tip down into the collection tube.	28	0	2
30	I understood that if I do not put the swab tip down in the collection tube (media), I might get false results or no results.	29	1	0

*These responses were Not Applicable, because the user did not watch the video.

Further supporting the users' ability to collect adequate sample, all of the samples tested positive for RNase P. These results indicate sufficient sample collection was performed for all users who participated in the study.

LIMITATIONS:

- A false negative result may occur if a specimen is improperly collected, transported or handled. False negative results may also occur if amplification inhibitors are present in the specimen or if inadequate numbers of organisms are present in the specimen.
- The clinical performance has not been established in all circulating variants but is anticipated to be reflective of the prevalent variants in circulation at the time and location of the clinical evaluation. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of SARS-CoV-2 and their prevalence, which change over time.

WARNINGS:

- This product has not been FDA cleared or approved, but, has been authorized by FDA under an Emergency Use Authorization (EUA) for use by laboratories designated by Assurance Scientific Laboratories that are also certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, and meet the requirements to perform high complexity tests.
- This product has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.
- The emergency use of this product is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated or authorization is revoked sooner.

FDA SARS-CoV-2 Reference Panel Testing

The evaluation of sensitivity and MERS-CoV cross-reactivity was performed using reference material (T1), blinded samples and a standard protocol provided by the FDA. 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 method and instrument used were Abnova Precipitor32 and BioRad CFX96 respectively. The results are summarized in the following Table.

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

Reference Materials Provide d by FDA	Specimen Type	Product LoD	Cross-Reactivity
SARS-CoV-2	Nasopharyngeal and Nasal Swabs	$5.4x10^3$ NDU/mL	N/A
MERS-CoV		N/A	ND

NDU/mL = RNA NAAT detectable units/mL
N/A: Not applicable
ND: Not detected