ACCELERATED EMERGENCY USE AUTHORIZATION (EUA) SUMMARY covidSHIELD Assay

(University of Illinois, Office of the Vice President for Economic Development and Innovation)

For *In vitro* Diagnostic Use
Rx Only
For use under Emergency Use Authorization (EUA) only

(The covidSHIELD assay will be performed at laboratories designated by the University of Illinois Office of the Vice President for Economic Development and Innovation, that are also certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a and meet the requirements to perform high complexity tests, as described in the Laboratory Instructions for Use that was reviewed by the FDA under this EUA.)

INTENDED USE

covidSHIELD is a real-time reverse transcription polymerase chain reaction (RT-qPCR) test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in saliva specimens that are collected without preservatives in a sterile collection tube or into a sterile collection tube with straw, in the presence of a trained observer (adult trained on how to collect saliva samples), from individuals who are suspected of COVID-19 by their healthcare provider. Testing is limited to laboratories designated by the University of Illinois Office of the Vice President for Economic Development and Innovation, that includes the University of Illinois Veterinary Diagnostic Laboratory, University of Illinois Urbana Champaign School of Veterinary Medicine, located at 2001 S. Lincoln Ave, Urbana, IL 61802, that are also 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. The SARS-CoV-2 RNA is generally detectable in saliva 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 test results to the appropriate public health authorities.

Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information. Negative results for SARS-CoV-2 RNA from saliva should be confirmed by testing of an alternative specimen type if clinically indicated.

covidSHIELD is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the techniques of RT-qPCR and *in vitro* diagnostic procedures. The covidSHIELD is only for use under the Food and Drug Administration's Emergency Use Authorization.

DEVICE DESCRIPTION AND TEST PRINCIPLE

The covidSHIELD assay is a direct saliva-to-RT-qPCR assay that detects three genes (ORF1ab (replicase), N-gene (nucleocapsid), and S-gene (spike) of SARS-CoV-2) after heat inactivation and addition of TBE buffer and Tween20. The test method does not require RNA isolation, purification or extraction. Heat treatment (at 95°C for 30 minutes) and treatment with buffer TBE and detergent Tween20 disrupts viral particles and releases viral RNA. SARS-CoV-2 RNA is then detected using one of the RT-qPCR systems authorized for use with the covidSHIELD assay. The TaqPath COVID-19 Combo Kit consists of 1) COVID-19 real-time PCR multiplex assays containing three primer/probe sets specific to different SARS-CoV-2 genomic regions (ORF1ab, S, and N) and primers/probe for bacteriophage MS2, 2) bacteriophage MS2 control template. The three SARS-CoV-2 targets and one MS2 control target are detected using probes specifically labeled by different dyes (ORF1ab, FAM dye; N, VIC dye; S, ABY dye; MS2, JUN dye). Therefore, if SARS-CoV-2 viral genome is present, it will be detected by labeled probes during PCR. Each of the targets are determined to be positive or negative, and the test result will be interpreted as positive, negative, or inconclusive.

INSTRUMENTS USED WITH TEST

covidSHIELD can be used with the following RT-qPCR Instruments:

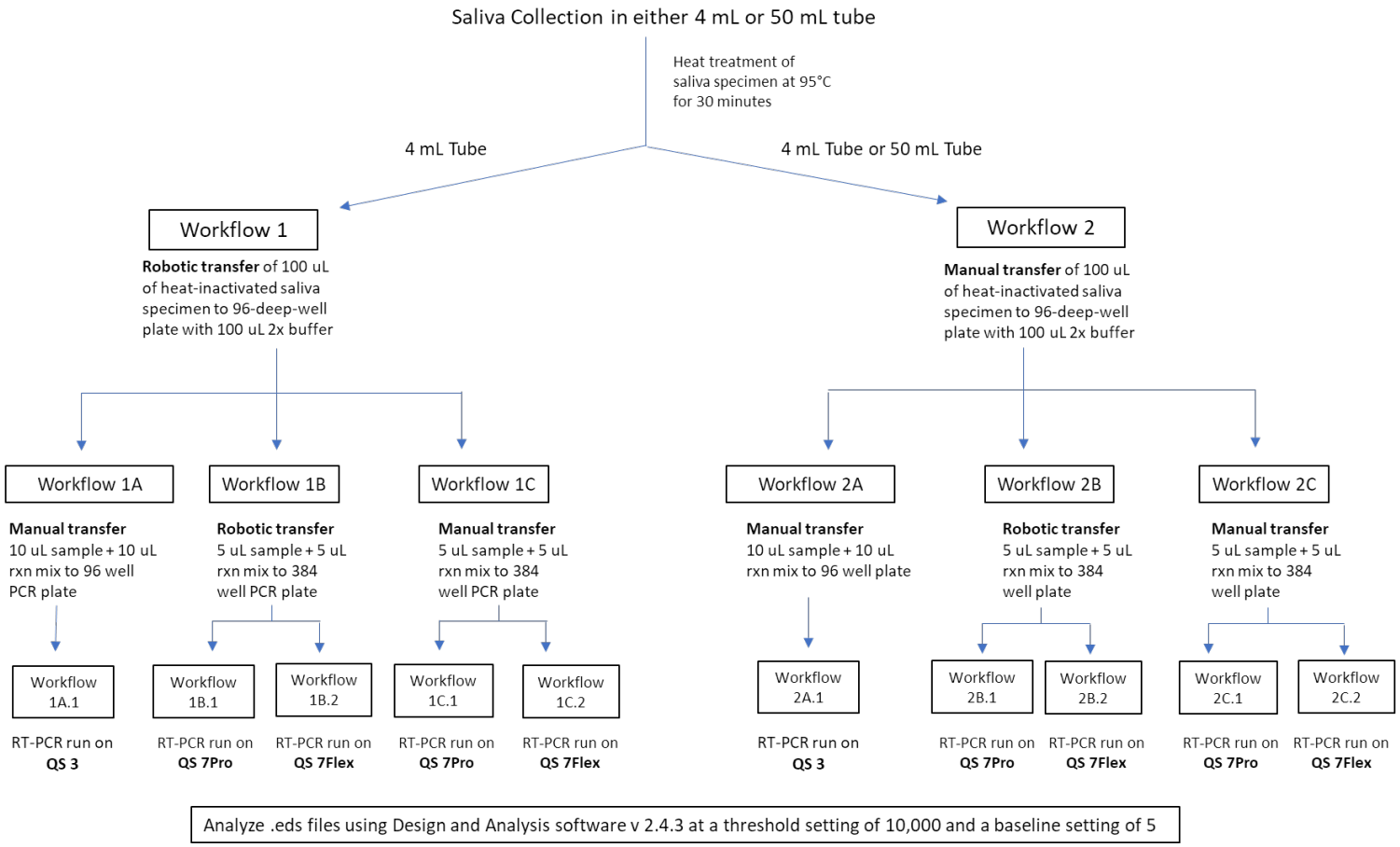
RT-qPCR instrument	ThermoFisher Scientific	QuantStudio 3 (96-well)_
	ThermoFisher Scientific	QuantStudio 7 Flex (384-well)
	ThermoFisher Scientific	QuantStudio 7 Pro (384-well)

Designated laboratories will receive an FDA accepted instrument qualification protocol included as part of the covidSHIELD Instructions for Use (IFU) and will be directed to execute the protocol prior to testing clinical samples. Designated laboratories must follow the authorized IFU, which includes the instrument qualification protocol.

Software analysis is conducted with QuantStudio 7 Design and Analysis Software v2.4.3 and QuantStudio Real- Time PCR Software v1.3.

Sample handling can be enhanced with Beckman Biomek i5 Span 8, which can be used to transfer saliva from the 4 mL sample tubes (but not the 50 mL sample tubes) to the 96-deep-well sample plates, and Beckman Biomek i5 Multichannel robotic systems, which can be used to transfer samples from the 96-deep-well sample plates to the 384-well PCR plates used for the Quant Studio 7Pro (QS 7Pro) and QuantStudio 7Flex (QS 7Flex) systems.

Below is a flow diagram illustrating the different workflows that can be used with the covidSHIELD assay:



REAGENTS AND MATERIALS

Sample collection materials

- Barcoded 50 mL sterile conical tubes, or equivalent, & 4 mL sterile cryotubes with straw.
- Biohazard specimen bag
- Plastic (non-absorbent) tube rack
- Secondary transport container (plastic bin)

Sample processing materials

- 10X Tris-Borate-EDTA (TBE) buffer
- Tween20
- 2X TBE with 1% Tween 20
- Biohazard waste container
- Hot water bath or heating block (capable of reaching 95°C)
- Thermometer

- Timer
- Micropipettes
- RNase-free, sterile pipette tips
- RNase-free, sterile 1.5 microcentrifuge tubes
- Tube racks

RT-qPCR materials

- Thermo Fisher Scientific TaqPath COVID-19 Combo Kit
- Thermo Fisher Scientific TaqPath Multiplex 1-Step MasterMix (no ROX)

COMPONENT	VOLUME	LONG AND SHORT TERM STORAGE
TaqPath Multiplex 1-step Master Mix (no ROX)	200 µl/80=2.5 µl per sample	Bottle of 10 ml at -20°C Bottle kept on ice for daily use
Primer/Probe mix	40 µl/80=0.5 µl per sample	Tubes of 1.5 ml at -20°C 40 µl aliquots at -20°C for daily use
UltraPure DNase and RNase free water	92 µl/80=1.15 µl per sample	Bottle of 500 ml at room temperature 500 µl aliquots on ice for daily use
MS2 bacteriophage	82 µl/80=1.025 µl per sample	Tubes of 1 ml at -20°C

- Ice buckets
- Vortex
- Plate centrifuge
- 96- and 384-well RT-qPCR reaction plates
- Calibration plates for ABY, JUN, VIC, and FAM

CONTROLS RUN WITH THE covidSHIELD ASSAY

For every run performed, a known positive and negative control will be included in the reaction plate, as well as internal controls for each sample. The negative control consists of UltraPure DNase- and RNase-free water, the positive control consists of the Thermo Fisher Scientific TaqPath COVID-19 Combo Kit provided positive control, and the internal control consists of the Thermo Fisher Scientific TaqPath COVID-19 Combo Kit provided MS2, which will be spiked into the MasterMix reaction, per manufacturer's instructions.

INTERPRETATION OF RESULTS

1) SARS-CoV-2 RT-PCR test Controls – Positive, Negative, and Internal:

Any test plate is considered valid if: the positive control yields detectable signal (Ct value between 1 and 39) in all three SARS-CoV-2 genes (ORF1ab, S-gene, N-gene) and the MS2 gene; and if the negative control yields no detectable signal in all three SARS-CoV-2 genes while the MS2 gene is detected.

Control	ORF1ab (FAM)	N-gene (VIC)	S-gene (ABY)	MS2 (JUN)	Interpretation
Negative	Undetermined	Undetermined	Undetermined	Ct < 39	Valid
	Any Ct value measured in any gene			Ct < 39	Invalid
Positive	Ct < 39	Ct < 39	Ct < 39	Ct < 39	Valid
	Any single gene yielding an undetermined Ct value				Invalid

2) Examination and Interpretation of Patient Specimen Results:

All test controls must be examined prior to interpretation of patient results. If the controls are not valid, the patient results cannot be interpreted. Results will be interpreted according to the tables below. Note that any individual sample result is considered valid if the MS2 internal control alone is detected.

ORF1ab (FAM)	N-gene (VIC)	S-gene (ABY)	MS2 (JUN)	Interpretation	Action
Undetermined	Undetermined	Undetermined	Ct < 39	Negative	Report result
Any 2-3 genes yielding Ct < 39			Ct < 39 or Undetermined	Positive	Report result
Any 1 gene yielding Ct < 39 (2 genes undetermined)			Ct < 39 or Undetermined	Inconclusive	Repeat test*
Undetermined	Undetermined	Undetermined	Undetermined	Invalid	Repeat test*

*Retest the sample one time, report results to the healthcare provider and appropriate public health authorities. If the repeat result remains inconclusive/invalid, the healthcare provider should consider conducting additional confirmation testing with a new specimen, if clinically indicated.

PERFORMANCE EVALUATION

1) Analytical Sensitivity:

Limit of Detection (LoD):

LoD for Workflow 2A.1:

The preliminary limit of detection (LoD) for covidSHIELD was determined using manual pipetting and a Thermo Fisher QuantStudio 3 (QS 3) using Gamma-irradiated SARS-CoV-2 spiked into fresh human saliva (SARS-CoV-2 negative) at $1.0x10^2$, $5.0x10^2$, $1.0x10^3$, $2.5x10^3$, $5.0x10^3$, $1.0x10^4$, $5.0x10^4$, $1.0x10^5$, and $5.0x10^5$ viral copies/mL; four replicates per concentration. Final LoD for covidSHIELD was tested using 30 individual extraction replicates at 1,000 copies/mL in different sources of saliva using manual pipetting and the QS 3; all 30 samples tested positive with 3 of 3 genes being detected. Using manual pipetting and the QS 3, at the LoD of 1000 copies/mL, the mean Ct of the ORF1ab gene is 31.7, for the N gene is 30.4, and for the S gene is 31.2.

LoD for Workflows 1B.1 and 1B.2:

The preliminary LoD using robotic systems and the Fisher Scientific QuantStudio 7 Flex (QS 7Flex) and 7 Pro (QS 7Pro) systems was determined as follows: 2mL saliva in 4mL tubes were spiked with gamma-irradiated SARS-CoV-2 at 0, 250, 500, 1000, 1500, 2000, 3000, and 4000 copies/mL. Following heat-inactivation at 95ºC for 30mins, 100uL of cooled saliva samples were transferred to 96-deep well plates pre-loaded with 100uL 2xTBE and 1% Tween-20 using a Biomek Span 8 robot, and subsequently transferred to 384-well plates pre-loaded with reaction mix using a Biomek Multichannel robot. Samples were loaded into either a QS 7Pro or QS 7Flex system using the standard protocol. Data analysis was performed using Design and Analysis software v. 2.4.3 with a threshold setting of 10,000 and baseline of 5. The LoD for QS 7Pro and QS 7Flex was 500 copies/mL and 1000 copies/mL, respectively. Final LoD for covidSHIELD was tested using 20 individual extraction replicates at 500 copies/mL for the QS 7Pro and 1,000 copies/mL for the QS 7Flex using robotic sample transfer; for each QS system, all 20 samples tested positive at the LoD for that system. Using the robotics systems and the QS 7Pro, at the LoD of 500 copies/mL, the mean Ct of the ORF1ab gene is 35.6, for the N gene is 34.6, and for the S gene is 36.3. Using the robotics systems and the QS 7Flex, at the LoD of 1000 copies/mL, the mean Ct of the ORF1ab gene is 33.9, for the N gene is 33.7, and for the S gene is 34.4.

LoD Confirmation for Workflows 2B.1 and 2C.1:

The LoD was also confirmed for two additional workflows using the QS 7Pro: 1) the transfer of the saliva sample into the 96-deep-well plate using manual pipetting and the transfer from the 96-deep-well plate to the 384-well PCR plate using robotics; and 2) the transfer of the saliva sample into the 96-deep-well plate using manual pipetting and the transfer from the 96-deep-well plate to the 384-well PCR plate using manual pipetting. Fresh saliva was spiked with gamma-irradiated SARS-CoV-2 virus at a concentration of 500 viral copies/mL. Following heating at 95ºC for 30mins, samples were manually loaded to 96-deep well plates. Samples were loaded either manually or robotically to 384-well PCR plates and run on a QS 7Pro; 20 replicates were tested for each workflow. Data analysis was performed using Design and Analysis software v.2.4.3 with a threshold setting of 10,000 and baseline of 5. Samples were called positive if 2 out of 3 genes were detected per the "Interpretation of Results" table above. The LoD for the QS 7Pro yielded positive results for 20 of 20 samples using both workflows.

The below table summarizes the LoD study results for the different instrument systems and manual/automated pipetting strategies:

Thermocycler	**Sample Transfer to buffer plate**	**Sample transfer to PCR plate**	**Workflow Number**	**LoD**	**ORF1ab-gene Ct**	**N-gene Ct**	**S-gene Ct**
QuantStudio 3	manual	manual	2A.1	1000 copies/mL	31.7	30.4	31.2
QuantStudio 7 Pro	robotic	robotic	1B.1	500 copies/mL	35.6	34.6	36.3
	manual	robotic	2B.1		35.01	34.01	35.15
	manual	manual	2C.1		34.6	33.87	34.76

QuantStudio 7 Flex	robotic	robotic	1B.2	1000 copies/mL	33.9	33.7	34.4

2) Inclusivity (Analytical Sensitivity):

covidSHIELD is a modification of the previously Emergency Use Authorized Thermo Fisher Scientific Applied Biosystems TaqPath COVID-19 Combo Kit. The University of Illinois has received a Right of Reference (ROR) from Thermo Fisher. As reported, this assay targets specific genomic regions of the SARS-CoV-2; specifically, the assay targets ORF1ab, nucleocapsid (N) gene, and spike (S) gene. Thermo Fisher Scientific's "*in silico*" analysis was updated on October 6, 2020. Based upon BLAST analysis, the TaqPath COVID-19 Combo Kit maps with 100% homology to >99.99% of known SARS-CoV-2 isolates in GISAID and 100% of known isolates in GenBank databases. Mapping was deemed successful for a given isolate if at least two of the three targets (ORF1ab, S gene, and N gene) showed 100% identity."

Mutations within the target regions of the Thermo Fisher Scientific TaqPath COVID-19 Combo Kit used by covidSHIELD could affect primer and/or probe binding resulting in a failure to detect the presence of viral RNA. However, because the TF Combo kit detects 3 genes (ORF1ab, N, and S) a mutation to only one of those genes can yield a positive result with dropout of the mutated gene. As of the publication of this EUA Summary, S-gene drop-outs have been detected when patients are infected with variant B.1.1.7, see here and here.

3) Cross-Reactivity (Analytical Specificity):

The analytical specificity of covidSHIELD was demonstrated *in silico* under the EUA for the Thermo Fisher Scientific Applied Biosystems TaqPath COVID-19 Combo Kit. Based on this analysis, significant amplification of non-target sequences that could result in cross-reaction (false-positive results) or interference (false-negative results) was considered unlikely to occur. In addition, the University of Illinois conducted additional wet testing to validate the specificity of the covidSHIELD detection system to SARS-CoV-2. Saliva was spiked with or without SARS-CoV-2 (gamma-irradiated virus, synthetic N- transcript), two other human coronaviruses (OC43, 229E), and SARS and MERS synthetic RNA. Two replicates were tested for each. Among these samples, SARS-CoV-2 genes were only detected in the positive control and SARS-CoV-2 samples, supporting specificity of the detection platform for SARS-CoV-2.

4) Interfering substances

Testing was done to determine the extent to which endogenous and exogenous substances interfered with the performance of the test. Potentially interfering substances tested were:

1. Nasal congestion spray (15% v/v),
2. NeilMed Nasogel (1.25% v/v),
3. Cepacol Lozenges (benzocaine/menthol) (3mg/mL),
4. Chloroseptic Sore Throat spray (5% v/v),
5. Crest/Listerine Mouth Wash (5% v/v/),
6. Act dry mouth lozenges (3mg/mL),

7. Toothpaste (Colgate) (0.5% v/v) ,
8. Mucin: bovine submaxillary gland, type I-S (2.5 mg/mL),
9. Human Genomic DNA (10ng/uL),
10. White blood cells/Leukocytes (1-5 x 10^6 cells/mL), and
11. Nicotine (0.03 mg/mL).

Three naturally occurring saliva samples were collected from SARS-CoV-2 negative subjects (subject A, subject B, and subject C) in 50 mL conical tubes. Each of the three saliva samples was divided into sets of aliquots (one set for the positive samples and one for the negative samples). The positive samples were created by spiking the saliva with gamma irradiated SARS-CoV-2 (BEI cat# NR-52287) at 3000 viral copies/mL. Both positive and negative samples were analyzed using the covidSHIELD assay run on the QuantStudio 7 Pro (workflow 2B.1) in triplicate as follows: a. Control samples: no addition of endogenous or exogenous interfering substances; b. Experimental samples: addition of one endogenous or one exogenous interfering substance via the addition of the substance into the saliva sample at the concentration indicated above. Results were reported as "positive" or "negative" based upon the measured Ct values for the viral genes and MS2 for each sample tested.

With one exception (Colgate toothpaste), no endogenous or exogenous substances interfered with the test at the concentration of the substances that were added into the saliva for either the negative samples or the low positive samples spiked with SARS-CoV-2 for covidSHIELD. The one exception was Colgate toothpaste at 0.5% v/v which caused one positive sample to test negative.

5) Clinical Evaluation

A prospective study was conducted to assess the clinical performance of the covidSHIELD assay. 120 study participants who were symptomatic for COVID-19 infection provided saliva samples (self-collected in either a 50 mL conical tube or 4 mL tube with straw in the presence of a trained observer) and either a nasopharyngeal (NP) or mid-turbinate (MT) sample collected by a healthcare professional. Additionally, matched saliva and MT samples were collected from convalescent patients to assess performance of covidSHIELD in low viral load samples, yielding a total of 137 matched saliva and upper respiratory (MT or NP) specimens. The NP and MT swab specimens were run on one of two different highly sensitive EUA authorized comparator assays, and the paired saliva specimen was run using the covidSHIELD. All three analytically validated thermocyclers (Quantstudio 3, Quantstudio 7 Flex, and Quantstudio 7 Pro) were utilized in the study using both the manual and robotic pipetting workflows.

Of the 137 upper respiratory specimens tested, 48 were positive and 89 were negative according to comparator results on the MT or NP swabs. Of the 48 positives, the candidate assay run on the matched saliva specimens identified 46 of the samples as positive, with two discordant positive results. Of the 89 negatives, the candidate assay run on the matched saliva specimen identified 88 of the samples as negative, with one discordant negative result. The positive percent agreement (PPA) and negative percent agreement (NPA) are therefore 95.8% and 98.9%, respectively. The lower bound of the two-sided 95% confidence interval for PPA is 85.1%. The lower bound of the two-sided 95% confidence interval for NPA is 93.2%.

		EUA authorized Comparator (NP/MT swabs)	
		Positive	Negative
covidSHIELD (saliva)	Positive	46	1
	Negative	2	88
	Total	48	89

PPA: 95.8% (46/48)
NPA: 98.9% (88/89)

LIMITATIONS:

- 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.
- This test only detects the presence of nucleic acids from SARS-CoV-2.
- Performance of covidSHIELD has only been established with saliva specimens from symptomatic individuals. Use of covidSHIELD with other specimen types has not been assessed and performance with these sample types is not known. Performance of this test was not evaluated in an asymptomatic patient population from individuals suspected of COVID-19 by their healthcare provider.
- Results depend on proper sample collection: patients should have taken nothing by mouth for 60 minutes prior to providing a saliva sample (that is, patients should not eat, drink, use tobacco products, brush their teeth, use mouthwash, or chew gum for at least 60 minutes prior to providing the saliva sample). Patients should drool to produce saliva; patients who spit forcibly can produce sputum, which is not a specimen type that has been assessed.
- Toothpaste was shown to cause inhibition of low positive samples at a concentration of 0.5% v/v.
- Detection of SARS-CoV-2 RNA may be affected by sample collection methods that differ from those described in the Instructions for Use (IFU).
- Sample storage and handling procedures other than those provided in the IFU have not been assessed.
- covidSHIELD provides a qualitative assessment of samples that are positive for SARS-CoV-2 RNA. The user of covidSHIELD can assess the RT-PCR results to make a qualitative decision of whether SARS-CoV-2 RNA is detected or not. covidSHIELD results should not be interpreted or used quantitatively.
- Mutations within the target regions of the Thermo Fisher Scientific TaqPath COVID-19 Combo Kit used by covidSHIELD could affect primer and/or probe binding resulting in a failure to detect the presence of viral RNA. However, because the TF Combo kit detects 3 genes (ORF1ab, N, and S) a mutation to only one of those genes can yield a positive result with dropout of the mutated gene. As of the publication of the IFU, S-gene drop-outs have been detected when patients are infected with variant B.1.1.7, see here and here.
- covidSHIELD has only been evaluated for use on the Thermo Fisher Quant Studio 3, 7 Flex, and 7 Pro PCR Systems. covidSHIELD has only been evaluated in a CLIA-certified laboratory.

- covidSHIELD has only been evaluated when using the collection systems described in the IFU.

WARNINGS:

- This test has not been FDA cleared or approved, but has been authorized for emergency use by FDA under an EUA for use by authorized laboratories;
- This test 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 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 declaration is terminated or authorization is revoked sooner.