EMERGENCY USE AUTHORIZATION (EUA) SUMMARY
iC SARS-CoV-2 Test
(Tempus Labs, Inc.)

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

(The iC SARS-CoV-2 Test will be performed at laboratories designated by Tempus Labs, Inc. which are certified under Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a and meet requirements to perform high-complexity tests, as described in the Laboratory Standard Operating Procedure that was reviewed by the FDA under this EUA).

INTENDED USE

The iC SARS-CoV-2 Test is a reverse transcription, real-time polymerase chain reaction (RT-PCR) test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in nasopharyngeal (NP), anterior nares (AN or anterior nasal), mid-turbinate nasal, and oropharyngeal (OP) swab specimens) collected from individuals suspected of COVID-19 by their healthcare provider.

This test is also for use with AN swab specimens that are self-collected unsupervised at home by individuals 18 years of age or older using the Tempus Nasal Sample Collection Kit, when determined to be appropriate by their healthcare provider.

Testing is limited to laboratories designated by Tempus Labs, Inc. which are certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, and meet requirements to perform high complexity tests.

Results are for the identification of SARS-CoV-2 RNA. 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 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 epidemiologic information.

The iC SARS-CoV2 Test is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the techniques of Real-Time PCR and in vitro diagnostic

procedures. The iC SARS-CoV-2 Test is only for use under the Food and Drug Administration's Emergency Use Authorization.

DEVICE DESCRIPTION AND TEST PRINCIPLE

The Tempus Labs, Inc iC SARS-CoV-2 Test is a modification of the Applied Biosystems TaqPath COVID-19 Combo Kit (Thermo Fisher Cat # A47814), authorized under EUA200010. The test is a Real-Time reverse transcription polymerase chain reaction (RT-PCR) assay. The iC SARS-CoV-2 test uses the TaqPath COVID-19 Combo kit primer and probe sets, which are designed to detect RNA from SARS-CoV-2 nucleocapsid (N) gene, spike (S) gene and open reading frame 1ab (ORF1ab) region in respiratory specimens collected from individuals suspected of COVID-19 by their healthcare provider.

Specimens including NP, AN, mid-turbinate nasal, and OP swab specimens collected from individuals suspected of COVID-19 by their healthcare provider may be stored in VTM, Mawi medium, or 0.85% saline.

Samples collected in VTM are stored according to CDC recommendations. Samples collected in Mawi medium are stored at room temperature for up to 7 days.

AN samples collected in saline are stored at ambient temperature (18-25ºC) and must be extracted within 48 hours of collection. Samples not extracted within 48 hours of collection will be deemed unsuitable for processing and will not be further processed.

AN samples may be self-collected "unsupervised" in a home setting by individuals 18 years of age or older using the Tempus Nasal Collection Kit.

The Tempus labs, Inc iC SARS-CoV-2 test differs from the Applied Biosystems TaqPath COVID-19 Combo Kit specifically in that HCP collected specimens are collected in MAWI DNA Technologies iSWAB-Microbiome collection tubes, VTM, or in 0.85% saline. Nucleic acids are isolated and purified using the Chemagic Viral DNA/RNA 300 H96 kit on the PerkinElmer Chemagic 360 instrument (software version 6.3.0.3) for automated RNA extraction. The purified RNA is reverse transcribed into cDNA and amplified and detected using TaqPath COVID-19 Combo Kit on the Applied Biosystem QuantStudio 7 Flex 384 instrument (software version 1.3). Patient results are communicated directly to the ordering physician via secure email and/or online portal.

Home Collection Kit Ordering and Processing

Nasal specimens (NA) must be self-collected in the home setting using the Tempus Nasal Sample Collection Kit which can be ordered via one of two workflows:

1. The individual is screened for testing eligibility by their healthcare professional (HCP). After HCP determination of testing eligibility, the HCP places a test order with Tempus Labs, Inc for their patient. The HCP provides the patient's address to Tempus Labs, Inc. Tempus labs, Inc (via a fulfillment center) ships the Tempus Nasal Sample Collection Kit

to the individual suspected of COVID-19, as determined to be appropriate by the HCP. In this ordering process, Tempus Labs, Inc does not accept requests for kits from patients directly and does not provide a questionnaire to determine eligibility for COVID testing, since this is left to the ordering HCP to consider appropriate guidelines in determining the best treatment for their patient.

2. Alternatively, the individual can request a test on Tempus Labs, Inc website (www. Tempuslabs.com/infectiousdiseases.com) after completing a required health COVID-19 eligibility questionnaire via a secure online portal (based on current CDC testing guidelines). A healthcare provider (from an independent physician organization contracted by Tempus (e.g., PWN) will review the individual response to the questionnaire, determine eligibility and issue an order for the home collection kit as appropriate. Tempus (via a fulfillment center) ships the Tempus Nasal Sample Collection Kit to the individual suspected of COVID-19 as determined to be appropriate by the HCP. Individuals exhibiting severe COVID warning symptoms should seek emergency medical care.

An individual deemed to be appropriate for testing receives the kit and proceeds with unsupervised anterior nasal swab specimen collection following the Instructions for Use (IFU) provided inside the kit, and returns the kit to the Tempus Labs, Inc. designated laboratory via FedEx. All results are provided to the ordering health care provider and the patient via secure email and/or online portal. An HCP will directly contact individuals with positive or invalid/inconclusive test results. In addition, a link to the fact sheets for both HCP and patient for the test is included in the test report that goes back to the patient via the online portal.

The Tempus Nasal Sample Collection Kit contains a 3 inch polyester swab, 5 mL polypropylene transport tube with 1.2 mL 0.85% saline, biohazard specimen bag with absorbent material, alcohol prep pad, adhesive label, return box and pre-labeled FedEx envelope along with Instructions for Use. The Instructions for Use outlines the kit handling procedures, the self-collection process, and step-by-step instructions to ship samples on the same day of collection for next day delivery to the laboratory.

After opening of the Tempus Nasal Sample Collection Kit, the patient is first instructed to activate their kit online at activate.tempus.com, following the instructions on the web page to fill in the necessary registration information and sample collection date and time. Next, the patient is instructed to insert the swab into one nostril, swab 4 times and hold for 15 seconds. Then, the patient repeats the same process in the other nostril using the same swab. The patient then places the swab, tip first, into the provided transport tube containing 1.2mL of 0.85% saline and screws the cap onto the sample transport tube until it is closed tightly. The patient is instructed to place the sample transport tube into the biohazard specimen bag, seal the bag tightly, then place in the provided box and seal inside the provided, self-addressed shipping envelope. Finally, the patient is instructed to drop off the sealed collected specimen at a FedEx drop off location or arranged pickup on the same day that it was collected.

The specimen collection instructions for use provide an outline of the kit handling procedures, the self-collection process, and step-by-step instructions to ship samples on the same day of

collection for next day delivery to the laboratory to support 48 h specimen stability claim. Specimens are shipped at ambient temperature (18-25ºC) and received at the clinical laboratory for testing with the iC SARS-CoV-2 test within 48h.

Specimens received for testing at authorized laboratory will undergo a thorough review and accessioning prior to acceptance for testing with the iC SARS-CoV-2 Test which was authorized on October 1, 2020. Specimens that arrive in the laboratory after 48 hours of sample collection will not be processed.

Home-collected specimens received at laboratories designated by Tempus labs, Inc. for testing with the iC SARS-CoV-2 Test undergo the following accessioning prior to acceptance for testing:

- Date of shipment received must be less than or equal to 48 hours from the date of sample collection
- If more than one swab is present in the tube, the sample will be deemed unsuitable for processing and will not be extracted.
- Tubes that are open or damaged with leaked media, and empty tubes are rejected
- Tubes with missing or damaged identifiers are rejected
- Non-Tempus tubes and swabs for unsupervised collection are rejected

Specimen information (e.g. date and time of receipt) and verified patient information is logged into the Tempus Labs, Inc. Information Management System (LIMS) system for tracking. Specimens are accessioned and tracked with unique sample and patient identifiers. Identifiers are verified on all test orders. Acceptable specimens proceed to extraction after secondary peer review confirming all sample information is correct and matched to the appropriate clinical order.

MATERIALS AND INSTRUMENTS FOR USE WITH THE TEST

The iC SARS-CoV-2 test is to be used with the instruments in **Table 1** below.

Table 1. Instruments Used for the iC SARS-CoV-2 Test

Instrument	Manufacturer	Software Version
Chemagic 360 * using 96 well plates	PerkinElmer	v6.3.0.3
Applied Biosystems QuantStudio 7 Flex Real-Time PCR system using 384 well plates *[a]	Thermo Fisher	v1.3

* These instruments were used for the validation studies presented herein.

[a] Thermo Fisher has granted a right of reference to any sponsor wishing to pursue an EUA to leverage their data addressing the RUO qualification of this instrument.

REAGENTS AND MATERIALS

Table 2. Reagents and Materials Required to Perform the iC SARS-CoV-2 Test

Reagent	Manufacturer	Catalog #
Chemagic Viral DNA/RNA 300 H96 Kit[1,2]	PerkinElmer	CMG-1033-S
Applied Biosystems TaqPath COVID-19 Combo Kit[1,2]	Thermo Fisher	A47814
TaqMan SARS-CoV-2 RNase P Assay Kit[2]	Thermo Fisher	A49564
Chemagic 360 96-deep well plates[12]	PerkinElmer	CMG-555
TaqPath 1-Step Multiplex Master Mix (No ROX) [12]	Thermo Fisher	A28523
iSWAB-Microbiome collection tubes (HCP Collection at healthcare setting)[1]	Mawi DNA Technologies	ISWAB-MB-1200
Swabs in accordance with FDA recommendations: nylon flocked or spun polyester (HCP Collection)[1]	Various suppliers	Various catalog numbers
Viral Transport Medium (HCP Collection)[1]	Commercially available or prepared according to CDC SOP#: DSR-052-04	N/A
5 ml transport tube prefilled with 1.2ml saline fill[2]	Wuxi NEST Biotechnology or	202141
	Apollo Renal Therapeutics (DBA - Artemis Plastics), or	SFV-2021-5
	Ningbo Dasky Life Science	VSM03-8011923

[1] These components are applicable for HCP collection in a healthcare setting only.
[2]These components are applicable for unsupervised home collection and/or HCP collection in a healthcare setting.

HOME COLLECTION KIT USED WITH THE TEST

The Tempus Nasal Sample Collection Kit collection device consists of a polyester swab, transport tube with saline, a biohazard bag, an alcohol prep pad, adhesive label, a prepaid shipping envelope, a cardboard box, and instructions.

Table 3. Reagents and Materials Included in the Tempus Nasal Sample Collection Kit

Supply	Manufacturer	Catalog #
3 inch polyester swab	SteriPack USA or other manufacturer	60564-RevA
5 ml transport tube with 1.2ml saline fill	Wuxi NEST Biotechnology, or	202141
	Apollo Renal Therapeutics (DBA - Artemis Plastics), or	SFV-2021-5
	Ningbo Dasky Life Science	VSM03-8011923
95kPa Biohazard specimen bag with absorbent pad for transport	Vonco Products or other manufacturer	N/A
Individually wrapped 70% isopropyl alcohol prep pad	Various suppliers	N/A
Custom 8x6x1” shipping box and FedEx UN3373 Pak	Federal Industries, FedEx or other manufacturer	N/A
Instructions for specimen collection and return of samples to Tempus	Tempus Labs	N/A
Adhesive label	Various suppliers	N/A

CONTROLS

The controls run with the iC SARS-CoV-2 Test are described in **Table 4**.

Table 4. Controls used with the iC SARS-CoV-2 Test

Control Type	Description	Purpose	Frequency of Testing
Positive	COVID-19 control RNA (ORF1ab, gene for the S protein, and gene for the N protein), Applied Biosystems TaqPath COVID-19 Combo Kit	Monitor the integrity of the PCR reagents and process.	Used on every PCR plate
Negative	Molecular grade, nuclease-free water plus MS2 bacteriophage spike-in control	Monitor background noise and/or contamination	Used on every extraction and PCR plate
Internal	MS2 bacteriophage spike-in control from TaqPath COVID-19 Combo Kit	Monitor RNA extraction and RT-PCR	Used in every patient sample and negative control
Internal	RNase P is in human specimens.	Verify the presence of human specimen in the collected sample	Used in every patient sample collected with the Tempus Nasal Sample Collection Kit

INTERPRETATION OF RESULTS

All test controls must be examined prior to interpretation of patient results. If the positive and negative controls are not valid, the patient results cannot be interpreted. The results from the controls are interpreted according to the criteria shown in **Table 5.**

Table 5. Interpretation of iC SARS-CoV-2 Test Controls

Control Type	Used to Monitor	N gene	S gene	ORF1ab	MS2	RNase P[2]
No Template Control	To check for contamination of PCR reagents or contamination occurring during PCR plate set-up.	Not detected	Not detected	Not detected	Not detected	Not detected
Positive	To validate the integrity of the RT-PCR reagents and process.	Detected Ct[1] ≤37	Detected Ct ≤37	Detected Ct ≤37	Not detected	Not detected
Negative Extraction Control	To validate a successful RNA extraction and monitor for any cross contamination that occurs during the extraction process.	Not detected	Not detected	Not detected	Detected Ct ≤32	Not detected
Internal Controls (MS2)	To verify successful extraction of the sample, proper assay setup, sample integrity, and efficient sample collection.	N/A	N/A	N/A	Detected Ct ≤32	N/A

RNase P Internal Control	Verify the presence of human specimen in collected sample	**N/A**	**N/A**	**N/A**	**N/A**	**Detected Ct ≤33**

[1] Cycle threshold (Ct) is defined as the number of cycles required for the fluorescent signal to cross the threshold.
[2]RNAse P is only tested for nasal specimens that are self-collected by individuals at home using the TempusNasal Sample Collection Kit. RNAse P is not required for testing upper respiratory specimens that are collected in healthcare facilities.

The results from testing of patient samples are interpreted according to the criteria described in Table 6 using the Applied Biosystems COVID-19 Interpretive Software version 2.5. An internal control targeting RNase P is used to verify that nucleic acid is present in every sample that is self-collected with the Tempus Nasal Sample Collection Kit in a home setting (**Table 6**).

Table 6. Interpretation of iC SARS-CoV-2 Test

ORF1ab	N gene	S gene	MS2	RNase P[2]	Status	Result	Action
NEG	NEG	NEG	NEG	NEG	INVALID	N/A	Repeat test. If the repeat result remains invalid, consider collecting a new specimen.
NEG	NEG	NEG	NEG	POS[1]	INVALID	N/A	Repeat test. If the repeat result remains invalid, consider collecting a new specimen.
NEG	NEG	NEG	POS[1]	POS[1]	VALID	SARS-CoV-2 Not Detected	Report results. Consider testing for other viruses.
NEG	NEG	NEG	POS[1]	NEG	INVALID	N/A	Repeat test. If the repeat result remains invalid, consider collecting a new specimen.
Only one SARS-CoV-2 target = POS[1]			POS or NEG		VALID	SARS-CoV-2 Inconclusive	Repeat test on original sample. If the repeat result remains inconclusive, report results. Additional confirmation testing should be conducted if clinically indicated.
Two or more SARS-CoV-2 targets = POS[1]			POS or NEG		VALID	SARS-CoV-2 Detected	Report results.

[1] Interpretation of results is based on the cycle threshold (Ct) cutoff values provided in the most recent authorized labeling for the TaqPath COVID-19 Combo Kit (EUA200010). Cycle threshold (Ct) is defined as the number of cycles required for the fluorescent signal to cross the threshold.
[2]RNAse P is only tested for nasal specimens that are self-collected by individuals at home using the TempusNasal Sample Collection Kit. RNAse P is not required for testing upper respiratory specimens that are collected in healthcare facilities.

PERFORMANCE EVALUATION

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

The LoD of the iC SARS-CoV-2 test was determined using heat inactivated SARS-CoV-2 (ATCC) spiked into negative nasopharyngeal swab matrix collected in Mawi medium or VTM.

a. Preliminary LoD

The preliminary LoD for the iC SARS-CoV7 test was determined using serial dilutions with 5 replicates per dilution using known titers (shown in copies/mL and GCE/reaction) of heat inactivated SARS-CoV-2 (ATCC) in negative nasopharyngeal swab matrix collected in Mawi medium or VTM. Spiked samples were tested with the iC SARS-CoV-2 test (N gene, S gene and ORF1ab detection) following the laboratory Standard Operating Procedure (SOP). Results are summarized in **Table 7**.

Table 7. Preliminary LoD for Mawi media and VTM

Concentration		Mawi Media					VTM				
GCE / reaction	Copies / mL	ORF1ab Mean Ct	N gene Mean Ct	S gene Mean Ct	MS2 Mean Ct	SARS-CoV-2 detected rate	ORF1ab Mean Ct	N gene Mean Ct	S gene Mean Ct	MS2 Mean Ct	SARS-CoV-2 detected rate
450	10000	27.1	27.5	27.0	23.2	100%	27.7	28.2	27.9	20.7	100%
225	5000	28.2	28.5	27.9	23.1	100%	28.3	29.2	28.5	20.6	100%
113	2500	28.9	29.5	28.9	22.1	100%	28.5	30.0	29.2	20.4	100%
45	1000	30.4	30.6	30.0	22.7	100%	30.5	31.1	31.2	20.6	100%
23	500	31.5	31.7	31.0	22.8	100%	31.6	32.3	32.3	20.5	100%
11	**250**	**32.3**	**33.3**	**33.3**	**22.6**	**100%**	**35.0**	**33.7**	**35.8**	**21.0**	**100%**
6	125	38.3	33.7	39.8	22.3	40%	37.1	35.8	38.4	21.0	60%
0	0	Und.[1]	Und.[1]	Und.[1]	22.9	0%	Und.[1]	Und.[1]	Und.[1]	20.9	0%

Und.[1]: Undetermined

b. Confirmatory LoD

To validate the preliminary LoD, an additional 32 replicates at 1x LoD were tested. Dilutions were generated as above using heat inactivated SARS-CoV-2 (ATCC) spiked into negative nasopharyngeal swab matrix collected in Mawi medium or VTM. Spiked samples were tested with the iC SARS-CoV-2 Test following the laboratory SOP. Results are summarized in **Table 8.**

The LoD of the iC SARS-CoV-2 Test was determined to be 250 copies/mL (0.25 copies/µL) or 11 GCE/reaction for both Mawi media and VTM.

Table 8. Confirmatory LoD of the iC SARS-CoV2 test for Mawi media and VTM

Transport Medium	SARS-CoV-2 GCE/reaction	SARS-CoV-2 copies/mL	Positives	N gene Mean Ct	N gene Std Dev (Ct)	S gene Mean Ct	S gene Std Dev (Ct)	ORF1ab Mean Ct	ORF1ab Std Dev (Ct)	MS2 Mean Ct	MS2 Std Dev (Ct)
Mawi	**11**	**250**	32/32	33.0	1.0	33.2	1.7	32.3	1.3	21.3	0.6
VTM	**11**	**250**	32/32	31.6	0.5	32.0	0.6	31.4	1.0	20.8	0.4

Limit of Detection in Saline:
The preliminary LoD for saline was established by a 2-fold dilution series with three (3) pooled saline nasopharyngeal (NP) specimen replicates inoculated with heat-inactivated SARS-CoV-2 virus (**Table 9**).

Table 9. Preliminary LoD for Saline

Concentration		Saline				
GCE/ reaction	Copies/mL	ORF1ab Mean Ct	N gene Mean Ct	S gene Mean Ct	MS2 Mean Ct	SARS-CoV-2 detected rate
450	10000	28.8	29.4	29.0	24.0	100%
225	5000	29.7	30.3	30.1	23.7	100%
113	2500	30.4	31.3	31.0	23.6	100%
45	1000	31.9	33.4	32.9	23.4	100%
23	500	32.4	34.9	35.8	23.3	100%
11	250	35.6	37.5	38.3	23.5	33%
6	125	36.1	37.3	Und.[1]	23.5	33%
0	0	Und.[1]	Und.[1]	Und.[1]	23.7	0%

[1]Undetermined.

To confirm the LoD, 20 replicates from the LoD determined in the preliminary range finding study were tested by spiking heat-inactivated SARS-CoV-2 virus (ATCC) into negative saline nasopharyngeal specimen replicates (**Table 10**). Spiked samples were tested with the iC SARS-CoV-2 Test following the laboratory SOP. The LoD for saline was determined to be 500 copies/mL (**Table 10**).

Table 10. Confirmatory LoD for Saline

Transport Medium	SARS-CoV-2 GCE/	SARS-CoV-2 copies/mL	Positives	N gene	N gene Std	S gene	S gene Std	ORF1ab Mean Ct	ORF1ab Std Dev (Ct)	MS2 Mean Ct	MS2 Std

	reaction			Mean Ct	Dev (Ct)	Mean Ct	Dev (Ct)				Dev (Ct)
Saline	23	500	20/20	33.2	0.5	33.2	1.5	32.5	1.6	24.8	0.3

2) Inclusivity (analytical sensitivity):

The iC SARS-CoV-2 Test uses the primers/probes included in the Thermo Fisher TaqPath COVID-19 combo kit. Please refer to EUA20010 and amendments for analytical inclusivity analysis.

3) Cross-reactivity (analytical specificity):

The iC SARS-CoV-2 Test uses the primers/probes included in the ThermoFisher TaqPath COVID-19 combo kit. Cross-reactivity has been established in EUA200010 in the "*in silico* cross reactivity analysis".

4) Clinical Evaluation

a) Contrived Sample Testing:

Performance of the iC SARS-CoV-2 Test was first evaluated using a total of 38 non-reactive (negative) and 37 contrived reactive (positive) clinical samples. Contrived-reactive specimens were prepared by spiking Twist Bioscience synthetic SARS-CoV-2 RNA Control 1 into leftover non-reactive clinical specimens at 2X LoD (i.e., nasopharyngeal swab (NP), anterior nares (AN) swab) collected in Mawi or VTM (**Table 11A**).

Table 11A. Non-reactive and Contrived-Reactive Specimens

Collection Media	Swab type	Non-reactive	Contrived-Reactive (2X LoD)
Mawi	AN	11	13
Mawi	NP	10	5
VTM	NP	17	19
Total Samples:		38	37

The samples were processed per Tempus Labs, Inc protocol with the Applied Biosystems™ TaqPath COVID-19 Combo Kit using the Chemagic 360 and Applied Biosystems™ QuantStudio 7 Flex Real-Time PCR system.

The results demonstrated 100% agreement against the expected results with all contrived samples at 2X LoD and all negative clinical specimen (**Table 11B**).

Table 11B. Summary of Contrived Sample Testing

Concentration	Collection Media & Swab Type	# of Swabs	N Gene			S Gene			ORF1ab			MS2 Internal Control		
			Positive	%	Ct avg	Positive	%	Ct avg	Positive	%	Ct avg	Positive	%	Ct avg
2x LOD	VTM-NP	19	19/19	100	30.6	19/19	100	29.7	19/19	100	30.0	19/19	100	22.7
2x LOD	Mawi-NP	5	5/5	100	30.1	5/5	100	29.2	5/5	100	29.4	5/5	100	23.1
2x LOD	Mawi-AN	13	13/13	100	30.8	13/13	100	29.9	13/13	100	30.2	13/13	100	27.5
Negative	VTM-NP	17	0/17	0	Und[.1]	0/17	0	Und[.1]	0/17	0	Und[.1]	17/17	100	23.1
Negative	Mawi-NP	10	0/10	0	Und[.1]	0/10	0	Und[.1]	0/10	0	Und[.1]	10/10	100	24.2
Negative	Mawi-AN	11	0/11	0	Und[.1]	0/11	0	Und[.1]	0/11[.1]	0	Und[.1]	11/11	100	28.8

Und[.1]: Undetermined

b) Clinical Study against FDA authorized Test #1

The clinical performance of the iC SARS-CoV-2 Test was further evaluated by testing a total of 61 clinical nasopharyngeal (NP) swab specimens collected in VTM that were previously determined to be negative or positive for SARS-CoV-2 by an FDA authorized, highly sensitive molecular test.

The samples were processed per the iC SARS-CoV-2 Test protocol using the Chemagic 360 and Applied Biosystems™ QuantStudio 7 Flex Real-Time PCR system.

Performance of the iC SARS-CoV-2 Test when compared to the comparator was 100% positive agreement (30/30 samples) and 96.8% negative agreement (30/31 samples), as summarized in **Table 12A**.

Table 12A. Performance of iC SARS-CoV-2 Test vs. FDA authorized Test #1

Patient NP Specimens		Comparator: FDA EUA Test#1		
		Positive	Negative	Total
iC SARS-CoV-2 Test	Positive	30	1	31
	Negative	0	30*	30
	Total	30	31	61
Positive Percent Agreement (PPA)		100% (30/30) (95% CI: 88.7%-100%)[1]		

Negative Percent Agreement (NPA)	96.8% (30/31) (95% CI: 83.8%-99.4%)[1]

[1]95% confidence interval calculated using the score method.
*One sample that was inconclusive was re-run as per protocol and determined to be negative. It was included in the statistical analysis.

c) Clinical Study against FDA authorized Test #2

The clinical performance of the iC SARS-CoV-2 Test was also evaluated by testing a total of 60 clinical NP swab specimens collected in MAWI medium that were previously determined to be negative or positive for SARS-CoV-2 by a second FDA authorized test.

Performance of the iC SARS-CoV-2 Test when compared to the second comparator was 100% positive agreement (29/29 samples) and 100% negative agreement (30/30 samples), as summarized in **Table 12B**.

Table 12B. Performance of iC SARS-CoV-2 Test vs. FDA authorized Test#2

Patient NP Specimens		**Comparator: FDA EUA Test#2**		
		Positive	**Negative**	**Total**
iC SARS-CoV-2 Test	**Positive**	29	0	29
	Negative	0	30	30
	Inconclusive*	1	0	1
	Total	30	30	60
Positive Percent Agreement (PPA)		100% (29/29) (95% CI 88.3% 100%)[1]		
Negative Percent Agreement (NPA)		100% (30/30) (95% CI 88.3% 100%)[1]		

[1]95% confidence interval calculated using the score method.
*One sample that was inconclusive (*ORF1ab:37.5, N gene:36.1, S gene: Und.1, MS2: 22.9)* was re-run as per protocol and remained inconclusive. It was not included in the agreement calculations.

d) Post-authorization Performance of iC SARS-CoV-2 Test vs. FDA authorized Test#3:

The clinical performance of the iC SARS-CoV2 test was evaluated by testing a total of 80 clinical NP swab specimens collected in viral transport medium (VTM) that were previously determined to be negative or positive for SARS-CoV-2 by a third FDA authorized, highly sensitive molecular test.

Performance of the iC SARS-CoV-2 Test when compared to the third comparator was 95% positive agreement (38/40 samples) and 100% negative agreement (40/40 samples), as summarized in **Table 12C**.

This study included 50% low positives for Target 1 (20/40 positive samples) and 42.5% low positives for Target 2 (17/40 positive samples) based on the limit of detection (LoD) Ct values for the comparator FDA authorized test.

Table 12C. Performance of iC SARS-CoV-2 Test vs. FDA authorized Test #3

Patient NP Specimens		Comparator: FDA EUA Test#3		
		Positive	Negative	Total
iC SARS-CoV-2 Test	**Positive**	38	0	38
	Negative	2	40	42
	Total	40	40	80
Positive Percent Agreement (PPA)		95.00% (38/40); (95% CI 83% 99%)[1]		
Negative Percent Agreement (NPA)		100% (40/40); (100% CI 91% 100%)[1]		

[1]The confidence interval was calculated using the Clopper-Pearson method.

5) Specimen Stability Study for Mawi iSWAB Microbiome Medium

To verify that SARS-CoV-2 samples are stable at room temperature (25°C) for 8 days in Mawi iSwab Microbiome medium, an 8-day stability study was conducted to confirm minimal loss in positive signal and no change in negative signal.

Twenty (20) negative nasopharyngeal (NP) clinical specimens in Mawi iSwab Microbiome medium were spiked at 2x LoD (500 copies/mL) with heat-inactivated SARS-CoV-2 virions to simulate low-positive SARS-CoV-2 clinical samples. The study also included 5 negative NP clinical specimens. On Days 0, 1, 4, and 8, a 300µL aliquot was taken from each of the 20 positive replicates and each of the 5 negative replicates.

Extraction was performed using the Chemagic Viral DNA/RNA 300 H96 Kit (PerkinElmer CMG-1033-S) on the Chemagic 360 instrument. RT-qPCR was performed per the Tempus Labs, Inc protocol using the Applied Biosystems™ TaqPath COVID-19 Combo Kit (Thermo Fisher A47814) on the Applied Biosystems™ QuantStudio 7 Flex Real-Time PCR system, software version 1.3.

There was 100% agreement with expected results as all samples remained positive on days 0, 4, 8. On day 1, one replicate result produced inconclusive result due to a borderline Ct value (37.0) on the S gene and an undetermined Ct value on ORF1ab; however, this was determined to be acceptable since SARS-COV-2 was detected on days 4 and 8 for this replicate with all three targets. At each time point 100% (5/5) of the negative replicates were negative (**Table 13**).

Table 13. Specimen Stability in Mawi iSwab Microbiome Medium

Sample type	Time (days)	ORF1ab		N gene		S gene		MS2 (IC)		% Agreement
		Ave Ct	SD	Avg Ct	SD	Avg Ct	SD	Avg Ct	SD	
SARS-CoV-2 Positive	0	32.0	1.3	32.2	0.7	31.7	0.7	21.7	0.7	100 (20/20), 95% CI (83.2-100)[2]
	1	35.6	2.7	33.5	1.2	34.5	3.1	21.9	0.4	95 (19/20), 95% CI (75.1-99.9)[2]
	4	32.3	2.8	33.4	1.1	33.1	3.0	20.9	0.6	100 (20/20), 95% CI (83.2-100)[2]
	8	34.7	3.2	33.3	0.9	33.4	1.6	22.3	0.5	100 (20/20), 95% CI (83.2-100)[2]
SARS-CoV-2 Negative	0	Und.[1]	N/A	Und.[1]	N/A	Und.[1]	N/A	21.9	1.0	100 (5/5), 95% CI (47.8-100)[2]
	1	Und.[1]	N/A	Und.[1]	N/A	Und.[1]	N/A	21.7	0.5	100 (5/5), 95% CI (47.8-100)[2]
	4	Und.[1]	N/A	Und.[1]	N/A	Und.[1]	N/A	21.5	1.0	100 (5/5), 95% CI (47.8-100)[2]
	8	Und.[1]	N/A	Und.[1]	N/A	Und.[1]	N/A	23.9	2.0	100 (5/5), 95% CI (47.8-100)[2]

[1]Undetermined.
[2]Clopper-pearson CI calculation

The results of this study demonstrate that SARS-CoV-2 samples are stable at room temperature (25°C) for 8 days in Mawi iSwab Microbiome medium with no impact on SARS-CoV-2 detection.

6) 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 Chemagic Viral DNA/RNA 300 H96 Kit (PerkinElmer CMG-1033-S) on the Chemagic 360 instrument and RT-qPCR using the FDA EUA authorized Applied Biosystems™ TaqPath COVID-19 Combo Kit (Thermo Fisher A47814) on the Applied Biosystems™ QuantStudio 7 Flex Real-Time PCR, system version 1.3. Data in Table 14A and Table 14B was analyzed using the Applied Biosystems COVID19 Interpretive Software v2.2. The results are summarized below.

Table 14A: 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 Swabs in Mawi media	$2.4x10^3$ NDU/mL	N/A
MERS-CoV		N/A	ND

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

Table 14B: 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 Swabs in VTM media	$1.2 x10^3$ NDU/mL	N/A
MERS-CoV		N/A	ND

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

7) ***Media Equivalency Study***

To show equivalent performance of normal saline (saline) to viral transport medium (VTM), a media equivalency study was performed comparing SARS-CoV-2 negative nasopharyngeal (NP) swab specimens collected in saline and SARS-CoV-2 negative NP swab specimens collected in VTM. The study was run using VTM as the comparison medium because VTM demonstrated higher sensitivity ($1.2 x10^3$ NDU/mL) than Mawi medium ($2.4x10^3$ NDU/mL) on the FDA reference panel. Comparing saline to the higher sensitivity medium was considered the more rigorous study design.

The LoD of saline was determined to be 500 copies/mL and the LoD of VTM was determined to be 250 copies/mL. This study was conducted at 500 copies/mL, which is 2X LoD in VTM. The acceptance criteria for the media equivalency study was the same for saline and VTM at ≥95% positive concordance.

Contrived positive replicates were generated using heat inactivated SARS-CoV-2 (ATCC) spiked into negative NP saline and negative NP VTM pools at 2X VTM LoD (500 copies/mL). Twenty (20) contrived positive replicates in each media type were tested in parallel. Five (5) SARS-CoV-2 negative replicates in each media type were also tested in parallel.

Extraction was performed using the Chemagic Viral DNA/RNA 300 H96 Kit (PerkinElmer CMG-1033-S) on the Chemagic 360 instrument. RT-qPCR was performed per the iC SARS-CoV-2 Test protocol on the Applied Biosystems QuantStudio 7 Flex Real-Time PCR system,

software version 1.3. Data was analyzed using the Applied Biosystems COVID19 Interpretive Software v2.3 (**Table 15**).

The results of this study showed that both saline and VTM spiked at 2x VTM LoD (500 copies/mL) had 100% positivity (20/20), and the negative replicates had 100% negativity for both media types (5/5) (**Table 15**). These results demonstrate that the LoD for saline is comparable to that of VTM.

Table 15. Overall Results for NP Replicates Collected in Saline and VTM

Media	SARS-CoV-2 copies/mL	ORF1ab		N gene		S gene		MS2		Result
		Avg Ct	SD	Avg Ct	SD	Avg Ct	SD	Avg Ct	SD	
Saline	500	32.5	1.6	33.2	0.5	33.2	1.5	24.8	0.3	20/20 Positive
	Negative	Und.[1]	n/a	Und.[1]	n/a	Und.[1]	n/a	23.2	0.3	5/5 Negative
VTM	500	32.2	1.6	32.4	0.6	31.9	0.6	24.8	0.4	20/20 Positive
	Negative	Und.[1]	n/a	Und.[1]	n/a	Und.[1]	n/a	23.2	0.6	5/5 Negative

[1]Undetermined.

8) *Collection Device Stability*

The Tempus Nasal Sample Collection Kit uses wrapped polyester nasal swabs transported in tubes prefilled by the manufacturer with 0.85% saline. The collection device is stored according to the manufacturer's expiration dating.

9) *Human Usability Study*

To support home specimen collection using the Tempus Nasal Sample Collection Kit, a Human Usability Study was conducted. Thirty-six (36) individuals were screened; however, one participant with prior self-collection experience was excluded from the study and another individual enrolled in the study but did not complete a study session or otherwise participated in the study and was lost to follow up. Therefore 34 users participated in the study. Participants represented varying education levels and ages.

After enrollment in the study, the collection kit was shipped to each participant. Each participant collected the nasal sample according the instructions for use at home setting while under observation via video conference, and the observer recorded any difficulties with the registration and sample collection process (refer to summary of these observations below) .

After each sample collection session was completed, the user was given a questionnaire to indicate the ease of use of the kit and sample collection (**Table 16**).

Two out of the 34 participants declined to answer any usability survey questions and were thus excluded from this analysis. Of the 32 participants who responded, 97% (31/32) indicated that

they understood the instructions, and 100% (32/32) responded that they were able to able to complete the full process for sample collection.

Thirty-four samples were shipped via FedEx with the provided pre-paid return envelope and received at Tempus labs, Inc.
Upon receipt, laboratory personnel inspected the packaging, recorded any packaging errors, and noted acceptability of each of the 34 samples for testing. One of the 34 samples was deemed unacceptable for sample testing and was rejected due to a missing second identifier. Thus, 33 out of 34 samples were deemed acceptable for testing.

To ensure that all samples were processed in the same final batch, samples were frozen at -80 degrees Celsius upon receipt at Tempus Labs until all 33 samples were received which is consistent with CDC storage recommendations. Once the full set of 33 samples was received, they were gently thawed and tested for RNase P.

RNase P was detected in all but one (32/33) of the tested samples. Negative control detected no amplification for RNase P and positive control detected amplification for RNase P at 25.89 Ct value. This indicates that the controls were successful.

See **Table 16** below for a summary of the usability study.

Table 16. Human usability study results summary

Participant Summary	**N**	**%**
Participants screened	36	100% (36/36)
Participants with no prior medical or laboratory training	35	97% (35/36)
Kit summary		
Kits distributed	35	100% (35/35)
Kits received for testing	34	97% (34/35)
Participant responses		
Participants who provided feedback	32	91% (32/35)
Participants answering yes to: "Did you understand the instructions provided for the sample collection?"	31	97% (31/32)
Participants answering yes to: "Were you able to complete the full process for sample collection, including shipping the kit back to Tempus?"	31	97% (31/32)
Packing		
Package intact	34	100% (34/34)
Package sealed	34	100% (34/34)
No visible signs of sample leakage	34	100% (34/34)
Sample adequacy		
Sample acceptable for testing	33	97% (33/34)
Sample not acceptable for testing (missing second identifier)	1	3% (1/34)
RNase P test positive	32	97% (32/33)
Observations at sample accessioning – did not preclude testing		
Cardboard box (inside FedEx UN3373 Pak) partially ripped	6	18% (6/33)

Biohazard bag partially open	2	6% (2/33)
Patient DOB sticker on outside of biohazard bag, rather than on tube	1	3% (1/33)
Handwriting for patient DOB difficult to read	1	3% (1/33)

10) Specimen Shipping/Stability

Nasal Samples collected at home are stored in saline at ambient temperature (18-25ºC). RNA extraction occurs within 48 hours following specimen collection.

Quantigen Biosciences has granted a right of reference to any sponsor wishing to pursue an EUA to leverage their COVID-19 summer shipping specimen stability data as part of that sponsor's EUA request. Tempus labs, Inc conducted a winter specimen stability study.

Winter Specimen Stability Study

A study was performed to test the stability of SARS-CoV-2 (SC2) nasal specimen collected in 0.85% saline solution under simulated winter shipping conditions. The study included 10 replicates at 10x LoD, 20 replicates at 2x LoD, and 10 negative replicates.

To prepare the contrived positive replicates, negative nasal specimens were collected in 0.85% saline solution and combined into a single pool. The pooled negative nasal specimen was spiked with irradiated SC2 virus (BEI resources catalog NR-52287; Lot # 70039068, original concentration of 7.85x 10^8 copies/mL) and serially diluted to 10x LoD and 2x LoD. Tubes were then filled with 1.5ml of the following: 2x LoD (low positive sample), 10x LoD (high positive sample) or negative nasal sample. The same polyester swab in the home collection kit was included in each tube.

A 0.3 mL aliquot was removed to test for T = 0 hr. The remaining 1.2 mL of (20) low positive, (10) high positive and (10) pooled negative nasal samples were placed in the incubator and cycled through the Winter Shipping temperatures according to **Table 17**. After 56 hours elapsed, the samples were removed from the incubator, thawed at room temperature (8-25°C), and then tested according to the iC SARS-CoV-2 Test laboratory SOP.

The results are summarized in **Table 18**. This study demonstrates saline-collected nasal specimens are stable for 56 hours after undergoing a simulated winter shipping study, with Ct values remaining within 3 Cts compared to time point zero. Therefore, a 48 hour winter shipping period is substantiated.

Table 17. Winter Shipping Profile

Temperature	Cycle Period	Cycle Period Hours	Total Time Hours
-10 C	1	8	8
18 C	2	4	12
-10 C	3	2	14

10 C	4	36	50
-10 C	5	6	56

Table 18. Winter Profile Results

Sample Group	Total Time	ORF1ab Avg Ct	N gene Avg Ct	S gene Avg Ct	RNaseP Avg Ct	Percent Positive
2x LoD	0 hr	30.9	30.3	31.4	27.5	100% (20/20)
	56 hr	30.8	30.9	31.3	26.1	100% (20/20)
10x LoD	0 hr	28.6	28.4	29.0	27.3	100% (10/10)
	56 hr	29.0	28.9	29.3	26.3	100% (10/10)
Negative	0 hr	Und.[1]	Und.[1]	Und.[1]	27.3	100% (10/10)
	56 hr	Und.[1]	Und.[1]	Und.[1]	26.5	100% (10/10)

[1]Undetermined

Assay Limitations:

- The use of this assay as an In vitro diagnostic under the FDA Emergency Use Authorization (EUA) is limited to laboratories designated by Tempus Labs Inc. which are certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. § 263a, and meet requirements to perform high complexity tests.
- The iC SARS-CoV-2 Test performance was established using nasopharyngeal swab specimens only. Testing using oropharyngeal swab, anterior nasal swabs and mid-turbinate nasal swabs are considered acceptable specimen types for use with the iC SARS-CoV-2 Test, but performance with these specimen types has not been established. Refer to FDA's FAQs on Diagnostic Testing for SARS-CoV-2 for additional information. Specimen types other than nasopharyngeal, oropharyngeal, anterior nasal and mid-turbinate nasal swabs should not be tested with this assay.
- Specimens submitted using the Tempus Nasal Collection Kit must be tested using the TaqMan SARS-CoV-2 RNase P Assay Kit and the iC SARS-CoV-2 Test.
- 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.
- 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 to the specified methods listed in this procedure. Other extraction approaches and processing systems have not been evaluated.
- False-negative results may arise from:

a) Improper sample collection
b) Degradation of the SARS-CoV-2 RNA during shipping/storage
c) Specimen collection after SARS-CoV-2 RNA can no longer be found in the specimen matrix
d) Using unauthorized extraction or assay reagents
e) The presence of RT-PCR inhibitors
f) Mutation in the SARS-CoV-2 virus
g) Failure to follow instructions for use

- False-positive results may arise from:
  a) Cross contamination during specimen handling or preparation
  b) Cross contamination between patient samples
  c) Specimen mix-up
  d) RNA contamination during product handling
- The impacts of vaccines, antiviral therapeutics, antibiotics, chemotherapeutic or immunosuppressant drugs have not been evaluated. The iC SARS-CoV-2 test cannot rule out diseases caused by other bacterial or viral pathogens.
- Negative results do not preclude infection with SARS-CoV-2 virus and should not be the sole basis of a patient management decision.
- Laboratories are required to report all results to the appropriate public health authorities.
- The performance of this test was established based on the evaluation of a limited number of clinical specimens. The clinical performance has not been established in 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.

WARNINGS:

- Caution: Federal Law restricts this device to sale by or on the order of a licensed practitioner.
- The iC SARS-CoV-2 test workflow should be performed by qualified and trained staff to avoid the risk of erroneous results. Use separate areas for the preparation of patient samples and controls to prevent false positive results. Samples and reagents must be handled in a biological safety cabinet.
- For *in vitro* diagnostic use.
- For prescription use only.
- This product has not been FDA cleared or approved but has been authorized by FDA under an EUA for use by authorized laboratories; use by laboratories designated by Tempus Labs, Inc. which are certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. § 263a, and meet requirements to perform high complexity tests.
- This product has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens; and

- 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 Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated or authorization is revoked sooner.
- Individuals exhibiting severe COVID-19 warning symptoms are not allowed to proceed with kit registration and instead directed to seek emergency medical care.
- Federal law restricts this device to be sold by or on the order of a licensed practitioner.
- The Tempus Nasal Sample Collection Kit is intended for individuals 18 years of age and older to self-collect anterior nares specimens unsupervised at home when determined to be appropriate by their Healthcare provider.