EMERGENCY USE AUTHORIZATION (EUA) SUMMARY

OraRisk COVID-19 RT-PCR

(Access Genetics, LLC)

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

(The OraRisk COVID-19 RT-PCR test will be performed at the Access Genetics, LLC laboratory, located at 7400 Flying Cloud Drive, Eden Prairie, MN 55344, that is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, and meets the requirements to perform high complexity tests. The Laboratory Standard Operating Procedure was reviewed by the FDA under this EUA.)

INTENDED USE

The OraRisk COVID-19 RT-PCR test is a real-time (rt) reverse transcriptase (RT) polymerase chain reaction (PCR) intended for the qualitative detection of nucleic acid from the SARS-CoV-2 in nasopharyngeal swab and nasal swab specimens collected in universal transport media, and nasal swabs collected in oral saline rinse, from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to Access Genetics, LLC laboratory, located at 7400 Flying Cloud Drive, Eden Prairie, MN 55344, that is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, and meets the 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 upper respiratory swab 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 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 OraRisk COVID-19 RT-PCR test is intended for use by qualified and trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures. The OraRisk COVID-19 RT-PCR test is only for use under the Food and Drug Administration's Emergency Use Authorization within the United States and territories.

DEVICE DESCRIPTION AND TEST PRINCIPLE

The OraRisk COVID-19 RT-PCR test 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 upper respiratory specimens from individuals suspected of COVID-19 by their healthcare provider. The test measures the presence or absence of RNA encoding the RdRp (polymerase) of the SARS-CoV-2. For detection of RdRp the Smart Logix Coronavirus 2019 assay authorized on April 3, 2020 is used in the OraRisk COVID-19 RT-PCR test. The test also co-extracts and amplifies sequences from the human RNase P gene detected by a differently labeled fluorophore.

RNA is isolated from claimed specimens and then reverse transcribed to cDNA and subsequently amplified using the LightCycler 480 II instrument with Sequence Detection Software version 1.5.1.62. During the amplification process, the probe anneals to a specific target sequence located between the forward and reverse primers. During the extension phase of the PCR cycle, the 5' nuclease activity of Taq polymerase degrades the bound probe, causing the reporter dye (FAM and CAL Fluor Red 610) to separate from the quencher dye (BHQ), generating a fluorescent signal. Fluorescence intensity is monitored at each PCR cycle by the LightCycler 480 II instrument.

INSTRUMENTS USED WITH TEST

For extraction of viral RNA, the PerkinElmer Chemagic MSM I automated specimen processing system (PerkinElmer, Waltham, MA) is used with the CMG-1033-S kit.

For thermocycling and detection of amplified DNA products the LightCycler 480 II qPCR System running the LightCycler 480 Software version 1.5.1.62 (Roche Molecular System) is used.

EQUIPMENT, REAGENTS AND MATERIALS

The following equipment/reagents/materials are required to run this test:

1. Swab and universal transport media for nasal swab collection, or
2. iClean (for nasopharyngeal swabs; Hcy, Huachenyang Technology, LTD, China; #CY-96000T) and universal transport media
3. Optional: Access Genetics Convenience Kit for nasal swab in oral rinse collection (PathTec, Midland, GA; #BM-000674; sterilized) consisting of:
    a. Swab for Specimen Collection (e.g., Dacron swab, Puritan Medical Products Company, LLC, Guilford, ME; #25-3406-H)
    b. Sarstedt tube filled with 5mL of sterile saline, Inc, Newton, NC; #86.290.104)
4. RNA Extraction Reagents (PerkinElmer; CMG-1033-S)
5. AccuPlex SARS-CoV-2 Reference Material Kit (Sercare #0505126)
6. SARS-CoV-2 Assay Kit (Co-Diagnostics, Inc., Salt Lake City, UT; #Covid K-001)
7. PCR reaction plate (Roche Lightcycler Multiwell Plate, Clear; #05102413001)

CONTROLS TO BE USED WITH THE COVID-19 RT-PCR

- **Internal Control:** Each sample that contains nucleic acid (positive control, negative control, LOD control, and patient samples) must demonstrate the presence of the internal control (IC) amplicon. The IC is created from PCR amplification of a locus within the RNase P human gene and monitors adequate amounts and quality of RNA in the sample and correct sample processing.
- **No template control (NTC)**: A no template control is comprised of normal saline. This control is carried through all aspects of sample processing including the extraction and monitors for reagent contamination. It should be negative for amplification of SARS-CoV-2 (FAM) and RNase P (IC- CAL Fluor Red 610).
- **Negative Extraction Control**: The RNase P containing control pseudovirus provided in the AccuPlex SARS-CoV-2 Reference Material Kit (Sercare #0505126) is used as a Negative Extraction Control. The pseudovirus is combined with saline and is included in each extraction run with a concentration of 30 copies/µL. This control is subjected to all processing steps including heat inactivation, RNA extraction, reverse transcription and PCR.
- **LOD Extraction Control**: Pseudovirus containing SARS-CoV-2 target sequence (Sercare #0505126) is assayed with each extraction run of the test. Briefly, this reagent is diluted to the Limit of Detection (LoD) of the OraRisk COVID-19 RT-PCR test in a diluent of saline. This control is subjected to all processing steps including RNA extraction, RT-PCR set up, and thermocycling.
- **Positive PCR Control**: The positive PCR control consist of a proprietary blend of SARS-CoV-2 synthetic templates provided by the LogixSmart test kit and is included in the PCR only with each batch of samples. The positive control is used to verify that the PCR run is performing as intended. The positive control contains targets for RdRP and RP. The positive control is used once on every PCR plate.

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. Result interpretation for patient samples was established based on a cutoff of 40 Ct for SARS-CoV-2 target.

a. Control Result Interpretation

Table 1: Expected Performance of Controls

Control Name	Ct Value		Interpretation	Action
	COVID (RdRp) FAM	**RNase P (Internal Control) CAL Fluor Red 610**		
Positive PCR control (SeraCare)	**25.8-26.6**	**<38**	**Pass**	PCR is valid
	None	<38	Fail	Run is invalid and needs to be repeated
No template control (NTC)	**None**	**None**	**Pass**	Run is valid
	>0	>0	Fail	Run is invalid. Investigate contamination and repeat run
Negative Extraction control	**None**	**<38**	**Pass**	Run is valid
	>0	<38	Fail	Run is invalid. Investigate contamination and repeat run
	None	>38	Fail	Investigate failed RT-PCR
LoD Extraction Control	**35.2- 38.3**	**<38**	**Pass**	Run is reported
	>38.3	<38	Fail	Low sensitivity run, repeat

If any of the above controls do not exhibit the expected performance as described, the assay may have been improperly set up and/or executed improperly, or reagent or equipment malfunction could have occurred. Invalidate the run and re-test.

b. Examination and Interpretation of Patient Specimen Results:

Assessment of clinical specimen test results should only be performed after the positive and negative controls have been examined and determined to be valid and acceptable. If the controls are not valid, the patient results cannot be interpreted, and testing needs to be repeated after a root cause is identified and eliminated.

Table 2: Interpretation of Sample Results

COVID-19 FAM	RNase-P Red-610	Test Result	Interpretation (reported)	Action
-	-	Invalid	None	Do not report result. Repeat extraction and PCR assay. If no internal control is detected in the repeat test, re-sampling is required
-	+	SARS-CoV-2 RNA Not Detected	The submitted sample is negative (absence of the RNA) from SARS-CoV-2, the virus that causes the disease called COVID-19.	Report results to healthcare provider. Consider test for other viruses that cause similar symptoms
Ct≤40	+	SARS-CoV-2 RNA Detected	The submitted sample is positive (presence of the RNA) from SARS-CoV-2, the virus that causes the disease called COVID-19.	Report results to healthcare provider and appropriate public health authorities
<50 Ct>40	+	Inconclusive	Initially inconclusive results are not reported. If repeat inconclusive it is reported as “repeat inconclusive. Absence or presence of SARS CoV-2 RNA could not be established. Additional testing on a new specimen is required”	Repeat extraction and PCR assay. If repeat test has COVID FAM <40 report as *SARS-CoV-2 Detected* and report results to healthcare provider and appropriate public health authorities If the result is repeatedly inconclusive. additional testing is required with a new sample and/or a different test.

PERFORMANCE EVALUATION

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

a. Tentative LoD Study: Nasal swab combined with saline oral rinse
To establish the limit of detection (LOD) for the OraRisk COVID-19 RT-PCR test , a tentative LoD study was performed. A dilution series was performed with the SeraCare SARS-CoV-2 RNA based pseudovirus diluted into a pool of nasal swab in oral rinse previously tested negative for infection with the SARS-CoV-2 virus.

b. Confirmation of the LoD: Nasal swab combined with saline oral rinse
Confirmation of the LoD for the nasal swab combined with a saline oral rinse was performed by testing a various number of replicates of the SARS-CoV-2 RNA based pseudovirus diluted into clinical matrix (i.e., nasal swabs combined with saline oral rinse) at slightly above (1.5x LoD), and slightly below (0.5x LoD and 0.75x LoD) the tentative LoD. Testing was performed according to the Laboratory SOP; all replicates were individual extraction replicates. Data are summarized in the table below and support a final LoD of 15 copies/µL for Nasal Swab in oral rinse for this test.

Table 3: Confirmatory LoD study in Nasal Swab in Oral Rinse Matrix

SARS-CoV-2 RNA (copies/µL)	Number Tested	Positive	Ct Value			
			SARS-CoV-2		RNase P IC	
			Mean	SD	Mean	SD
10	40	30 (75%)	37.8	0.80	24.1	1.20
15	**20**	**19 (95%)**	**37.1**	**0.79**	**23.8**	**1.05**
20	**60**	**57 (95%)**	**36.7**	**0.56**	**25.2**	**0.40**
30	20	20 (100%)	36.3	0.90	24.8	0.37

c. Confirmation of the LOD: Nasopharyngeal (NP) Swab
An identical study design as described above was used, testing a narrow range of virus concentrations, each condition in 20 individual extraction replicates using nasopharyngeal swab in 5 mL of saline (no oral rinse). The data are summarized in the table below and support a final LOD for this test in Nasopharyngeal swab specimens of 15 viral copies/µL.

Table 4: Confirmatory LoD study in NP Swabs

SARS-CoV-2 RNA (copies/µL)	Number Tested	Positive	Ct Value			
			SARS-CoV-2		RNase P IC	
			Mean	SD	Mean	SD
5	20	8 (40%)	38.7	0.82	28.9	0.27
15	**20**	**19 (95%)**	**38.5**	**0.63**	**28.7**	**0.24**
20	20	20 (100%)	38.1	0.70	29.0	0.29

2) ***Analytical Inclusivity/Specificity:***

a. Inclusivity

The sponsor uses a proprietary product for which primers and probe sequences are not available to the laboratory, namely the Logix Smart Coronavirus Disease 2019 (COVID-19) Kit. The manufacturer of the Logix Smart Coronavirus Disease 2019 (COVID-19) Kit has granted right-to-reference to Access Genetics, LLC to leverage their inclusivity data. Accordingly, an inclusivity analysis was not repeated.

b. Cross-reactivity

The sponsor uses a proprietary product for which primers and probe sequences are not available to the laboratory, namely the Logix Smart Coronavirus Disease 2019 (COVID-19) Kit. The manufacturer of the Logix Smart Coronavirus Disease 2019 (COVID-19) Kit has granted right-to-reference to Access Genetics, LLC. Accordingly, a cross reactivity study was not repeated.

3) ***Clinical Evaluation:***

Four different clinical studies were performed testing clinical samples in comparison to nucleocapsid gene (N1/N2) based EUA authorized RT-PCR comparator tests by two different laboratories. Both comparators are using a Ct cutoff of 40.0, with Cts ≤40 being scored positive. The investigational tests were processed, and the results interpreted per laboratory SOP using a cutoff of Ct 40 including the heat-inactivation step.

A total of 77 samples were collected from patients with signs and symptoms of an acute respiratory illness (30 nasopharyngeal swab specimens in VTM and 46 nasal swabs in VTM or saline oral rinse).

Test results for nasopharyngeal swab samples collected in VTM are summarized in the Table below.

Table 5: Clinical Performance in Nasopharyngeal Swab Specimens

NP swab in VTM		**EUA Authorized Comparator (N1/N2)**			**Total**
		Positive	**Inconclusive**	**Negative**	
OraRisk COVID-19 Test	**Positive**	15	0	0	15
	Negative	0	0	15	15
Total		**15**	**0**	**15**	**30**

Performance for nasopharyngeal swabs in VTM is calculated as:
Positive Percent Agreement (PPA): 15/15 = 100% (95% CI: 79.6% - 100%)
Negative Percent Agreement (NPA): 15/15 = 100% (95% CI: 79.6% - 100%)

Test results for nasal swab samples collected in VTM or saline oral rinse are summarized in the Table below. The table combines nasal swabs in VTM and in saline oral rinse because the LoDs of the OraRisk COVID-19 RT-PCR test in swabs in VTM and in saline oral rinse are comparable (within 3x LoD; please refer to the LoD study above).

Table 6: Clinical Performance in Nasal Swab Specimens (in VTM or Oral Rinse)

Nasal swab in VTM or Saline Oral Rinse		**EUA Authorized Comparator (N1/N2)**			**Total**
		Positive	**Inconclusive**	**Negative**	
OraRisk COVID-19 Test	**Positive**	22	1*	1**	24
	Negative	0	0	22	22
Total		**22**	**1**	**23**	**46**

*One sample detected as positive by the investigational device was detected by the comparator test only in N2 target channel (with Ct >39). Since no amplification was detected in second N1 target channel of the comparator test, this sample was inconclusive by the comparator, which would typically reflex to re-testing. Retesting could not be performed for this sample. The sample was excluded from the analysis because with one positive and one negative target detection, it is considered to be neither Positive nor Negative.
**One sample was detected as positive by the investigational test from Access Genetics. This sample had a Ct values of 38.7, indicative of a low positive result; this sample was not detected by the comparator test.

Performance for Nasal swabs is calculated as:
Positive Percent Agreement (PPA): 22/22 = 100% (95% CI: 85.1% - 100%)
Negative Percent Agreement (NPA): 22/23 = 95.7% (95% CI: 79.0% - 100%)

WARNINGS:

- For *in vitro* diagnostic use.
- Rx only.
- For use under Emergency Use Authorization (EUA) only.
- This test has not been FDA cleared or approved.
- This test has been authorized by FDA under an EUA for use by the authorized laboratory.
- 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* diagnostic tests for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug and Cosmetic Act, 21U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.
- Handle all specimens as if infectious using safe laboratory procedures. Refer to Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with 2019-nCoV https://www.cdc.gov/coronavirus/2019-nCoV/lab-biosafety-guidelines.html.
- Use personal protective equipment such as (but not limited to) gloves, eye protection, and lab coats when handling kit reagents while performing this assay and handling materials including samples, reagents, pipettes, and other equipment and reagents.
- Amplification technologies such as PCR are sensitive to accidental introduction of PCR product from previous amplifications reactions. Incorrect results could occur if either the clinical specimen or the real-time reagents used in the amplification step become contaminated by accidental introduction of amplification product (amplicon). Workflow in the laboratory should proceed in a unidirectional manner.
- Dispose of unused kit reagents and human specimens according to local, state, and federal regulations.

LMITATIONS:

- Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for treatment or other patient management decisions.
- 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.
- This test cannot rule out diseases caused by other bacterial or viral pathogens.