ACCELERATED EMERGENCY USE AUTHORIZATION (EUA) SUMMARY SARS-COV-2 RNA DETECTR ASSAY (UCSF Health Clinical Laboratories, UCSF Clinical Labs at China Basin)

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

(The SARS-CoV-2 RNA DETECTR Assay will be performed in the UCSF Clinical Labs at China Basin located at 185 Berry Street, San Francisco, CA 94107, which is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a and meets requirements to perform high-complexity tests, per the laboratory procedures that were reviewed by the FDA under this EUA).

INTENDED USE

The SARS-CoV-2 RNA DETECTR Assay is a molecular diagnostic test intended for the qualitative detection of nucleic acid from the SARS-CoV-2 in upper respiratory specimens (nasopharyngeal swabs, oropharyngeal (throat) swabs, mid-turbinate nasal swabs, anterior nasal swabs, nasopharyngeal wash/aspirate or nasal aspirate) from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to UCSF Clinical Labs at China Basin located at 185 Berry Street, San Francisco, CA 94107, which is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a and meets requirements to perform high-complexity tests.

Results are for the detection 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 SARS-CoV-2 RNA DETECTR Assay is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the techniques of molecular assays and *in vitro* diagnostic procedures. The SARS-CoV-2 RNA DETECTR Assay is only for use under the Food and Drug Administration's Emergency Use Authorization.

DEVICE DESCRIPTION AND TEST PRINCIPLE

University of California, San Francisco (UCSF) and Mammoth Biosciences have developed a rapid (45 min) and accurate CRISPR-Cas12 based assay for qualitative

detection of SARS-CoV-2 from respiratory swab RNA extracts. The assay, SARS-CoV-2 RNA DNA Endonuclease-Targeted CRISPR Trans Reporter (DETECTR), performs simultaneous reverse transcription and isothermal amplification using loop-mediated amplification (RT-LAMP) for RNA extracted from nasopharyngeal or oropharyngeal swabs in universal transport media (UTM), followed by the addition of the Cas12 enzyme, after which cleavage of a reporter molecule confirms detection of the virus. The assay uses fluorescence measurements on the ABI 7500 Fast Dx instrument (Applied Biosciences) with a total run time of 45 minutes (30 minutes RT-LAMP, followed by 15 minutes Cas12 detection) as the test readout.

This test uses primer sets and CRISPR guide RNAs (gRNAs) specific for the detection SARS-CoV-2 using reverse transcription-loop-mediated isothermal amplification (RT-LAMP) with the *Bst* DNA polymerase and Cas12-mediated cleavage of the amplified product. The target is the N gene, along with primers and gRNAs for the human RNaseP (RP) gene as a sample internal control. The target region of the N gene overlaps with the N2 region of the FDA Emergency Use Authorization (EUA)-authorized CDC assay.

Extractions are performed using the Qiagen EZ1 DSP virus kit on the Qiagen EZ1 Advanced benchtop automated extraction instrument according to the manufacturer's instructions.

INSTRUMENTS USED WITH THE TEST

The SARS-CoV-2 RNA DETECTR Assay test is to be used with the Thermo Scientific ABI 7500 Fast Dx Real-Time PCR System, Software Version 1.4.1.

REAGENTS AND MATERIALS

Table 1. SARS-CoV-2 RNA DETECTR Reagent Set v.1.1

Amplification Set Component	Vial Color	# of vials	Volume (mL/vial)
Amp-N	**Red**	**1**	**1000**
Amp-RP	**Yellow**	**1**	**1000**
Amp-A	**Clear**	**1**	**150**
Positive Control	**Vial Color**	**# of vials**	**Volume (µL/vial)**
PC-RP	**Lavender**	**2**	**100**
DETECTR Set Component	**Vial Color**	**# of vials**	**Volume (µL/vial)**
Det-N	**Red**	**1**	**1000**
Det-RP	**Yellow**	**1**	**1000**

Reagents and Materials Required but Not Included

A. Nuclease-Free Water, not DEPC treated (NFW) (5 x 100 mL bottles)
   1. Stored at room temperature. In use vials are stored at 2-8°C.
   2. This water is used as a No Template Control (NTC)
B. Qiagen EZ1 DSP virus kit (Catalog #955134) on the Qiagen EZ1 Advanced benchtop automated extraction
C. ABI 7500 Fast Dx Real-Time PCR system (Thermo Scientific), Software version 1.4.1

D. Mini Centrifuges
E. Multichannel or Single channel pipette and barrier tips (10 µL – 200 µL)
F. PCR plate/tube strip support frame/racks
G. PCR cooler rack

LIMITATIONS

The performance of the SARS-CoV-2 RNA DETECTR Assay was established using nasopharyngeal swabs. Oropharyngeal (throat) swabs, mid-turbinate nasal swabs, anterior nasal swabs, nasopharyngeal wash/aspirate and nasal aspirate are considered acceptable specimen types for use with the SARS-CoV-2 RNA DETECTR Assay but performance has not been established.

CONTROLS

Controls for the assay include positive control (PC), human specimen control (HSC), universal transport medium (UTM) and negative template/extraction water control (NTC). NTC, PC, HSC and UTM are extracted with each extraction run along with patient samples.

The PC consists of AccuPlex SARS-CoV-2 Verification Panel reference material (SeraCare, Catalog # 0505-0129) spiked at 18,000 copies/mL in negative patient matrix (corresponding to 30 copies/µL or 1.5X the claimed LoD of 20 copies/µL. The AccuPlex SARS-CoV-2 Verification Panel reference material consists of a target synthetic viral RNA, including the N gene, encapsulated in a viral protein coat. As the reference material spiked into negative matrix is fully extractable, this reference material serves as a full-process control for the DETECTR assay. The PC serves as a positive control for amplification of the N gene target.

The HSC serves as a positive control for the presence of human nucleic acid in the sample, which is targeted by the RP gene.

The UTM serves as a control for contamination in the extraction or amplification.

The NTC serves as a control for contamination during both the extraction and PCR reagent preparation.

The RP target also serves as an internal control for each patient sample, ensuring that human nucleic acid is present in the sample.

The results of the expected control reactions are shown in **Table 2.**

Table 2. Expected Control Reactions

Control	Target	N gene	RP gene
HSC	Human RNase P	Negative (<500,000 FU*)	Positive (≥500,000 FU)
PC	N gene	Positive (≥500,000 FU)	Positive (≥500,000 FU)
UTM	None	Negative (<500,000 FU)	Negative (<500,000 FU
NTC	None	Negative (<500,000 FU)	Negative (<500,000 FU

*FU, Fluorescence units

1. NTC should be negative and exhibit no fluorescence.
    a. If a false positive occurs, sample contamination may have occurred.
2. UTM should be negative and exhibit no fluorescence.
    a. If a false positive occurs, sample contamination may have occurred
3. PC reaction should produce a positive result and exhibit fluorescence
    a. If the PC does not exhibit positivity, the run is invalid.
4. RP should be positive for all negative clinical samples and HSC. Acceptable results for the RP in patient samples indicates the presence of sufficient nucleic acid from the human RP-gene, and thus, acceptable specimen quality.
    a. Failure of RP detection in HSC may indicate improper assay set up or instrument malfunction.
    b. Failure of RP in patient samples but detection in HSC may indicate extraction failure, assay inhibition, or absence of sufficient human cellular material.
    c. Samples that are positive for viral detection do not require amplification of RP target to be valid.
5. HSC should be negative for N-gene.
    a. Fluorescence may indicate contamination of reagents or cross contamination of samples.
    b. If the HSC exhibits positive signal, the run is invalid.

INTERPRETATION OF RESULTS

The range of possible assay results and interpretation are provided in **Table 3.**

Table 3. Result Interpretation

N gene	RP gene	Interpretation	Action
Positive (≥500,000 FU*)	Positive (≥500,000 FU) or Negative (<500,000 FU)	Detected	Report as Detected
Negative (<500,000 FU)	Positive (≥500,000 FU)	Not Detected	Report as Not Detected
Negative <500,000 FU)	Negative (<500,000 FU)	Invalid	Repeat with new extraction; if same result Report as Invalid[#]

*FU, fluorescence units

[#]If the sample result is invalid, the test is repeated. If repeat test also yields an invalid result, the result is reported as inhibitory to nucleic acid amplification, suggest recollection of sample

PERFORMANCE EVALUATION

I. Analytical Sensitivity

Quantified AccuPlex SARS-CoV-2 Verification Panel reference material (SeraCare) at a stock concentration of 100,000 copies/mL was diluted in pooled negative patient NPS/OPS in UTM (dilutions shown in Table 4 below) and run in 20 contrived extraction replicates of pooled extracted RNA per dilution. The limit of detection was defined as the lowest concentration where at least 19 of 20 replicates were positive. Therefore, the LoD was confirmed to be 20 copies/µL for the SARS-CoV-2 RNA DETECTR Assay, Table 4.

Table 4. LoD Based on AccuPlex Reference Material (Spiked into NPS Matrix and Extracted)

Sample ID	Concentration Equivalent in UTM (copies/mL)	Copies / Reaction	Copies / µL	Total # of Reactions	# of Positive Reactions	# of Negative Reactions
Dil2	18,000	150	30	20	20	0
Dil3	12,000	100	20	20	20	0
Dil4	9,000	75	15	20	16	4
Dil5	6,000	50	10	20	16	4

II. Analytical specificity

Inclusivity

To demonstrate the predicted inclusivity, *in silico* analysis of the primer and gRNA sequences with 2,072 SARS-CoV-2 genomes available on GISAID as of March 30th, 2020 (defined as >29 kbp of sequence) was performed using NC_045512 from GenBank as a reference for SARS-CoV-2. A total of 15 variants were found containing single nucleotide variants (SNVs) in the primer and gRNA regions in the N gene target amplicon used by the DETECTR assay, representing 0.77% (16 of 2,072) of all sequences in GSAID. Among the 15 variants, 14 of 15 have a single SNV within one of the 6 primer regions (F3, B3, LF, LB, FIP (F2-F1c), or BIP (B2-B1c)), while 1 of 16 has a single SNV within the gRNA region (hCoV-19/Chongqing/YC01/2020).

A single SNV in a primer region is unlikely to significantly affect the sensitivity of the assay unless it is at the 3’ end of the primer. Among the 14 of 15 variants with a single SNV within one of the 6 primer regions, only one has an SNV at the 3’ end of the primer (hCoV-19/Netherlands/NA_8/2020). A sequence containing a SNV in the gRNA region may also affect sensitivity given the single nucleotide specificity of the gRNA for a CRISPR-Cas12 reaction. Thus, the sensitivity of detection of the DETECTR assay is likely to be affected in only 2 sequences out of 2,072, constituting only 0.096% of all available sequences in GISAID as of March 30th, 2020. Note that the SNV in the gRNA region for (hCoV-19/Netherlands/NA_8/2020) may also affect sensitivity of the detection of the CDC N2 assay as well, as it also overlaps with the N2 probe region.

Cross-reactivity

In Silico BLASTn analysis queries of the SARS-CoV-2 DETECTR assay N gene primers and gRNAs were performed against public domain nucleotide sequences in NCBI (National Center for Biotechnology Information) nucleotide collection (nt) using default parameters. Sequences from the following organisms were analyzed (Table 5):

Table 5: List of Organisms Analyzed *in silico* for Cross-Reactivity

Other high priority pathogens from the same genetic family	**High priority organisms likely in circulating areas**
SARS-CoV-2	Adenovirus
bat-SL-CoVCZ45	Influenza A virus
SARS-CoV	Influenza B virus
MERS-CoV	Influenza C virus
HCoV-OC43	RSV (A + B)
HCoV-NL63	Human parainfluenza virus (1-4)
HCoV-229E	Enterovirus
HCoV-HKU1	Rhinovirus
	Bacillus anthracis
	Bordetella pertussis
	Candida albicans
	Chlamydia pneumoniae
	Chlamydia psittaci
	Chlamydia trachomatis
	Corynebacterium diphtheriae
	Coxiella burnetii
	Haemophilus influenzae
	Human metapneumovirus (hMPV)
	Legionella longbeachae
	Legionella pneumophila
	Leptospira interrogans
	Moraxella catarrhalis
	Mycobacterium tuberculosis
	Mycoplasma pneumoniae
	Neisseria elongata
	Neisseria gonorrhoeae
	Parechovirus
	Pneumocystis jirovecii

Other high priority pathogens from the same genetic family	**High priority organisms likely in circulating areas**
	Pseudomonas aeruginosa
	Staphylococcus aureus
	Staphylococcus epidermidis
	Streptococcus pneumoniae
	Streptococcus pyogenes
	Streptococcus salivarius

Summary of the results are shown below:

N gene primers and gRNAs:

- F3 → 83.3% homology to a sequence in the *Haemophilus influenzae* genome and the *Homo sapiens* genome. No significant homology to other organisms of interest.
- B3 → 81.8% identity to a sequence in the *Homo sapiens* genome.
- FIP (F2-F1c) → 100% homology to bat coronaviruses and SARS-CoV. No significant homology to other organisms of interest.
- BIP (B2-B1c) → 100% homology for the B1c portion of the BIP primer to bat coronaviruses and SARS-CoV. No significant homology to other organisms of interest.
- LF → homology to SARS-CoV (94%), *Chlamydia pneumoniae* (84%), *Streptococcus pyogenes* (84%), and *Homo sapiens* (89% genomes).
- LB → 88.8% homology to bat coronaviruses. No significant homology to other organisms of interest.
- N-gene gRNA → 80% homology to a sequence in the *Homo sapiens* genome. This sequence lacks the PAM required for Cas12 activity.

Although some primers have partial homology to the organisms of interest, it is unlikely for cross-reactivity to occur with these organisms as RT-LAMP requires complementarity to at least 4 of the 6 primers. In addition, the specificity of the RT-LAMP amplicon is benefited by the sequence specificity of the Cas enzyme.

Although most of the N gene primers (B3, BIP, FIP, LF, and LB) have 100% identity to SARS coronavirus and other bat coronavirus, cross-reactivity with these other coronaviruses would not be expected given the lack of sequence homology in F3 and the N-gene gRNA. The gRNA sequence, in particular, has single-nucleotide specificity.

Cross-reactivity with human, other coronaviruses, and bacterial sequences would also not be expected given the lack of sequence homology in several primers (B3, BIP, FIP) and in the N- gene gRNA.

Cross-reactivity was also assessed by wet-testing 18 negative nasopharyngeal swab patient specimens, positive for the organisms listed in Table 6. Viruses were detected by Luminex NxTAG Respiratory Pathogen Panel and CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel, Influenza A/B Typing Kit. Each sample was then assayed using the SARS-CoV-2 RNA DETECTR Assay. All 18 clinical specimens were negative for SARS-CoV-2.

Table 6: Summary of Wet-Testing for the Analytical Specificity of the SARS-CoV-2 RNA DETECTR Assay

Sample ID	Viruses Detected	SARS-CoV-2 DETECTR Result
RespV-001	Human coronavirus OC43	Negative
RespV-002	Human rhinovirus/enterovirus	Negative
RespV-003	Influenza B virus	Negative
RespV-004	Human coronavirus NL63	Negative
RespV-005	Respiratory syncytial virus A	Negative
	Respiratory syncytial virus B	
RespV-006	Respiratory syncytial virus B	Negative
RespV-007	Human rhinovirus/enterovirus	Negative
RespV-008	Human metapneumovirus	Negative
RespV-009	Human coronavirus HKU1	Negative
RespV-010	Human coronavirus HKU1	Negative
	Human adenovirus	
	Human bocavirus	
RespV-011	Human metapneumovirus	Negative
RespV-012	Respiratory syncytial virus A	Negative
RespV-013	Human metapneumovirus	Negative
RespV-014	Human bocavirus	Negative
RespV-015	Human rhinovirus/enterovirus	Negative
RespV-016	Human metapneumovirus	Negative
RespV-017	Human bocavirus	Negative

Three additional potentially cross-reactive organisms were spiked into negative clinical matrix and tested with the SARS-CoV-2 RNA DETECTR Assay. All specimens were negative **(Table 7)**.

Table 7: Contrived Specimens Spiked with Potentially Cross-Reactive Species

Sample ID	Organism Tested	Final Concentration (CFU/mL)	SARS-CoV-2 DETECTR Result
RespV-019	*Staphylococcus epidermidis*	1.5 x 10^7	Negative
RespV-020	*Pseudomonas aeruginosa*	1.5 x 10^7	Negative
RespV-021	*Candida albicans*	1.5 x 10^7	Negative

III. Clinical evaluation

A clinical study was performed to evaluate the performance of the SARS-CoV-2 RNA DETECTR Assay. A total of 102 individual nasopharyngeal swabs (NPS) specimens were tested with the SARS-CoV-2 RNA DETECTR Assay. Positive and negative agreement were based on the comparator result. RNA was extracted using Qiagen EZ1 DSP virus kit on the Qiagen EZ1 Advanced benchtop automated extraction instrument. Of the 40 positive NPS patient samples, 38 (95.0%) were detected by the DETECTR assay and 62/62 (100%) negative NPS samples were confirmed negative. Results are summarized in Table 8.

Table 8. Evaluation with Clinical NPS Specimens

		Comparator assay	
		Positive	**Negative**
DETECTR™ Assay	**Positive**	38	0
	Negative	2	62
	Total	40	62
Positive Agreement		38/40 = 95.0% (95% CI: 83.5% – 98.6%)	
Negative Agreement		62/62 = 100% (95% CI: 94.2% - 100.0%)	

Warnings:

- 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 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.