ACCELERATED EMERGENCY USE AUTHORIZATION (EUA) SUMMARY SARS-CoV-2 ASSAY (Rutgers Clinical Genomics Laboratory)

For in vitro diagnostic use
Rx only
For use under Emergency Use Authorization (EUA) Only

(The Rutgers Clinical Genomics Laboratory TaqPath SARS-CoV-2 Assay will be performed in the Rutgers Clinical Genomics Laboratory, a Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a certified high-complexity laboratory, per the Instructions for Use that were reviewed by the FDA under this EUA).

INTENDED USE

The Rutgers Clinical Genomics Laboratory TaqPath SARS-CoV-2 Assay is intended for the qualitative detection of nucleic acid from SARS-CoV-2 in oropharyngeal (throat) swab, nasopharyngeal swab, anterior nasal swab, mid-turbinate nasal swab and saliva specimens from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to Rutgers Clinical Genomics Laboratory (RCGL) at RUCDR Infinite Biologics – Rutgers University, Piscataway, NJ, that is a Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a certified high-complexity laboratory.

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 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 under the Food and Drug Administration's Emergency Use Authorization.

Testing of self-collected or healthcare provider-collected anterior and mid-turbinate nasal swabs is limited to patients with symptoms of COVID-19.

Collection of saliva specimens is limited to patients with symptoms of COVID-19 and should be performed in a healthcare setting under the supervision of a trained healthcare provider using the Spectrum Solutions LLC SDNA-1000 Saliva Collection Device. Negative results for SARS-CoV-2 RNA from saliva should be confirmed by testing of an alternative specimen type if clinically indicated.

Please refer to FDA's FAQs on Diagnostic Testing for SARS-CoV-2 for additional information regarding the collection appropriate specimen types for the detection of SARS-CoV-2.

DEVICE DESCRIPTION AND TEST PRINCIPLE

The Rutgers Clinical Genomics Laboratory TaqPath SARS-CoV-2 Assay is a real-time reverse transcription polymerase chain reaction (rRT-PCR) test. The assay uses primers and probes that were developed and validated under the Emergency Use Authorization (EUA) for the TaqPath COVID-19 Combo Kit and are designed to detect RNA from SARS-CoV-2 in respiratory specimens from patients as recommended for testing by public health authority guidelines. The purpose of this EUA request is to enable testing of additional specimen types, including saliva, and use of alternative nucleic acid extraction and amplification systems available in the Rutgers Clinical Genomics Laboratory.

Anterior nasal swabs, mid-turbinate nasal swabs, oropharyngeal (throat) swabs and nasopharyngeal swabs should be collected, transported and stored according to standard procedures. Saliva specimens must be collected, transported and stored using the Spectrum Solutions LLC SDNA-1000 Saliva Collection Device. Saliva specimens must be transported and stored at ambient temperature and tested within 48 hours of collection.

RNA extraction for all specimen types is performed using the PerkinElmer Chemagic 360 automated specimen processing system with the Chemagic Viral DNA/RNA 300 Kit H96. The input sample volume is 300µL, the elution volume is 50µL.

Reverse transcriptase-PCR (RT-PCR) is performed using the Applied Biosystems TaqPath COVID-19 Combo Kit with 5µL of the extracted sample.

INSTRUMENTS USED WITH THE TEST

The Rutgers Clinical Genomics Laboratory TaqPath SARS-CoV-2 Assay is for use with the ThermoFisher Applied Biosystems QuantStudio 5 Real-Time PCR System equipped with software v1.3, or the Applied Biosystems ViiA7 Real-Time PCR System with the Applied Biosystems QuantStudio 5 software v1.3 for data analysis, and Perkin Elmer Chemagic 360 extraction instrument (software v6.3.0.3).

REAGENTS AND MATERIALS

Table 1. Reagents and materials required for use of the Rutgers Clinical Genomics Laboratory TaqPath SARS-CoV-2 Assay

Reagent	Manufacturer	Catalogue #
Chemagic Viral DNA/RNA 300 Kit H96	PerkinElmer	CMG-1033-S
96 well Deep Well Plates	PerkinElmer	43001-0120
TaqPath COVID-19 Combo Kit	ThermoFisher Scientific	A147814
384 well PCR plate	ThermoFisher Scientific	4483273
Optical adhesive PCR plate cover	ThermoFisher Scientific	4311971
Nuclease-free water	--	--
Ethanol (96-100%)	--	--

CONTROLS

The controls supplied with the ThermoFisher - Applied Biosystems TaqPath COVID-19 Combo Kit are described in **Table 2**.

Table 2. Controls supplied with the Applied Biosystems TaqPath COVID-19 Combo Kit

Control Type	Purpose	Frequency of Testing
Negative	To monitor for cross-contamination during RNA extraction and RT-PCR	Once per batch of specimens
Positive	To monitor the integrity of the RT-PCR reagents and process	Once per run of RT-PCR
Internal (MS2 Phage)	To monitor the integrity of nucleic acid extraction and RT-PCR for each specimen	Added to each specimen and the Negative Control prior to extraction

In addition to these controls, a No Template Control containing none of the SARS-CoV-2 targets or the Internal Control is included in every PCR run. The results from the controls are interpreted according to the criteria shown in **Table 3**. If the results obtained with the Positive, Negative and No Template Controls do not meet the criteria shown, the results from the entire batch of samples are considered invalid and repeat testing must be performed.

Table 3. Ct values for controls that must be observed to obtain valid results

Control	Ct Value (Optical Channel)			
	N Gene (VIC)	S Gene (ABY)	ORF1ab (FAM)	MS2 Phage (JUN)
Negative	>40	>40	>40	≤37
Positive	<37	<37	<37	Undetermined [1]
No Template	Undetermined	Undetermined	Undetermined	Undetermined [1]
Internal	Any	Any	Any	<37

[1] The MS2 Phage Internal Control is not added to the Positive Control or No Template Control and no signal should be obtained

INTERPRETATION OF RESULTS

The results from testing of patient samples are interpreted according to the criteria described in **Table 4**.

Table 4. Result interpretation for patient samples

Ct Value (Optical Channel)				Result Interpretation
N Gene (VIC)	**S Gene (ABY)**	**ORF1ab (FAM)**	**MS2 Phage (JUN)**	
Undetermined	Undetermined	Undetermined	<37	Negative
Two of three <37			**<37**	Positive
One of three <37			**<37**	Re-test [1]
Undetermined	Undetermined	Undetermined	Undetermined	Re-test [1]

[1] Re-test required from the residual extracted sample and by processing a new aliquot of the original sample if volume permits; if the re-test result is the same as the original then report result as "inconclusive"

PERFORMANCE EVALUATION

1) Analytical Sensitivity

The LoD was determined using *in vitro* transcripts from Exact Diagnostics (SARS-CoV-2 Standard) that were diluted in SARS-CoV-2 negative nasopharyngeal swab matrix. An initial estimate of the LoD with the Applied Biosystems QuantStudio 5 Real-Time PCR System was obtained by testing three replicates at each of four different target levels: 1000, 500, 200 and 100 copies/mL. The lowest level at which all three replicates were positive for all three SARS-CoV-2 targets was 200 copies/mL. 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, and the LoD was therefore confirmed to be 200 copies/mL.

To validate use of the Applied Biosystems ViiA7 Real-Time PCR System for PCR amplification, an additional study was performed by testing 20 nasopharyngeal and 10 saliva samples that were each spiked with 400 copies/mL of the Exact Diagnostics SARS-CoV-2 transcripts. Positive results were obtained for each of the samples for all three target genes and the MS2 internal control, demonstrating that the ViiA7 Real-Time PCR system performed similarly to the QuantStudio 5. These results are acceptable.

2) Analytical Specificity

Inclusivity

The Rutgers Clinical Genomics Laboratory TaqPath SARS-CoV-2 Assay is a modification of the previously authorized ThermoFisher Applied Biosystems TaqPath COVID-19 Combo Kit. The assay targets specific genomic regions of the SARS-CoV-2 nucleocapsid (N) gene, spike (S) gene, and ORF1ab region. Inclusivity was demonstrated under the original EUA by mapping the primers and probes to 185 complete SARS-CoV-2 genomes that were available in the GenBank and GISAID (Global Initiative on Sharing All Influenza Data) databases as of March 5, 2020. For all primers and probes, there was 100% homology to each of the SARS-CoV-2 sequences analyzed, with one exception; a single base mismatch (95.6% homology) with the reverse primer for ORF1ab in sequence EPI_ISL_407084

(BetaCoronavirus/Japan/AI/I-004/2020). The mismatch is located at the 5' end of the primer and is not expected to affect test performance

Cross-reactivity

The analytical specificity of the Rutgers Clinical Genomics Laboratory TaqPath SARS-CoV-2 Assay was demonstrated *in silico* under the original EUA for the ThermoFisher Applied Biosystems TaqPath COVID-19 Combo Kit. The analysis included evaluation of the primer and probe homology with the 43 organisms and viruses listed in **Table 5**. Based on this analysis, significant amplification of non-target sequences that could result in cross-reaction (false-positive results) or interference (false-negative results) was considered unlikely to occur.

Table 5. Organisms and viruses evaluated for potential cross-reaction and/or interference with the Applied Biosystems TaqPath COVID-19 Combo Kit

Viruses	**Bacteria**
Adenovirus	*Bacillus anthracis*
Enterovirus	*Bordetella pertussis*
Human coronavirus 229E	*Chlamydophila pneumoniae*
Human coronavirus HKU1	*Chlamydophila psittaci*
Human coronavirus NL63	*Corynebacterium diphtheriae*
Human coronavirus OC43	*Coxiella burnetii*
Human Metapneumovirus (hMPV)	*Haemophilus influenzae*
Influenza A, B and C	*Legionella* (non-pneumophila)
MERS-coronavirus	*Legionella pneumophila*
Parainfluenza 1-4	*Leptospira* sp.
Parechovirus	*Moraxella catarrhalis*
Respiratory Syncytial Virus A and B	*Mycobacterium tuberculosis*
Rhinovirus/Enterovirus	*Mycoplasma pneumoniae*
SARS-coronavirus	*Neisseria elongata* and *Neisseria meningitidis*
Yeast/Fungus	*Pseudomonas aeruginosa*
Candida albicans	*Staphylococcus aureus*
Pneumocystis jirovecii	*Staphylococcus epidermidis*
	Streptococcus pneumoniae
	Streptococcus pyogenes
	Streptococcus salivarius

3) Clinical Evaluation

Nasopharyngeal Swabs

The performance of the Rutgers Clinical Genomics Laboratory TaqPath SARS-CoV-2 Assay with nasopharyngeal swabs was evaluated using contrived specimens composed of leftover nasopharyngeal swab samples that were spiked with SARS-CoV-2 *in vitro* transcripts or human DNA (both Exact Diagnostics). A total of 30 contrived positive and contrived negative samples were tested. A summary of the results of the study is provided in **Tables 6** and **7**. All 30 (100%) contrived negative samples produced the expected results. Of the 30 contrived positive samples, all 30 (100%) produced positive results for

the N and S genes, whereas the ORF1ab target was positive for 25/30 samples (83.3%). No amplification of the ORF1ab target was observed with 1/10 samples (10.0%) at 200 copies/mL and 4/10 samples (40.0%) at 400 copies/mL. According to the result algorithm described in **Table 4**, above, a sample is considered positive for SARS-CoV-2 RNA if amplification is detected with at least two of the three SARS-CoV-2-specific target sequences. The results of the Clinical Evaluation with contrived nasopharyngeal swabs were therefore considered acceptable.

Table 6. Summary of results from the contrived specimen study with nasopharyngeal swabs, stratified by target level and measurand

Transcript Copies/mL	Number Tested	Analysis	Target (Optical Channel)			
			N Gene (VIC)	S Gene (ABY)	ORF1ab (FAM)	MS2 (JUN)
0	30	Positive (%)	0 (0)	0 (0)	0 (0)	0 (0)
		Mean Ct (SD)	N/A	N/A	N/A	24.4 (0.4)
200	10	Positive (%)	10 (100)	10 (100)	9 (100)	10 (100)
		Mean Ct (SD)	21.7 (4.3)	22.1 (6.0)	19.7 (1.6	27.1 (1.2)
400	10	Positive (%)	10 (100)	10 (100)	6 (60.0)	10 (100)
		Mean Ct (SD)	27.0 (6.7)	26.6 (6.8)	21.1 (2.3)	26.1 (1.2)
600	4	Positive (%)	4 (100)	4 (100)	4 (100)	4 (100)
		Mean Ct (SD)	28.5 (5.2)	27.2 (4.5)	27.4 (6.2)	25.7 (0.9)
800	3	Positive (%)	3 (100)	3 (100)	3 (100)	3 (100)
		Mean Ct (SD)	33.0 (1.6)	30.5 (0.4)	35.0 (3.9)	25.0 (0.9)
1000	3	Positive (%)	3 (100)	3 (100)	3 (100)	3 (100)
		Mean Ct (SD)	28.8 (6.6)	27.7 (5.6)	29.0 (7.3)	25.8 (1.2)
All Positives	30	Positive (%)	30 (100)	30 (100)	25 (83.3)	30 (100)
		Mean Ct (SD)	26.2 (6.3)	25.7 (5.1)	24.2 (6.4)	26.2 (1.3)

N/A: Not applicable; SD: Standard Deviation

Table 7. Summary of positive and negative agreement with contrived nasopharyngeal swab specimens

		Contrived Specimen Type		
		Positive	**Negative**	**Total**
TaqPath SARS-CoV-2 Assay	**Positive**	30	0	**30**
	Negative	0	30	**30**
	Total	**30**	**30**	**60**
Positive Agreement		100% (30/30); 88.7-100% [1]		
Negative Agreement		100% (30/30); 88.7-100%		

[1] Two-sided 95% score confidence intervals

Saliva

A study was performed to evaluate the use of saliva as a specimen type for detection of SARS-CoV-2 in patients who are suspected of COVID-19. The study was conducted with symptomatic patients from three ambulatory care centers who were each provided with instructions for self-collection of saliva using the Spectrum Solutions LLC SDNA-1000 Saliva Collection Device. Self-collection of saliva samples was performed under the observation of a healthcare provider who subsequently (within 10 minutes) also collected either a nasopharyngeal or oropharyngeal swab from each patient for parallel testing for

SARS-CoV-2. The swabs were placed in viral transport medium for shipment to the testing laboratory. Both the saliva and swabs were transported at ambient temperature and tested using the Rutgers Clinical Genomics Laboratory TaqPath SARS-CoV-2 Assay within 48 hours of collection. A summary of the results of the study is presented in **Tables 8** and **9**.

There was 100% positive and negative agreement between the results obtained from testing of saliva and those obtained from nasopharyngeal and oropharyngeal swabs. Overall mean Ct values were similar for saliva and either nasopharyngeal or oropharyngeal swabs, there was no correlation between Ct values from different samples from the same patient. Nevertheless, the results support the use of saliva as a specimen type for use with the Rutgers Clinical Genomics Laboratory TaqPath SARS-CoV-2 Assay.

Table 8. Summary of qualitative results obtained from parallel testing of nasopharyngeal and oropharyngeal swab samples and saliva from patients suspected of COVID-19

		Nasopharyngeal Swab		
		Positive	**Negative**	**Total**
Saliva	**Positive**	26	0	**26**
	Negative	0	27	**27**
	Total	**26**	**27**	**53**
Positive Agreement		100% (26/26); 87.1-100% [1]		
Negative Agreement		100% (27/27); 87.5-100%		
		Oropharyngeal Swab		
		Positive	**Negative**	**Total**
Saliva	**Positive**	4	0	**4**
	Negative	0	3	**3**
	Total	**4**	**3**	**7**
Positive Agreement		100% (4/4); 51.0-100% [1]		
Negative Agreement		100% (3/3); 43.9-100%		
		Nasopharyngeal or Oropharyngeal Swab		
		Positive	**Negative**	**Total**
Saliva	**Positive**	30	0	**30**
	Negative	0	30	**30**
	Total	**30**	**30**	**60**
Positive Agreement		100% (30/30); 88.7-100% [1]		
Negative Agreement		100% (30/30); 88.7-100%		

[1] Two-sided 95% score confidence intervals

Table 9. Summary of results obtained from parallel testing of nasopharyngeal and oropharyngeal swab samples and saliva from patients suspected of COVID-19, stratified by measurand

Number of Patients	Sample Type	Analysis	Target (Optical Channel)			
			N Gene (VIC)	S Gene (ABY)	ORF1ab (FAM)	MS2 (JUN)
26 NP positive	NP swab	Positive (%)	26 (100)	26 (100)	26 (100)	26 (100)
		Mean Ct (SD)	24.4 (4.0)	24.5 (3.9)	23.6 (3.7)	24.3(2.6)
	Saliva	Positive (%)	26 (100)	26 (100)	26 (100)	26 (100)
		Mean Ct (SD)	23.5 (6.2)	24.6 (6.0)	23.6 (5.7)	26.0 (4.1)
27 NP negative	NP swab	Positive (%)	0 (0)	0 (0)	0 (0)	27 (100)
		Mean Ct (SD)	N/A	N/A	N/A	24.4 (1.2)
	Saliva	Positive (%)	0 (0)	0 (0)	0 (0)	27 (100)
		Mean Ct (SD)	N/A	N/A	N/A	25.0 (1.9)
4 OP positive	OP swab	Positive (%)	4 (100)	4 (100)	4 (100)	4 (100)
		Mean Ct (SD)	24.7 (4.0)	24.3 (3.9)	23.5 (4.4)	25.4 (1.8)
	Saliva	Positive (%)	4 (100)	4 (100)	4 (100)	4 (100)
		Mean Ct (SD)	22.0 (7.1)	22.3 (7.2)	21.4 (7.1)	29.6 (5.6)
3 OP negative	OP Swab	Positive (%)	0 (0)	0 (0)	0 (0)	23.5 (1.5)
		Mean Ct (SD)	N/A	N/A	N/A	3 (100)
	Saliva	Positive (%)	0 (0)	0 (0)	0 (0)	3 (100)
		Mean Ct (SD)	N/A	N/A	N/A	23.1 (1.4)

NP: Nasopharyngeal; OP: Oropharyngeal; N/A: Not applicable; SD: Standard Deviation

Clinical Confirmation

The first 5 positive and first 5 negative nasopharyngeal specimens as determined by Rutgers Clinical Genomic Laboratory using the Rutgers TaqPath SARS-CoV-2 Assay were also tested by the New Jersey State Health Department using the previously authorized CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel. There was 100% (5/5) positive and negative agreement for the specimens tested. These results are acceptable and support use of the by Rutgers Clinical Genomic Laboratory TaqPath SARS-CoV-2 Assay for testing clinical specimens.