CSI COVID RT-PCR Assay Validation Summary

INTENDED USE

CSI utilizes the TaqPath COVID-19 Combo Kit (an EUA approved assay) which is a real-time reverse transcription polymerase chain reaction test for the qualitative detection of nucleic acid from SARS-CoV-2 in respiratory tract specimens including nasopharyngeal (NP) swabs, oropharyngeal (OP) swabs, as well as buccal swabs collected from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to Contamination Source Identification LLC in Huntingdon, PA which is a Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, registered high-complexity laboratory.

Results indicate the detection and identification of SARS-CoV-2 RNA. SARS-CoV-2 RNA is generally detectable in respiratory specimens in the acute phase of infections. Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.

Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient treatment or other patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information. Testing with the TaqPath COVID-19 Combo Kit is intended for use within CSI testing laboratories by qualified laboratory personnel trained in the use of molecular techniques.

DEVICE DESCRIPTION AND TEST PRINCIPLE

The TaqPath COVID-19 Combo Kit is a real-time reverse transcription polymerase chain reaction (RT-PCR) test. The test detects three specific regions of the SARS-CoV-2 genome including the ORF1ab region and the N (nucleocapsid) and S (Spike protein) genes. The assay also includes one primer and probe set to detect the MS2 phage internal control in both the negative extraction control and clinical samples. RNA is isolated from upper respiratory specimens nasopharyngeal (NP) swabs, oropharyngeal (OP) swabs, as well as buccal specimens using the QIAamp Viral RNA Mini Kit (Cat # 52906) performed manually by CSI testing personnel. RNA is reverse transcribed to cDNA using the TaqPath 1-Step Multiplex Master Mix and subsequently amplified using the Applied Biosystems (ABI) 7500 Fast Dx real time PCR system instrument with SDS software version 1.4.1. During the amplification process, the probe anneals to a specific target sequence located between the forward and reverse primers. During the extension phase of the PCR cycle, the 5’ nuclease activity of Taq polymerase degrades the bound probe, causing the reporter dye (VIC, ABY, and FAM for the N, S, and ORF1ab targets, respectively) to separate from the quencher dye, generating a fluorescent signal. Fluorescence intensity is monitored at each PCR cycle by the 7500 Fast Dx real time PCR system instrument.

INSTRUMENTS AND REAGENTS USED WITH TEST

Key Instrument/Reagent/Consumable	Supplier	Cat no.
ABI 7500 Fast Dx Real Time PCR System plus COVID-19 Interpretive Software	Thermofisher	4406985
TaqPath COVID-19 Combo Kit	Thermofisher	A47814
TaqPath 1-Step Multiplex Master Mix (No ROX™)	Thermofisher	A28522
Optical 96-Well Fast Clear Reaction Plates with Barcode	Thermofisher	A28522
MicroAmp Optical Adhesive Film	Thermofisher	4360954
QIAamp Viral RNA Mini Kit	Qiagen	52906

CONTROLS TO BE USED WITH THE TaqPath COVID-19 Assay

Table 1: Controls used by CSI in the TaqPath COVID-19 Assay

Control	Purpose	Frequency of Testing
Negative Control (NTC)	To monitor for cross contamination during extraction and RT-PCR	In each batch of specimens that are extracted and RT-PCR set up.
External Positive Control (nCoV-PC)	To monitor for failures in the RT-PCR assay	One per run of the RT-PCR assay
Internal Positive Control (MS2 Phage)	To monitor extraction efficiency and RT-PCR success in each run	Spiked into each specimen and NTC
Human Specimen Control (HSC)	To monitor for extraction efficiency via previously tested samples	One per run of the RT-PCR assay

The results from the controls are interpreted according to the criteria shown in Table 2. If the results obtained with the Positive, Negative, Internal, and HSC do not meet the criteria shown, the results from the entire batch of samples are considered invalid and repeat testing must be performed using residual extracted nucleic acid. If any of the above controls do not exhibit the expected performance as described, the assay may have been set up improperly and is re-set up to correct any set up errors. Continued control failure may indicate reagent/equipment failures and triggers system QC to ensure all components are functioning properly.

External Positive Control
The positive control (TaqPath COVID-19 Control) is used to verify proper assay set-up and SARS-CoV-2 reagent integrity. The positive control is composed of RNA that contains targets specific to the SARS-CoV-2 genomic regions targeted by the assays.

Negative Control (NTC)
The extraction control monitors for any potential cross-contamination that could occur during the nucleic acid extraction process or RT-PCR assay. This control is not included in the TaqPath COVID-19 Combo Kit; however, CSI uses UltraPure RNase/DNase free H2O with the MS2 control spiked into it per one batch of specimens that are extracted.

MS2 Phage Internal Control
The MS2 internal control serves as an internal process control for nucleic acid extraction to ensure that clinical samples and controls contain sufficient and quality RNA to be used in the RT-PCR reactions.

Human Specimen Control
The Human Specimen Control serves as a process control by extracting a previously isolated specimen and confirming it is successfully isolated again and is performed at least once per day. This control confirms consistency in the test system and that the system is operating as expected.

INTERPRETATION OF RESULTS

All test controls are examined prior to interpretation of patient results. If the controls are not valid, the patient results cannot be interpreted (Refer to Table 2 for a summary of control results).

1) COVID-19 RT-PCR Test Controls – Positive (internal and external), Negative, HSC:

- **MS2 (Internal Positive Control):** MS2 in a patient sample indicates that PCR amplification occurred in the well. The presence of MS2 and no detectable SARS-CoV-2 during the analysis indicates that proper RNA extraction and amplification occurred, however, no SARS-CoV-2 is present. If SARS-CoV-2 is present in the specimen, amplification of the target RNA may reduce or abrogate MS2 amplification. In this case, the amplified SARS-CoV-2 indicates proper RNA extraction and amplification. Therefore, MS2 may or may not be detectable in a valid test on patient specimens.

- **External Positive Control:** The positive control must be positive for all three SARS-CoV-2 targets, i.e., the ORF1ab, the N Protein, and the S Protein genes and amplification must have a Ct <37 in order for the test result to be valid. The positive control does not contain MS2.

- **Negative Control (NTC):** The negative control must be negative except for MS2 for the test result to be valid.

- **Human Specimen Control (HSC):** The human specimen control must have at least MS2 amplification to indicate that the sample was successfully extracted and able to be amplified during RT-PCR.

Table 2. Expected Results of Controls as Reported By COVID-19 Interpretive Software

Control	Ct Value (Optical Channel)			
	N Gene (VIC)	S Gene (ABY)	ORF1ab (FAM)	MS2 Phage (JUN)
Negative	Undetermined**	Undetermined**	Undetermined **	Positive
Positive Control	Positive	Positive	Positive	Undetermined*
MS2 Phage	Any	Any	Any	Positive
HSC	Any	Any	Any	Positive

* The MS2 Phage Internal Control is not added to the Positive Control or No Template Control and no signal should be obtained.
** Undetermined (No Positive Signal in Interpretive Software)

If any control does not perform as described above, the run is considered invalid and all specimens in the invalid assay are repeated using residual extracted specimen RNA.

2) Examination and Interpretation of Patient Specimen Results:

Assessment of clinical specimen test results should be performed after the positive and negative controls have been examined and determined to be valid and acceptable. If the controls are not valid, the patient results cannot be interpreted. Please see the table below (Table 3) for guidance on interpretation and reporting of results.

- If all three SARS-CoV-2 specific targets (ORF1ab, N, S) are negative (undetermined) and the MS2 control is also negative (undetermined), the result is invalid. The extracted RNA from the patient specimen should be re-tested. If the repeat result is invalid (negative for all markers), reisolation of RNA and/or collection of a new patient sample should be considered.

- If all three SARS-CoV-2 specific targets (ORF1ab, N, S) are negative (undetermined) and the MS2 control is positive, the patient sample is reported as negative.
- If only one SARS-CoV-2 specific target is positive, and the MS2 control is positive or negative (undetermined), the patient sample is reported as inconclusive.
- If all three SARS-CoV-2 specific targets (ORF1ab, N, and S) are positive, and the MS2 control is positive or negative (undetermined), the patient sample is reported as positive.

Table 3: Interpretation of Patient Results Using the TaqPath COVID-19 Assay

ORF1ab	N gene	S gene	MS2 Control	Status	Result	Action
NEG	NEG	NEG	NEG	INVALID	NA	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 to healthcare provider and appropriate public health authorities.
Only one SARS-CoV-2 target POS			POS or NEG	VALID	SARS-CoV-2 INCONCLUSIVE	Repeat test. If the repeat result remains inconclusive, additional confirmation testing should be conducted if clinically indicated.
2-3 SARS-CoV-2 target POS			POS or NEG	VALID	SARS-CoV-2 POSITIVE	Report results to healthcare provider and appropriate public health authorities.

NEG: Negative in COVID-19 Interpretive Software
POS: Positive in COVID-19 Interpretive Software

PERFORMANCE EVALUATION

1) Analytical Sensitivity: Limit of Detection (LoD):
The LoD of the TaqPath COVID-19 assay in the CSI test system was determined using quantified, SARS-CoV-2 Genomic RNA (ATCC VR-1986D) obtained from ATCC. A preliminary LoD was determined by testing five

concentrations of a half log-fold dilution series (1 genome copy/µL to 100 genome copies/µL of specimen) of RNA spiked into pooled clinical negative swab matrices and extracted in quadruplicate at each level with the QIAamp Viral RNA Mini Kit and tested with the TaqPath COVID-19 RT-PCR Assay on the ABI 7500 Fast Dx instrument.

The initial LoD determination of the TaqPath COVID-19 assay in the CSI test system was between 1 and 32 copies/µL depending on the swab type (Table 4 in green). The LoD was verified by testing 20 additional extraction replicates consisting of pooled clinical, negative nasopharyngeal swab matrix spiked at 2X of each swab types preliminary LOD and 20 known-negative replicates. Samples were spiked with SARS-CoV-2 RNA prior to extraction with the QIAamp kit. The LoD of the TaqPath COVID-19 assay in the CSI test system was confirmed for each swab with >95% overall accuracy on each swab type. A total of 40 contrived positives and 40 known-negative samples were tested at 2X LOD for the OP captiswab in DNA/RNA shield sample type. The results are summarized in Table 4.

Table 4. LoD and LOD Confirmatory Results

Specimen Type	**100 copies/ µL**	**32 copies/ µL**	**10 copies/ µL**	**3.2 copies/ µL**	**1 copies/ µL**	**Confirmation at 2X LOD**
	N Positive/ Total	N Positive /Total	N Positive /Total	N Positive /Total	N Positive /Total	N Accurately Identified/Total (% accuracy)
NP in VTM	**4/4**	**4/4**	**2/4**	**0/4**	**0/4**	**40/40 (100%)**
OP in VTM	**4/4**	**4/4**	**4/4**	**2/4**	**0/4**	**39/40 (97.5%)**
OP (captiswab) in DNA/RNA Shield	**4/4**	**4/4**	**4/4**	**4/4**	**4/4**	**78/80 (97.5%)***

***2 <u>inconclusive</u> results were observed for negative samples, which would result in retesting as per current CSI protocols.**

2) Analytical Inclusivity/Specificity:

In Silico Analysis of Primer and Probe Inclusivity:

CSI utilizes the identical oligonucleotide sequences for the N and S genes and ORF1ab region as those used in the ThermoFisher TaqPath COVID-19 Combo Kit. In silico testing of the SARS-CoV-2 assay was previously performed by ThermoFisher as part of their EUA authorization (EUA200010) and this information has been provided in the FDA authorized EUA granted to this manufacturer.