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 either suspected of COVID-19 by their healthcare provider or from individuals without symptoms or other epidemiological reasons to suspect COVID-19 when tested at least weekly and with no more than 168 hours between tests. This test is also for use with saliva specimens that are collected at home or in a community-based setting by individuals age 16 years and older (self-collected) or 6 years and older (collected with adult assistance) using the SHIELD Saliva Collection Kit, when determined to be appropriate by a 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.

This test is also for the qualitative detection of nucleic acid from SARS-CoV-2 in pooled samples containing up to eight individual saliva specimens (using specified workflows) that are collected without preservatives in a sterile collection tube or into a sterile collection tube with a straw, in the presence of a trained observer (adult trained on how to collect saliva samples), from individuals who are either suspected of COVID-19 by their healthcare provider or from individuals without symptoms or other epidemiological reasons to suspect COVID-19 when tested at least weekly with no more than 168 hours between tests or saliva specimens collected using the SHIELD Saliva Collection Kit. Negative results from pooled testing should not be treated as definitive. If a patient's clinical signs and symptoms are inconsistent with a negative result or results are necessary for patient management, then the patient should be considered for individual testing. Specimens included in pools with a positive or invalid result must be tested

individually prior to reporting a result. Specimens with low viral loads may not be detected in sample pools due to the decreased sensitivity of pooled testing.

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.

SHIELD SALIVA COLLECTION KIT:

The SHIELD Saliva Collection Kit is intended for the collection of saliva specimens at home by individuals age 16 years and older (self-collected) or for adult assisted collection of saliva samples from individuals 6 years and older. The saliva specimen will be collected in a sterile collection tube that will be sent to a laboratory designated by University of Illinois when determined to be appropriate by a healthcare provider. The single collection kit consists of a collection vial, funnel, biohazard bag with absorbent material, alcohol wipe, and instructions for use. The multipack collection kit contains up to five (5) of the following items: collection vial,

funnel, biohazard bag with absorbent material, alcohol wipe, and instructions for use. All items in the collection kit are placed in the kit bag.

The SHIELD Saliva Collection Kit includes the following components:

Name	Description	Quantity
Funnel	Plastic Funnel	1
Transport Tube and Cap	2 ml tube. Manufactured sterile	1
Biohazard Bag	4922 Bag, Biohazard Specimen Transport, 6" x 9", Ziplock with Score Line, Document Pouch and Absorbent Pad	1
Alcohol Wipe	Alcohol prep pad containing alcohol wipe, 70% alcohol	1
Instructions for Use	Instructions for Use brochure with link to video instruction and Patient and Healthcare provider EUA Information	1
Kit Bag	5 x 7" 4 Mil White Block Reclosable Bags	1

SHIELD SALIVA COLLECTION KIT ORDERING, PROCESSING, AND MEDICAL OVERSIGHT:

Individuals are screened by their healthcare provider utilizing resources available to the healthcare practice (e.g., telehealth, in-person). When a healthcare provider determines that an individual should be evaluated with the covidSHIELD test, a SHIELD Saliva Collection Kit is given to the patient. The SHIELD Saliva Collection Kit consists of a collection transport tube and cap, funnel, biohazard bag with absorbent material, alcohol wipe, and Instructions For Use. The collection kit materials are assembled by the designated laboratory and can be assembled into the kit bag for either the collection of a single sample or so that a patient receives sufficient supplies for submission of samples on five separate occasions (i.e., a multi-pack required for asymptomatic screening). Each multipack kit contains up to five (5) of the following items: collection transport tube and cap, funnel, biohazard bag with absorbent material, alcohol wipe, and instructions for use. The healthcare provider will determine which assembly is appropriate. For the multipack, the individual activates each collection vial prior to use.

The Instructions For Use provide detailed instructions to the patient to wait at least 60 minutes after taking something by mouth to provide the sample, sanitize hands, assemble the funnel and transport tube, drool saliva into the tube, remove the funnel, affix the enclosed cap, properly dispose of the funnel, and place the tube in the biohazard bag. The sample is to be dropped off at a location designated by the healthcare provider for testing by the Authorized Laboratory. The individual is instructed to collect and return the sample on the same day. The specimen then undergoes accessioning by the Authorized Laboratory to ensure acceptability for testing. The

Authorized Laboratory compares the date of the prescription order to the date the specimen is returned to the Authorized Laboratory; specimens returned 56 hours after sample collection are not processed.

INSTRUMENTS USED WITH TEST

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

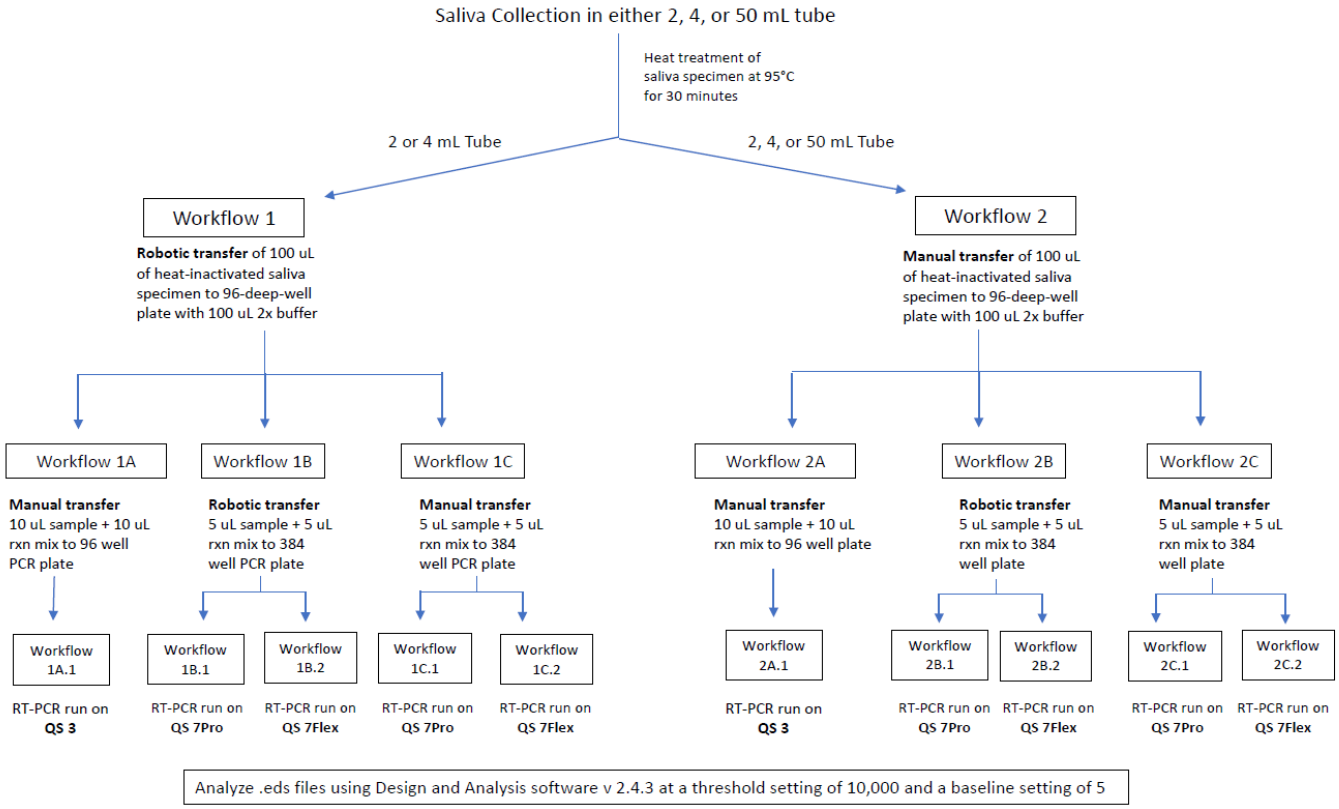
RT-qPCR instrument	ThermoFisher Scientific	QuantStudio 3 (96-well)_
	ThermoFisher Scientific	QuantStudio 7 Flex (384-well)
	ThermoFisher Scientific	QuantStudio 7 Pro (384-well)

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. Additionally, sample handling can also be performed with a Gilson PipetMax, which can be used to transfer saliva from 2 or 4 mL sample tubes to the 96-deep-well sample plates. Gilson PipetMax can also be used to pool samples at a 4:1 or 8:1 ratio in the 96-deep-well sample plates.

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, OR 4 mL sterile cryotubes with straw, OR 4 mL sterile tubes with funnel, OR 2 mL sterile collection tube with funnel.
- Biohazard specimen bag
- Plastic (non-absorbent) tube rack
- Secondary transport container (plastic bin)

The SHIELD Saliva Collection Kit should include the following materials:

a. Collection vial with unique bar code
b. Funnel
c. Biohazard bag with absorbent pad
d. Alcohol wipe
e. Instructions

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.

Interpretation of test results for samples that are not pooled:

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.

Results Interpretation of pooled sample output

For pooled specimens, amplification of a single gene target will result in the pooled sample being deemed positive, as shown in the table immediately below. This is different than result interpretation for individual samples that are not pooled (shown in the table immediately above) which requires amplification of two genes for a declaration of a positive result. A positive pool interpretation or an invalid pool interpretation upon retest (see table below) will result in reflex testing of each individual sample in the pool for deconvolution to identify the positive specimen(s) within that pool. For example, once a positive pool is identified from 8 pooled samples, all 8 samples will be tested individually. Therefore, the resulting patient report for a positive test will not change – the individual sample will need to test positive per the table above

entitled “Interpretation of test results for samples that are not pooled” for the patient to receive a report of a positive result.

Interpretation of test results for samples that are pooled:

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

* Reflex testing for deconvolution to identify positive specimens within the positive pool
**Repeat testing of the pooled specimen once to conclude whether positive or negative; a second invalid result of the same pool will require reflex testing of each individual sample in the pool

Pool Negative:
No further testing is required and each sample in the pool can individually be reported as Negative.

Pool Positive:
For pooled sample testing, if any gene target yields a Ct <39, then the pool is interpreted as positive and all samples from the positive pool should be reflex tested to deconvolve the positive sample(s) within the pool. Deconvolution involves individually testing each sample in the pool from their original sample vial using the individual covidSHIELD testing workflow and interpreting the result via the table above entitled “Interpretation of results for samples that are not pooled”.

When the pooled sample returns a positive result and one or more of the individual samples yields a positive interpretation per the table above entitled “Interpretation of results for samples that are not pooled”, then the result from each of the individually tested samples should be reported.

In the case where the pooled sample returns a positive result with only one gene target amplified, , all samples in the pool should be retested individually according to the above table. If upon retest, that same gene target is the only positive gene target in an individual sample or samples, then results for that samples(s) should be reported according to the results interpretation for individual samples (i.e., inconclusive). For all other cases where the pooled sample returns a positive result but all individual samples return a negative interpretation, each individual sample should be tested a second time. When the result interpretations of an individual sample’s first and second test are concordant, then that result should be reported. When the result interpretations of individual sample’s first and second test are discordant, then the result should be reported as inconclusive and the healthcare provider should consider conducting additional confirmation testing with a new specimen, if clinically indicated.POOLING MONITORING:

Instructions for Pooling Implementation, Pooling Monitoring, and Pooling Re-assessment were added to the covidSHIELD IFU for implementation by designated laboratories.

SPECIMEN COLLECTION CONTROL FOR SAMPLES COLLECTED WITH THE SHIELD SALIVA COLLECTION KIT:

In order to verify whether a patient has provided an adequate volume of saliva in the sterile collection device, visual inspection is conducted to ensure the specimen meets minimum volume requirements. The collection vial has fill line markings to indicate the volume of specimen collected.

SHIELD SALVIA COLLECTION KIT SAMPLE ACCESSIONING:

Specimens received at the clinical laboratory for testing with the covidSHIELD assay undergo the following accessioning prior to acceptance for testing.

Upon accessioning, each sample tube is manually removed from the transport bag and visually inspected to verify:

- The biohazard bag was sealed
- The cap was sealed properly, and the vial has no apparent leak
- The tube was filled to a sufficient level as indicated by fill line markings on the vial
- The saliva is satisfactorily clear
- The saliva is satisfactorily untainted with food particles
- The timestamp from sample collection to testing is less than 56 hours

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:

Biomek Robotics:
The preliminary LoD using the Biomek 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.

Gilson PipetMax Robotics:
The Gilson PipetMax robotics system was also validated with the QS7 Flex (Workflow 1B.2) and QS7 Pro (Workflow 1B.1) systems:

For QS7 Flex systems using Gilson robotics, 2 mL of negative saliva in 4 mL tubes were spiked with gamma-irradiated SARS-CoV-2 (BEI control material; 7.65 x 10^8 genome copies per mL), to final concentrations of 2,000 and 6,000 copies/mL (20 samples each). Following heat-inactivation in a water bath at 95ºC for 30 minutes, 100uL of cooled saliva samples were transferred to 96-deep well plates pre-loaded with 100uL 2xTBE and 1% Tween-20 using a Gilson PipetMax, and subsequently transferred to 384-well plates pre-loaded with Reaction mix using a Gilson Plate Master. The viral concentration in each well was now 1000 and 3000 copies/mL, which is 1x and 3x the LoD. The 384-well plates were loaded into a QS7 Flex 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. Samples were called positive if 2 out of 3 gene targets were detected per the "Interpretation of Results for samples that are not pooled" table in the IFU. All 20 samples at 1,000 copies/mL (1 x LoD) and all 20 samples at 3,000 copies/mL (3x LoD) were positive. Using the Gilson PipetMax robotics systems and the QS 7Flex, at 1x LoD of 1,000 copies/mL, the mean Ct of the ORF1ab gene target is 33.2$\pm$0.7, for the N gene target is 32.8$\pm$0.4, and for the S gene target is 33.4$\pm$0.8 (mean $\pm$ S.D., N=20).

For QS7 Pro systems using Gilson robotics, 1 mL saliva in 2 mL tubes were spiked with gamma-irradiated SARS-CoV-2 (BEI control material; 7.65 x 10^8 genome copies per mL), to final concentrations of 1,000 copies/mL (20 samples each). Following heat-inactivation at 95ºC for 30 minutes, 100uL of cooled saliva samples were transferred to 96-deep well plates pre-loaded with 100uL 2xTBE and 1% Tween-20 using a Gilson PipetMax, and subsequently transferred to 384-well plates pre-loaded with Reaction mix using a Gilson Plate Master. The viral concentrations in each well were now 500 copies/mL, which is 1x LoD. The 384-well plates were loaded into a QS 7 Pro 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. Samples were called positive if 2 out of 3 genes were detected per the "Interpretation of Results for samples that are not pooled" table in the IFU. All 20 samples at 500 copies/mL (1 x LoD) were positive. Using the Gilson PipetMax robotics systems and the QS 7Pro, at 1x LoD of 500 copies/mL, the mean Ct of the ORF1ab gene target is 33.2±0.7, for the N gene target is 33.3±0.6, and for the S gene target is 34.2±1.0 (mean ± S.D., N=20).

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 95°C 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 (Biomek)	robotic	1B.1	500 copies/mL	35.6	34.6	36.3
	robotic (Gilson)	robotic	1B.1		33.2	33.3	34.2
	manual	robotic	2B.1		35.01	34.01	35.15
	manual	manual	2C.1		34.6	33.87	34.76
QuantStudio 7 Flex	robotic (Biomek)	robotic	1B.2	1000 copies/mL	33.9	33.7	34.4
	robotic (Gilson)	robotic	1B.2	1000 copies/mL	33.2	32.8	33.4

Forced Air Oven Validation:

A study comparing heat inactivation of saliva samples using either a water bath at 95°C for 30 minutes or a forced air oven at 95°C for 50 minutes was conducted. Positive samples were

generated by spiking negative saliva with inactivated virus to target a concentration of 1X and 3X LoD. Eight samples of each concentration were generated for each heating method, with each sample tested in 4 replicates on the Quantstudio 7 Flex with manual sample handling (workflow 2C.2). All replicates for both concentrations generated positive results using either the oven or water bath for heat inactivation. Ct values for the ORF1b gene, N gene, and S gene targets were similar for both heat inactivation methods and both virus concentrations, as indicated below:

	ORF1ab Gene - Average (StDev)		N Gene - Average (StDev)		S Gene - Average (StDev)	
	Water Bath	Oven	Water Bath	Oven	Water Bath	Oven
1X LoD (1,000 copies/mL)	33.79 (1.06)	33.17 (0.77)	32.69 (0.46)	32.15 (0.40)	33.05 (0.96)	32.44 (0.58)
3x LoD (3,000 copies/mL)	32.71 (0.62)	31.74 (0.82)	31.46 (0.38)	30.90 (0.43)	31.69 (0.253)	30.93 (0.61)

These results indicate that heat inactivation of saliva specimens may be performed using either a water bath at 95°C for 30 minutes or a forced air oven at 95°C for 50 minutes.

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

<u>Individual Testing:</u>
A prospective study was conducted to assess the clinical performance of the covidSHIELD assay. 120 study participants who were symptomatic for COVID-19 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)

Pooling evaluation (4- and 8-sample pooling)

To validate pooling of saliva specimens, an evaluation was performed with 23 separate, deidentified, positive samples that had previously tested positive using the covidSHIELD individual assay. Samples were thawed and diluted with saliva that had been confirmed as negative to ensure a minimum of 25% of samples had Ct values within 2-3 of the LoD as defined in the covidSHIELD IFU. Diluted samples were tested individually using the EUA authorized covidSHIELD SARS-CoV-2 assay and again using the proposed pooled test workflow at pooling ratios of 4:1 and 8:1. The covidSHIELD Pooled Test combined 4-sample pools comprised of 1 positive sample and 3 negative samples, or 8-sample pools comprised of 1 positive sample and 7 negative samples, in equal volumes of 100uL per sample. Each pooled sample was then tested according to workflow 1B.1 using the Gilson automated robotics systems for sample transfer. The results for each pooled sample were interpreted using the results interpretation algorithm for pooled testing, where detection of at least one target generated a positive result. All 23 positive 4- and 8-sample pools yielded positive results by the covidSHIELD assay, generating a positive percent agreement (PPA) of 100%.

A regression analysis of the wet testing results from this study indicated that the following Ct shifts were exhibited for each of the three gene targets, confirming a slight loss of assay sensitivity upon 4- and 8-sample pooling:

	Ct Shift		
	N-gene	**ORF1ab**	**S-gene**
4-sample pools	2.4	2.8	2.8
8-sample pools	3.3	3.8	4.9

To assess the clinical impact of this loss in sensitivity with pooled testing, an *in-silico* sensitivity analysis was conducted using historical data collected from laboratories designated to run the covidSHIELD assay. In this analysis, the above Ct shifts were applied to the individual positive results from this historical dataset to determine the percent of positive results that would remain positive upon 4- and 8- sample pooling. A total of 595 historical positive results from five different high complexity CLIA labs designated to run the covidSHIELD assay and located in geographical diverse locations were used in this analysis. This *in-silico* analysis predicted that 96.6% and 95.6% of samples would remain positive upon 4- and 8- sample pooling, respectively.

6) Usability Study

A usability study was conducted to assess user interactions with the SHIELD Saliva Collection Kit. The study was conducted in a simulated at-home environment. The participants asked to enroll in the usability study were consented (per the IRB-approved protocol) and were provided the collection materials and Instructions For Use (IFU). The participants' ability to follow the instructions in the kit was observed by a trained study observer. The study observer was not allowed to answer questions or aid the participant, and any difficulties in the sample collection were noted. After providing their sample, participants completed a questionnaire to assess their comprehension of the IFU. Participants were asked to provide feedback on any areas of confusion or difficulty using the kit.

A total of 126 persons aged 6 years and older completed the study, none of whom had prior experience with saliva collection. Participants from a variety or ages, genders, and education levels were included in the study. Both adult and children participants were enrolled at three sites. Collection in children aged 6-15 years was supervised by a parent, guardian, teacher, or other responsible adult. The age distribution of the participants is broken down below:

	Age group	**N**
Adults	16-24	10
	25-44	39
	45+	28
Children	6-9	15
	10-12	17
	13-15	17
Total		126

The adult participants included those with a range of educational backgrounds, described below:

	N	Percentage
Less than High School or GED	4	5%
High School or GED	7	9%
Some College	11	12%
College Degree	35	45%
Post college degree	21	26%

All 126 saliva samples that were collected tested positive for RNaseP and negative for COVID-19 using covidSHIELD. There were no rejections, invalids, or inconclusives for either test. RNaseP values are summarized below:

Count	126
RNase P Ct Min	18.70
RNase P Ct Max	26.53
RNase P Ct Average	22.76

Additionally, upon accessioning, 95% of specimens had an adequate sample volume, and 99% had no leaks. Participant responses on the questionnaire as well as observations made by the study observers demonstrated that the users were able to comprehend the instructions for use and properly collect an adequate saliva specimen. The responses to the participant questionnaire exceeded the 90% acceptance criteria for correct responses to questions assessing the steps critical for sample collection. Responses on the observer survey met the 95% acceptance criteria when assessing participant performance of the critical tasks.

The study identified two minor areas of user-related risk:

1. 31% of participants neglected to sanitize their hands after cleaning the test kit
2. 11% of participants did not indicate on the questionnaire that samples must be returned the same day they are created.

Based on these results, two minor enhancements were made to the Instructions for Use to emphasize more strongly the need to sanitize hands after providing the sample and to return the sample to the designated collection site on the same day it is collected.

The results of this usability study support adequate comprehension and usability of the instructions for use included in the SHIELD Saliva Collection Kit.

LIMITATIONS:

- The performance of this test was established based on the evaluation of a limited number of clinical specimens. Clinical performance has not been established with all circulating variants but is anticipated to be reflective of the prevalent variants in circulation at the

time and location of the clinical evaluation. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of SARS-CoV-2 and their prevalence, which change over time.

- Negative results for SARS-CoV-2 RNA from saliva should be confirmed by testing of an alternative specimen type if clinically indicated.
- 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.
- Samples should only be pooled when testing volume (demand) exceeds laboratory capacity and/or when testing reagents are in short supply.
- Sample pooling has only been validated using saliva specimens.

WARNINGS:

- This product 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 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.