EMERGENCY USE AUTHORIZATION (EUA) SUMMARY
ASSURANCE SARS-COV-2 PANEL
(Assurance Scientific Laboratories)

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

INTENDED USE

The Assurance SARS-CoV-2 Panel is a real-time RT-PCR test intended for the qualitative detection of nucleic acid from the SARS-CoV-2 in nasal, nasopharyngeal or oropharyngeal swabs, from individuals suspected of COVID-19 by their healthcare provider.

This test is also for use with nasal swab specimens that are self-collected at home or in a healthcare setting by individuals using an authorized home-collection kit specified in this authorized test's authorized labeling when determined to be appropriate by a healthcare provider.

Testing is limited to laboratories designated by Assurance Scientific Laboratories, that are also certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263, and meet the requirements to perform high-complexity tests.

Results are for the detection and identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in respiratory specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2. 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 positive 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 Assurance SARS-CoV-2 Panel is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the techniques of real-time RT-PCR and *in vitro* diagnostic procedures. The Assurance SARS-CoV-2 Panel test is only for use under the Food and Drug Administration's Emergency Use Authorization.

2) Special Conditions for Use Statements

For Emergency Use Authorization (EUA) only
For prescription use only
For *in vitro* diagnostic use

DEVICE DESCRIPTION AND TEST PRINCIPLE

The assay 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 patients as recommended for testing by public health authority guidelines.

The RT-PCR test for the Assurance COVID-19 Home Collection Kit will be performed using the FDA-authorized Assurance SARS-CoV-2 Panel from Assurance Scientific Laboratories (EUA200522).

The Assurance COVID-19 Home collection kit collection device consists 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.

Sample Preparation

Two extraction chemistries are validated for COVID-19 PCR testing: 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 (BBQ). 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 SARS-CoV-2 Panel, 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.

Collection Kits

- This assay can be used with the Everlywell COVID-19 test home collection kit. Everlywell has granted Assurance Scientific Laboratories a right of reference to the data supporting the use of this authorized home collection kit.

- This test is also for use with the Assurance COVID-19 Home collection kit to self-collect nasal swab specimens at home by individuals when determined by a healthcare provider to be appropriate based on the results of an online COVID-19 questionnaire, or telephone or online video appointment, etc. with a healthcare provider, described in more detail below.

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 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	

Assurance COVID-19 At-Home Kit Components:

The test kits sent to users include the following components.

Name	Description	Quantity	Material Supplier
Flocked Nasal Swab	Flocked Nasal Swab	1	Miraclean or Puritan
Collection Tube	Saline tube	1	Edge Biologicals or equivalent
Tube Label	Label for patient 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 COVID-19 RT-PCR

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.

SARS-CoV-2 N1	RP	Result Interpretation	Ct	Report	Actions
+	±	SARS-CoV-2 detected	<40	Positive SARS-CoV-2	Report results to state health department and provider*.
-	+	2019-nCoV not detected	<40	Not Detected	Report results to provider. Consider testing for other respiratory viruses.
-	-	Invalid Result	≥40	Invalid	Repeat extraction and rRT-PCR. If the repeated result remains invalid, request a new specimen from the patient.

* For at home collection from Everlywell, reporting will be done through an Application Program Interface to PWN. For details on this process, please refer to Everlywell's EUA by right of reference.

CLINICIAN INVOLVEMENT AND OVERSIGHT

Individuals may request the Assurance COVID-19 Home collection kit collection device. The individual's eligibility will be determined by either direct evaluation by a health care provider HCP either locally or via telemedicine. If an online questionnaire is used, then it will be evaluated by a HCP, who will provide the necessary prescription for the home collection kit and test. Appropriateness for at home nasal swab collection will use the CDC screening guidelines (https://www.cdc.gov/coronavirus/2019-nCoV/hcp/clinical-criteria.html). Once a patient has a HCP order, they will be eligible to receive and collect their sample via the Assurance COVID-19 Home Collection Kit. The collected sample will be shipped overnight in the provided pre-labeled packaging to Assurance Scientific Laboratories. Any sample that is received without a HCP order will be rejected. The presentation of the patient may occur in a variety of ways.

1. A patient request to be tested via an internet site (Digital Healthcare Company). An online questionnaire will determine eligibility. If the patient is eligible the they will receive prescription from HCP either locally or via telemedicine. Once confirmed, the home collection kit will be shipped to the patient. The lab will process the sample and make results available to ordering provider and Digital Healthcare Company. The Digital Health Care company will provide results to the patient via an online portal.
2. A patient presents to a pharmacy and requests to be tested. They will have opportunity to fill out questionnaire to determine eligibility. If the patient is eligible, they will receive prescription from HCP either locally or via telemedicine. The pharmacy then will allow the patient to purchase the home collection kit. The lab will process the sample and make results available to ordering provider. Results are made available to the patient via the pharmacy or ordering provider by email or phone call.
3. A patient presents to their employer and requests to be tested. The HCP determined by the employer assesses for eligibility and provides prescription for the test. Once confirmed, the home collection kit will be shipped to the patient. The lab will process the sample and make results available to ordering provider and employer. Results are made available to the patient via the employer or ordering provider by email or phone call.
4. A patient presents to their HCP, and HCP determines the patient is eligible. A prescription is provided to the lab for test. Once confirmed, the home collection kit will be shipped to the patient. The lab will process the sample and make results available to ordering provider. The HCP will report results to patient per their usual practice policy for lab result reporting.

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 Assurance 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 patient'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 patient
2. Insufficient volume or leakage
3. The label does not contain, the patient name, date of birth and collection date
4. The information on the label does not match the electronic record

Test results are communicated back to individuals that used the Assurance COVID-19 Home collection kit via the ordering provider.

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 My Lab Box manufacturing facility.

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 timeframe.

PERFORMANCE EVALUATION

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

The LoD study was performed using viral genomic RNA from BEI using the CFX96. 10-fold serial dilutions of genomic RNA were spiked into pooled respiratory matrix (NP and OP swabs collected in liquid Amies) to obtain the LoD range. It was confirmed by 2-fold dilutions of RNA into matrix. The concentrations of RNA show the amount of RNA spiked into the matrix so the LoD was determined assuming 100% extraction efficiency.

Table 1. Limit of Detection Confirmation of the Assurance SARS-CoV-2 Panel with Abnova Total Nucleic Acid Purification Kit

Targets	**2019-nCoV_N1**	
Concentration (genomic copies/µL)	9	5
Concentration (genomic copies/ reaction)	37	18
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 Research Quick-DNA/RNA Viral MagBead Kit

Targets	**2019-nCoV_N1**	
Concentration (genomic copies/µL)	29	9
Concentration (genomic copies/reaction)	116	37
Positives/Total	20/20	20/20
Mean Ct[1]	30.29	31.57
Standard Deviation (Ct)	0.33	0.35

The LoD was confirmed using the CFX384 with the 384 well plate as shown in Table 3.

Table 3. Limit of Detection Evaluation of the Assurance SARS-CoV-2 Panel with the CFX384.

	Zymo		**Precipitor**
Targets	**2019-nCoV_N1**		**2019-nCoV_N1**
Concentration (genomic copies/µL)	29	9	29
Concentration (genomic copies/reaction)	116	37	116
Positives/Total	19/19	20/20	20/20
Mean Ct[1]	26.86	27.84	26.40
Standard Deviation (Ct)	0.36	0.32	0.34

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 (EUA200001). 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.

3) Cross-reactivity (Analytical Specificity):

***In silico,* analysis has been performed and was reviewed by FDA (not shown because of large data set).**

In addition to the *in silico* analysis, nucleic acids were extracted from several organisms and tested with the CDC 2019-nCoV Real-Time RT-PCR Diagnostic panel to demonstrate analytical specificity and exclusivity. Studies were performed with nucleic acids extracted using the Abnova Precipitor instrument using the Viral Total Nucleic Acid Purification Kit. Testing was performed using the ThermoFisher Scientific TaqPath 1-Step RT-qPCR Master Mix, CG on the BioRad CFX96 Real-Time PCR instrument. The data demonstrate the expected results are obtained for each organism when tested with the CDC N1 and N2 primers and probes.

Wet testing was performed with any organism that has greater than 80% homology to any primer or probe.

Wet testing results

Pathogens	Assays Evaluated		
	2019-nCoV N1	2019-nCoV N2	Final Result
Human coronavirus 229E	**0/3**	**0/3**	**Neg.**
Human coronavirus OC43	**0/3**	**0/3**	**Neg.**
Human coronavirus HKU1	**0/3**	**0/3**	**Neg.**
Human coronavirus NL63	**0/3**	**0/3**	**Neg.**
Adenovirus (e.g. C1 Ad. 71)	**0/3**	**0/3**	**Neg.**
Human Metapneumovirus (hMPV)	**0/3**	**0/3**	**Neg.**
Parainfluenza virus 1	**0/3**	**0/3**	**Neg.**
Parainfluenza virus 2	**0/3**	**0/3**	**Neg.**
Parainfluenza virus 3	**0/3**	**0/3**	**Neg.**
Parainfluenza virus 4	**0/3**	**0/3**	**Neg.**
Influenza A	**0/3**	**0/3**	**Neg.**
Influenza B	**0/3**	**0/3**	**Neg.**
Enterovirus (e.g. EV68)	**0/3**	**0/3**	**Neg.**
Respiratory syncytial virus	**0/3**	**0/3**	**Neg.**
Rhinovirus	**0/3**	**0/3**	**Neg.**
Chlamydia pneumoniae	**0/3**	**0/3**	**Neg.**
Haemophilus influenzae	**0/3**	**0/3**	**Neg.**
Legionella pneumophila	**0/3**	**0/3**	**Neg.**

Mycobacterium tuberculosis	**0/3**	**0/3**	**Neg.**
Streptococcus pneumoniae	**0/3**	**0/3**	**Neg.**
Streptococcus pyogenes	**0/3**	**0/3**	**Neg.**
Bordetella pertussis	**0/3**	**0/3**	**Neg.**
Mycoplasma pneumoniae	**0/3**	**0/3**	**Neg.**
Staphylococcus epidermidis	**0/3**	**0/3**	**Neg.**
Candida albicans	**0/3**	**0/3**	**Neg.**

4) Clinical Evaluation:

The experiments were performed using contrived samples generated by spiking viral genomic RNA into the pooled negative matrix (NP, OP and nasal swabs in liquid amies) from patients that were negative for SARS-CoV-2. For the non-reactive specimens, negative matrix was extracted without any additional spike.

For the Abnova Preciptor study 16 samples were prepared at LoD, 12 samples at 2xLoD and 10 samples were prepared across the range of the curve. Similarly, for the IndiMag 48, 24 samples were prepared at LoD and 11 samples were prepared across the range of the curve.

100% agreement was observed between the predicted results and actual results. All samples were run on the CFX96.

Contrived Samples Extracted with Abnova Precipitor

Assurance SARS-CoV-2 Panel Result	**Composite Comparator Result – Abnova Precipitor**	
	N1	
	Positive	**Negative**
Positive	**38**	**0**
Inconclusive	**0**	**0**
Negative	**0**	**30**

Positive percent agreement = 38/38 = 100%

Negative percent agreement = 30/30 = 100%

Contrived Samples Extracted with Zymo Research kit on the IndiMag

Assurance SARS-CoV-2 Panel Result	**Composite Comparator Result – Zymo Research**	
	N1	
	Positive	**Negative**
Positive	**34**	**0**
Inconclusive	**0**	**0**
Negative	**0**	**48**

Positive percent agreement = 34/34 = 100%

Negative percent agreement = 48/48 = 100%

Clinical specimens received by Assurance Scientific Laboratories were tested by the Assurance Scientific Laboratories SARS-CoV-2 assay were confirmed by another clinical laboratory; Devansh Lab Werks Inc. using the CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel. Results are below.

Assurance SARS-CoV-2 Panel Result	**Reference result**	
	Positive	Negative
Positive	5	0
Negative	0	5

5) Self-Collection Validation

A usability study was conducted using the Assurance 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 Assurance COVID-19 Home Collection Kits. Each participant received a kit and followed the instructions in the kit to register the sample, collect the sample, 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 be noted in the results. The samples collected during the study were tested for specimen adequacy using our 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 a 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 Assurance 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	6	24	0

	collection tube?			
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
23	I understood that if any liquid is spilled or leaks from the collection tube, I cannot 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.

Conclusion

Positive and negative percent agreements to expected result was 100% for the contrived swab specimens. Positive and Negative clinical specimens were also confirmed by secondary testing.

6) Retrospective Data Analysis of Clinical Samples for Removing N2 target:

Clinical sample test results were analyzed for N1 and N2 target detection from 03/11/20 to 04/29/20. 26,233 samples were analyzed with 2,256 samples positive for at least one target. 2,084 samples were positive by both targets, 172 had only one target positive which would be "Presumptive Positive" by the previously authorized results interpretation algorithm. Further analysis indicated that 157 samples were positive by N1 only and 15 were positive by N2 only. This data analysis demonstrates that switching to only one target (N1 target) does not

significantly affect (less than 1% drop in positive percent agreement with the authorized version) the performance of the Assurance SARS-CoV-2 Panel.

WARNINGS:

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

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 Provided 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