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 anterior nasal, mid-turbinate nasal, nasopharyngeal or oropharyngeal swabs, from individuals suspected of COVID-19 by their healthcare provider.

This test is also for use with anterior nasal swab specimens that are collected using the Everlywell COVID-19 Test Home Collection Kit when used consistent with its authorization.

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 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.

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 (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 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.

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	

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	SARS-CoV-2 N1 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.

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

Clinical Performance of the Assurance SARS-CoV-2 Panel as Assessed in Swabs Collected with the Everlywell COVID-19 Test Home Collection Kit

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°C 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.

		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%)

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.

5) 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.

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 authorized laboratories; 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 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 Pane l

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