

**ACCELERATED EMERGENCY USE AUTHORIZATION (EUA) SUMMARY  
ASPIRUS SARS-COV-2 rRT-PCR ASSAY  
(ASPIRUS LABORATORY)**

For *In vitro* Diagnostic Use  
Rx Only

For use under Emergency Use Authorization (EUA) only

**(The Aspirus SARS-CoV-2 rRT-PCR Assay will be performed at Aspirus Laboratory, certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, as per the Instructions of Use that were reviewed by the FDA under this EUA.)**

**INTENDED USE**

The Aspirus SARS-CoV-2 rRT-PCR Assay is a real-time RT-PCR assay intended for the qualitative detection of nucleic acid from SARS-CoV-2 RNA in upper respiratory specimens (such as nasal, mid-turbinate, nasopharyngeal, and oropharyngeal swab specimens) and bronchoalveolar lavage specimens from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to the Aspirus Reference Laboratory, which 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 upper 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 infective 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 Aspirus SARS-CoV-2 rRT-PCR 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 SARS-CoV-2 Assay is only for use under the Food and Drug Administration's Emergency Use Authorization.

## **DEVICE DESCRIPTION AND TEST PRINCIPLE**

The Aspirus SARS-CoV-2 rRT-PCR Assay is a lab developed test by Aspirus Reference Laboratory for implementation on the BD MAX for the qualitative real-time detection by reverse transcriptase polymerase chain reaction of SARS-CoV-2 RNA from upper respiratory specimens (such as nasal swabs, nasopharyngeal swabs, oropharyngeal swabs) and bronchoalveolar lavage specimens.

The oligonucleotide primers and probe for detection of SARS-CoV-2 was selected from regions of the virus nucleocapsid (N) gene as specified by the CDC. The panel is designed for specific detection of the SARS-CoV-2.

The test incorporates automated total nucleic acid (TNA) extraction and concentration from viral transport media using the BD MAX TNA-3 kit. 500µL of the specimen is added to the BD MAX Sample Buffer Tube and processed using the BD MAX System. The BD MAX System contains a combination of lytic and extraction reagents designed to perform cell lysis, nucleic acid extraction, and removal of inhibitors. Following cell lysis, the released nucleic acids are captured by magnetic affinity beads. The beads, with the bound nucleic acids, are washed and the TNA is eluted using 12.5pL of Elution Buffer.

Isolated nucleic acids are added to the BD MAX TNA MMK (a complete PCR reagent mix for amplification of total nucleic acid (TNA)) master mix which contains the dNTPs, Hot Start DNA polymerase with a reverse transcriptase function, and buffers, as well as forward and reverse primers and a fluorophore labeled TaqMan Probe (Excitation: 500nm: Emission 715nm) which are used to amplify a proprietary armored RNA QUANT™ (Asuragen, Inc.) used as a specimen processing control (SPC).

RNA isolated and purified from upper respiratory specimens is reverse transcribed to cDNA and subsequently amplified in the 80D Max Real-Time PCR Instrument. 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 Tag 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 the BD MAX.

## **INSTRUMENTS USED WITH TEST**

The Aspirus SARS-CoV-2 rRT-PCR Assay is to be used with the following PCR and nucleic acid extraction instrument:

- BD MAX, software version 4.72 A

## REAGENTS AND MATERIALS

The Aspirus SARS-CoV-2 rRT-PCR Assay has been validated using only the components referenced in this submission.

**Table 1: Aspirus SARS-CoV-2 rRT-PCR Assay Reagents**

Reagent	Manufacturer	Catalog #
2019-nCoV CDC EUA, Kit, 500rxn	IDT	10006606
BD MAX ExK TNA-3 Kit	BD	4428247
BD MAX TNA MMK(SPC) Kit	BD	442830
2019-CoV_N Positive Control	IDT	10006625
Molecular Grade Water		

## CONTROLS TO BE USED WITH THE ASPIRUS SARS-COV-2 rRT-PCR ASSAY

Controls that provided with the test kit are as follows:

**Sample Processing Control (SPC):** The SPC is present in the BD MAX TNA-3 extraction reagent and verifies the adequacy of the assay throughout the entire process to include processing of the sample, extraction and purification of the nucleic acids, and efficacy of the PCR reaction. The SPC should be positive in a negative sample and can be negative or positive in a positive sample. The SPC passes if it meets the validated acceptance criteria, i.e.  $C_T$  value of 10-36 in the SPC channel 680/715.

**Negative Control:** A no template control (NTC) is used as the negative control in which nothing is added to a sample buffer tube in the assay and is processed in the same manner as patient samples throughout the entire process to include processing of the sample, extraction and purification of the nucleic acids, and efficacy of the PCR reaction. The NTC should give a  $C_T$  value of -1 (indicating no amplification crossing threshold detected) in channels 475/520 and a  $C_T$  value of 10-36 in the SPC channel 680/715. If this is not observed, QC is invalid and should be repeated. Patient results will not be released until QC issues are resolved.

**SARS-CoV-2 Positive/Sensitivity Control:** The plasmid positive control contains the complete nucleocapsid gene from 2019-nCoV virus. Forty  $\mu$ L of the control is added to a sample buffer tube. The SARS-CoV-2 control should give a  $C_T$  value of 10-40 in channel 475/520. The SPC channel, 680/715, can have a  $C_T$  value of -1 or 10-40. If this is not observed, QC is invalid and should be repeated. Patient results will not be released until QC issues are resolved.

External positive and negative surrogate sample controls must be run according to the following schedule:

- With every new preparation of primer and probe master mix and at least daily.
- With every new lot of BD MAX MMK (SPC) kits
- After replacement of critical instrument parts
- After major software updates

## 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.

**Table 2: Interpretation of Results for Quality Controls**

Control Type	Used to Monitor	SARS-CoV-N1 Target (475/520 channel)	RNase P (680/715 channel)
			The SPC is incorporated in every sample and used to monitor failure in lysis, extraction, and amplification.  CT value of 10-36
<b>SARS-CoV-2 Positive Control</b>	Substantial reagent failure including primer and probe integrity	$C_T$ between 10 and 40	+/-
<b>SARS-CoV-2 Negative Control</b>	Reagent and/or environmental contamination	$C_T < 0$	$C_T$ between 10 and 36

If the 2019-nCoV N1 is positive even in the absence of a positive RNase P, 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 RNase P signal does not preclude the presence of 2019-nCoV virus RNA in a clinical specimen.

**Table 3: Interpretation of test results for the Aspirus SARS-CoV-2 rRT-PCR Assay**

Interpretation	SARS-CoV-N1 Target (475/520 channel)	RNase P (680/715 channel)	Resolution
SARS-CoV-2 RNA Detected	$C_T$ between 1 and 40	+/-	Result as Detected
SARS-CoV-2 RNA Not Detected	$C_T < 0$	$C_T$ between 10 and 36	Result as Not Detected
INVALID (SARS-CoV-2 RNA indeterminate)	$C_T < 0$	$C_T < 0$ or $> 36$	Repeat up to 2x, if not resolved, send to reference laboratory
	$C_T > 40$	+/-	

If the 2019-nCoV N1 is positive even in the absence of a positive RNase P, 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 RNase P signal does not preclude the presence of 2019-nCoV virus RNA in a clinical specimen.

## LIMITATIONS

The use of this assay as an *in vitro* diagnostic under the FDA Emergency Use Authorization (EUA) is limited to laboratories that are certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. § 263a, to perform high complexity tests.

Use of this assay is limited to personnel who are trained in the procedure. Failure to follow these instructions may result in erroneous results.

The performance of Aspirus SARS-CoV-2 rRT-PCR Assay was established using nasopharyngeal swabs. 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. Please refer to FDA's [FAQs on Diagnostic Testing for SARS-CoV-2](#) for additional information.

Samples must be collected, transported, and stored using appropriate procedures and conditions. Improper collection, transport, or storage of specimens may hinder the ability of the assay to detect the target sequences.

Extraction and amplification of nucleic acid from clinical samples must be performed according the specified methods listed in this procedure. Other extraction approaches and processing systems have not been evaluated.

False-negative results may arise from:

- Improper sample collection
- Degradation of the viral RNA during shipping/storage
- Using unauthorized extraction or assay reagents

- The presence of RT-PCR inhibitors
- Mutation in the SARS-CoV-2 virus
- Failure to follow instructions for use

False-positive results may arise from:

- Cross contamination during specimen handling or preparation
- Cross contamination between patient samples
- Specimen mix-up
- RNA contamination during product handling

The effect of vaccines, antiviral therapeutics, antibiotics, chemotherapeutic or immunosuppressant drugs have not yet been evaluated.

Please note, Negative results do not preclude infection of SARS-CoV-2 virus and should not be the sole basis of a patient management decision. A positive result indicates detection of nucleic acid from the relevant virus. Nucleic acid may persist even after the virus is no longer viable.

Laboratories are required to report all positive results to the appropriate public health authorities.

## PERFORMANCE EVALUATION

### 1) Analytical Sensitivity:

The Limit of Detection (LOD) was determined for the Aspirus SARS-CoV-2 rRT-PCR Assay. The Limit of Detection is the lowest detectable concentration of SARS-CoV-2 at which greater or equal to 95% of all replicates test positive. Nasopharyngeal swabs were collected from individuals negative for SARS-CoV-2. SARS-CoV-2 RNA positive control (SeraCare's AccuPlex SARS-CoV-2 Reference Material, #0505-0126) was diluted into the sample matrix in dilutions/concentration of 50 copies/ $\mu$ L down to 0.1 copies/ $\mu$ L and tested in triplicate. The preliminary LoD was determined to be 0.5 copies/ $\mu$ L.

To confirm the Limit of Detection, 20 replicates at the lowest level detected were tested at 0.5 copies/ $\mu$ L. The replicates were prepared the same way as described above for the preliminary LoD by spiking the SARS-CoV-2 RNA positive control in nasopharyngeal swabs collected from individuals negative for SARS-CoV-2.

**Table 4: LoD Confirmation Study Summary**

Target	SARS-CoV-2 target in NP
RNA Concentration	0.5 copies/ $\mu$ L
Positives/Total	20/20
Mean Ct	32.8
SD (Ct)	0.7
CV	0.47

The LoD was confirmed to be 0.5 copies/ $\mu$ L based on a positivity rate of  $\geq 95\%$  for 19/20 replicates.

2) **Analytical Inclusivity:**

An alignment was performed with the oligonucleotide primer and probe sequence of the Aspirus SARS-CoV-2 rRT-PCR Assay with all publicly available nucleic acid sequences for SARS-CoV-2 in GenBank as of March 27, 2020 to demonstrate the predicted inclusivity of the assay. All the alignments show 100% identity of the assay to the top 100 available SARS-CoV-2 sequences.

Subsequent *in silico* analysis of primers and probe used in the assay was performed on April 23, 2020 which showed 100% alignment with all published SARS-CoV-2 sequences.

3) **Cross-Reactivity:**

The Aspirus SARS-CoV-2 rRT-PCR Assay utilizes identical oligonucleotide sequences for the N1 and N2 SARS-CoV-2 target genes as those used in the CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel.

As reported under the CDC EUA, *in silico* analysis of the N1 probe sequence showed high sequence homology of the N1 probe with SARS coronavirus and Bat SARS-like coronavirus genome. However, forward and reverse primers showed no sequence homology with SARS coronavirus and Bat SARS-like coronavirus genome. Combining primers and probe, there is no significant homologies with human genome, other coronaviruses or human microflora that would predict potential false positive rRT-PCR results.

#### 4) Clinical Evaluation

##### *Clinical Evaluation of Aspirus SARS-CoV-2 rRT-PCR Assay Using Contrived Samples*

The performance evaluation of Aspirus SARS-CoV-2 rRT-PCR Assay was evaluated using contrived samples. Ten positive samples were prepared by spiking SARS-CoV-2 positive control (SeraCare's AccuPlex SARS-CoV-2 Reference Material, #0505-0126) at 1-2x LoD into NP specimens confirmed negative for SARS-CoV-2. Thirty contrived negative samples were prepared with NP specimens confirmed negative for SARS-CoV-2. Samples were extracted and amplified on the BD Max System. The results from the SARS-CoV-2 assay are shown in Table 5 below.

**Table 5: Summary of Contrived Clinical Sample Evaluation**

Specimen Type	SARS-CoV-2	Performance Agreement	95% CI
	N +		
Positive Contrived	10/10	100%	72.3-100%
Negative Contrived	0/30	100%	88.7-100%

##### *Clinical Evaluation of the Aspirus SARS-CoV-2 rRT-PCR Assay*

A clinical study was performed to evaluate the performance of the Aspirus SARS-CoV-2 rRT-PCR Assay. Results obtained with a total of 60 clinical NP swab specimens (30 negatives and 30 positives) from Wisconsin State Laboratory of Hygiene (WSLH) tested with the BD BioGX SARS-CoV-2 EUA Assay (EUA200020) were compared to results obtained the Aspirus SARS-CoV-2 rRT-PCR Assay. Samples were extracted and amplified on the BD Max System. The results are summarized in Table 6 below.

**Table 6: Clinical Evaluation of the Aspirus SARS-CoV-2 rRT-PCR Assay**

Aspirus SARS-CoV-2 rRT-PCR Assay	BD BioGX SARS-CoV-2		Total	% Performance Agreement	95% CI
	Detected	Not Detected			
Detected	30	0	30	PPA 100%	88.7-100%
Not Detected	0	30	30	NPA 100%	88.7-100%
Total	30/30	30/30	60		

#### **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.