ACCELERATED EMERGENCY USE AUTHORIZATION (EUA) SUMMARY CHILDRENS-ALTONA-SARS-COV-2 ASSAY (BOSTON CHILDREN'S HOSPITAL)

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

(The Childrens-Altona-SARS-CoV-2 Assay will be performed at Boston Children's Hospital, Infectious Diseases Diagnostics 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 Childrens-Altona-SARS-CoV-2 Assay is a real-time reverse transcription polymerase chain reaction (RT-PCR) test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in nasopharyngeal (NP), oropharyngeal (OP), NP/OP, anterior nasal, and mid-turbinate nasal swab specimens from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to the Infectious Diseases Diagnostics Laboratory (IDDL) at Boston Children's Hospital located in Boston, MA, 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 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 treatment or other patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

Testing with the Childrens-Altona-SARS-CoV-2 Assay is intended for use by qualified and trained laboratory personnel specifically instructed and trained in the techniques of real-time PCR assays. The Childrens-Altona-SARS-CoV-2 Assay is only 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. Please refer to FDA's FAQs on Diagnostic Testing for SARS-CoV-2 for additional information.

DEVICE DESCRIPTION AND TEST PRINCIPLE

The Childrens-Altona-SARS-CoV-2 Assay is a real-time reverse transcription polymerase chain reaction (RT-PCR) test. The SARS-CoV-2 primer and probe set is designed to detect RNA from SARS-CoV-2 in nasopharyngeal (NP), oropharyngeal (OP), NP/OP, anterior nasal, and mid-turbinate nasal swabs from patients as recommended for testing by their healthcare providers.

The oligonucleotide primers and probes in the Childrens-Altona-SARS-CoV-2 Assay are designed to detect one target sequence of the 2019-nCoV virus spike glycoprotein (S) gene. The test also includes a primer and probe sequence for the universal detection of SARS-like coronaviruses (B-βcoronavirus; B-βCoV) (E gene). Oligonucleotide primers and probe to detect the internal control (IC) gene (artificial RNA and DNA) in control samples and clinical specimens is also included.

RNA is isolated from upper respiratory specimens including nasopharyngeal (NP), oropharyngeal (OP), NP/OP, anterior nasal, and mid-turbinate nasal swabs using the Qiagen Virus MinElute Spin kit on the QiaCube extraction platform and is reverse transcribed to cDNA and subsequently amplified using the Qiagen Rotor-Gene Q 5Plex RT-PCR Platform with software version 2.3.1 During the amplification 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 Taq polymerase degrades the bound probe, causing the reporter dye (FAM, Cy5, JOE) to separate from the quencher dye (BHQ-1, BHQ-2), generating a fluorescent signal. Fluorescence intensity is monitored at each PCR cycle.

INSTRUMENTS USED WITH TEST

The Childrens-Altona-SARS-CoV-2 Assay is to be used with the Qiagen Virus MinElute Spin kit performed on the QiaCube extraction platform, Qiagility with software version 4.17.1, and Rotor-Gene Q 5Plex instrument with software version 2.3.1.

REAGENTS AND MATERIALS

Reagent Manufacturer	Description	Catalog #	Manufacturer
Qiagen Virus MinElute Spin Kit	Kit for purification of viral RNA/DNA using spin-column method	57704	Qiagen
QiaCube	Nucleic acid purification device	9001292	Qiagen
Qiagility	Automated PCR setup device	9001532	Qiagen
Rotor-Gene Q 5Plex RT-PCR System	Real-time PCR cycler	9001570	Qiagen
RNase OUT	Ribonuclease inhibitor	10-777-019	Invitrogen
RealStar SARS-CoV-2 RT-PCR Kit 1.0 • Master A (240 µL) • Master B (720 µL) • Positive control (250 µL) • Internal Control (1000 µL) • Water (PCR grade) (500 µL)	Kit for the qualitative detection and differentiation of lineage B-βCoV and SARS-CoV-2 specific RNA in respiratory samples by real-time PCR	821003	Altona Diagnostics

Universal Transport Medium	Specimen collection and transport medium	403C	Quidel

CONTROLS TO BE USED WITH THE COVID-19 RT-PCR

1) **No Template Control (NTC)**
A "no template" (negative) control (NTC) is needed to check for contamination of extraction and assay reagents. Universal Transport Medium (UTM) or Viral Transport Medium (VTM) is used in place of sample nucleic acid for this control. The NTC is run with master mix containing no internal control and is used on every assay plate.

2) **Positive Assay Control/Positive Extraction Control**
A patient sample that previously tested positive for SARS-CoV-2 RNA (at either the Massachusetts State Laboratory or Boston Children's Hospital) serves as a positive extraction/assay control. The confirmed clinical positive sample is included in each extraction batch and on every assay plate.

3) **Negative Assay Control/Extraction Control**
The negative extraction control consists of universal transport medium and monitors for any cross-contamination that could occur during the extraction or PCR process.

4) **Internal Control**
The internal control contains a defined copy number of an "artificial" RNA) as well as of an "artificial" DNA molecule with no homologies to each other or to any other known sequences. It is included in the master mix. The RNA component of the internal control is reverse transcribed, amplified and detected in parallel to the lineage B-βCoV and SARS-CoV-2 specific RNA. Detection of the internal control in patient test samples verifies proper assay setup.

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 (Refer to Table 1 for a summary of control results).

1) <u>COVID-19 RT-PCR test Controls – Positive, Negative, and Internal</u>:

- **Negative (No Template [NTC]) Control**
All NTC reactions should be negative for the S, E, and internal control targets, meaning no amplification curves cross the PCR cycle threshold (Ct) (Ct < 45). If the S, E, or internal control NTC reactions exhibit positive fluorescence above the threshold, it is possible that contamination occurred, or that the assay was setup improperly. The RT-PCR run is invalid. Repeat from the RT-

PCR step using residual extraction material. If the repeat test result is positive for these targets, re-extract and re-test all samples.

- **Positive Assay/Extraction Control**
  Positive control reactions for the S and E assays should yield positive results with a Ct value between 30 and 34 and positive (Ct < 34)/negative (Ct Not Detected) results for the internal control. Negative results with either the S or E primer/probe sets invalidates the run and suggests the assay may have been set up incorrectly, or the integrity of the primers/probes is compromised. The RT-PCR run is invalid. Repeat from the RT-PCR step using residual extraction material. If the repeat test result is negative for S/E gene targets, re-extract and re-test all samples.

- **Negative Assay/Extraction Control**
  The negative extraction/amplification control should be negative for SARS-CoV-2 (S gene) and B-βCoV (E gene) (Ct Not Detected) and positive for the internal control (Ct < 34). If these conditions are not met, the run is invalidated and is repeated using residual patient material.

- **Internal Control**
  If the internal control is positive (Ct < 34), this indicates that the specimen did not contain any interfering substances and the result can be considered valid provided all other controls work as expected. It is possible for the internal control to be negative due to competition in the presence of a high viral load. However, the internal control should always be positive for a specimen that is negative for the target analyte(s) in order to be considered a valid run.

Table 1: Expected Results of Controls Used in the Childrens-Altona-SARS-CoV-2 Assay

Control Type/ Name	Used to Monitor	Expected Results and Ct Values					
		SARS-CoV-2 E gene		B-βCoV S gene		Internal Control	
		Call	Ct	Call	Ct	Call	Ct
No Template Control (NTC)	Contamination during PCR process	Negative	ND*	Negative	ND	Negative	ND
Positive Assay/Extraction Control	Successful extraction; assay inhibition	Positive	30-34	Positive	30-34	Positive/ Negative	< 34 for positive/ND for negative
Negative Assay/Extraction control	Contamination during extraction or PCR process; assay inhibition	Negative	ND	Negative	ND	Positive	< 34

*ND = Not Detected

2) *Examination and Interpretation of Patient Specimen Results:*

Assessment of clinical specimen test results should be performed after the positive and negative controls have been examined and determined to be valid and acceptable.

If the controls are not valid, the patient results cannot be interpreted. Please see the table below (Table 2) for guidance on interpretation and reporting of results.

- If both the SARS-CoV-2 S and B-βCoV E assays are positive (Ct value < 45), and the internal control is positive (Ct value < 34) or negative (Ct Not Detected), the patient sample is reported as positive. It is possible that some samples may have a negative internal control due to competition in the presence of a high titer positive. A negative internal control does not preclude the presence of SARS-CoV-2 viral RNA in a clinical specimen.
- If the SARS-CoV-2 S target is positive (Ct value < 45), the B-βCoV E assay is negative (Ct Not Detected), and the internal control is positive (Ct < 34)/negative (Ct Not Detected), the patient sample is reported as positive.
- If the SARS-CoV-2 S target is negative (Ct Not Detected), the B-βCoV E assay is positive (Ct < 45), and the internal control is positive (Ct < 34)/negative (Ct Not Detected), the result is considered inconclusive. Repeat the RT-PCR using residual extract and email Medical Director and include initial Ct value. If the repeat result is the same, discuss results with Medical Director before reporting. If the B-βCoV target only is positive, additional confirmatory testing may be conducted to differentiate between SARS-CoV-2 and other SARS-like viruses, for epidemiological purposes or clinical management.
- If the SARS-CoV-2 S and B-βCoV E assay is negative (Ct Not Detected), and the internal control result is positive (Ct value < 34), the patient sample is reported as negative.
- If both the S and E assay targets and internal control are negative (Ct Not Detected), the result is inconclusive. The RT-PCR is repeated using residual extract. If the repeat result remains inconclusive, it is recommended to re-collect a new patient sample.

Table 2: Interpretation of Patient Results Using the Childrens-Altona-SARS-CoV-2 Assay

SARS-CoV-2 S target	B-βCoV E target	Internal Control	Interpretation	Report Result	Actions
+	+	+/-	SARS-CoV-2 **Detected**	**POSITIVE**	Report to sender and appropriate public health authorities.
+	-	+/-	SARS-CoV-2 **Detected**	**POSITIVE**	Report to sender and appropriate public health authorities.
-	+	+/-	**Inconclusive**	**INCONCLUSIVE**	Sample is repeated once. Additional confirmatory testing may be conducted.
-	-	+	SARS-CoV-2 **Not Detected**	**NEGATIVE**	Report to sender.
-	-	-	**Inconclusive**	**INCONCLUSIVE**	Sample is repeated once. If a second failure occurs, it is reported to sender as inconclusive and recollection is recommended if clinically indicated.

PERFORMANCE EVALUATION

1) ***Analytical Sensitivity:***

Limit of Detection (LoD):

The LoD of the Childrens-Altona-SARS-CoV-2 Assay was determined using quantified whole viral SARS-related coronavirus 2 (USA-WA1/2020) RNA obtained from BEI Resources (NR-52285) with a starting concentration of 5.5 x10^7 genome equivalents/mL. Genomic RNA was spiked into pooled clinical nasopharyngeal matrix that had tested negative on an in-house Influenza A/B/RSV testing prior to December 2019 and was anticipated to all be negative for SARS-CoV-2, based on dates of specimen collection. The pooled sample was prepared with RNase inhibitor (RNase OUT, 25 µL per 5.0 mL pooled sample).

A preliminary LoD was determined by testing 3-fold dilutions (550 copies/µL-0.08 copies/µL) of RNA spiked into pooled material. For each concentration, three extraction replicates were prepared. Spiked samples were tested with the Childrens-Altona-SARS-CoV-2 Assay following extraction with the Qiagen Virus MinElute Spin Kit performed on the QiaCube instrument. The initial LoD was 0.77 copies/µL

The LoD was verified by testing 20 additional extraction replicates consisting of pooled negative clinical nasopharyngeal swab matrix However, E and S gene target amplification in all 20 replicates at 0.77 copies/µL failed, indicating that this concentration was not reliably amplifiable. Therefore, dilutions were repeated to create 20 extraction replicates at 2.3 copies/µL and 20 replicates at 6.8 copies/µL. Samples were spiked with RNA prior to extraction with the Qiagen Virus MinElute kit. 20/20 replicates tested positive at concentrations of 2.3 and 6.8 copies/µL. The results of the study are summarized below in Table 3.

Table 3: LoD Verification Study Results

Concentration (copies/µL)	Average Ct Values			Detection Rate		
	E-gene	S-gene	Internal Control	E-gene	S-gene	Internal Control
6.8	30.5	31.2	27.5	20/20	20/20	20/20
2.3	32.4	34.2	27.1	20/20	20/20	20/20

The LoD of the Childrens-Altona-SARS-CoV-2 Assay was established at 2.3 copies/µL.

2) ***Analytical Inclusivity:***

In Silico Analysis of Primer and Probe Inclusivity:

An in silico inclusivity analysis for E and S gene targets using whole genome sequences published on GISAID and NCBI as of March 27, 2020 was performed. Of the 1916 whole genome sequences of SARS-CoV-2, 1809 were published via GISAID and 107 were published via NCBI. The primers and probes included in the RealStar SARS-CoV-2 RT-PCR Kit showed 100% homology with >99% of E and S gene sequences evaluated. The results of the inclusivity analysis were therefore considered acceptable.

3) ***Analytical Specificity:***

In Silico Analysis of Primer and Probe Exclusivity:

An in silico cross-reactivity study was performed on all primer and probe sequences against the following respiratory organisms in Table 4 below.

Table 4: Organisms Assessed In Silico for Potential Cross-Reactivity

Viruses	Bacteria/Candida
Human coronavirus 229E	*Chlamydia pneumoniae*
Human coronavirus OC43	*Haemophilus influenzae*
Human coronavirus HKUl	*Legionella pneumophila*
Human coronavirus NL63	*Mycobacterium tuberculosis*
SARS-coronavirus	*Streptococcus pneumoniae*
MERS-coronavirus	*Streptococcus pyogenes*
Adenovirus (e.g. Cl Ad.71)	*Bordetella pertussis*
Human Metapneumovirus	*Mycoplasma pneumoniae*
Parainfluenza virus 1-4	*Pneumocystis jirovecii* (PJP)
Influenza A & B	Pooled human nasal wash -to represent diverse microbial flora in the human respiratory tract
Enterovirus (e.g. EV68)	*Candida albicans*
Respiratory syncytial virus	*Pseudomonas aeruginosa*
Rhinovirus	*Staphylococcus epidermidis*
	Staphylococcus salivarius

No significant homology was detected with the S and E gene assays that is likely to result in amplification and/or detection.

4) ***Clinical Evaluation:***

Performance of the Childrens-Altona-SARS-CoV-2 Assay was evaluated using individual clinical nasopharyngeal swab and nasopharyngeal/oropharyngeal swab clinical specimens previously tested by the Massachusetts State Public Health Laboratory using the CDC EUA SARS-CoV-2 assay. In total, 30 positive (20 NP, 10 NP/OP) and 30 negative (19 NP, 11 NP/OP) clinical samples were tested for clinical validation of the Childrens-Altona-SARS-CoV-2 Assay.

Prepared samples were randomized and blinded, and RNA was extracted using the Qiagen Virus MinElute Kit on the QiaCube extraction platform. Testing was performed in one RT-PCR run on the Rotor-Gene Q 5Plex with one positive control, one negative extraction control, and one NTC included per plate. Results of the study are summarized below in Table 5.

Table 5: Clinical Evaluation Summary Data

Sample	Number of samples	% Positive (95% CI	% Negative (95% CI)
Positive clinical samples	30	30/30;100% (88%-100%)	N/A

Negative clinical samples	30	NA	30/30; 100% (88%-100%)

Positive percent agreement (PPA%): 30/30 = 100% (95: CI: 88%-100%)
Negative percent agreement (NPA%): 30/30 = 100% (95% CI: 88%-100%)

The testing on these clinical specimens performed at Boston Children's Hospital and at the alternate testing laboratory fulfills the requirement for confirmatory testing for at least five positive and five negative specimens.

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 Qiacube Connect using the Qiagen Virus MinElute spin kit and Rotor-Gene Q 5Plex RT-PCR System. The results are summarized in Table 6.

Table 6: Summary of LoD Confirmation Result using the FDA SARS-CoV-2 Reference Panel

Reference Materials Provided by FDA	Specimen Type	Product LoD	Cross-Reactivity
SARS-CoV-2	Nasopharyngeal Swab	$1.8x10^4$ NDU/mL	N/A
MERS-CoV		N/A	ND

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