EMERGENCY USE AUTHORIZATION (EUA) SUMMARY
ICTC SARS-CoV-2 RT-PCR Assay
(University of Massachusetts)

For *in vitro* Diagnostic Use
Rx Only
For Use Under Emergency Use Authorization (EUA) Only

The ICTC SARS-CoV-2 RT-PCR Assay will be performed at IALS Clinical Testing Center, located at 240 Thatcher Road, Amherst, MA 01003, 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, as described in the Laboratory Standard Operating Procedures that were reviewed by the FDA under this EUA.

INTENDED USE

The ICTC SARS-CoV-2 RT-PCR Assay is an in vitro real-time reverse transcription polymerase chain reaction (rRT-PCR) test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in anterior nasal (AN) swab specimens from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to IALS Clinical Testing Center, located at 240 Thatcher Road, Amherst, MA 01003, 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 identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in anterior nasal swab 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 definitive 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/or epidemiological information.

The ICTC SARS-CoV-2 RT-PCR Assay is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the techniques of real-time RT-PCR and in vitro diagnostic procedures. The ICTC SARS-CoV-2 RT-PCR Assay is only for use under the Food and Drug Administration's Emergency Use Authorization.

DEVICE DESCRIPTION AND TEST PRINCIPLE

Device Description

The ICTC SARS-CoV-2 RT-PCR Assay is a nucleic acid amplification in vitro diagnostic test intended for the qualitative detection of SARS-CoV-2 RNA isolated and purified from anterior nasal (AN) swab specimens obtained from individuals who meet COVID-19 clinical and/or epidemiological criteria.

The ICTC SARS-CoV-2 RT-PCR Assay uses a primer and probe set (Integrated DNA Technologies) targeting SARS-CoV-2 specific genetic sequences (N1 and N2) and one human cellular material control (RP) per sample. Overall performance of the assay requires incorporation of specific external run controls, including a positive template control (A549 cells/SARS-Cov-2 plasmid or positive pooled AN-nasal swab) and a negative template control (NTC). External run controls must be valid to report results. Nucleic acid from respiratory samples is extracted and purified using a MagMAX Viral/Pathogen II (MVP II) Nucleic Acid Isolation Kit on a Hamilton MagEx STAR AL 8/96 Automated Liquid Handler Robot. Reagents and samples to perform qPCR reactions are assembled in PCR (Polymerase Chain Reaction) plates using a Hamilton PCR Prep STAR ML 8/96 automated liquid handling robot. Amplification and detection of the targets is performed on the CFX384 Touch Real-Time PCR Detection System (Bio-Rad).

Description of Test Steps

1. Specimen Collection

Anterior nasal (AN) swab specimens will be collected from individuals via self-collection with instructions from a healthcare provider in a healthcare setting. Samples can be placed into sterile tubes as dry swabs for up to 24 hours and re-hydrated in 1% SDS-saline buffer before immediately processing. Samples collected can also be placed directly in sample tubes containing 1 mL of VTM can be stored following CDC guidelines or immediately run on the assay. Each sample tube is labelled with a bar-code that relates back to the patient the sample was collected from based on identification information presented during the nasal swab collection.

2. Specimen Testing

RNA from the anterior nasal samples are extracted and purified using a MagMAX Viral/Pathogen II (MVP II) Nucleic Acid Isolation Kit Hamilton MagEx STAR AL 8/96 Automated Liquid Handler Robot. Reagents and samples to perform qPCR reactions are assembled in PCR (Polymerase Chain Reaction) plates again using a Hamilton PCR Prep STAR ML 8/96 automated liquid handling robot. qPCR reactions are performed using three probes - the N1 and N2 viral probes defining the presence of SARS-CoV-2 and the human RNase P (RP) probe ensuring that a sufficient nasal swab sample was collected to amplify RNA coding for this human gene. Amplification and detection of the targets is performed on the CFX384 Touch Real-Time PCR Detection System (Bio-Rad). Samples that pass quality control tests and are positive for the presence of SARS-CoV-2 RNA are flagged as COVID-19 positive samples.

INSTRUMENTS USED WITH THE TEST

Table 1. Instruments and Software for Use with the ICTC SARS-CoV-2 RT-PCR Assay

Instrument	Manufacturer
MagEx STAR AL 8/96	Hamilton Company
PCR Prep STAR ML 8/96	Hamilton Company
CFX384 Touch Real-Time PCR Detection System	Bio-Rad Laboratories

REAGENTS AND MATERIALS

Table 2. Reagents and Materials Used for Sample Preparation and to Perform the ICTC SARS-CoV-2 RT-PCR Assay

Reagent/Material	Manufacturer/ Supplier	Catalogue/Part Number
MagMAX Viral/Pathogen II Nucleic Acid Isolation Kit (MVP II)	ThermoFisher Scientific Inc	A48383
Luna Probe One-Step RT-qPCR Kit (No ROX)	New England Biolabs, Inc.	E3007E
1000 µL Conductive Sterile Filter Tips	Hamilton Company	235940
300 µL Conductive Sterile Filter Tips	Hamilton Company	235938
50µL Conductive Sterile Filter Tips	Hamilton Company	235979
60mL Reagent Reservoir Self-Standing with Lid	Hamilton Company	56694-01
nCov2-N1 Primers and nCov2-N1-Cy5 Probe	Integrated DNA Technologies	Custom
nCov2-N2 Primers and nCov2-N1-FAM Probe	Integrated DNA Technologies	Custom
RPP Primers and RPP-HEX Probe	Integrated DNA Technologies	Custom
2019-nCoV_N_Positive Control Plasmid	Integrated DNA Technologies	10006625
A549 cells	ATCC	CCL-185
96W 2 ml Deep Well Plate, round wells (U) bottom	Stellar Scientific	DWP-76-51-S0-C
96W 2 ml Deep Well Plate, square well	Olympus Plastics	27-413S
96W PCR Plate	AlphaGem	PCR-96-PRD-FS-C
384W PCR Plate	Bio-Rad Laboratories	HSP3905
Plate Seal	Genesee Scientific	12-157
PX1 Plate Sealer	Bio-Rad Laboratories	1814000
Heat Plate Seal	Bio-Rad Laboratories	1814030

CONTROLS

Controls are included with each batch of 384 samples tested. The two controls are transferred to the qPCR plate during the qPCR setup steps. The extraction process will result in 384 purified RNA samples that are collected into four 96-well plates on the MagEx STAR. Each 96 well plate includes 94 nasal samples, one 'Positive Template Control' (PTC) located in well G12, and one 'Negative Template Control' (NTC) located in well H12. The PTC acts as a positive control for the extraction and reverse transcription of human RP gene target (the cells) as well as a positive control for amplification of nCOV N1 and N2 gene target (the nCoV plasmid). The NTC well acts as a negative control that serves to check QC of the sample batch.

Two types of Positive Template Controls can be used. The first is a combination of human-derived cells (A549 cells) with a plasmid encoding the SARS-CoV-2 nucleocapsid protein (N1 and N2 genes). A second source of PTC is a pool of AN-nasal swab samples previously identified as having DETECTED levels of SARS-CoV-2 from a CLIA-approved laboratory. Each such pool has been inactivated in 1% SDS and validated by this ICTC SARS-CoV-2 RT-PCR Assay as previously demonstrated to be SARS-CoV-2 positive and resulting in N1, N2, and RP amplification with a Ct (cycle threshold) <36.

Table 3. Assay Controls Used With the ICTC SARS-CoV-2 RT-PCR Assay

Control	Description	Purpose	Expected Results	Frequency of Use
Negative Template Controls (NTC)	1 ml of buffered saline 1 ml of VTM or 1 ml of TE buffer	Test for the presence of any amplified RNA in the absence of adding a nasal sample	Baseline levels of fluorescence following the PCR amplification (Cq >36 or N/A).	Each batch of 384 samples to be tested
Positive Template Controls (PTC)	A549 cells/SARS-CoV-2 plasmid (final concentration 40,000 copies/mL) or Pooled AN-Nasal Swab samples	Test to ensure that extraction and amplification has occurred.	Significant increase in the fluorescence following the PCR amplification (Cq values <36)	Each batch of 384 samples to be tested

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 4: Plate QC Interpretation Matrix for the SARS-CoV-2 RT-PCR Assay: NTC and PTC

Well Type	N1 primers	N2 primers	RP primers	Result Interpretation	Action
NTC wells (4 RP and 4 N1 and N2)	≥2 out of 4 wells NEGATIVE	≥2 out of 4 wells NEGATIVE	≥2 out of 4 wells NEGATIVE	**PASS:** Plate passes Extraction and Assay NTC QC	Plate sent for review and reporting
	>2 wells POSITIVE			**FAIL:** Plate fails Extraction and Assay NTC QC	Plate reworked from RNA extraction
Well Type	**N1 primers**	**N2 primers**	**RP primers**	**Result Interpretation**	**Action**
PTC wells (4 RP and 4 N1 and N2)	≥2 out of 4 wells are POSITIVE	≥2 out of 4 wells are POSITIVE	≥2 out of 4 wells are POSITIVE	**PASS:** Plate passes Extraction and Assay PTC QC	Plate sent for review and reporting
	>2 wells NEGATIVE			**FAIL:** Plate fails Extraction and Assay PTC QC	Plate reworked from RNA extraction

Table 5: Plate Interpretation Matrix for the SARS-CoV-2 RT-PCR Assay: Patients Samples

Well Type	N1 primers	N2 primers	RP primers	Result Interpretation	Action
Test wells	POSITIVE	POSITIVE	POSITIVE	**PASS:** SARS-CoV-2 detected Valid Result	Score: **POSITIVE**
	POSITIVE	POSITIVE	NEGATIVE	**PASS:** SARS-CoV-2 detected Valid Result	Score: **POSITIVE**
	NEGATIVE	NEGATIVE	POSITIVE	**PASS:** No SARS-CoV-2 Valid Result	Score: **NEGATIVE**
	POSITIVE or NEGATIVE	NEGATIVE	NEGATIVE	**FAIL:** Insufficient RP detected, possibly due to insufficient sample on swab	Score: **INVALID** Rerun sample as STAT. If the repeat result remains invalid, approve and report the result as INVALID to the healthcare provider and public health authorities. The healthcare provider should recommend confirmation testing with a new specimen ASAP.
	NEGATIVE	POSITIVE or NEGATIVE	NEGATIVE	**FAIL:** Insufficient RP detected, possibly due to insufficient sample on swab	Score: **INVALID** Rerun sample as STAT. If the repeat result remains invalid, approve and report the result as INVALID to the healthcare provider and public health authorities. The healthcare provider should recommend confirmation testing with a new specimen ASAP.
	NEGATIVE	POSITIVE	POSITIVE	**FAIL:** Insufficient N1 viral gene detected, possibly due to insufficient sample on swab	Score: **INVALID** Rerun sample as STAT. If the repeat result remains invalid, approve and report the result as INVALID to the healthcare provider and public health authorities. The healthcare provider should recommend confirmation testing with a new specimen ASAP.
	POSITIVE	NEGATIVE	POSITIVE	**FAIL:** Insufficient N2 viral gene detected, possibly due to insufficient sample on swab	Score: **INVALID** Rerun sample as STAT. If the repeat result remains invalid, approve and report the result as INVALID to the healthcare provider and public health authorities. The healthcare provider should recommend confirmation testing with a new specimen ASAP.

PERFORMANCE EVALUATION

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

The LoD of the ICTC SARS-CoV-2 RT-PCR Assay was determined by measuring the lowest concentration of SARS-CoV-2 viral particles (obtained through BEI Resources, NIAID, NIH: SARS-Related Coronavirus 2, Isolate USA-WA1/2020, Gamma-Irradiated, NR-52287) that can be measured or distinguished from a dry swab containing real human clinical matrix suspended in saline/1% SDS assay buffer and VTM. The initial LOD for both saline/1% SDS assay buffer and VTM used a total of 25 samples, at 5 different concentrations: 750 Genome Copy Equivalents (GCE)/mL, 200 GCE/mL, 70 GCE/mL, 50 GCE/mL, and 20 GCE/mL. The preliminary LoD for saline/1% SDS assay buffer and VTM was determined to be 50 GCE/mL. A confirmation study of both materials was completed by running 20 replicate samples at 3 different concentrations: 70 GCE/mL, 50 GCE/mL, and 20 GCE/mL. The final LoD was confirmed to be 50 GCE/mL.

Table 6: LoD Study Summary for saline/1% SDS assay buffer

Target Concentration (GCE/mL)	Number Replicates (n)	SARS-CoV-2 N1	SARS-CoV-2 N2	RP (Internal Control)
		# Positive/ Total	# Positive/ Total	# Positive/ Total
750	5	5/5 (100%)	5/5 (100%)	5/5 (100%)
200	5	5/5 (100%)	5/5 (100%)	5/5 (100%)
70	5	5/5 (100%)	5/5 (100%)	5/5 (100%)
50	**5**	**5/5 (100%)**	**5/5 (100%)**	**5/5 (100%)**
20	5	4/5 (90%)	5/5 (100%)	5/5 (100%)
70	20	20/20 (100%)	20/20 (100%)	20/20 (100%)
50	**20**	**20/20 (100%)**	**20/20 (100%)**	**20/20 (100%)**
20	20	16/20 (80%)	17/20 (85%)	20/20 (100%)

Table 7: LoD Study Summary for VTM

Target Concentration (GCE/mL)	Number Replicates (n)	SARS-CoV-2 N1	SARS-CoV-2 N2	RP (Internal Control)
		# Positive/ Total	# Positive/ Total	# Positive/ Total
750	5	5/5 (100%)	5/5 (100%)	5/5 (100%)
200	5	5/5 (100%)	5/5 (100%)	5/5 (100%)
70	5	5/5 (100%)	5/5 (100%)	5/5 (100%)
50	**5**	**5/5 (100%)**	**5/5 (100%)**	**5/5 (100%)**
20	5	4/5 (90%)	4/5 (90%)	5/5 (100%)
70	20	20/20 (100%)	20/20 (100%)	20/20 (100%)
50	**20**	**20/20 (100%)**	**20/20 (100%)**	**20/20 (100%)**
20	20	14/20 (70%)	16/20 (80%)	19/20 (95%)

2) Inclusivity (Analytical Reactivity):

The ICTC SARS-CoV-2 RT-PCR Assay targets specific genomic regions of the SARS-CoV-2 nucleocapsid (N) gene. The inclusivity of the N1 and N2 primer and probe sequences was evaluated in silico using the 6,658,946 coronavirus strains available in the NCBI database as of January 25th, 2023. Sequence homology was compared using the Basic Local Alignment Search Tool (BLAST, NCBI). No mismatches were identified among the 5000 strains evaluated for any of the N1 or N2 primer or probe sequences. From this analysis it was determined that well above 95% of the current circulating SARS-CoV-2 sequences aligned using the NCBI BLASTn tool showed a 100% sequence match to all primers/probes utilized in the ICTC SARS-CoV-2 RT-PCR Assay. In order to account for newly emerging SARS-CoV-2 variants, inclusivity analyses will be conducted monthly.

3) Cross-Reactivity (Analytical Specificity), Microbial Interference,

The N1 and N2 primers utilized in the ICTC SARS-CoV-2 RT-PCR Assay are identical to those of the CDC nCoV Real-Time RT-PCR Diagnostic Panel. The CDC performed both an *in silico* analysis and wet-testing to evaluate cross-reactivity. The CDC has granted right of reference to use the performance data submitted to the FDA as part of EUA200001.

4) Interfering Substances:

The interference of various substances on the performance of the extraction methods utilized in the ICTC SARS-CoV-2 RT-PCR Assay was previously assessed by ThermoFisher (EUA200010) for the same isolation kit used in the ICTC assay - MagMAX Viral/Pathogen II Nucleic Acid Isolation Kit. ThermoFisher has provided a right of reference to ICTC for use of this study data as part of the ICTC EUA submission.

5) Specimen Stability:

The ICTC SARS-CoV-2 RT-PCR Assay may be used with AN dry swabs that can be placed directly in sample tubes containing 1 mL of VTM and stored following CDC guidelines or immediately run on the assay. Additionally, AN samples can be placed into sterile tubes as dry swabs for up to 24 hours and re-hydrated in 1% SDS-saline buffer before immediately processing. If a delay in testing is expected, samples are to be stored at 2-8°C for up to 72 hours or frozen at -80°C prior to testing as recommended by the CDC's Guidelines for Collecting and Handling of Clinical Specimens for COVID-19 Testing.

A stability study was completed to demonstrate the stability of the dry AN swabs held at room temperature for 24 hours post collection and prior to resuspension. Forty (40) dry swabs containing human clinical matrix were spiked with irradiated virus (SARS-Related Coronavirus 2, Isolate USA-WA1/2020, Gamma-Irradiated, NR-52287) at a concentration of 3x the LoD (150GCE/mL) for the ICTC SARS-CoV-2 RT-PCR Assay and left to incubate at room temperature in sterile sample collection tubes. After 24 hours, the samples were tested using the ICTC SARS-CoV-2 RT-PCR Assay. Results of the testing showed 39/40 samples were determined to be positive after the 24 hour incubation period.

6) Clinical Evaluation:

Clinical performance of the ICTC SARS-CoV-2 RT-PCR Assay evaluated concordance of results from AN swab specimens in Viral Transport Media (VTM), collected from individuals suspected of infection with SARS-CoV-2 with those of an EUA authorized molecular comparator test. A total of 85 specimens, 45 positive and 40 negative, were collected in local healthcare offices from individuals suspected of infection with SARS-CoV-2 and transported to the Baystate Reference Laboratories (BRL) in Holyoke, MA. After testing at BRL, specimens were then stored at 2-8C for up to 48 hours before being de-identified and transported to the IALS Clinical Testing Center (ICTC), and immediately run on the ICTC SARS-CoV-2 RT-PCR Assay. All negative specimens were negative for both the candidate and comparator test. All positive specimens, including 19 specimens determined to be low positives (i.e., within 3 Ct of the mean Ct at the LOD of the comparator test), were determined to be positive for both the candidate and comparator test.

Table 8. Clinical Evaluation Results

		EUA Authorized Comparator Test	
		Positive	**Negative**
ICTC SARS-CoV-2 RT-PCR Assay	**Positive**	45	0
	Negative	0	40
Positive Agreement		100% (45/45), 95% CI [92.1%, 100%]	
Negative Agreement		100% (40/40), 95% CI [91.2%, 100%]	
Overall Agreement		100% (85/85), 95% CI [95.7%, 100%]	

WARNINGS

- For *in vitro* diagnostic use.

- For prescription use only.
- For use under Emergency Use Authorization (EUA) only.
- This product has not been FDA cleared or approved, but has been authorized for emergency use by FDA under an EUA for use by the authorized laboratory.
- This product has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.
- The emergency use of this product is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of *in vitro* diagnostics 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 declaration is terminated or authorization is revoked sooner.
- This assay is for testing anterior nasal specimens from individuals 2 years of age and older.

LIMITATIONS

- The use of this assay as an in vitro diagnostic is limited to IALS (Institute for Applied Life Sciences) Clinical Testing Center, LLC (ICTC) at the University of Massachusetts, Amherst MA 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. 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 clinical performance of the ICTC SARS-CoV-2 RT-PCR Assay was assessed using anterior nasal swabs (ANS) samples collected in VTM. Analytical performance of the assay was further assessed using both ANS samples collected in VTM and dry ANS samples suspended in saline/1% SDS buffer.
- The performance of this test was established based on the evaluation of a limited number of clinical specimens. Clinical performance has not been established with all circulating variants but is anticipated to be reflective of the prevalent variants in circulation at the time and location of the clinical evaluation. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of SARS-CoV-2 and their prevalence, which change over time.
- 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.
- Detection of RNase P indicates that human nucleic acid is present and implies that human biological material was collected and successfully extracted and amplified. It does not necessarily indicate that the specimen is of appropriate quality to enable detection of SARS-CoV-2.