ACCELERATED EMERGENCY USE AUTHORIZATION (EUA) SUMMARY SARS-CoV-2 RT-PCR Assay

(Diatherix Eurofins Laboratory)

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

(The SARS-CoV-2 RT-PCR assay will be performed at the Diatherix Eurofins Laboratory, certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a as per Laboratory Instructions for Use that was reviewed by the FDA under this EUA.)

INTENDED USE

The SARS-CoV-2 assay is a PCR test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in nasal swabs, nasopharyngeal swabs/wash, oropharyngeal swabs/throat swabs, bronchial aspirates, or sputum from individuals in which identification of SARS-CoV-2 would provide relevant clinical information to the individual and to the healthcare provider. Testing is limited to Diatherix Laboratories, LLC, a College of American Pathologists (CAP)-accredited, and 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. 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.

DEVICE DESCRIPTION AND TEST PRINCIPLE

Diatherix's Target-Enriched Polymerase Chain Reaction is a nested, end-point PCR technology that allows SARS-CoV-2 detection through target enrichment and amplification.

The reaction process includes enrichment and tagging of the target, followed by traditional PCR amplification. First, nucleic acid is extracted from the sample. Each targeted pathogen is then amplified by low concentration nested gene-specific primers that are designed to enrich the targets during the initial PCR cycles. Inside nested primers have a unique tag sequence complementary to proprietary SuperPrimers (Fs and Rs), which are included in the primer mix. The universal SuperPrimers are used to amplify all targets by annealing to the complementary tag sequence on the inside nested primers. The reverse primer of the SuperPrimer set is labeled with biotin, which is incorporated into the resultant PCR product. The schematic below describes this procedure:

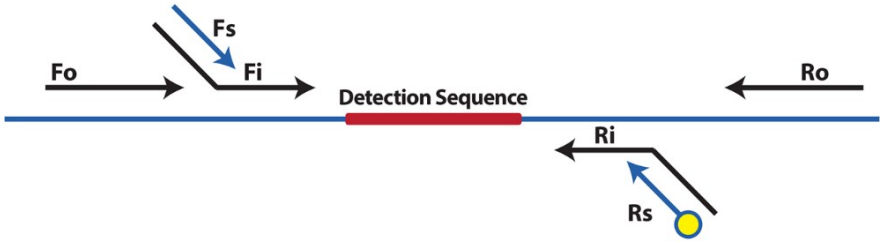

Figure 1. Reaction Process. Low concentration nested gene-specific primers are (Fo – forward out; Fi – forward in; Ri – reverse in; and Ro – reverse out) designed to enrich the targets during the initial PCR cycles. Later in the procedure, a pair of universal SuperPrimers (Fs and Rs) is used to amplify all targets. The Rs primer is labeled with biotin for subsequent detection.

Following PCR, amplicons are hybridized onto a Diatherix microarray in which a detection probe specific to the assay target is attached. Hybridization is followed by incubation with streptavidin-phycoerythrin (SA-PE), which binds to the biotinylated amplicon and emits fluorescence upon excitation. Results for SARS-CoV-2 are reported as 'Detected' or 'Not Detected' based on fluorescence intensity above background.

INSTRUMENTS USED WITH TEST

The Diatherix SARS-CoV-2 assay has been validated to be used with the KingFisher Flex System (Thermo Fisher Scientific, Waltham, MA) utilizing extraction reagents from either Qiagen (ClearMag) or Omega Bio-tek (MagBind Viral DNA/RNA). RT-PCR reactions have been validated on and can be performed on the Applied Biosystems (ABI) GeneAmp 9700 or the Veriti 96-well Thermal Cycler (Thermo Fisher Scientific). Post-amplification hybridization washes are performed using a Wellwash Versa Microplate Washer (Thermo Fisher Scientific). Fluorescence signals are analyzed on the SensoSpot Fluorescence Low Density Microarray Analyzer (Sensovation AG, Radolfzell, Germany).

REAGENTS AND MATERIALS

Table 1. Preferred vendors and catalog numbers.

Item	Vendor (Manufacturer)	Catalog Number(s)
Blotting pads, Grade 707, 11.4 x 7.7cm	Fisher Scientific	NC9775795
ClearMag Beads	Qiagen	27510-4-50
ClearMag Binding Solution	Qiagen	27510-3-1000
ClearMag Wash Solution	Qiagen	27510-5-1000
Deepwell plates for KingFisher™	Fisher Scientific (Thermo)	95040460
Elution Buffer	Omega Bio-tek	Elution-1000DTX
Heratherm Microbiological Incubator (or similar; at least 117L with port)	Fisher Scientific (Thermo)	51028131
Lint free cloth	Fisher Scientific	19-200-734
Luna Universal One-Step qRT-PCR Kit	New England Biolabs	E3005X
Lysis Solution PM3	Qiagen	27510-1-500
Mag-Bind Viral DNA/RNA 96 Kit	Omega	M6246-03
Microarray alignment tool (jig)	Grace Bio	RD476875
Microarray plates or slides	Diatherix	---
Microarray plate press (Storage Mat Applicator)	Corning	3081
Microplates (elution plates) for KingFisher™	Fisher Scientific (Thermo)	97002540
Nuclease-free PCR plates, tall chimney	Fisher Scientific	14-230-242
Plate seals, breathable rayon	VWR or Fisher	60941-084 (VWR) or 13-882-95 (Fisher)
Plate seals, heat-sealing foils, easy-pierce	Fisher Scientific (ABgene)	AB-1720
Plate seals, self-adhesive	Fisher Scientific	07-200-683
ProPlate® gasket	Grace Bio	204960
SensoSpot® Fluorescence Microarray Analyzer (FLAIR)	Sensovation AG	SVAR06-MB-2002
Streptavidin-phycoerythrin (SA-PE)	Moss Substrates	SAPE-001
Incubating microplate shakers	Troemner or VWR	ISLDMPHDGLUS (Troemner) or 97043-606 (VWR)
Tip combs, KingFisher 96 for DW Magnets	Fisher Scientific (Thermo)	97002534
UltraPure nuclease-free water	Life Technologies	10977015
Wellwash Versa Microplate Washer, 2x8	Fisher Scientific (Thermo)	5165010

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

For SARS-CoV-2 patient sample testing, the following controls are required: Negative Extraction control consisting of H1N1-09 organism, Positive Control consisting of SARS-CoV-2 IVT RNA, and an Internal Amplification Control. A PCR negative control must also be run with every batch.

A description of each control is below:

SARS-CoV-2 Positive Control:
One positive control is run on each plate. This control is designed to assess the integrity of the PCR run. The positive control consists of SARS-CoV-2 *in vitro* transcribed RNA from a partial sequence of the S gene diluted in Simulated Lung Fluid (SLF, also known as Gamble's Solution)[1] for a final concentration of 100 copies/µL.

Internal Control (IC):
An internal control is run with every patient sample from extraction. This control is a synthetic oligonucleotide sequence that is complementary to universal primers included in the assay. The control is spiked into the elution buffer used during nucleic acid extraction and is intermingled with the nucleic acids of patient samples during the final step of nucleic acid extraction. The IC is carried throughout the remainder of the assay steps.

NTC (No Template Control):
This control is run in every batch (up to 96 samples) to rule out contamination of reagents with target nucleic acid. The no template control is a PCR negative control and consists of 4 µL of the nuclease-free water provided in the Luna Universal One-Step RT-qPCR enzyme kit.

SARS-CoV-2 Negative Extraction Control:
The SARS-CoV-2 negative extraction control is run on each plate. This control consists of the H1N1-09 organism. This is a process control that monitors for the entire lysis, extraction, amplification, and detection reactions.

INTERPRETATION OF RESULTS

1) SARS-CoV-2 RT-PCR test Controls – Positive, Negative, and Internal:

Positive Control: Amplification must yield a positive signal (above cutoff) for the SARS-CoV-2 target (NCOVDe2 Detection sequence) and yield negative signal for all other targets. If the extraction control fails, the cause of the failure will be investigated and the run repeated, if warranted.

[1] Marques, M., Loebenberg, R., and Almukainzi, M. 2011. *Simulated Biological Fluids with Possible Application in Dissolution Testing*. Dissolution Technologies.15-28. <dx.doi.org/10.14227/DT180311P15>

Internal Control: The internal control must be detected for a negative result to be reported. For specimens in which the IC is not detected, it will be assumed that the sample includes a PCR inhibitor, and the sample will be repeated from extraction.

No Template Control: The PCR negative control must have all target signals below their respective cutoff for the run to pass. If the negative control contains positive signals for any of the targets, the run will be repeated.

SARS-CoV-2 Negative Extraction Control: This control should only be positive for the H1N1 targets and negative for all other targets. If the extraction control fails, the cause of failure will be investigated and the run repeated, if warranted.

If any batch control (NTC or Extraction Control) fails, the Laboratory Manager or Technical Supervisor will be notified. If an individual patient sample control fails, the patient report will be held and repeated. If the sample fails upon repeat testing, the affected sample will be reported as a failed test.

2) Examination and Interpretation of Patient Specimen Results:

Results for the SARS-CoV-2 target are reported as "Detected" or "Not Detected" based on signal intensity above background (cutoff), measured in Relative Fluorescence Units (RFU).

1. Positive Specimens:
   Specimens with a SARS-CoV-2 signal intensity above the pre-defined cutoff will be considered detected.

2. Negative Specimens:
   Specimens with a SARS-CoV-2 signal intensity level below the pre-defined cutoff will be considered not detected only if the internal control is also detected.

3. Failed Test:
   If a signal intensity cannot be generated for a well, the patient sample is considered a "hybridization failure" and the run must be repeated from hybridization. If the sample fails upon retesting, the affected sample will be reported as a failed test.

Table 2. Interpretation of Patient Results.

Target	IC	Result
Signal intensity above predefined cutoff	Detected	SARS-CoV-2 Detected
Signal intensity below predefined cutoff	Detected	SARS-CoV-2 Not Detected
Signal intensity below predefined cutoff	Not Detected	Sample repeated from extraction
Hybridization failure	N/A	Testing must be repeated from hybridization. If hybridization fails again, report as failed test

PERFORMANCE EVALUATION

1) Analytical Sensitivity:

Limit of Detection (LoD):

The analytical sensitivity of the SARS-CoV-2 assay was determined in Limit of Detection (LoD_{95}) studies. LoD_{95} is defined as the concentration that can accurately be detected 95% of the time. Since no quantified viral isolates were available at the time of the study, assays were tested from extraction with characterized genomic RNA (Human 2019-nCoV strain 2019-nCoV/Italy-INMI1[2]) and stocks of *in vitro* transcribed (IVT) RNA for a partial sequence of the spike (S) gene. Genomic RNA and IVT RNA of known titers (copies/µL) were spiked into non-reactive, pooled sputum clinical matrix and simulated lung fluid (SLF) to mimic respiratory swab specimens. Nucleic acid extraction was performed on the KingFisher Flex extraction platform.

To first determine performance of the assay, 10-fold serial dilutions of IVT RNA in SLF were prepared (ranging from 10^{10} copies/µL to 10^{0} copies/µL) and extracted in triplicate using Qiagen ClearMag extraction reagents. PCR was performed on the Veriti 96 well thermocycler.

Once assay performance was assessed, the same test conditions were used to determine broad-range LoD for the assay in clinical matrix. Broad range LoD was determined by testing triplicate samples of IVT RNA and genomic RNA spiked in pooled sputum from 10^{3} to 10^{0} copies/µL on the ABI 9700 thermocycler.

The LoD was confirmed to be 1 copy/µL based on a positivity rate of 95% upon testing 60 replicates in pooled sputum matrix (57/60). Samples for the LoD confirmation study were extracted using the Qiagen ClearMag or Omega Bio-tek kits.

2) Analytical Inclusivity

An alignment was performed with the oligonucleotide primer and detection sequences designed for the Diatherix SARS-CoV-2 assay using all publicly available sequences for SARS-CoV-2 found in NCBI Virus Resource and GenBank as of March 15, 2020 to predict the inclusivity of the target. All alignments show 100% identity to the primer sequences utilized by the SARS-CoV-2 assay.

3) Cross-Reactivity

A total of 31 viral strains, 34 bacterial strains, and two fungal strains were wet tested for cross-reactivity against the SARS-CoV-2 assay (Table 3). The organisms were

[2] This work was supported by the European Virus Archive Goes Global (EVAg) project that has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 653316.

selected for their likelihood to be found in the respiratory tract, for genetic similarities, or for likelihood of carriage within the clinical matrix. For this testing, titered organisms were prepared in mixes containing up to six microorganisms at the concentrations listed below, spiked into SLF and extracted utilizing Qiagen ClearMag reagents in at least triplicate. RT-PCR reactions were performed on the Veriti 96-well thermocycler. No false positive results indicating cross-reactivity were observed.

Table 3. List of organisms analyzed by wet testing.

Strain	Vendor	Catalog #	Concentration Tested
Adenovirus Type 4	Zeptometrix	0810070CF	10^3 $TCID_{50}$/mL
Coxsackie Virus Type A16	Zeptometrix	0810107CF	10^2 $TCID_{50}$/mL
Influenza A H3N2 Texas/50/12	Zeptometrix	0810238CF	10^2 $TCID_{50}$/mL
Human metapneumovirus Type B2 Strain IA18-2003	Zeptometrix	0810162CF	10^3 $TCID_{50}$/mL
Parainfluenza virus Type 4A	Zeptometrix	0810060CF	10^2 $TCID_{50}$/mL
Parainfluenza virus Type 4B	Zeptometrix	0810060BCF	10^2 $TCID_{50}$/mL
Human metapneumovirus Type A2 Strain IA27-2004	Zeptometrix	0810164CF	10^3 $TCID_{50}$/mL
Adenovirus Type 7A	Zeptometrix	0810021CF	10^4 $TCID_{50}$/mL
Enterovirus D68 2014 Isolate 1	Zeptometrix	0810300CF	10^2 $TCID_{50}$/mL
Influenza A H1N1 Michigan/45/15	Zeptometrix	0810538CF	10^2 $TCID_{50}$/mL
Parainfluenza Virus Type 1	Zeptometrix	0810014CF	10^2 $TCID_{50}$/mL
Adenovirus Type 1	Zeptometrix	0810062CF	10^3 $TCID_{50}$/mL
Echovirus Type 30	Zeptometrix	0810078CF	10^3 $TCID_{50}$/mL
Human Coronavirus NL63	Zeptometrix	0810228CF	10^2 $TCID_{50}$/mL
Influenza A H7N9 Shanghai/2/2013 (BPL Treated)	IRR	FR-1281	10^2 $TCID_{50}$/mL
Influenza B/Phuket/3073/2013 (Yamagata lineage)	IRR	FR-1364	10^6 $CEID_{50}$/mL
MERS-CoV (Heat-inactivated)	BEI	NR-50171	10^3 $TCID_{50}$/mL
Human Coronavirus OC43	Zeptometrix	0810024CF	10^2 $TCID_{50}$/mL
Parainfluenza Virus Type 2	Zeptometrix	0810015CF	10^3 $TCID_{50}$/mL
Respiratory Syncytial Virus Type B 3/2015 Isolate #2	Zeptometrix	0810480CF	10^2 $TCID_{50}$/mL
Human Coronavirus 229E	Zeptometrix	0810229CF	10^2 $TCID_{50}$/mL
Influenza B Brisbane/33/08	Zeptometrix	0810253CF	10^3 $TCID_{50}$/mL
Parainfluenza virus Type 3	Zeptometrix	0810016CF	10^3 $TCID_{50}$/mL
Rhinovirus A16	Zeptometrix	0810285CF	10^4 $TCID_{50}$/mL
Respiratory Syncytial Virus Type A 3/2015 Isolate #3	Zeptometrix	0810482CF	10^3 $TCID_{50}$/mL
Acinetobacter baumannii	Zeptometrix	801597	10^6 cfu/mL
Bordetella parapertussis	ATCC	BAA-587	10^6 cfu/mL
Bordetella pertussis	ATCC	BAA-1335	10^6 cfu/mL
Candida albicans	ATCC	11006	10^6 cfu/mL
Candida glabrata	ATCC	32554	10^6 cfu/mL
Chlamydia trachomatis	Zeptometrix	801775	10^6 ifu/mL
Chlamydophila pneumoniae	IRR	FR-319	10^4 $TCID_{50}$/mL

Corynebacterium sp.	ATCC	35515	10^6 cfu/mL
Escherichia coli	ATCC	11775	10^6 cfu/mL
Fusobacterium necrophorum	ATCC	51357	10^6 cfu/mL
Haemophilus influenzae	Zeptometrix	801680	10^6 cfu/mL
Herpes simplex virus 1	Zeptometrix	0810005CF	10^4 $TCID_{50}$/mL
Herpes simplex virus 2	Zeptometrix	0810006CF	10^4 $TCID_{50}$/mL
Herpesvirus 3 (VZV)	Zeptometrix	0810172CF	10^3 $TCID_{50}$/mL
Klebsiella pneumoniae	ATCC	35555	10^6 cfu/mL
Lactobacillus acidophilus	Zeptometrix	801540	10^6 cfu/mL
Lactobacillus plantarum	IRR	FR-324	10^6 cfu/mL
Legionella pneumophila	IRR	FR-317	10^6 cfu/mL
Listeria monocytogenes	Zeptometrix	801534	10^6 cfu/mL
Moraxella catarrhalis	IRR	FR-327	10^6 cfu/mL
Mycoplasma genitalium	Zeptometrix	0804094-1	10^5 ccu/mL
Mycoplasma hominis	Zeptometrix	804011	10^6 ccu/mL
Mycoplasma pneumoniae	IRR	FR-318	10^6 ccu/mL
Neisseria elongata	ATCC	25295	10^6 cfu/mL
Neisseria gonorrhoeae	Zeptometrix	801482	10^6 cfu/mL
Neisseria meningitidis	Zeptometrix	801511	10^6 cfu/mL
Neisseria sicca	Zeptometrix	801754	10^5 cfu/mL
Parechovirus 1	IRR	FR-308	10^4 $TCID_{50}$/mL
Parechovirus 2	Zeptometrix	0810146CF	10^4 $TCID_{50}$/mL
Parechovirus 3	Zeptometrix	0810147CF	10^4 $TCID_{50}$/mL
Proteus vulgaris	ATCC	33420	10^6 cfu/mL
Pseudomonas aeruginosa	Zeptometrix	801519	10^6 cfu/mL
Serratia marcescens	ATCC	43861	10^6 cfu/mL
Staphylococcus aureus	Zeptometrix	801531	10^6 cfu/mL
Staphylococcus epidermidis	Zeptometrix	801651	10^6 cfu/mL
Staphylococcus haemolyticus	Zeptometrix	801591	10^6 cfu/mL
Streptococcus agalactiae	Zeptometrix	801545	10^6 cfu/mL
Streptococcus dysgalactiae	Zeptometrix	801516	10^6 cfu/mL
Streptococcus pneumoniae	Zeptometrix	801439	10^6 cfu/mL
Streptococcus pyogenes	Zeptometrix	801512	10^6 cfu/mL
Streptococcus salivarius	ATCC	13419	10^6 cfu/mL
Ureaplasma urealyticum	ATCC	33699	10^6 cfu/mL

Additionally, a total of 183 clinical specimens from individual patients exhibiting signs and symptoms of respiratory infection were tested against the SARS-CoV-2 assay (Table 4). No positive signals for SARS-CoV-2 were observed.

Table 4. Specimens tested for cross-reactivity.

Sample Type	Number tested
Bronchial	1
Nares	1
Nasal	92
Nasopharyngeal	10
Sputum	10
Throat	54
Throat/nasal	1
Other	3
Not given	11
Total	**183**

For strains not available for wet testing, *in silico* analysis of the SARS-CoV-2 amplicon sequences were performed (Table 5).

Table 5. Organisms analyzed *in silico*.

SARS-CoV
Human coronavirus HKU1
Human bocavirus
Chlamydia psittaci
Corynebacterium diptheriae
Coxiella burnetii
Legionella spp.
Leptospira
Mycobacterium tuberculosis
Pneumocystis jirovecii

Results from the *in silico* analysis showed that the only organisms in Table 5 with significant homology (>80%) to the SARS-CoV-2 assay are *Legionella pneumophila*, SARS-coronavirus, and bat-like coronavirus. Despite having >80% homology of one assay component (primers or detection sequence), there is no anticipated amplification or detection of SARS-CoV or *L. pneumophila* because hybridization and extension of three assay components are required to generate a detection signal.

4) Clinical Evaluation:

A clinical evaluation study with contrived samples was performed to evaluate the performance of the SARS-CoV-2 assay. A total of 271 individual respiratory specimens, comprised of nasal swabs, nasopharyngeal swabs, throat swabs, bronchial washes, and sputum, were collected from patients exhibiting signs and symptoms of respiratory infection. A total of 183 negative specimens were tested with the assay (these were the same specimens used for the cross-reactivity study in Table 4). The remaining 88 specimens, consisting of 27 sputum samples, 25 NP swabs, 11 nasal swabs, 14 throat swabs, and 11 BALs, were contrived by spiking in either genomic SARS-CoV-2 RNA (32 samples) or *in vitro* transcribed RNA (56 samples), as limited genomic RNA material was available. Of these 88 samples, a total of 40 specimens were spiked at the 2X LoD concentration (2 copies/µL) and 48 specimens were spiked at a concentration of >2X LoD. Samples for this study were extracted using Qiagen ClearMag reagents, and RT-PCR reactions were performed on the ABI 9700 or the Veriti 96 well thermocycler, depending on batch run.

The positive percent agreement (PPA) for all contrived positive samples was 98.9% (87/88). The PPA was 100% (40/40) for the samples spiked at 2X LoD and 98% (47/48) for samples spiked at >2X LoD. No positive signals for SARS-CoV-2 were observed in the negative samples, yielding 100% negative percent agreement (NPA) with expected results. Table 6 below summarizes the data for the positive samples tested:

Table 6. Samples tested and positivity rates.

Sample Type	Concentration	Positivity rate
Sputum	2X LoD	11/11
	>2XLoD	16/16
NP Swab	2X LoD	11/11
	>2XLoD	13/14
Nasal Swab	2X LoD	5/5
	>2XLoD	6/6
Throat Swab	2X LoD	8/8
	>2XLoD	6/6
BAL	2X LoD	5/5
	>2XLoD	6/6
Total		87/88

In addition, the first 5 positive and 5 negative patient results obtained with this test were sent to Alabama Public Laboratory, where all results were confirmed.

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 the Omega Biotek viral extraction on a Kingfisher Flex instrument, and a Veriti 96 thermocycler for PCR. The results are summarized in the following Table.

Table 7: Summary of LoD Confirmation Result using the FDA SARS-CoV-2 Reference Panel

Reference Materials Provide d by FDA	**Spe cimen Type**	**Product LoD**	**Cross-Reactivity**
SARS-CoV-2	Nasopharyngeal Swab	$1.8x10^5$ NDU/mL	N/A
MERS-CoV		N/A	ND

NDU/mL = RNA NAAT detectable units/mL
N/A: Not applicable
ND: Not detected