

**EMERGENCY USE AUTHORIZATION (EUA) SUMMARY
THE AKRON CHILDREN'S HOSPITAL
SARS-CoV-2 ASSAY**

For *in vitro* Diagnostic Use
Rx Only

For Use Under Emergency Use Authorization (EUA) Only

(The Akron Children's Hospital SARS-Cov-2 Assay will be performed at the Akron Children's Hospital Molecular Diagnostics Laboratory, One Perkins Square, Akron, OH 44308 that is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a and meets the requirements to perform high complexity tests as described in the Laboratory Standard Operating Procedures that were reviewed by the FDA under this EUA).

INTENDED USE

The Akron Children's Hospital SARS-CoV-2 Assay is a real-time reverse transcription (RT)-PCR test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in nasopharyngeal swabs, oropharyngeal (throat) swabs, anterior nasal swabs, mid-turbinate nasal swabs, nasal aspirates, nasal washes and bronchoalveolar lavage (BAL) fluid specimens from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to the Akron Children's Hospital Molecular Diagnostics Laboratory located at One Perkins Square, Akron, OH 44308, which is certified under 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 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 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 Akron Children's Hospital SARS-CoV-2 Assay is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR assays and *in vitro* diagnostic procedures. The Akron Children's Hospital SARS-CoV-2 Assay is only for use under the Food and Drug Administration's Emergency Use Authorization.

DEVICE DESCRIPTION AND TEST PRINCIPLE

The Akron Children's Hospital SARS-CoV-2 Assay is a real-time reverse transcription polymerase chain reaction (RT-PCR) test. The assay is performed using the QIAGEN Rotor-Gene Q MDx PCR instrument with reagents from the Altona Diagnostics RealStar SARS-CoV-

2 RT-PCR Kit 1.0. The components and formulation of this kit are the same as those of the FDA-authorized Altona Diagnostics RealStar SARS-CoV-2 RT-PCR Kit U.S. and the two kits may be used interchangeably in the Akron Children’s Hospital SARS-CoV-2 Assay. The assay is designed to detect a SARS-CoV-2-specific region of the spike protein (S) gene in addition to a region of the envelope (E) gene that is specific for lineage B-β-coronaviruses, and an exogenous internal control.

Nucleic acid extraction is performed using the Maxwell 16 MDx and Maxwell 16 Viral Total Nucleic Acid Purification Kit or Maxwell RSC 48 System and Maxwell RSC Viral Total Nucleic Acid Purification Kit (all from Promega Corporation).

The assay is for use with respiratory specimens from patients suspected of COVID-19 by their healthcare provider.

INSTRUMENTS USED WITH THE TEST

The Akron Children’s Hospital SARS-CoV-2 Assay is for use with the QIAGEN Rotor-Gene Q MDx Instrument and Rotor-Gene Software V2.1.0.9.

Nucleic acid extraction is performed using Promega Maxwell 16 MDx Instruments with firmware v1.4 or v1.6 or the Promega Maxwell RSC 48 System with software v3.0.1.

REAGENTS AND MATERIALS

Nucleic Acid Extraction		
Reagent	Catalogue Number	Storage Temperature
Maxwell 16 Viral Total Nucleic Acid Purification Kit	Promega AS1150	15 to 30 °C
Maxwell RSC Viral Total Nucleic Acid Purification Kit	Promega AS1330	15 to 30 °C
Equipment and Supplies		
Promega Maxwell 16 MDx (firmware v1.4 or v1.6)		
Promega Maxwell RSC 48 System (with software 3.0.1)		
Pipettes with aerosol resistant tips		
Class II Biosafety Cabinet		
2mL Sarstedt tubes (DNase/RNase-free) with screw cap		
Mini centrifuge		
Sorvall microcentrifuge		
Sand Heat Block (95 °C)		
Thermo Fisher heat blocks (56 °C)		
Water Bath (70 °C)		

PCR Amplification		
Reagent	Catalogue Number	Storage Temperature
Altona Diagnostics RealStar SARS-CoV-2 RT-PCR Kit 1.0	Altona Diagnostics 821005	-20 °C

altona Diagnostics RealStar SARS-CoV-2 RT-PCR Kit U.S.	altona Diagnostics 821025	-20 °C
Phosphate Buffered Saline (PBS, 1X), sterile-filtered	Alfa Aesar by Thermo Fisher Scientific J61196	Room temperature
UltraPure DNase/RNase-Free Distilled Water	Thermo Fisher Scientific 10977-015	--
Equipment and Supplies		
QIAGEN Rotor-Gene Q MDx System (Serial #0814318 and #0714304)		
Micropipettes: 20 µL and 10 µL with filter tips		
0.2 mL PCR tubes for Rotor-Gene Q MDx (QIAGEN 3001-001)		
Mini vortex		
Mini centrifuge		
Chilled tube holder		
PCR workstations		
Thermo Fisher heat blocks (56 °C)		
Class II Biosafety Cabinet		

Notes:

- a) altona Diagnostics attested that the chemical composition of the RealStar SARS-CoV-2 RT-PCR Kit 1.0 (Cat. #821005) and RealStar SARS-CoV-2 RT-PCR Kit U.S. (Cat. #821025) is identical. Therefore, the two kits may be used interchangeably with the Akron Children’s Hospital SAR-CoV-2 Assay.
- b) Promega Corporation attested that the Maxwell 16 Viral Total Nucleic Acid Purification Kit (Cat. #AS1150) and Maxwell RSC Viral Total Nucleic Acid Purification Kit (Cat. #AS1330) are chemically equivalent. The performance of the Akron Children’s Hospital SAR-CoV-2 Assay was validated for use with both the Maxwell 16 MDx using the Maxwell 16 Viral Total Nucleic Acid Purification Kit and the Maxwell RSC 48 System using the Maxwell RSC Viral Total Nucleic Acid Purification Kit (see below).

CONTROLS

The altona Diagnostics RealStar SARS-CoV-2 RT-PCR Kits include the following control materials that are used with the Akron Children’s Hospital SARS-CoV-2 Assay:

- a) *Negative Control*
 PCR grade water is used as a Negative (“no template”) Control for the RT-PCR. Its function is to indicate contamination of RT-PCR reagents. One Negative Control must be tested in each RT-PCR run. The Negative Control is for the RT-PCR step and does not go through nucleic acid extraction.
- b) *Positive Control*
 The Positive Control is used to verify the integrity of the SARS-CoV-2 and lineage B-β-coronavirus specific RT-PCR amplification process and reagents.
 The Positive Control consists of a mixture of two *in vitro* transcripts (IVTs): one

corresponding to the S gene of the SARS-CoV-2 genome and the other the E gene of lineage B-β-coronavirus. One Positive Control must be tested in each RT-PCR run. The Positive Control is for the RT-PCR step and does not go through nucleic acid extraction.

c) *Internal Control*

The RealStar SARS-CoV-2 RT-PCR Kit includes a heterologous Internal Control that is used in the Akron Children’s Hospital SARS-CoV-2 Assay as both a control for nucleic acid extraction and RT-PCR. The Internal Control is added to each patient sample or Negative Process Control after the lysis/proteinase K digestion.

External Process Controls:

One External Positive and one External Negative Control must be processed with each new lot of extraction reagents for the Maxwell 16 MDx and Maxwell RSC 48 instruments and the expected results must be obtained in order to qualify the reagents for use with patient samples (**Table 1**).

Table 1. External Controls used with the Akron Children’s SARS-CoV-2 Assay

External Process Control	Control Material
Positive	SARS-CoV-2 Positive patient sample ^{1, 2}
Negative	Phosphate Buffered Saline

¹ Because the Maxwell 16 MDx Instrument and Maxwell 16 Viral Total Nucleic Acid Purification Kit are used by Akron Children’s Hospital to prepare patient samples for detection of analytes other than SARS-CoV-2, other analyte-specific controls may be substituted as appropriate to qualify a new lot of extraction reagents. Each analyte-specific Positive Process Control must meet a pre-defined acceptance criterion for the reported Ct value, as defined in the laboratory SOP.

² For SARS-CoV-2 the Ct values for the S and E genes must be within ± 3 of those obtained when the sample was originally tested

INTERPRETATION OF RESULTS

The results from the controls for the Akron Children’s Hospital SARS-CoV-2 Assay are interpreted according to the criteria shown in **Table 2**. If the results obtained with the controls do not meet the criteria shown, the results from the entire batch of samples are considered invalid and repeat testing must be performed.

Table 2. Acceptable RT-PCR results for the controls for the Akron Children’s Hospital SARS-CoV-2 Assay

Control Type	Acceptable Ct Value ¹		
	E Gene (B-β-coronavirus)	S Gene (SARS-Cov-2)	Internal Control
Negative Control (No Template)	Not Detected	Not Detected	Not Detected
Positive Control	26.78-32.78	25.63-31.63	Not Detected
Negative Process Control ²	Not Detected	Not Detected	26.02-32.02
Positive Process Control ^{2,3}	± 3 Ct of original result	± 3 Ct of original result	26.02-32.02

¹ For each target, the Ct threshold value = 0.1

² Tested with each new lot of extraction reagents (refer also to **Table 1**)

³ Known SARS-CoV-2 positive patient sample

The results from testing of patient samples are interpreted according to the criteria described in **Table 3**.

Table 3. Summary of results interpretation for patient samples tested with the Akron Children’s Hospital SARS-CoV-2 Assay

Ct Value			Interpretation
E Gene (B-β-coronavirus)	S Gene (SARS-Cov-2)	Internal Control	
< 45	< 45	< 45 or Not Detected ¹	B-β-CoV (E gene target) and SARS-CoV-2 (S gene target) specific RNA detected. Positive for SARS-CoV-2. Report result to healthcare provider and appropriate public health authorities.
< 45	Not Detected	< 45 or Not Detected ¹	Only B-β-CoV (E gene target) specific RNA was detected. Presumptive positive for SARS-CoV-2. Repeat extraction and RT-PCR. If the repeated result remains positive for B-β-CoV only, report as presumptive positive for SARS-CoV-2 and contact the responsible national reference center. Repeated presumptive positive results should be confirmed if clinically needed.
Not Detected	< 45	< 45 or Not Detected ¹	Only SARS-CoV-2 (S gene target) specific RNA was

Ct Value			Interpretation
E Gene (B-β-coronavirus)	S Gene (SARS-Cov-2)	Internal Control	
			detected. Positive for SARS-CoV-2. Report result to healthcare provider and appropriate public health authorities.
Not Detected	Not Detected	< 45	Neither B-β-CoV (E gene target) nor SARS-CoV-2 (S gene target) specific RNA was detected.
Not Detected	Not Detected	Not Detected	RT-PCR inhibition or reagent failure.

¹ Detection of the Internal Control is not required. A high level of B-β-CoV and/or SARS-CoV-2 RNA in the sample can lead to reduced or absent Internal Control signal

PERFORMANCE EVALUATION

1) **Limit of Detection (LoD) - Analytical Sensitivity:**

LoD Determination

The Limit of Detection (LoD) of the Akron Children’s Hospital SARS-CoV-2 Assay was determined by testing dilutions of *in vitro* transcripts of viral RNA (Exact Diagnostics Cat. #COV019) in M4 transport medium containing nasopharyngeal swab matrix. Nucleic acid extraction was performed using the Maxwell 16 MDx Instrument and Maxwell 16 Viral Total Nucleic Acid Purification Kit. The LoD was initially estimated by testing five different concentrations of the *in vitro* transcripts (**Table 4**). The LoD was estimated to be the lowest concentration at which all three replicates for the SARS-CoV-2-specific S gene target produced positive results (i.e., 250 copies/mL). The estimated LoD was then confirmed by testing a further 20 replicates at the same concentration. The LoD of the Akron Children’s Hospital SARS-CoV-2 Assay was confirmed to be 250 copies of *in vitro* transcribed RNA/mL of nasopharyngeal swab matrix.

Table 4. Determination of the LoD of the Akron Children’s Hospital SARS-CoV-2 Assay

Study	Copies/mL	E Gene		S Gene	
		Positive (%)	Mean Ct (SD)	Positive (%)	Mean Ct (SD)
Preliminary Titration	1250	3/3 (100)	34.7 (0.32)	3/3 (100)	31.7 (0.20)
	500	2/3 (67)	36.7 (0.06)	3/3 (100)	33.3 (0.66)
	250	1/3 (33)	36.9 (NA)	3/3 (100)	36.3 (1.80)
	125	1/3 (33)	40.2 (0.31)	3/3 (100)	36.3 (1.50)
	50	1/3 (33)	40.6 (NA)	2/3 (67)	38.0 (0.14)
Confirmation	250	15/20 (75.0)	39.1 (1.99)	20/20 (100)	34.3 (1.21)

SD: Standard Deviation; NA: Not Applicable

Validation of the Maxwell RSC 48 Instrument for Nucleic Acid Extraction

To validate use of the higher throughput Maxwell RSC 48 Instrument for nucleic acid extraction, Exact Diagnostics *in vitro* transcripts were diluted to the LoD target concentration in M4 medium containing nasopharyngeal swab matrix and tested following extraction with the Maxwell RSC Viral Total Nucleic Acid Purification Kit. Of 20 replicates, 18 (90.0%) produced positive results for the SARS-CoV-2-specific S gene, whereas 12/20 (60.0%) were positive for the E gene (**Table 5**). The two replicates that were negative for the S gene gave positive results for the E gene target and were reported as “presumptive positive for SARS-CoV-2” according to the algorithm described in **Table 3**. A subsequent study to evaluate the precision of the Akron Children’s Hospital SARS-CoV-2 Assay demonstrated that samples containing 1250 copies of *in vitro* transcripts/mL (5X LoD) that were processed using the Maxwell RSC 48 Instrument produced 100% positive results both the E and S gene targets.

Table 5. Comparison of performance with the Akron Children’s Hospital SARS-CoV-2 Assay using alternative nucleic acid extraction instruments

Nucleic Acid Extraction Instrument	Copies/mL	E Gene		S Gene		Positive or Presumptive Positive
		Positive (%)	Mean Ct (SD)	Positive (%)	Mean Ct (SD)	
Maxwell 16 ¹	250	15/20 (75.0)	39.1 (1.99)	20/20 (100)	34.3 (1.21)	20/20 (100)
Maxwell RSC	250	12/20 (60.0)	40.1 (2.28)	18/20 (90.0)	36.7 (1.73)	20/20 (100) ³
	250 ²	6/8 (75.0)	40.9 (1.62)	7/8 (87.5)	36.2 (2.22)	8/8 (100) ⁴
	1250 ²	8/8 (100)	36.3 (0.62)	8/8 (100)	32.8 (0.57)	8/8 (100)

SD: Standard Deviation; NA: Not Applicable

¹ Data from the original LoD Confirmation Study (**Table 4**)

² Data from additional Precision Study

³ 2 samples were positive only for the E gene and reported as “presumptive positive for SARS-CoV-2”

⁴ 1 sample was positive only for the E gene and reported as “presumptive positive for SARS-CoV-2”

Because the analytical sensitivity of the Akron Children’s Hospital SARS-CoV-2 Assay was shown to be similar with samples processed using the Maxwell 16 MDx and Maxwell RSV 48 instruments, both these systems were used interchangeably in the Clinical Evaluation Studies described below.

Validation of Alternative Transport Media

A study was conducted to validate the use of samples collected in alternative transport media and to demonstrate the stability of such samples under different storage conditions. Samples for the study were prepared by diluting two known SARS-CoV-2 positive clinical specimens 1:100 in the desired transport medium and by testing each dilution at time 0 and after storage for 24, 48 or 72 hours at 4 °C. An aliquot of each sample was also stored at -70 °C and tested at the end of the study. The samples were extracted using the Maxwell 16 MDx Instrument and Maxwell 16 Viral Total Nucleic Acid Purification Kit.

All samples produced the expected results at each test point in the study and there was no evidence of a trend in Ct values that could be indicative of specimen instability (**Table 6**). The results of the study demonstrated compatibility of the Akron Children’s Hospital SARS-CoV-2 Assay with each of the transport media tested (M4, CDC Viral Transport Medium and Phosphate Buffered Saline) and that samples could be stored for up to 72 hours at either 4 °C or -70 °C.

Table 6. Results from evaluation of alternative transport media with the Akron Children’s Hospital SARS-CoV-2 Assay

Target	Storage Temp. (°C)	Hours	Mean Ct Value					
			M4		CDC-VTM		PBS	
			Sample 1	Sample 2	Sample 1	Sample 2	Sample 1	Sample 2
E Gene	NA	0	27.7	28.5	27.5	28.0	27.8	28.1
	4	24	27.1	28.4	27.5	27.3	27.7	27.6
		48	28.0	28.6	27.7	27.5	27.2	27.7
		72	27.7	28.1	27.1	27.0	26.3	27.3
	-70	72	28.1	27.6	27.4	26.6	27.5	27.6
S Gene	NA	0	27.0	26.9	26.3	26.0	26.7	26.4
	4	24	26.4	27.3	26.9	26.1	27.0	26.4
		48	27.2	27.2	26.8	26.1	26.3	26.4
		72	27.0	27.0	26.4	25.9	25.7	26.2
	-70	72	27.5	26.5	26.8	25.5	26.8	26.5

M4: Remel M4 Medium

CDC-VTM: Viral Transport Medium prepared according to the formulation and methodology recommended by CDC (<https://www.cdc.gov/coronavirus/2019-ncov/downloads/Viral-Transport-Medium.pdf>)

PBS: Sterile filtered Phosphate Buffered Saline (Alfa Aesar)

2) Inclusivity (Analytical Sensitivity):

The Akron Children’s Hospital SARS-CoV-2 Assay uses PCR reagents from the Altona Diagnostics RealStar SARS-CoV-2 RT-PCR Kit 1.0 which, according to the kit manufacturer, is identical in composition to the FDA-authorized RealStar SARS-CoV-2 RT-PCR Kit U.S. The assay targets a SARS-CoV-2-specific region of the spike protein (S) gene and a B-β-coronavirus-specific segment of the envelop (E) gene.

The inclusivity of the assay was demonstrated by Altona Diagnostics through *in silico* analysis of the available SARS-CoV-2 whole genome sequences in the databases of the National Center for Biotechnology Information (NCBI) and Global Initiative on Sharing All Influenza Data (GISAID), as of March 27, 2020. Details of this analysis are held by FDA as a Master File to which Altona Diagnostics has granted a Right of Reference for laboratories seeking to validate the RealStar SARS-CoV-2 RT-PCR Kit 1.0 for clinical use. The same analysis was used by Altona Diagnostics to support FDA authorization of the RealStar SARS-CoV-2 RT-PCR Kit U.S. As a result, Akron Children’s Hospital did not perform any additional analysis of the inclusivity of the RealStar assay primers and probes.

3) Cross-reactivity (Analytical Specificity)

As noted above, the Akron Children’s Hospital SARS CoV-2 Assay uses PCR reagents from the Altona Diagnostics RealStar SARS-CoV-2 RT-PCR Kit 1.0 which is identical in composition to the FDA-authorized RealStar SARS-CoV-2 RT-PCR Kit U.S.

The analytical specificity of the RealStar assay primers and probes was demonstrated through a combination of *in silico* analysis and laboratory testing, the details of which are

held by FDA as a Master File to which Altona Diagnostics has granted a Right of Reference to laboratories seeking to validate the RealStar SARS-CoV-2 RT-PCR Kit 1.0 for clinical use. The same analysis was used by Altona Diagnostics to support FDA authorization of the RealStar SARS-CoV-2 RT-PCR Kit U.S.

In addition to citing the analysis conducted by Altona Diagnostics, to demonstrate the analytical specificity of the Akron Children’s Hospital SARS-CoV-2 Assay, a study was performed using nasopharyngeal specimens that were known to be positive for other coronaviruses as determined using an FDA-cleared multiplex assay for respiratory pathogens. A summary of the results of the study is presented in **Table 7**. No cross-reaction or interference was observed.

Table 7. Evaluation of nasopharyngeal swab specimens known to be positive for non-SARS-CoV-2 coronaviruses

#	Non-SARS-CoV-2 Coronavirus	Ct Value		
		E Gene	S Gene	Internal Control
1	OC43 ¹	Not Detected	Not Detected	32.9
2	NL63	Not Detected	Not Detected	32.8
3	HKU1	Not Detected	Not Detected	33.2
4	NL63	Not Detected	Not Detected	33.3
5	229E	Not Detected	Not Detected	33.0
6	HKU1	Not Detected	Not Detected	33.1

¹ Specimen also positive for rhinovirus and enterovirus as determined by the comparator FDA-cleared assay

4) **Clinical Evaluation:**

The performance of the Akron Children’s Hospital SARS-CoV-2 Assay was evaluated using a combination of contrived positive samples and previously characterized clinical specimens.

Evaluation of Contrived Samples

Contrived SARS-CoV-2 positive clinical samples were prepared by spiking *in vitro* transcripts of SARS-CoV-2 RNA (Exact Diagnostics Cat. #COV019) into pooled SARS-CoV-2 negative nasopharyngeal swab matrix. Twenty samples were tested at a concentration of 250 copies/mL (1X LoD) and a further 16 were tested at a concentration of 1250 copies/mL (5X LoD). All the contrived positive specimens produced the expected positive results for SARS-CoV-2 although, while 100% (20/20) were positive for the SARS-CoV-2 specific S gene at the LoD concentration of 250 copies/mL, only 75% (15/20) were positive for the E gene (B-β-coronavirus) at the same target level (**Table 8**).

Table 8. Summary of results from testing contrived positive nasopharyngeal specimens with the Akron Children’s Hospital SARS-CoV-2 Assay

Copies/mL	E Gene		S Gene	
	Positive (%)	Mean Ct (SD)	Positive (%)	Mean Ct (SD)
250 ^{1,2}	15/20 (75.0)	39.1 (1.99)	20/20 (100)	34.3 (1.21)
1250 ³	16/16 (100)	35.1 (0.82)	16/16 (100)	31.6 (0.26)

SD: Standard Deviation; NA: Not Applicable

¹ Data from the LoD confirmation study as described in **Table 4**

² Nucleic acid extraction performed using the MaxWell 16 MDx instrument

³ Nucleic acid extraction performed using the MaxWell RSC 48 instrument

Evaluation of Known SARS-CoV-2 Positive/Negative Clinical Specimens

Evaluation of known SARS-CoV-2 positive and negative clinical specimens was conducted in two phases as described below:

Phase I:

Testing was performed with 5 SARS-CoV-2 positive and 5 SARS-CoV-2 negative clinical nasopharyngeal swab specimens that had previously been tested by the Ohio Department of Health using the FDA-authorized CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel. All 5 (100%) of the SARS-CoV-2 positive specimens produced the expected result; however, 2/5 (40%) of the specimens that were SARS-CoV-2 negative by the CDC assay were reported as SARS-CoV-2 positive using the Akron Children’s Hospital SARS-CoV-2 Assay (**Table 9**).

Table 9. Results from evaluation of known SARS-CoV-2 positive/negative clinical specimens with the Akron Children’s Hospital SARS-CoV-2 Assay

Sample	CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel				Akron Children’s Hospital SARS-CoV-2 Assay ¹			
	Result	N1	N2	RNase P	Result	E Gene	S Gene	Internal Control
ODH1	Positive	21.1	20.8	24.8	Positive	17.2	15.8	28.7
ODH2	Positive	27.2	27.2	23.9	Positive	24.3	23.3	28.2
ODH3	Positive	18.7	18.0	28.6	Positive	14.8	13.5	29.8
ODH4	Negative	ND	ND	24.3	Positive	32.7	32.3	29.0
ODH5	Negative	ND	ND	25.3	Negative	ND	ND	28.9
ODH6	Positive	19.8	19.6	23.9	Positive	17.3	16.2	28.4
ODH7	Negative	ND	ND	24.3	Positive	32.8	31.8	28.9
ODH8	Negative	ND	ND	25.5	Negative	ND	ND	29.1
ODH9	Positive	19.0	18.0	23.7	Positive	15.4	14.3	29.3
ODH10	Negative	ND	ND	25.0	Negative	ND	ND	29.0

ND: Not Detected

¹ Nucleic acid extraction was performed using the Maxwell 16 Dx Instrument and Maxwell 16 Viral Total Nucleic Acid Purification Kit

Subsequent investigation of the apparent false positive results with the Akron Children’s Hospital SARS-CoV-2 Assay identified a 56 °C water bath and the Maxwell 16 MDx instrument that were used during sample preparation as potential sources of contamination. To address this, the sample processing procedure was modified to

replace the water bath with a dry heat block. The Maxwell 16 MDx Instrument was also thoroughly cleaned and routine cleaning was implemented as part of the operating procedure.

Following the investigation, insufficient material remained from the two specimens that originally produced false-positive results to repeat the original Clinical Evaluation. Instead, two additional SARS-CoV-2 negative specimens were obtained from the Ohio Department of Health and these were included in a second Clinical Evaluation. All 10 specimens produced the expected results with the Akron Children’s Hospital SARS-CoV-2 Assay (**Table 10**).

Table 10. Repeat results from evaluation of known SARS-CoV-2 positive/negative clinical specimens with the Akron Children’s Hospital SARS-CoV-2 Assay

Sample	CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel				Akron Children’s Hospital SARS-CoV-2 Assay ¹			
	Result	N1	N2	RNase P	Result	E Gene	S Gene	Internal Control
ODH1	Positive	21.1	20.8	24.8	Positive	17.0	15.4	28.3
ODH11	Negative	ND	ND	26.4	Negative	ND	ND	29.1
ODH2	Positive	27.2	27.2	23.9	Positive	24.4	23.2	28.6
ODH5	Negative	ND	ND	25.3	Negative	ND	ND	29.2
ODH3	Positive	18.7	18.0	28.6	Positive	15.0	13.6	29.5
ODH12	Negative	ND	ND	24.6	Negative	ND	ND	28.4
ODH6	Positive	19.8	19.6	23.9	Positive	17.9	16.8	29.6
ODH10	Negative	ND	ND	25.0	Negative	ND	ND	29.0
ODH9	Positive	19.0	18.0	23.7	Positive	15.5	14.3	32.1
ODH8	Negative	ND	ND	25.5	Negative	ND	ND	32.7

ND: Not Detected

¹ Nucleic acid extraction was performed using the Maxwell 16 Dx Instrument and Maxwell 16 Viral Total Nucleic Acid Purification Kit following procedural changes to reduce the risk of contamination

Phase II

Additional testing was performed with 31 nasopharyngeal swabs specimens that were presumed to be negative for SARS-CoV-2. These swabs were collected between March 16 and March 19, 2020, prior to evidence of SARS-CoV-2 infection in the patient population served by the Akron Children’s Hospital. As expected, all 31 samples (100%) produced negative results with the Akron Children’s Hospital SARS-CoV-2 Assay.

Phase III

To evaluate the performance of the Akron Children’s Hospital SARS-CoV-2 Assay further, an additional study was performed with 25 SARS-CoV-2 positive and 25 SARS-CoV-2 negative clinical specimens that had previously been characterized using the Cepheid Xpert Xpress SARS-CoV-2 Assay. Nucleic acid extraction for the Akron Children’s assay was performed using the Maxwell RSC 48 Instrument and Maxwell RSC Viral Total Nucleic Acid Purification Kit. A summary of the results of the study is presented in **Table 11**.

Table 11. Evaluation of the Akron Children’s Hospital SARS-CoV-2 Assay with natural clinical specimens

		Cepheid Xpert Xpress SARS-CoV-2 Assay		
		Positive	Negative	Total
ACH SARS-CoV-2 Assay	Positive	24	1 ¹	25
	Negative	1 ^{2,3}	24	25
	Total	25 ⁴	25	50
Positive Agreement		96.0% (24/25); 80.5-99.3%⁵		
Negative Agreement		96.0% (24/25); 80.5-99.3%		

ACH: Akron Children’s Hospital

¹ Reported negative by the Akron Children’s Hospital SARS-CoV-2 Assay upon retesting

² Confirmed as positive by the Xpert Xpress SARS-CoV-2 Assay and negative by the Akron Children’s Hospital SARS-CoV-2 Assay upon retesting

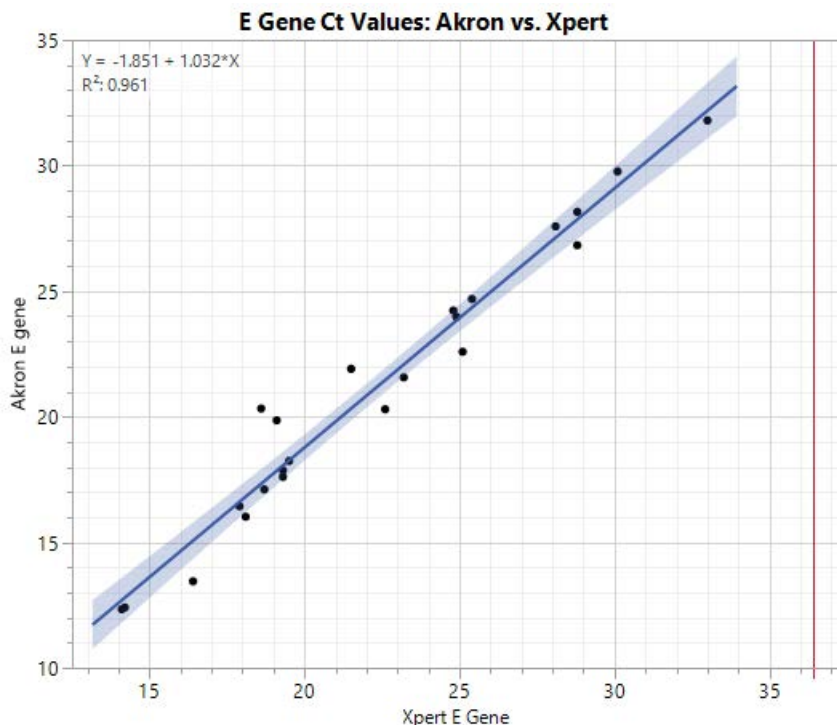
³ Also reported as SARS-CoV-2 negative using the FDA-authorized Luminex ARIES SARS-CoV-2 Assay

⁴ Ct values for the E gene with the comparator ranged from 0 (not detected) to 42.8 (**Figure 1**)

⁵ Two-sided 95% score confidence interval

Both the Akron Children’s Hospital SARS-CoV-2 Assay and the Xpert Xpress SARS-CoV-2 Assay amplify regions of the SARS-CoV-2 E gene. A comparison of the Ct values for the E gene obtained with the two assays is shown in **Figure 1**. The distribution of Ct values for samples reported as SARS-CoV-2 E-gene positive by the Xpert Xpress assay ranged from 14.1 to 42.8 and 4/25 (16%) had E gene Ct values >30 or were E gene negative.

Figure 1. Comparison of Ct values for the E gene obtained with the Akron Children’s Hospital SARS-CoV-2 Assay and the Cepheid Xpert Xpress SARS-CoV-2 Assay



Red line indicates the mean Ct value at the LoD of the Xpert assay (36.4)
 2 samples that were reported as SARS-CoV-2 positive by the Xpert Xpress assay were excluded from the chart:
 1 sample was Xpert positive for the E gene (Ct value = 42.8) but SARS-CoV-2 negative by the Akron Children’s assay and 1 sample was positive for the N gene by the Xpert Assay but negative for the E gene
 4/25 (16%) samples had E gene Ct values >30 using the Xpert Xpress Assay or were E gene negative

Table 12 summarizes the overall positive and negative agreement observed by combining the results from retrospective testing of known SARS-CoV-2 positive and negative specimens from Phases II and III of the Clinical Evaluation with the Akron Children’s Hospital SARS-CoV-2 Assay.

Table 12. Cumulative results from testing natural clinical specimens with the Akron Children’s Hospital SARS-CoV-2 Assay

		Comparator ¹		
		Positive	Negative	Total
ACH SARS-CoV-2 Assay	Positive	29	1 ²	30
	Negative	1 ³	29	30
	Total	30	30	60
Positive Agreement		96.7% (29/30); 83.3-99.4% ⁴		
Negative Agreement		96.7% (29/30); 88.3-99.4%		

ACH: Akron Children's Hospital

- ¹ Either CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel (n = 10; 5 positive, 5 negative) or Cepheid Xpert SARS-CoV-2 Assay (n = 50; 25 positive, 25 negative)
- ² Negative by the Cepheid Xpert SARS-CoV-2 Assay
- ³ Positive by the Cepheid Xpert SARS-CoV-2 Assay
- ⁴ Two-sided 95% score confidence interval

WARNINGS

- Caution: Federal Law restricts this device to sale by or on the order of a licensed practitioner.
- For *in vitro* diagnostic use.
- This test has not been FDA cleared or approved.
- This test has been authorized by FDA under an EUA for use by Akron Children's Hospital Molecular Diagnostics Laboratory, located at One Perkins Square, Akron, OH 44308.
- This test has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.
- 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 Federal Food, Drug and Cosmetics Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.