Department of Pathology and Laboratory Medicine Hospital of the University of Pennsylvania SARS-CoV-2 Assay EUA Summary
Updated: January 20, 2021

**ACCELERATED EMERGENCY USE AUTHORIZATION (EUA) SUMMARY SARS-CoV-2 ASSAY (DEPARTMENT OF PATHOLOGY AND LABORATORY MEDICINE HOSPITAL OF THE UNIVERSITY OF PENNSYLVANIA)**

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

The SARS-CoV-2 Assay will be performed at Department of Pathology and Laboratory Medicine Hospital of the University of Pennsylvania in Philadelphia, PA certified under the Clinical Laboratory Improvement Amendments of 1988(CLIA), 42 U.S.C. §263a, as per the Laboratory Instructions for Use reviewed by the Office of the Assistant Secretary for Health at the Department of Health and Human Services (DHHS) under this EUA.

## Intended Use:

The SARS-CoV-2 assay is a real-time RT-PCR test intended for the qualitative detection of nucleic acid from the SARS-CoV-2 in upper respiratory specimens and tracheal aspirates from individuals suspected of COVID- 19 by their healthcare provider. Testing is limited to laboratories of the Hospital of the University of Pennsylvania that are Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a certified high-complexity laboratories.

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 active infection with SARS-CoV-2 but 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 test 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 CLIA certified high-complexity laboratories with experience in developing molecular diagnostics and is only for use under the U.S. Department of Health and Human Services-issued Emergency Use Authorization (HHS-EUA).

## DEVICE DESCRIPTION AND TEST PRINCIPLE

The assay is a real-time reverse transcription polymerase chain reaction (rRT -PCR) test that is performed using the BD MAX open platform system. The SARS-CoV-2 primer and probe set is designed to detect RNA (ORF1a) from the SARS-CoV-2 in nasopharyngeal swab specimens from patients as recommended for testing by public health authority guidelines.

The workflow the assay is as follows. VTM from a patient nasopharyngeal swab tube and internal processing control (MS2 Phage; Zeptometrix) is added to a BD MAX Erk TNA-2 sample buffer tube. The BD MAX reaction strip, including reverse transcriptase, RT-PCR master mix tube (Luna Universal One-Step RT-qPCR Kit, New England Biolabs), SARS-CoV-2 ORF1a primer/probe and M2 primer/probe tube

and beta-actin primer/probe tube are assembled. The reaction strip is loaded onto BD MAX for inline extraction and real-time RT-PCR

**Control Material(s) to be Used:**

The master mix containing the SARS-CoV-2 primers and probe will also include the following primer and probe sets to be used as internal controls for each sample:

- **MS2** – RNA bacteriophage used as an internal processing control. Extract and reverse transcription RT-PCR control. The reaction should amplify at a pre- specified Ct range (e.g. 20-25) to establish the presence of PCR inhibitors in the specimen.
- **Beta-actin** – human DNA target to establish specimen quality. We have previously observed a small fraction of nasopharyngeal swabs yield invalid results due to improper collection technique. Beta-actin confirms that sufficient specimen has been collected by the swab to deem a specimen as a true negative for SARSCoV-2 nucleic acid. Beta-actin must be detected to provide a valid SARS-CoV-2 result.

Additional negative and positive control reactions will be run in parallel with patient specimens:

- **Positive control** – Contrived from confirmed negative patient samples spiked with synthetic linear DNA (G-Block; IDT) designed for the ORF1a region that our SARS-CoV-2 primers target. Positive control is run daily. Positive control must amplify all assay targets (SARS-CoV-2, MS2, Beta-actin) to pass.
- **Negative control** – Viral transport medium added directly to a BD MAX sample buffer tube containing MS2. Negative control must amplify MS2 in the specified Ct range, but not SARS-CoV-2 and Beta-actin. A negative control is run with every batch of specimens to verify patient samples are true positives and not the result of environmental contamination.

**Assay results and interpretation**

COVID-19 BD MAX Assay Result Interpretations

| | **RT-PCR Amplification Targets** | | |
|---|---|---|---|
| Result Interpretation | **SARS-CoV-2** | **Beta-actin** | **MS2** |
| Positive | + | +/- | + |
| Negative | - | + | + |
| Inconclusive | - | + | - |
| Inconclusive | - | - | + |

To be considered positive for the target in Table 1, the amplified targets must fall in the following Ct ranges:

SARS-CoV-2: 10-40
Beta-actin: 24-37
MS2: 20-25

## PERFORMANCE EVALUATION

### ***Analytical Sensitivity:***

Analytical sensitivity of the assay for detection of SARS-CoV-2 was demonstrated by spiking quantified SARS-CoV-2 virus from a patient sample into COVID-19 RT-PCR negative nasopharyngeal specimens collected in viral transport medium (BD Universal Viral Transport Kit). For quantification, the patient sample was extracted (MagNaPure, Biomerieux) and Ct values determined using the CDC COVID-19 RT-PCR assay modified for the Quantstudio platform (Thermo Scientific Fisher). A dilution series of the patient sample $10^1$-$10^3$ was analyzed against a standard curve generated using plasmid containing the SARS-CoV-2 sequence purchased from IDT. Results from these quantification experiments demonstrated the undiluted specimen to be 736,506,250 copies/ml (Ct 20.7).

Using the quantified patient specimen, a 10-fold series dilution was performed to $1/10^6$ for three extraction replicates on the BD Max.

Table 1: Summary of limit of detection determination through dilution series of three extraction replicates.

| Dilution | #positive/total tested | % Positive | Copies/mL |
|---|---|---|---|
| 1/10 | 3/3 | 100% | 73,650,625 |
| 1/100 | 3/3 | 100% | 7,365,062 |
| 1/1000 | 3/3 | 100% | 736,506 |
| 1/10,000 | 3/3 | 100% | 73,650 |
| 1/100,000 | 3/3 | 100% | 7,365 |
| $1/10^6$ | 0/3 | 0% | 736 |

Findings from Table 1 indicate that the limit of detection is between 7,365 copies/mL and 736 copies/mL. The studies confirming the LoD with 20 replicates (where virus can be detected in 95% of samples) was performed at 2x the LoD observed (20,000 copies/mL).

Summary of limit of detection confirmatory studies for 20 replicates.

| Total Tested | # Positive | % Positive | Copies/mL |
|---|---|---|---|
| 20 | 20 | 100% | 20,000 |

### ***Inclusivity (analytical sensitivity):***

The NextStrain database of 3953 SARS-Cov-2 strains was accessed on 01/14/2021 and showed no polymorphisms in the amplification region for the BD MAX COVID-19 assay.

### ***Cross-reactivity (Analytical Specificity)***

A combination of *in silico* analysis and wet lab confirmation was performed to determine analytical specificity. *In silico* analysis for cross-reactivity of the primers/probes did not demonstrate >80% homology with other common human respiratory viruses with the exception of SARS-CoV-1.

Analytical specificity was also carried with laboratory-based cross-reactivity studies. Respiratory pathogens were tested for specificity. Viral pathogens were tested from previously characterized patient samples. Bacterial pathogens were spiked into COVID-19 negative patient samples at a concentration of 1.0x10^6 CFU/mL. No cross reactivity was detected.

***Clinical Evaluation:***

Accuracy of the COVID-19 PCR BD MAX assay was determined by comparison to the GenMark SARS-CoV-2 ePlex assay (Emergency Use Authorization FDA cleared). A total of 43 patient NP swab specimens collected in viral transport media were stored at -80°C for retrospective analysis by the COVID-19 BD Max Assay. These specimens were previously characterized by the SARS-CoV-2 GenMark ePlex assay (positive n=13; negative n=30). Twenty of the negative clinical specimens were also used to create 17 contrived reactive specimens at a concentration 1-2x the LoD.

Table 6. Summary of accuracy results performed in triplicate over a 7-day period.

| Previous PCR Result | # of Samples | Positive by BD MAX | Negative by BD MAX |
|---|---|---|---|
| SARS-CoV-2 Positive | 30 | 30 | 0 |
| SARS-CoV-2 Negative | 30 | 0 | 30 |

The BD MAX COVID-19 assay demonstrated 100% accuracy among runs compared to the GenMark ePlex SARS-CoV-2 assay. All SARS-CoV-2 results were in concordance with the expected results and no discrepancies were observed. These findings suggest that the BD MAX COVID-19 assay has equivalent performance to the Emergency Use Authorized FDA-cleared ePlex assay. The State of Pennsylvania Bureau of Laboratories confirmed an additional 5 positives and 5 negatives as suggested by the FDA EUA guidance.

**Limitation**

The performance of the BD MAX COVID-19 at the University of Pennsylvania was established using nasopharyngeal swab specimens and sputum specimens. Nasal swabs, mid-turbinate swabs, oropharyngeal swabs, nasopharyngeal wash/aspirates, nasal aspirates, bronchoalveolar lavage, and lower respiratory tract aspirates are also considered acceptable specimen types for use with the BD MAX COVID-19 at the University of Pennsylvania. Testing of nasal and mid-turbinate nasal swabs (self-collected at a healthcare site or collected by a healthcare provider) is limited to patients with symptoms of COVID-19.

**WARNINGS:**

- This test has not been FDA cleared or approved;
- This test has been authorized by the Office of the Assistant Secretary for Health, U.S. Department of Health and Human Services, 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.