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 Tempus Labs, Inc.'s laboratories located at 600 W Chicago Ave, Ste 510, Chicago, IL 60654 and 3155 Northwoods Place, Peachtree Corners, GA 30071, 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 upper respiratory tract specimens (including nasopharyngeal (NP), anterior nares (AN or nasal), mid-turbinate nasal, and oropharyngeal (OP) swab specimens) collected from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to Tempus Labs, Inc.'s laboratories located at 600 W Chicago Ave, Ste 510, Chicago, IL 60654 and 3155 Northwoods Place, Peachtree Corners, GA 30071, 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 generally detectable in respiratory tract 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-CoV2 test is only for use under the Food and Drug Administration's Emergency Use Authorization.

DEVICE DESCRIPTION AND TEST PRINCIPLE

The Tempus 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 from patients as recommended for testing by public health authority guidelines.

The Tempus iC SARS-CoV-2 test differs from the Applied Biosystems™ TaqPath COVID-19 Combo Kit in that specimens are collected in MAWI DNA Technologies iSWAB-Microbiome collection tubes or VTM. 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).

MATERIALS AND INSTRUMENTS FOR USE WITH THE TEST

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

Table 1. Reagents and Materials used for the Tempus iC SARS-CoV-2 Test

Reagent	Manufacturer	Catalog #
Chemagic Viral DNA/RNA 300 H96 Kit	PerkinElmer	CMG-1033-S
Applied Biosystems™ TaqPath COVID-19 Combo Kit	Thermo Fisher	A47814
Chemagic 360 96-deep well plates	PerkinElmer	CMG-555
TaqPath™ 1-Step Multiplex Master Mix (No ROX)	Thermo Fisher	A28523

Reagent	Manufacturer	Catalog #
iSWAB-Microbiome collection tubes	Mawi DNA Technologies	ISWAB-MB-1200
Swabs in accordance with FDA recommendations: nylon flocked or spun polyester	Various suppliers	Various catalog numbers
Viral Transport Medium	Commercially available or prepared according to CDC SOP#: DSR-052-04	N/A

Table 2. Instruments used for the Tempus iC SARS-CoV-2 Test

Instrument	Manufacturer	Software Version
Chemagic 360 *	PerkinElmer	v6.3.0.3
Applied Biosystems™ QuantStudio 7 Flex Real-Time PCR system *	Thermo Fisher	v1.3

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

CONTROLS

The controls run with the IC SARS-CoV2 Test are described in **Table 3**.

Table 3. 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), TaqPath COVID-19 Combo Kit	Monitor the integrity of the PCR reagents and process.	Used on every PCR plate

Control Type	Description	Purpose	Frequency of Testing
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

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 4.**

Table 4. Interpretation of iC SARS-CoV-2 Test Controls

Control Type	Used to Monitor	Target 1: N gene	Target 2: S gene	Target 3: ORF1ab	Target 4: MS2
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
Positive	To validate the integrity of the RT-PCR reagents and process.	Detected Ct ≤37	Detected Ct ≤37	Detected Ct ≤37	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 ≤37
Internal Control	To verify successful extraction of the sample, proper assay setup, sample integrity, and efficient sample collection.	N/A	N/A	N/A	Detected Ct ≤37

[1] Cycle threshold (Ct) is defined as the number of cycles required for the fluorescent signal to cross the threshold. Expected Ct cutoffs are based on Thermo Fisher's RUO protocol and communication with a Thermo Field Application Scientist.

The results from testing of patient samples are interpreted according to the criteria described in **Table 5.**

Table 5. Interpretation of iC SARS-CoV-2 Test Results for Patient Clinical Specimen

N gene	S gene	ORF1ab	MS2	Status	Result	Action
NEG	NEG	NEG	NEG	INVALID	N/A	Repeat test. If the repeat result remains invalid, consider collecting a new specimen.
NEG	NEG	NEG	POS	VALID	SARS-CoV-2 Not Detected	Report results. Consider testing for other viruses.
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			POS or NEG	VALID	SARS-CoV-2 Detected	Report results.

[1] A target gene (N, S, ORF1ab) or MS2 internal control is called POS at Ct ≤ 37.

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-CoV 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 6**.

Table 6. Preliminary LoD for Mawi media and VTM

Concentration		**Mawi Media**					**VTM Media**				
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 7.**

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

	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

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 8A**).

Table 8A. 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 8B**).

Table 8B. Summary of Contrived Sample Testing

			N Gene			S Gene			ORF1ab			MS2 Internal Control		
Concentration	**Collection Media & Swab Type**	**# of Swabs**	**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 Thermo Fisher Scientific TaqPath COVID-19 Combo Kit

The clinical performance of the iC SARS-CoV2 test was further evaluated and validated against previously authorized Thermo Fisher Scientific TaqPath COVID-19 Combo kit. A total of 61 clinical nasopharyngeal (NP) swab specimens collected in VTM were previously determined to be negative or positive for SARS-CoV-2 by Illinois Department of Public Health (IDPH).

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.

Overall concordance between IDPH test results and Tempus lab test results was 100% positive concordance (30/30 samples) and 96.8% negative concordance (30/31 samples).

Performance of the iC SARS-CoV2 test compared to the Thermo Fisher Scientific TaqPath COVID-19 COMBO Kit is summarized in **Table 9**.

Table 9. Performance of iC SARS-CoV-2 Test vs. TaqPath™ COVID-19 Combo Kit assay

Patient NP Specimens		**IDPH Applied Biosystems TaqPath™ COVID-19 Combo Kit**		
		Positive	**Negative**	**Total**
iC SARS-CoV-2 Test *Tempus Labs*	**Positive**	30	1	31
	Negative	0	30*	30
	Total	30	31	61
Positive Percent Agreement		100% (30/30) (95% CI: 88.7%-100%)[1]		
Negative Percent Agreement		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 AvellinoCoV2 test

The clinical performance of the iC SARS-CoV2 test was also evaluated against the AvellinoCoV2 FDA authorized test using a total of 60 clinical NP swab specimen collected in MAWI medium. Clinical NP swab specimens collected in MAWI medium were previously determined to be negative or positive for SARS-CoV-2 by Avellino LAB USA.

Overall concordance between Avellino Lab USA Inc results and Tempus lab test results was 100% positive concordance (29/29 samples) and 100% negative concordance (30/30 samples). Performance of the iC SARS-CoV2 test compared to the AvellinoCARS-CoV-2 assay is summarized in **Table 10**.

Table 10. Performance of iC SARS-CoV-2 Test vs. AVELLINOCoV2 Test

Patient NP Specimens		**Avellino CoV2 SARS-CoV-2 assay**		
		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.

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 11**).

Table 11. 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-	0	32.0	1.3	32.2	0.7	31.7	0.7	21.7	0.7	100 (20/20), 95%

Sample type	Time (days)	ORF1ab		N gene		S gene		MS2 (IC)		% Agreement
		Ave Ct	SD	Avg Ct	SD	Avg Ct	SD	Avg Ct	SD	
CoV-2 Positive										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 12A and Table 12B was analyzed using the Applied Biosystems™ COVID-19 Interpretive Software v2.2. The results are summarized below.

***Table 12A: S**ummary 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 12B**: 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

WARNINGS:

- This test has not been FDA cleared or approved;
- This test has been authorized by FDA under an EUA for use by Tempus Labs, Inc.'s laboratories located at 600 W Chicago Ave, Ste 510, Chicago, IL 60654 and 3155 Northwoods Place, Peachtree Corners, GA 30071;
- This test has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens; and
- This test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro 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 authorization is terminated or revoked sooner.