ACCELERATED EMERGENCY USE AUTHORIZATION (EUA) SUMMARY OF THE AVERA INSTITUTE for HUMAN GENETICS SARS-CoV-2 RT-PCR DIAGNOSTIC PANEL

For *In vitro* Diagnostic Use

Rx Only

For use under Emergency Use Authorization (EUA) only

(The SARS-CoV-2 Assay will be performed at the Avera Institute for Human Genetics laboratory located at 3720 W 69th Street, Sioux Falls, SD 57108 which is certified under the Clinical Laboratory Improvement Amendments of 1988(CLIA), 42 U.S.C. §263a, as per the Instructions for Use that were reviewed by the FDA under this EUA.)

INTENDED USE

The SARS-CoV-2 assay is a *Real-Time RT-PCR assay* intended for the qualitative detection of nucleic acid from the SARS-CoV-2 in upper respiratory specimens (e.g., nasopharyngeal, nasal, and oropharyngeal swab specimens) and BAL from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to *The Avera Institute for Human Genetics* that is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform high complexity tests.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in respiratory tract 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 assay 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 assay is only for use under the Food and Drug Administration's Emergency Use Authorization.

2) Instruments Used with Test:

The Avera Institute for Human Genetics SARS-CoV2 RT-PCR Diagnostic Panel test is to be used with the Applied Biosystems Quant Studio 7 Flex equipped with Quant Studio software v1.1, or the Applied Biosystems Viia 7 equipped with the Quant Studio software v1.1.

DEVICE DESCRIPTION AND TEST PRINCIPLE

The assay is a real-time reverse transcription polymerase chain reaction (rRT -PCR) test based on the EUA FDA issued for the CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel. 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 oligonucleotide primers and probes for detection of 2019-nCoV were selected from regions of the virus nucleocapsid (N) gene. The panel uses two primer and probe sets to detect two regions of the SARS 2019-nCoV-2 nucleocapsid (N) gene. An additional primer/probe set used to detect the human RNase P gene (RP) in control samples and clinical specimens is also included in the panel. RNA is isolated and purified from respiratory specimens utilizing the QIAGEN RNeasy mini kit (Cat. No.74104, 74106). The purified RNA is reverse transcribed to cDNA and subsequently amplified in the Applied Biosystems Quant Studio 7 Flex equipped with Quant Studio software v1.1, or the Applied Biosystems Viia 7 equipped with the Quant Studio software v1.1. In the 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 probe, causing the reporter dye to separate from the quencher dye, generating a fluorescent signal. With each cycle, additional reporter dye molecules are cleaved from their respective probes, increasing the fluorescence intensity. Fluorescence intensity is monitored at each PCR cycle by Applied Biosystems Quant Studio 7 Flex equipped with Quant Studio software v1.1, or the Applied Biosystems Viia 7 equipped with the Quant Studio software v1.1. Detection of viral RNA not only aids in the diagnosis of illness but also provides epidemiological and surveillance information. The primer and probe sequences for the SARS-CoV-2 Assay are listed in the table below:

Name	Description	Oligonucleotide Sequence
2019-nCoV_N1-F	2019 nCoV_N1 Forward Primer	5'-GAC CCC AAA ATC AGC GAA AT-3'
2019-nCoV_N1-R	2019 nCoV_N1 Reverse Primer	5'-TCT GGT TAC TGC CAG TTG AAT CTG-3'
2019-nCoV_N1-P	2019 nCoV_N1 Probe	5'-FAM-ACC CCG CAT TAC GTT TGG ACC-BHQ1-3'
2019-nCoV_N2-F	2019-nCoV_N2 Forward Primer	5'-TTA CAA ACA TTG GCC GCA AA-3'
2019-nCoV_N2-R	2019-nCoV_N2 Reverse Primer	5'-GCG CGA CAT TCC GAA GAA-3'
2019-nCoV_N2-P	2019-nCoV_N2 Probe	5'-FAM-ACA ATT TGC CCC CAG CGC TTC AG-BHQ1-3'
RP-F	RNAse P Forward Primer	5'-AGA TTT GGA CCT GCG AGC G-3'
RP-R	RNAse P Reverse Primer	5'-GAG CGG CTG TCT CCA CAA GT-3'
RP-P	RNAse P Probe	5'-FAM – TTC TGA CCT GAA GGC TCT GCG CG – BHQ-1-3'

INSTRUMENTS USED WITH TEST

The Avera Institute for Human Genetics SARS-CoV2 RT-PCR Diagnostic Panel test is to be used with the Applied Biosystems Quant Studio 7 Flex equipped with Quant Studio software v1.1, or the Applied Biosystems Viia 7 equipped with the Quant Studio software v1.1.

REAGENTS AND MATERIALS

MATERIALS:

1. Biological Safety Cabinet with fume hood
2. 1000µl pipette and tips
3. 100µl pipette and tips
4. 200µl pipette and tips
5. 1.5 mL microcentrifuge tubes (DNase/RNase free)
6. Falcon tubes (14mL round bottom tubes)
7. 0.5mL 2D barcode tubes and 96 count latch racks
8. Mix-mate
9. QIAshredder Spin Columns (2mL)
10. Biological Safety Cabinet with fume hood
11. Vortex mixer
12. Microcentrifuge
13. Micropipettes (10 µL, 20 µL, 100 µL, 200 µL and 1000 µL)
14. Racks for 1.5 mL microcentrifuge tubes
15. 2 x 96-well -20°C cold blocks
16. Molecular grade water, nuclease-free
17. 10% bleach (1:10 dilution of commercial 5.25-6.0% hypochlorite bleach)
18. DNA*Zap* (Invitrogen, cat. #AM9890) or equivalent
19. RNase Away (Fisher Scientific; cat. #14-375-35) or equivalent
20. Disposable powder-free gloves, surgical masks and lab coats
21. Nuclease Free/Filtered/Aerosol barrier pipette tips
22. MicroAmp EnduraPlate Optical 96-well clear reaction plates (Applied Biosystems; catalog #4483354)
23. MicroAmp Optical Adhesive Film (Applied Biosystems; catalog #4311971)
24. MicroAmp 8-cap Strips (Applied Biosystems; catalog #N8011535)
25. QuantStudio 7 Flex or ViiA 7 Real-Time PCR Systems and QuantStudio Real-Time PCR software (version 1.1) (Applied Biosystems; catalog #4485688 or #4453534)

REAGENTS:

1. QIAGEN RNeasy RNA Mini Kit (Cat. No 74104, 74106)
2. β-mercaptoethanol
3. 96-100% Ethanol
4. Human Specimen Control (HSC), if applicable.
5. Primers and Probes:

Integrated DNA Technologies 2019-nCoV CDC EUA Primer/Probe Kit (#10006606)

Reagent Label	Part #	Description	Reactions / Tube
2019-nCoV_N1	10006600	2019-nCoV_N1 Combined Primer/Probe Mix	500
2019-nCoV_N2	10006601	2019-nCoV_N2 Combined Primer/Probe Mix	500
RNaseP	10006603	Human RNase P Forward Primer/Probe Mix	500

6. **2019-nCoV Positive Control Material**

Integrated DNA Technologies 2019-nCoV_N_Positive Control Material

Reagent Label	Part #	Description
2019-nCoV_N_Positive Control	10006626	2019-nCoV Positive Control (nCoVPC) For use as a positive control with the Avera 2019- nCoV Real-Time RT-PCR Diagnostic Panel procedure. The 2019-nCoV_N_Positive Control contains noninfectious positive control material consisting of nCoV nucleocapsid gene plasmid. nCoVPC will yield a positive result with the N1 and N2 assays in the 2019-nCoV Real-Time RT-PCR Diagnostic Panel.

7. **Human Control Materials**

Description	Quantity
Negative human specimen material: Laboratories may prepare a volume of human specimen material (e.g., human sera or pooled leftover negative respiratory specimens) to extract and run alongside clinical samples as an extraction control. This material should be prepared in sufficient volume to be used across multiple runs. Material should be tested prior to use as the extraction control to ensure it generates the expected results for the HSC listed in these instructions for use.	> 100 µL per extraction

Acceptable alternatives to HSC:

- Materials manufactured by CDC: For use as an RNA extraction procedural control to demonstrate successful recovery of RNA as well as extraction reagent integrity. The HSC consists of noninfectious (beta-propiolactone treated) cultured human cell material supplied as a liquid suspended in 0.01 M PBS at pH 7.2-7.4.

- Contrived human specimen material: Laboratories may prepare contrived human specimen materials by suspending any human cell line (e.g., A549, Hela or 293) in PBS. This material should be prepared in sufficient volume to be used across multiple runs. Material should be tested prior to use as the extraction control to ensure it generates the expected results for the HSC listed in these instructions for use.

CONTROLS TO BE USED WITH THE SARS-CoV-2 ASSAY

A. **Positive Control**
- Integrated DNA Technologies 2019-nCoV_N_Positive Control Material, 2019-nCoV_N_Positive Control, Pru1 #10006626
- 1 positive control will be run on each analysis plate.

B. **Negative Control (NTC)**
- RNAse free molecular grade water
- 1 negative control will be run on each analysis plate.

C. **Extraction Control**
i. Negative Human Specimen Material (e.g. negative respiratory specimens)
- Specimen should be tested prior to use to ensure it generates the expected results for the HSC.

ii. Or UTM or VTM with CDC manufactured Human Specimen Control (HSC) spiked in.

iii. 1 extraction control will be run per extraction batch.

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. The run should be invalidated and re-tested with previously extracted RNA.

1) <u>SARS-CoV2- Assay-Controls – Positive (s), Negative, and Internal</u>

A. **RNase P (Extraction Control)**

i. All clinical samples should exhibit fluorescence growth curves in the RNase P reaction that cross the threshold line within 40.00 cycles (< 40.00 Ct), thus indicating the presence of the human RNase P gene. Failure to detect RNase P in any clinical specimens may indicate:
- Improper extraction of nucleic acid from clinical materials resulting in loss of RNA and/or RNA degradation.
- Absence of sufficient human cellular material due to poor collection or loss of specimen integrity.

- Improper assay set up and execution.
- Reagent or equipment malfunction.

ii. If the RP assay does not produce a positive result for human clinical specimens, interpret as follows:
- If the 2019-nCoV N1 and N2 are positive even in the absence of a positive RP, the result should be considered valid. It is possible that some samples may fail to exhibit RNase P growth curves due to low cell numbers in the original clinical sample. A negative RP signal does not preclude the presence of 2019-nCoV virus RNA in a clinical specimen.
- If all 2019-nCoV markers AND RNase P are negative for the specimen, the result should be considered invalid for the specimen. If residual specimen is available, repeat the extraction procedure and repeat the test. If all markers remain negative after re-test, report the results as invalid and a new specimen should be collected if possible.

B. **2019-nCoV Markers (N1 and N2)**

i. When all controls exhibit the expected performance, a specimen is considered negative if all 2019- nCoV marker (N1, N2) cycle threshold growth curves DO NOT cross the threshold line within 40.00 cycles (< 40.00 Ct) AND the RNase P growth curve DOES cross the threshold line within 40.00 cycles (< 40.00 Ct).

ii. When all controls exhibit the expected performance, a specimen is considered positive for 2019-nCoV if all 2019-nCoV marker (N1, N2) cycle threshold growth curves cross the threshold line within 40.00 cycles (< 40.00 Ct). The RNase P may or may not be positive as described above, but the 2019-nCoV result is still valid.

iii. When all controls exhibit the expected performance and the growth curves for the 2019-nCoV markers (N1, N2) AND the RNase P marker DO NOT cross the cycle threshold growth curve within 40.00 cycles (< 40.00 Ct), the result is invalid. The extracted RNA from the specimen should be re- tested. If residual RNA is not available, re-extract RNA from residual specimen and re-test. If the re- tested sample is negative for all markers and RNase P, the result is invalid and collection of a new specimen from the patient should be considered.

iv. When all controls exhibit the expected performance and the cycle threshold growth curve for any one marker (N1 or N2 but not both markers) crosses the threshold line within 40.00 cycles (< 40.00 Ct) the result is inconclusive. The extracted RNA should be retested. If residual RNA is not available, re- extract RNA from residual specimen and re-test. If the same result is obtained, the laboratory should coordinate transfer of the specimen to CDC for further analysis.

v. If HSC is positive for N1 or N2, then contamination may have occurred during extraction or sample processing. Invalidate all results for specimens extracted alongside the HSC. Re-extract specimens and HSC and re-test

Table 1. Expected Results of Controls Used in the SARS-CoV-2 Assay

Control Type	External Control Name	Used to Monitor	2019 nCoV_N1	2019 nCoV_N2	RP	Expected Ct Values
Positive	nCoVPC	Substantial reagent failure including primer and probe integrity	+	+	+	< 40.00 Ct[1]
Negative	NTC	Reagent and/or environmental contamination	-	-	-	None detected
Extraction	HSC	Failure in lysis and extraction procedure, potential contamination during extraction	-	-	+	< 40.00 Ct

[1]Cycle threshold is defined as the number of cycles required for the fluorescent signal to cross the threshold

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

2) ***Examination and Interpretation of Patient Specimen Results:***

Assessment of clinical specimen test results should 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. The table below lists the expected results for the SARS-CoV2 Assay.

Table 2. Expected Patient Specimen Results in the SARS-CoV-2 Assay

2019 nCoV_Nl	2019 nCoV_N2	RP	Result Interpretation	Report	Actions
+	+	±	2019-nCoV detected	Positive	Report results to sender and appropriate public health authorities.
If only one of the two targets is positive		±	Inconclusive Result	Inconclusive	Repeat testing of nucleic acid and/or re-extract and repeat rRT-PCR once. If the repeated result remains inconclusive, it is reported to the sender as inconclusive and recommend recollection if patient is still clinically indicated
-	-	+	2019-nCoV not detected	Not Detected	Report results to sender. Consider testing for other respiratory viruses.
-	-	-	Invalid Result	Invalid	Repeat extraction and rRT-PCR. If the repeated result remains invalid, consider collecting a new specimen from the patient.

G. PERFORMANCE EVALUATION

1) <u>Limit of Detection (LoD) -Analytical Sensitivity</u>:

The LoD study established the lowest concentration of SARS-CoV-2 (genome copies (copies)/µL) that can be detected by the COVID-19 RT-PCR test at least 95% of the time. The preliminary LoD was established by testing eight dilutions of BEI SARS-CoV-2 synthetic RNA (Cat. No NR-52358; Lot 70033953) utilizing the QIAGEN RNeasy mini kit (Cat. No. 74104, 74106). The BEI SARS-CoV-2 synthetic RNA is a Quantitative synthetic RNA from SARS-related coronavirus 2 [synthetic RNA in RNA stable (Biomatrica 52201-013)]. Preparation includes fragments from the ORF 1ab, Envelope (E) and Nucleocapsid (N) regions. The preliminary LoD was determined by testing three replicates of eight dilutions (655 copies/µL, 65.5 copies/µL, 32.7 copies/µL, 16.4 copies/µL, 8.2 copies/µL, 3.3 copies/µL, 1.6 copies/µL, and 0.3 copies/µL). The dilutions were prepared by spiking the quantified synthetic SARS-CoV-2 RNA into negative respiratory clinical matrices (NP swabs). The lowest dilution in which all three replicates returned a positive result for both targets was 1.6 copies/µl.

The estimated LoD was confirmed by testing an additional 20 replicates at the same target level. All 20 replicates produced the expected results for each SARS-CoV-2 target.

The study results showed that the LoD of the COVID-19 RT-PCR test is 1.6 copies/µL (20/20 positive)**.**The results are shown in the table below.

Table 3. LoD Confirmation Results

Sample	Concentration Specimen (cp/µL)	N1 Ct	N2 Ct	RP Ct
1	1.6	33.993	34.571	29.003
2	1.6	33.958	34.255	29.042
3	1.6	34.313	34.306	28.842
4	1.6	34.317	34.572	28.982
5	1.6	34.667	34.404	28.986
6	1.6	35.052	34.816	29.125
7	1.6	34.744	34.184	28.878
8	1.6	34.961	35.468	29.112
9	1.6	36.033	35.309	29.201
10	1.6	35.276	34.867	29.114
11	1.6	34.810	34.746	29.226
12	1.6	34.166	34.996	29.227
13	1.6	34.140	34.800	28.936
14	1.6	34.192	35.122	29.578
15	1.6	34.947	34.794	29.364
16	1.6	34.995	34.977	29.494
17	1.6	35.690	35.396	29.313
18	1.6	36.247	35.700	29.321
19	1.6	36.014	36.036	29.259
20	1.6	36.261	36.657	29.516

Table 4. Summary of LoD Confirmatory Testing

Targets	**2019-NCoV_N1**	**2019-NCoV_2**	**RP**
RNA Conc (copies/µl)	**1.6 copies/µl**	**1.6 copies/µl**	**1.6 copies/µl**
Pos/Total	**20/20**	**20/20**	**20/20**
Mean Ct	**34.937**	**34.999**	**29.176**
Standard Deviation	**0.763**	**0.625**	**0.212**

2) Inclusivity (analytical sensitivity):

The sequences for the N1, N2 primers/probes used in this assay are identical to the N1, N2 primers/probes sequences used in the FDA authorized original CDC 2019-Novel Coronavirus (2019-nCoV) real time RT-PCR Diagnostic Panel.

Given the dramatic increase in available genomic information pertaining to SARS-CoV-2 in publicly available databases, an *in silico* inclusivity analysis was performed, aimed at determining the potential alignment capability of the CDC-defined primers relative to the numerous publicly available SARS-CoV-2 sequences. Accession numbers pertaining to 2532 SARS-CoV-2 complete sequence entries were obtained from the Severe acute respiratory syndrome coronavirus 2 data hub. Assay primer sequences were aligned to the 2532 sequences using BLASTN suite available from NCBI with the default parameters

for aligning a short input sequence. Alignments were reduced to the highest scoring alignment for each sequence queried. This analysis was completed on May 16th, 2020 and determined to be acceptable.

3) Cross-reactivity (Analytical Specificity)

The analytic specificity of the Avera Institute for Human Genetics SARS-CoV2 RT-PCR Diagnostic Panel was demonstrated *in silico* under the original EUA for the CDC 2019-Novel Coronavirus (2019-nCov) Real-Time RT-PCR Diagnostic Panel. The analysis included evaluation of the primer and probe homology with the 20 organisms and viruses**.** Based on this analysis, significant amplification of non-target sequences that could result in cross-reaction (false positive results) or interference (false negative results) were considered unlikely to occur. The data demonstrated that the expected results were obtained for each organism when tested. Refer to the CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel FDA Emergency Use authorization.

4) Clinical Evaluation:

The performance of the Avera Institute for Human Genetics SARS-CoV2 RT-PCR Diagnostic Panel was evaluated using nasopharyngeal swabs samples which had previously been tested utilizing the Cepheid Xpert Xpress SARS-CoV-2, an FDA authorized EUA test. A total of 30 positive samples and 30 negative samples were tested. Samples were blinded of any comparator method result when being sent to the end user. All 30 (100%) of the negative samples produced the expected results. Of the 30 positive samples, all 30 (100%) produced positive results for the N1 and N2 genes. The results of the clinical evaluation with previously tested nasopharyngeal swabs is considered acceptable.

Table 5. Performance of the SARS-CoV-2 Assay Compared to the Cepheid EUA Authorized Assay

Nasopharyngeal Swabs		**Cepheid Xpert Xpress SARS-CoV-2**		
		Positive	**Negative**	**Total**
Avera Institute for Human Genetics SARS-CoV2 RT-PCR Diagnostic Panel	**Positive**	30	0	**30**
	Negative	0	30	**30**
	Total	**30**	**30**	**60**
Positive Agreement		100% (30/30), 95%CI: (88.7-100%)[1]		
Negative Agreement		100% (30/30); 95%CI: (88.7-100%)		

[1] Two-sided 95% score confidence intervals

Clinical Confirmation:

The first five positive and first five negative patient samples were sent to the South Dakota Department of Health (SDDOH) and tested CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel FDA approved EUA. All results were 100% concordant.

Table 6. Performance of SARS-CoV2 Assay Compared to the CDC EUA Authorized Assay

Nasopharyngeal Swabs		Avera Institute for Human Genetics SARS-CoV2 RT-PCR Diagnostic Panel		
		Positive	**Negative**	**Total**
CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel (CDC)	**Positive**	5	0	**5**
	Negative	0	5	**5**
	Total	**5**	**5**	**10**
Positive Agreement		100% (5/5), 95% CI (56.6%;100%)[1]		
Negative Agreement		100% (5/530);95% CI: (56.6%; 100%)		

[1]Two-sided 95% score confidence intervals

Warnings:

- This test has not been FDA cleared or approved;
- This test has been authorized by FDA under an EUA for use by authorized laboratories;
- This test has been authorized only for the detection of nucleic acid from SARSCoV-2, not for any other viruses or pathogens; and
- 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 Act, 21
- U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.