EMERGENCY USE AUTHORIZATION (EUA) OF THE AMAZON REAL-TIME RT-PCR TEST FOR DETECTING SARS-CoV-2

Amazon Real-Time RT-PCR Test for Detecting SARS-CoV-2

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

The Amazon Real-Time RT-PCR Test for Detecting SARS-CoV-2 ("Amazon Test") will be performed at laboratories designated by STS Lab Holdco (a subsidiary of Amazon.com Services LLC) ("Amazon") that are 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 Standard Operating Procedures that were reviewed by the FDA under this EUA).

INTENDED USE

The Amazon Real-Time RT-PCR Test for Detecting SARS-CoV-2 ("Amazon Test") is an in vitro diagnostic real-time reverse transcription polymerase chain reaction (rRT-PCR) test for the qualitative detection of nucleic acid from SARS-CoV-2 in anterior nasal swab specimens that are self-collected by any individuals (18 years of age or older), including individuals without symptoms or other reasons to suspect COVID-19, using either (1) the Amazon COVID-19 Collection Kit under the supervision of a healthcare provider (HCP) or, (2) the Amazon COVID-19 Test Collection Kit unsupervised at home.

The Amazon Test is also intended for the qualitative detection of nucleic acid from SARS-CoV-2 in pooled samples containing up to five individual anterior nasal swab specimens per pool that are self-collected using the Amazon COVID-19 Collection Kit under the supervision of a healthcare provider in individual vials containing transport medium by any individual (18 years of age or older), including individuals without symptoms or other reasons to suspect COVID-19.

Testing is limited to laboratories designated by STS Lab Holdco (a subsidiary of Amazon.com Services LLC) that 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. The SARS-CoV-2 RNA is generally detectable in nasal swab 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 epidemiological information. 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 and if 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.

The Amazon Test is intended for use by qualified and trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures. The Amazon Test with the Amazon COVID-19 Collection Kit and Amazon COVID-19 Test Collection Kit is only for use under the Food and Drug Administration's Emergency Use Authorization.

DEVICE DESCRIPTION AND TEST PRINCIPLE

Overview of Amazon Employee Screening Program
Amazon plans to use the Amazon Real-Time RT-PCR Test for Detecting SARS-CoV-2 (Amazon Test) as part of the company's overall Coronavirus Disease 2019 ("COVID-19") preparedness and response program. All Amazon facilities have health and safety measures in place that are consistent with current public health guidelines.[1]

Individuals who are permitted to enter Amazon facilities under these health and safety measures will be invited to be tested periodically using the Amazon Test. To implement the testing program, Amazon has partnered with a third-party healthcare provider who will issue the necessary prescriptions and individual test orders, as well as facilitate the reporting of results to test recipients.

Amazon employees may automatically be provided with a testing appointment. The appointment day and time will be determined by the testing cadence which is typically every 14 days depending on the individual's work schedule and previous test result. Appointments are voluntary (unless there is a government-mandated testing program in place) and individuals can elect whether to participate. Individuals will be informed of their appointment date and time on the preceding day via an email, text message, and/or other employee communications tool.

For individuals who are not automatically scheduled for testing, Amazon will communicate information about testing availability, frequency, and other aspects of Amazon's testing program through a variety of channels, including regular manager alerts, direct-to-employee notifications, text messages, and signage.

Depending on factors such as work schedule and location, employees may be scheduled for on-site, supervised specimen collection or provided with a collection kit for unsupervised collection at home. When an Amazon employee checks in (either online or in person) at the time of their appointment and requests a sample collection kit, Amazon will automatically verify that the employee meets the eligibility criteria for testing according to applicable risk factors and public health guidelines consistent with the standing order prescription, and, if they are eligible, a test order will be issued and transmitted to the laboratory. Under no circumstance does an employee receive a collection kit prior to issuance of an order for the collection kit and associated test under the blanket prescription.

[1] As of March 23, 2021, individuals at all Amazon facilities are subject to mandatory temperature checks, self-monitoring of clinical symptoms, and self-quarantining.

Workflow for the Amazon Test

a) *Electronic Orders*
Individuals who qualify for testing under the program may be tested on a regular basis. For each test, an individual electronic order will be authorized by a healthcare provider in accordance with a standing order prescription pursuant with the healthcare provider's practices and applicable public health guidelines.

b) *Specimen Collection*

Supervised Collection using the Amazon COVID-19 Collection Kit
Anterior nasal sample collection will be performed by the individual test recipient using a nylon flocked swab, following written or digital instructions provided at the collection location under the supervision of a healthcare worker, either in person or via telehealth link. When supervision is via remote telehealth link, audio-visual communication with the healthcare provider will be set up prior to arrival of the test recipient, so that no specific instruction regarding use of the telecommunications system is needed. Under both scenarios, trained Amazon personnel (a healthcare worker and a trained collection monitor) will be present to assist with the logistics of specimen collection, addition of the specimen to the transport medium and preparation for shipment. On site safety measures required under the sample collection protocol include the use of safety fixtures to secure sample collection tubes, gloves worn while at the sample collection station, clear and simple pictorial and text sample processing instructions, and clinician and Amazon collection monitor observation to ensure safe and effective sample collection.

Unsupervised Collection Using the Amazon COVID-19 Test Collection Kit
After the order for a test is authorized by a healthcare provider as described above, an individual will receive the Amazon COVID-19 Test Collection Kit by mail or pick it up from a designated Amazon facility. Prior to sample collection, individuals must register the kit using the website listed in the Instructions for Use (IFU) and verify their personal information and that the number on the sample tube matches the registration number. During the online registration process, individuals will be prompted to watch an instructional video and to read the printed IFU in their entirety before collecting their sample. According to the step-by-step instructions, after swabbing both nostrils, they will break the swab into the collection tube, cap the tube tightly and place it into the provided biohazard bag for return to a designated Amazon drop-off location within 24 hours.

Specimen Transport and Storage
Anterior nasal swabs in PrimeStore MTM (collected on-site under the supervision of an HCP) or Phosphate Buffered Saline (PBS) (collected on-site under the supervision of an HPC or unsupervised at home) for use with the Amazon Test may be transported and stored at between -20 °C and +40 °C for up to 120 hours (5 days) prior to testing. Automated procedures implemented within the laboratory preclude testing of any specimen that is received > 96 hours after collection in order to ensure that testing is completed within the specified interval from collection of 120 hours.

Following either on-site or at home collection, the collection tube is sealed in a plastic biohazard bag and deposited by the individual who provided the sample in a dedicated

Amazon drop-box at their workplace or other designated location. At prespecified intervals, samples within each drop-box are packaged by trained Amazon personnel for shipment to the testing laboratory in accordance with Department of Transport Hazardous Material Shipping Regulations and ICAO (International Civil Aviation Organization)/IATA (International Air Transportation Association) Packaging Instruction 650. All shipments are at ambient temperature.

c) *Specimen Testing*

The Amazon Test is a modification of the BGI Genomics Real-Time RT-PCR Test for Detection SARS-CoV-2 (EUA200034). Amazon has obtained a Right of Reference from BGI Genomics Co. Ltd. to information contained in the EUA submission for the BGI Genomics Real-Time RT-PCR Test for Detection SARS-CoV-2 and all current and future amendments. The assay includes primers and probes for the detection of the ORF1ab region of the SARS-CoV-2 genome, in addition to human β-actin RNA as an endogenous internal control. Nucleic acid extraction is performed using the MGI Easy Nucleic Acid Extraction Kit that was previously authorized for use with the BGI assay.

A Hamilton Microlab STARlet robot is used to transfer sample from the individual collection tubes to 96 well plates for automated sample processing. The Amazon Test has been validated for pooling of up to 5 samples in PrimeStore MTM. The pooling process is automated using the MGI SP-960RS instrument which also performs nucleic acid extraction using reagents aliquoted by an Agilent AssayMAP Bravo Protein Sample Prep Platform. RT-PCR amplification is performed on either an Applied Biosystems 7500 Fast Dx Real-Time PCR Instrument or QuantStudio 5.

Individual samples from any pool that produces a non-negative result (i.e., positive or invalid) are re-tested.

d) *Result Reporting*

Results will be communicated to the test recipients using established channels (e.g., electronic employee portal). Possible result outcomes, explanation and follow-up are described in **Table 1**.

Table 1. Result reporting and follow-up

Reported Result	Explanation/Follow-up
Positive for SARS-CoV-2 RNA	Subjects with a positive test result will receive the following message (or similar) via the employee portal: *"We'd like to inform you that your test result came back POSITIVE for the presence of SARS-CoV-2, the virus that causes COVID-19. This means that the virus was detected in your sample. Your safety is our number one priority. Stay home and do not come to the facility. If you are at work, please exit the site safely, using physical distancing. You do not need to check in with your manager before you collect your belongings and leave."* The message will also provide a link to the FDA-authorized Fact Sheet for Patients. A third-party healthcare provider will contact all subjects who receive a positive result by letter within 24 hours of receipt of the test result and direct the individual to contact their personal healthcare provider.
Negative for SARS-CoV-2 RNA	A negative result does not mean that the individual does not have COVID-19. If they have symptoms consistent with COVID-19, the individual should contact their healthcare provider and stay home from work. [1] The Fact Sheet provided to test recipients will explain the meaning and limitations of a negative test result.
Invalid	The test was invalid (a testing error occurred) and therefore the result could not be interpreted. [1] Recollection and re-testing is recommended.

[1] Individuals who receive negative or invalid results and who do not exhibit symptoms of COVID-19 will be permitted to continue to report to work and will be encouraged to be re-tested by collecting a new sample. Retesting will be performed with samples in pools.

INSTRUMENTS USED WITH THE TEST

Table 2. Instruments and software for use with the Amazon Test

Instrument	Manufacturer	Model Number	Software Version
BSC 1300 Series A2	Thermo Scientific	1300 A2 - 1347	N/A
BSC Purifier	Labconco	302619101	N/A
BSC Purifier	Labconco	302319101	N/A
Agilent NGS Bravo	Agilent Tech	G5563A	Version.A.1.0.2
Microlab StarLET	Hamilton	173000-058/J	Version.4.5.0.7977
Tip Carrier	Hamilton	182085	N/A
Base Carrier, Flat	Hamilton	93522-01	N/A
Raised Carrier	Hamilton	6601988-1	N/A

Instrument	Manufacturer	Model Number	Software Version
		660518-01	
Hamilton Carrier-1x32	Hamilton	173410	N/A
MGI Liquid Handler	MGI Tech	MGI SP-960RS	Version.1.2.0.163
QuantStudio 5 Real-Time PCR System (0.2 mL block)	Applied Biosystems	A28134R	Version.1.3.3
ABI 7500 Fast Dx Real-Time PCR Instrument (0.1 mL block)	Applied Biosystems	44047205	Version.1.4.1
Centrifuge Sorvall ST8	Thermo Scientific	75007200	N/A
Centrifuge Minifuge	Cole Parmer	C1008-B	N/A
Hirschman Pipetus Tool	Andwin Scientific	9907200	N/A
Vortex Genie 2	Scientific Industries	SI-0236	N/A
Iso-temp Oven 1	Thermo Scientific	151030512	N/A
Thermo Cube	Agilent Tech	10-400-1C-4-RS-LT-AR-37B	N/A

REAGENTS AND MATERIALS

Table 3. Reagents used to perform the Amazon Test

Reagent Kit	Manufacturer	Catalog Number	Kit Components	Storage Temperature
MGIEasy Nucleic Acid Extraction Kit	MGI Tech Co., Ltd.	1000020261	Buffer MW1	0°C to 30°C
			Buffer MW2	0°C to 30°C
			RNase Free water	0°C to 30°C
			Enhancer Buffer	-25°C to -15°C
			Magnetic Beads	2°C to 8°C
			Proteinase K	2°C to 8°C
Real-Time Fluorescent RT-PCR Kit for Detecting SARS-CoV-2	BGI Genomics	HW5105	Reaction Mix	-18°C
			Enzyme Mix	-18°C
			BGI Positive Control	-18°C
			BGI Negative Control	-18°C
ROX Reference Dye	Invitrogen	12223-012	Reference Dye	-18°C
Twist Synthetic SARS-CoV-2 RNA Control	Twist Bioscience	102024	Control 2 (MN908947.3)	-70°C to -90°C
Total RNA Control (Human)	Applied Biosystems	4307281	Control	-15 to -25°C
NATtrol SARS-Related Coronavirus-2 (SARS-Cov-	Zeptometrix Corporation	NATSARS (COV2)-ERC	Control	2°C to 8°C

Reagent Kit	Manufacturer	Catalog Number	Kit Components	Storage Temperature
2) External Run Controls				

Table 4. Consumables used to perform the Amazon Test

Consumables	Manufacturer	Catalog Number
Tweezers, Blue 4-1/2 in L Plastic, Sterile, Bag = 100 Tweezers	Dynarex Magnum Medical	491805-2391
Poxygrid Wire Test Tube Rack, Blue, 60 spaces for 13-16 mm tubes, Case = 24 Racks	Bel-Art	F187560160
PCR Plates, Hard-shell Thin-wall 96-well Skirted, White Shell/Clear, Sterile, RNase DNase Free, Box = 50 Plates	Bio-Rad	HSP9601
1000 µL CO-RE Disposable Tips, Sterile, RNase DNase Free, Pack = 5 Tip Racks	Hamilton	235905
Optical Adhesive Film, Pack = 100 Films	Applied Biosystems	4311971
96-Well 2 mL Polypropylene DeepWell Storage Plates, Sterile, RNase DNase Free, Case = 50 Plates	Thermo Scientific	AB0661
96-Well 2 mL Polypropylene DeepWell Storage Plates, Sterile, RNase DNase Free, Case = 10 Packs = 50 Plates	VWR International	75870-796
1.3 mL U-Bottom Deep-Well Plate, Sterile, RNase DNase Free, Pack = 2 Plates	DN Biotech	07350504
Ethanol, Absolute (200 Proof) Molecular Biology Grade, Bottle = 4 Liters	Thermo Fisher Sci.	BP28184
Distilled Water. RNase DNase Free, Case = 10 (500 mL) Bottles	Invitrogen	10977023
Pipette Tips TR LTS 1000 µL F 768A/8, Pre-Sterilized, Filter, RNase DNase Free, Box = 10 Tip Racks	Mettler Toledo Rainin LLC	TR-L1000F
Pipette Tips TR LTS 200 µL F 960A/10, Pre-Sterilized, Filter, RNase DNase Free, Box = 10 Tip Racks	Mettler Toledo Rainin LLC	TR-L200F
Pipette Tips TR LTS 20 µL F 960A/10, Pre-Sterilized, Filtered, RNase DNase Free, Box = 10 Tip Racks	Mettler Toledo Rainin LLC	TR-L10F
Pipette Tips RT LTS 5000 µL 192A/8, Box = 8 Tip Racks	Mettler Toledo Rainin LLC	30389256
Pipette Tips RT LTS 5000 µL, Box = 5 Tip Racks	Thermo Scientific	94052550
Single Well Robotic Reagent Reservoir, Sterile, RNase DNase Free, Each = 1 Reservoir	Corning	RESSW96HPSI

Consumables	Manufacturer	Catalog Number
Single Well Robotic Reagent Reservoir, Sterile, RNase DNase Free, Box = 100 Reservoirs	Integra	6328
Single Well Robotic Reagent Reservoir, Sterile, RNase DNase Free, Box = 20 Reservoirs	Scilutions	RES96DWHRS
Centrifuge Tubes (50 mL conical in racks), Sterile, RNase DNase Free, Case = 12 Racks of 25 Tubes	Thermo Scientific	339653
Serological Pipettes, 25 mL, Sterile, RNase DNase Free, Case = 4 Bags = 200 Pipettes	Thermo Scientific	170357N
Polycarbonate Erlenmeyer Flask w/Flat Cap, 250 mL, RNase/DNase Free, Case = 50 Bottles	Corning	431406
Multiple Well Reagent Reservoir with 12-Channel Trough, Sterile, RNase DNase Free, Case = 25 Reservoirs	Corning Inc.	RESMW12HPSI
Multiple Well Reagent Reservoir with 12-Channel Trough, Sterile, RNase DNase Free, Case = 5 Sleeves = 25 Reservoirs	Agilent Technologies	201256-100
Fast Optical 96-well Reaction Plate with Barcode (0.1 mL) I2070, RNase DNase Free, Box = 20 Plates	Applied Biosystems	4346906
Serological Pipettes (10 mL), Individually Wrapped, Sterile, RNase DNase Free, Case = 2 Bags = 200 Pipettes	Thermo Scientific	170356N
Sealing Tape, Clear Sterile Polyester Adhesive for 96 Well Plates, Pack = 200 Tapes	Thermo Scientific	236366
Pipette, 50 mL Polystyrene Serological, All-Plastic Wrapped, Sterile, RNase DNase Free, Bag = 100 Pipettes	Thermo Scientific	1367610R
Pipette, 100 mL Polystyrene Serological, All-Plastic Wrapped, Sterile, RNase DNase Free, Bag = 100 Pipettes	Corning	4484
Lint Wipers, Case = 60 Packs = 16,800 Wipes	Kimberly Clark	34155
Reagent Reservoirs 100 mL, RNase, DNase Free, Bag = 10 Reservoirs	Research Products Intl Corp	248225
Cooler Block, Aluminum 1.5/2.0 mL 15-Well, Each = 1 Block	Cole Parmer	6361501
Cooler Block, Aluminum 0.2 mL 96-Well, Each = 1 Block	Cole Parmer	6361504
Graduated Cylinder, PMP, 500 mL, Each = 1 Cylinder	Thermo Scientific	36630500
Carousel Stand for 7 pipettes, Each = 1 Stand	Toledo Mettler Rainin LTD	CR-7
96-Well Support Base, Box = 10 Bases	Applied Biosystems	4379590

Consumables	Manufacturer	Catalog Number
Reservoir Base, for Integra 6328, Box = 8 Bases	Integra	6305
E4 XLS+ 8-channel pipette, 20-200 µL, uses LTS LiteTouch tips	Toledo Mettler Rainin LTD	E8-200XLS+
E4 XLS+ 8-channel pipette, 100-1200 µL, uses LTS LiteTouch tips	Toledo Mettler Rainin LTD	E8-1200XLS+
E4 XLS+ 8-channel pipette, 2-20 µL, uses LTS LiteTouch tips	Toledo Mettler Rainin LTD	E8-20XLS+
Pipet-Lite XLS+ manual 8-channel pipette, 20-200 µL, uses LTS LiteTouch tips	Toledo Mettler Rainin LTD	L8-200XLS+
Pipet-Lite XLS+ manual 8-channel pipette, 100-1200 µL, uses LTS LiteTouch tips	Toledo Mettler Rainin LTD	L8-1200XLS+
Pipet-Lite XLS+ manual single-channel pipette, 20-200 µL, uses LTS LiteTouch tips	Toledo Mettler Rainin LTD	L-200XLS+
Pipet-Lite XLS+ manual single-channel pipette, 2-20 µL, uses LTS LiteTouch tips	Toledo Mettler Rainin LTD	L-20XLS+
Pipet-Lite XLS manual single-channel pipette, 500-5000 µL, uses LTS LiteTouch tips	Toledo Mettler Rainin LTD	L-5000XLS+
Cleaning Swabs For Micro Focus Cell and Other Cuvettes - Long Handled, with flexible knit polyester tip, Pack = 10 Swabs	Fireflysci	SWABMFC
96 WELL .2 ML SPECTRAL CALIBRATION PLATE 2	Applied Biosystems	A26332
ABY Dye Spectral Calibration Plate for Multiplex qPCR, Fast 96-well	Applied Biosystems	A24734
TaqMan RNase P Instrument Verification Plate, Fast 96-well (for 0.1 mL block)	Applied Biosystems	4351979
Optical 96-Well Reaction Plate with Barcode, for 0.2 mL Tubes, Box = 20 Plates	Applied Biosystems	4306737

Table 5. Amazon COVID-19 Collection Kit (supervised collection)

PrimeStore MTM [1]			
Component	**Description**	**Supplier**	**Part Number**
Nasal swab (1 ea.)	Individually-wrapped sterile flocked nylon swab for nasal specimen collection	ASP Global	8205
Collection Tube (1 ea.)	Prefilled tube containing 1.5 mL PrimeStore MTM	Longhorn Vaccines & Diagnostics	MTM-5
Biohazard bag (1 ea.)	Clear, 2 mm plastic 2-wall zip top bag	Various	Various
Phosphate Buffered Saline (PBS) [1]			
Component	**Description**	**Supplier**	**Part Number**
Nasal swab (1 ea.)	Individually-wrapped sterile flocked nylon swab for nasal specimen collection	Puritan Medical Products *or* Medico Technology	25-3306-U 96000BQ
Collection Tube (1 ea.)	Sterile plastic collection tube aseptically pre-filled with 1 mL sterile phosphate-buffered saline (PBS) solution	Tube: Corning Inc. PBS: Thermo Fisher	430663 BP2438
Biohazard bag (1 ea.)	Clear, 2 mm plastic 2-wall zip top bag	Various	Various

[1] The Amazon COVID-19 Collection Kit contains either a collection tube containing PrimeStore MTM or a collection tube containing PBS, together with the applicable listed components

Instructions for use of the Amazon COVID-19 Collection Kit are provided in hard copy or on a static digital screen at the collection station.

Table 6. Amazon COVID-19 Test Collection Kit (unsupervised collection)

Component	**Description**	**Supplier**	**Part Number**
Nasal swab (1 ea.)	Individually-wrapped sterile flocked nylon swab for nasal specimen collection	Puritan Medical Products *or* Medico Technology	25-3306-U 96000BQ
Collection Tube (1 ea.)	Sterile plastic collection tube aseptically pre-filled with 1 mL sterile phosphate-buffered saline (PBS) solution	Tube: Corning Inc. PBS: Thermo Fisher	430663 BP2438
Biohazard bag (1 ea.)	Clear, 2 mm plastic 2-wall zip top bag	Various	Various
Instructions for Use (1 ea.)	Kit registration, sample collection, and drop-off instructions	Amazon.com Services LLC	N/A

ACCESSIONING CRITERIA

Table 7. Accessioning criteria applied to specimens received for analysis with the Amazon Test

Rejection Reason	Definition
Insufficient sample volume (no leaking)	There is no evidence of leaking on the vial or biohazard bag but the vial does not contain the minimum volume.
Empty vial (no leaking)	There is no evidence of leaking on the vial or biohazard bag and the vial is empty.
Sample leaking	The vial is not damaged, but there is evidence of leaking.
Barcode damage (unreadable un-scannable)	There is damage to the barcode that makes it unreadable and unscannable.
Sample damaged (tube cracked)	The vial is cracked.
Sample damaged (other)	The sample is damaged in a way not specified by other rejection code options; damage prevents the sample from being processed.
Sample not received	Sample virtually received at dock but the sample is not physically in outer biohazard bag.
Tube not present in biohazard bag	Biohazard bag is empty.
More than 1 tube in biohazard bag	Biohazard bag contains multiple vials.
Incorrect tube or label type	Tube type is incompatible/incorrect and cannot be processed in lab machines.
Swab Missing	Swab is not present in the sample vial.
Swab inserted improperly	Swab is present in the sample vial, but it is was inserted improperly (e.g., upside down)
Swab issue – other	All other swab issues (i.e., swab damaged or cannot be removed)
Expired specimen	Received > 96 hours post collection

Specimens in PBS may be self-collected unobserved or under the supervision of a healthcare provider (HCP). Pooling of specimens in PBS is restricted to those collected under HCP supervision. Therefore, the following precautions have been implemented to prevent pooling of samples that are not collected in this manner:

1) Manual demarcation of shipping containers to indicate that the contents are from “unsupervised” collection.
2) Sorting of specimens upon receipt in the laboratory to segregate specimens according to transport medium and collection type (supervised/unsupervised).
3) Reconciliation of collection tube serial numbers and site information to ensure that all samples collected without HCP supervision have been received and routed for individual testing, prior to processing any PBS specimens collected under HCP observation.

CONTROLS

The assay controls used with the Amazon Test are described in **Table 8**. Amazon has validated alternative source materials for use as Positive and Negative Controls which may be used interchangeably. Two Positive Controls and two Negative Controls must be processed with each batch of up to 92 patient samples.

Table 8. Assay controls used with the Amazon Test

Control Type	Material	Description
Positive[1]	BGI SARS-CoV-2 Positive Control	Supplied with the BGI assay kit. Two aliquots of the BGI Positive Control material are processed with every plate of samples.
	Twist nCoV2 Synthetic Viral RNA	Prepared by diluting a working stock of 10,000 copies/µL in PrimeStore medium or Phosphate Buffered Saline (PBS) containing 5 ng/mL total human RNA to a final concentration of 100 copies/µL. The working stock is stored at -80 °C in single use aliquots.
	Zeptometrix Flu/RSV/SARS-CoV-2	Prepared in PrimeStore medium or PBS containing 5 ng/mL total human RNA to a final concentration of 200 copies/µL. The working stock is stored at -80 °C in single use aliquots.
Negative[1]	BGI SARS-CoV-2 No Template Control	Supplied with the BGI assay kit. Two aliquots of the BGI No Template Control material are processed with every plate of samples.
	RNase-free water	Prepared in aliquots from bulk. Stored at -80 °C.
Internal	β-actin	Endogenous Internal Control for the presence of human RNA in patient samples and Positive Controls.

[1] 2 Positive and 2 Negative Controls must be processed with each batch of up to 92 patient samples. Positive and Negative Controls formulated with different source materials may be used interchangeably.

Passive Reference Dye

Amazon has validated use of a passive reference dye (ROX, Invitrogen Cat #. 12223012) that is added to each amplification reaction to normalize fluorescent signals and thereby reduce variability instrument-to-instrument, run-to-run and well-to-well.

INTERPRETATION OF RESULTS

The Standard Operating Procedure for the Amazon Test recommends that the operator should verify the Confidence Score associated with each amplification curve. The Confidence Score refers to the QCCONF parameter that is an output of the result interpretation software for the ABI 7500 and QuantStudio 5 PCR instruments. In general, a Confidence Score value of > 0.75 is indicative of valid amplification but this value alone should not be used to either accept or reject a run. All runs must be reviewed and evaluated by the laboratory director or his/her designee to make a final judgement of the validity of the data taking into in consideration all the acceptance criteria.

Assay Controls

The criteria for interpretation of the results obtained with the assay controls are shown in **Table 9**. All controls must produce the expected results in order to interpret the results from testing of patient samples. If one or both of the controls of each type (Positive, Negative) do not meet specification, a retest of the whole run must be performed or, if sample quantity is insufficient, an "error" test result is reported.

Table 9. Interpretation of results for assay controls

Control	Ct Value		Interpretation
	ORF1ab (FAM)	β-actin (VIC)	
Positive Control	≤ 32	≤ 35	Pass [1]
	> 32	≤ 35	Fail [2]
	Any	> 35	Fail
Negative Control	Undetermined	> 35 or Undetermined	Pass
	Any	> 35 or Undetermined	Fail

[1] The Positive Controls must have Ct ≤ 32 and ΔCt < 3 for the SARS-CoV-2 target

[2] If the controls fail, the extraction/PCR run is considered invalid and all samples must be retested with fresh controls

Patient Specimens

Pooled Specimens (observed collection only)

If a pool returns a positive or failed (invalid) test result, each sample within the pool must be retested individually (**Table 10**). If a pool returns a negative result, all samples within the pool are reported as "Presumptive Negative."

Table 10. Interpretation of results from pooled specimens in PrimeStore MTM

Ct Value		Interpretation for the Pool	Action
ORF1ab (FAM)	β-actin (VIC)		
Any or Undetermined	> 35 or Undetermined	Failed	Repeat testing of each constituent specimen in the pool as a separate "hitpick" extraction.
≤ 38	≤ 35	Positive	
> 38	≤ 35	Negative	Report individual specimens as Presumptive Negative

Individual Specimens

The results from testing individual specimens either as a reflex to testing in a specimen pool ("hitpick") or when testing as a primary, individual specimen without a previous result from a pool, are interpreted as shown in **Tables 11** and **12**.

Table 11. Interpretation of results from individual specimens (initial run) [1]

Ct Value		Interpretation for the Specimen	Action
ORF1ab (FAM)	**β-actin (VIC)**		
Any or Undetermined	> 35 or Undetermined	Failed	Repeat test
≤37	≤ 35	Positive	Report as Positive
> 37	≤ 35	Negative	Report as Negative

[1] Either as a primary, individual specimen or as a reflex to the result obtained from a pool

Table 12. Interpretation of results from individual specimens upon re-test

Ct Value		Interpretation for the Specimen	Action
ORF1ab (FAM)	**β-actin (VIC)**		
> 37	> 35	Failed	Report as Invalid (Recollect) [1]
Undetermined	Undetermined	Failed	Report as Invalid (Recollect)
≤ 37	> 35	Failed	Report as Invalid (Recollect)
≤ 37	≤ 35	Positive	Report as Positive
> 37	≤ 35	Negative	Report as Negative

[1] Recollect: the subject will be informed of a test error and encouraged voluntarily to re-test; recollected samples will be tested according to the standard pooling workflow

If a pool is reported as positive but all five samples from the pool return negative test results when tested individually, the occurrence will be referred to the laboratory director and an investigation will be initiated, including assessment of the potential for:

a) Contamination / false positive pool result
b) Assay inhibition upon individual testing
c) Differences in assay reagents between pooled and individual testing

If no root cause is identified, the individual samples will be retested once (assuming adequate volume remains) and the results will be reported. If insufficient volume remains for retesting, the subjects will be informed of a test error and encouraged voluntarily to re-test. Re-collected samples will be processed according to the standard pooling workflow.

PERFORMANCE EVALUATION

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

LoD Determination with Samples in PrimeStore MTM

Determination of the LoD of the Amazon Test was performed using the ABI 7500 Fast Dx Real-Time PCR Instrument (software version 1.4.1) with PrimeStore MTM containing nasal swab matrix that was spiked with inactivated SARS-CoV-2 (BEI Resources; SARS-related Coronavirus, Isolate USA-WA1/202; Cat. # N52287). A preliminary titration was performed with 12 replicates at each of 10 different concentrations as shown in **Table 13**. The lowest level at which all replicates were reported as positive was 500 copies/mL, irrespective of whether a Ct cut-off of 37 (individual testing) or 38 (pooling) was used.

Table 13. LoD estimation using inactivated SARS-CoV-2 in PrimeStore MTM containing nasal swab matrix

Copies/mL	Total	Positive (%)		Ct Value	
		Ct ≤ 37	Ct ≤ 38	Mean	SD
2000	12	12 (100)	12 (100)	32.9	0.29
1000	11	11 (91.7)	11 (91.7)	34.2	0.55
500	**12**	**12 (100)**	**12 (100)**	**34.9**	**0.36**
250	12	9 (75.0)	11 (91.7)	36.3	0.82
125	12	6 (50.0)	8 (66.7)	36.9	0.63
62.5	12	3 (25.0)	6 (50.0)	37.3	0.57
31.3	12	1 (8.3)	3 (25.0)	36.7	1.51
15.6	12	1 (8.3)	1 (8.3)	35.7	3.31
7.8	12	0 (0)	0 (0)	39.7	N/A
3.9	12	0 (0)	1 (8.3)	37.9	N/A

N/A: Not applicable; SD: Standard Deviation
The lowest level at which all replicates were reported positive is highlighted in yellow/green with **bold** face text
A Ct cut-off of 37 is used for individual samples; a Ct cut-off of 38 is used for pooled specimens

The estimated LoD obtained was confirmed by testing an additional 20 independent extraction replicates at each of five target levels above and below the estimated value (**Table 14**). The confirmed LoD with inactivated virus PrimeStore medium containing nasal swab matrix, defined as the lowest level at which ≥ 95% of replicates were reported positive, was 500 copies/mL irrespective of which Ct cut-off was applied (for individual or pooled specimen testing).

Table 14. LoD confirmation with inactivated virus in PrimeStore MTM containing nasal swab matrix

Copies/mL	Total	Positive (%)		Ct Value	
		Ct ≤ 37	Ct ≤ 38	Mean	SD
2000	20	20 (100)	20 (100)	34.1	0.76
1000	20	18 (90.0)	18 (90.0)	35.2	0.78
500	**20**	**19 (95.0)**	**19 (95.0)**	**35.7**	**0.49**
250	20	11 (55.0)	18 (90.0)	37.0	0.61
125	20	4 (20.0)	15 (75.0)	37.3	0.60

SD: Standard Deviation
The lowest level at which ≥95% of replicates were reported positive is highlighted in yellow/green with **bold** face text
A Ct cut-off of 37 is used for individual samples; a Ct cut-off of 38 is used for pooled specimens

LoD Comparison: PrimeStore MTM vs Phosphate Buffered Saline
Phosphate Buffered Saline (PBS) offers advantages over PrimeStore MTM in terms of supply chain logistics and ease of use due to the absence of potentially hazardous preservative fluid, making it suitable for use as a transport medium for unsupervised home collection of specimens. A study was conducted to compare the analytical sensitivity of the Amazon Test with anterior nasal swab specimens collected in PBS and PrimeStore MTM. Pooled nasal swab matrix in each medium was spiked with inactivated SARS-CoV-2 (Zeptometrix Cat. # NATFRC-6C) at different concentrations and tested using the ABI 7500 Fast Dx Real-Time PCR Instrument. The LoD was initially estimated as the lowest concentration at which ≥ 7/8 replicates produced positive results. The LoD was then confirmed by testing 20 additional replicates at concentrations around the estimated LoD. Using a Ct cut-off of 37 (individual testing), the LoD for the Amazon Test was confirmed to be 100 copies/mL with samples in PBS and 75 copies/mL with samples in PrimeStore medium (**Table 15**). With a Ct cut-off of 38 that is used with pooled specimens, the confirmed LoD was 50 copies/mL with samples in PBS and 75 copies/mL with PrimeStore MTM (**Table 16**).

Table 15. Estimation of the Amazon Test LoD with nasal swab matrix in PBS and PrimeStore MTM

Copies/ mL	*N*	Phosphate Buffered Saline				PrimeStore MTM			
		Positive (%)		Ct value		Positive (%)		Ct value	
		Ct ≤ 37	Ct ≤ 38	Mean	SD	Ct ≤ 37	Ct ≤ 38	Mean	SD
10000	8	8 (100)	8 (100)	28.8	0.11	8 (100)	8 (100)	28.8	0.14
5000	8	8 (100)	8 (100)	29.8	0.20	8 (100)	8 (100)	29.8	0.11
2500	8	8 (100)	8 (100)	30.7	0.19	8 (100)	8 (100)	30.9	0.08
1500	8	8 (100)	8 (100)	31.5	0.19	8 (100)	8 (100)	31.7	0.21
1000	8	8 (100)	8 (100)	32.1	0.23	8 (100)	8 (100)	32.2	0.24
500	8	8 (100)	8 (100)	33.2	0.34	8 (100)	8 (100)	33.2	0.16
250	8	8 (100)	8 (100)	34.4	0.54	8 (100)	8 (100)	34.4	0.49
150	8	8 (100)	8 (100)	35.3	0.61	8 (100)	8 (100)	35.2	0.67
100	8	7 (88)	8 (100)	36.0	0.76	7 (88)	8 (100)	36.0	0.91
75	8	6 (75)	8 (100)	36.4	0.59	**8 (100)**	8 (100)	**35.5**	**0.74**
50	8	**8 (100)**	**8 (100)**	**36.2**	**0.50**	6 (75)	**8 (100)**	**36.7**	**0.86**
25	8	2 (25)	4 (50)	37.1	1.21	2 (25)	4 (50)	36.9	1.15

SD: Standard Deviation
A Ct cut-off of 37 is used for individual samples; a Ct cut-off of 38 is used for pooled specimens
Samples with no Ct value (undetermined) were excluded from calculation of the mean and SD
Results at the estimated LoD are highlighted in yellow (individual testing)/green (pooled specimens) with **bold** face text

Table 16. Confirmation of the Amazon Test LoD with nasal swab matrix in PBS and PrimeStore MTM

Copies/ mL	*N*	Phosphate Buffered Saline				PrimeStore MTM			
		Positive (%)		Ct Value		Positive %		Ct Value	
		Ct ≤ 37	Ct ≤ 38	Mean	SD	Ct ≤ 37	Ct ≤ 38	Mean	SD
300	20	20 (100)	20 (100)	33.5	0.58	20 (100)	20 (100)	33.5	0.34
200	20	20 (100)	20 (100)	34.2	0.48	20 (100)	20 (100)	34.3	0.41
150	20	20 (100)	20 (100)	34.6	0.58	20 (100)	20 (100)	34.8	0.52
100	20	**19 (95)**	19 (95)	**35.2**	**0.76**	20 (100)	20 (100)	35.2	0.46
75	20	18 (90)	20 (100)	35.9	1.01	**20 (100)**	**20 (100)**	**35.4**	**0.70**
50	20	16 (80)	**20 (100)**	36.3	0.98	12 (60)	16 (80)	36.6	0.92
37.5	20	8 (40)	14 (70)	36.8	0.88	11 (55)	17 (85)	36.8	0.94
25	20	8 (40)	16 (80)	36.7	1.12	9 (45)	17 (85)	36.8	1.10

SD: Standard Deviation
A Ct cut-off of 37 is used for individual samples; a Ct cut-off of 38 is used for pooled specimens
Samples with no Ct value (undetermined) were excluded from calculation of the mean and SD
Results at the estimated LoD are highlighted in yellow (individual testing)/green (pooled specimens) with **bold** face text

The analytical sensitivity of the Amazon Test was therefore shown to be similar with both types of transport medium (< 2-fold difference in LoD), supporting their use for individual testing and testing of pooled samples collected under supervision.

LoD Comparison: ABI 7500 Fast Dx vs QuantStudio 5
To support use of the ABI Fast Dx Real-Time PCR Instrument and QuantStudio 5 Real-Time PCR System interchangeably, a bridging study was which demonstrated that the analytical sensitivity of Amazon Test is similar when performed on each system.

2) Inclusivity (Analytical Sensitivity) and Cross-reactivity (Analytical Specificity):

Amazon has obtained a Right of Reference from BGI Genomics Co. Ltd. to information submitted under EUA200034 for the BGI Genomics Real-Time Fluorescent RT-PCR Test for Detection SARS-CoV-2, as well as all current and future amendments. As such, no additional inclusivity or cross-reactivity testing was performed in support of the EUA request for the Amazon Test.

To account for the dilution effect from specimen pooling, Amazon has implemented the use of a higher Ct cut-off for pooled nasal swab specimens than for testing of individual samples ($Ct \leq 38$ vs $Ct \leq 37$), which could lead to a reduction in specificity. However, all sample pools with non-negative test results are reflexed for individual testing. As such, because the cut-off for individual testing is the same as that which was validated originally by BGI under EUA200034, no adverse effect on analytical or clinical specificity is expected from this modification.

Independent *in silico* inclusivity analysis performed in February, 2021 predicted no significant impact from known SARS-CoV-2 variants on the inclusivity of the primers and probes used in the BGI Genomics Real-Time Fluorescent RT-PCR Test for Detection of SARS-CoV-2.

3) Specimen Stability in PrimeStore MTM

Amazon has obtained a Right of Reference from Longhorn Vaccines and Diagnostics to specimen stability data submitted in support of DEN170029 for PrimeStore MTM. Data contained within this submission demonstrate the stability of viral RNA in PrimeStore MTM for 29 days at 4 °C or 8 days at 27 °C. Additional studies performed on behalf of Amazon evaluated the stability of SARS-CoV-2 RNA in PrimeStore MTM at temperatures between -20 and 60 °C, including exposure to up to 10 freeze-thaw cycles.

In combination, the results of these studies support the transport and storage of nasal swab specimens in PrimeStore MTM for up to 120 hours (5 days) at -20 to +40 °C prior to testing.

4) Amazon COVID-19 Test Collection Kit

Specimen Stability in Phosphate Buffered Saline
The stability of specimens collected in PBS was evaluated using nasal swab matrix that was spiked with inactivated SARS-CoV-2 (Zeptometrix Cat. # NATFRC-6C) and held under different conditions prior to testing. In combination, the results of these studies support the transport and storage of nasal swab specimens in PBS for up to 120 hours (5 days) at -20 to +40 °C prior to testing.

Usability Study

Amazon conducted a study to evaluate the usability of the Amazon COVID-19 Test Collection Kit for unsupervised collection of anterior nasal swab specimens. The study was performed in a simulated home environment at a designated location at an Amazon facility. Each individual participant received a kit and performed the steps described in the IFU, including: (i) registration of the kit (using a prototype version of the registration software), (ii) sample collection, (iii) packaging of the sample, and (iv) returning the packaged sample to a designated location. Each participant also completed an online questionnaire regarding their experience.

There was a total of 65 study participants representing different age groups and levels of education. To participate in the study, individuals had to be at least 18 years of age, be willing to receive and perform specimen collection, and to fill out the participant questionnaire. Individuals with experience of at-home self-collection of specimens, medical or laboratory training, or who were unwilling to perform sample collection were excluded from participating.

During sample collection, each participant was watched by a remote passive observer who also completed a questionnaire based their observations. Samples collected in the Usability Study were shipped by Amazon personnel to Amazon's laboratory in Hebron, KY for testing. Upon arrival, the collection kit packaging and samples were inspected according to the laboratory accessioning criteria to determine whether they were acceptable for testing. Samples deemed acceptable were submitted for analysis using the Amazon Test.

The demographics of the usability study population are summarized in **Table 17**. The participants had a diverse range of age and education (ages 18+, with education from a high school degree to a doctoral level degree).

Table 17. Demographic characteristics of participant in the Usability Study for the Amazon COVID-19 Test Collection Kit

Age	**Participants**
18-29 years	19
30-39 years	28
40-49 years	13
50-59 years	4
60 years or older	1
Gender	**Participants**
Male	35
Female	29
Other	1
Highest Education	**Participants**
High School Diploma / Associate Degree	9
Bachelor's Degree	31
Master's Degree	22
Doctoral Degree / Post Graduate	3

Except for the questions with Yes/No answers, participants and passive observers answered their questionnaires using a 5-point Likert scale: 5-Strongly Agree, 4-Agree, 3-Neither Agree nor Disagree, 2-Disagree, 1-Strongly Disagree. Responses 3 (Neither Agree nor Disagree) and above were considered positive (favorable). Each participant responded to every survey question. Samples from all 65 study participants were received in the laboratory. One sample (1/65; 1.5%) did not have sufficient volume of PBS for processing. All 64 samples that were tested (100%) were found to have detectable levels of β-actin target (Ct < 35).

Although almost all study participants successfully collected their anterior nasal swab samples, their responses to the questionnaire indicated several areas for improvement in the procedure that were addressed by changing the swab supplier and packaging, clarifying the Instructions For Use and adding a weblink to an instructional video.

Amazon's steps to improve user experience with the Amazon COVID-19 Test Collection Kit were found to be acceptable to mitigate the risks associated with unobserved sample collection. Amazon has agreed to conduct an additional Usability Study with an appropriate number of participants within 30 days post-authorization to validate that the changes they have made to the IFU and swab packaging are effective.

5) Clinical Evaluation:

Comparison to FDA-authorized RT-PCR Assay
To validate the pooling strategy for the Amazon Test, a total of 339 clinical specimens in PrimeStore MTM were obtained from the intended use population of asymptomatic Amazon employees from subjects in 7 different U.S. states. These specimens had previously been characterized as SARS-CoV-2 positive or negative at a single third-party laboratory using a highly sensitive FDA-authorized test (comparator method). Among them were 69 consecutively collected SARS-CoV-2 positive specimens and 270 consecutively collected

SARS-CoV-2 negative specimens, as determined by the comparator method. All 339 specimens were tested individually using the Amazon Test (**Table 18**). Positive and negative agreement with the comparator were 95.7% and 100%, respectively. Among the positive specimens included in the study, > 46% were considered "weak positives" based on the Ct values obtained with the comparator method. The results of this study therefore support the use of the Amazon Test on individual samples obtained from the intended use asymptomatic population.

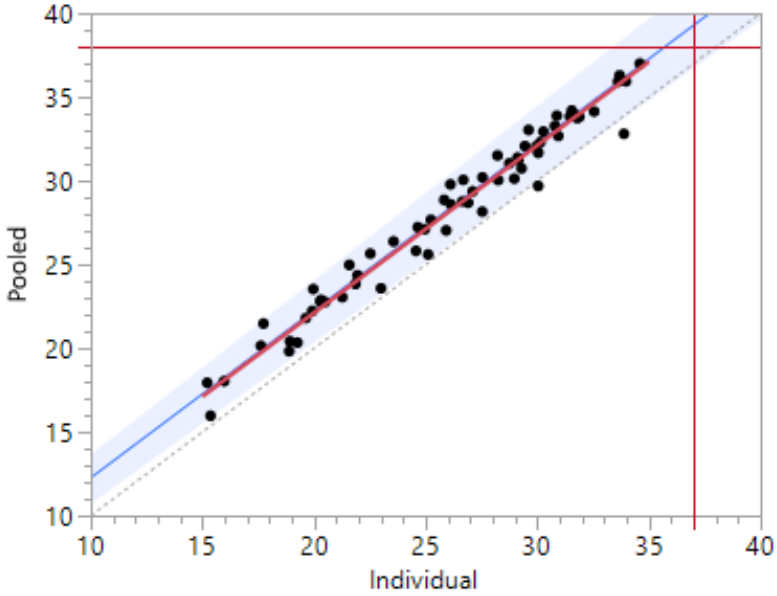
Table 18. Performance of the Amazon Test on individual nasal swab specimens in comparison to an FDA-authorized method

		FDA Authorized Comparator		
		Positive	**Negative**	**Total**
Amazon Test [1]	**Positive**	66	0	**66**
	Negative	3	270	**273**
	Total	**69** [2]	**270**	**339**
Positive Agreement		**95.7% (66/69); 88.0-98.5%**		
Negative Agreement		**100% (270/270); 98.6-100%**		

[1] As determined using the Ct cut-off for individual samples (Ct ≤ 37 = Positive)
[2] 32/69 specimens (46.3%) were considered "weak positive" based on analysis of the Ct values for the comparator assay

Analysis of Real-World Pooling Data with Samples in PrimeStore MTM
Amazon recorded the pooled and individual test results from 64 consecutive 5-sample pools from the intended use population that were positive for SARS-CoV-2 by the Amazon Test between September 13 and October 2, 2020. Passing-Bablok regression analysis based on this data set showed that the predicted shift in Ct from pooling at the individual sample cut-off of 37 was 2.28 (**Figure 1**), which is close to the predicted shift in Ct values due to the dilution factor caused by 5-sample pooling ($\log_2 5 = 2.32$).

Figure 1. Ct values obtained with the Amazon Test with 5-sample pooling compared with individual test results (September 13 to October 2, 2020)



Ct Cut-offs: individual testing ≤ 37; 5-sample pools ≤ 38
Passing-Bablok Regression:
Intercept: 2.25429
Slope: 1.0067
$Ct_{Pooled} = 2.25429 + 1.0067 \times Ct_{Individual}$

Application of the predicted Ct shift from the Passing-Bablok regression described above to the individual Amazon Test results obtained with the 69 known positive samples from **Table 18** demonstrated 89.9% positive agreement compared to the FDA-authorized comparator and 93.9% agreement with the individual Amazon Test results (**Table 19**).

Table 19. Agreement between individual and predicted test results for samples in 5-sample pools

Comparator for Individual Testing	Individual Positive	Predicted Amazon Test Result	
		Positive in 5-sample Pools [1]	Positive Agreement (%)
FDA-authorized assay	69	62	89.9
Amazon Test [1]	66	62 [2]	93.9

[1] As determined using the applicable Ct cut-off for individual or pooled samples (Ct ≤ 37 and ≤ 38, respectively)
[2] Of 4 samples that were predicted to produce negative Amazon Test results in 5-sample pools based on the applied Ct shift, 3 were tested in pools of 1 positive and 4 negative samples and all 3 produced positive results

Validation of 5-Sample Pooling Strategy
Thirty SARS-CoV-2 positive specimens from among those that were identified as positive by

Amazon Test and the FDA-authorized comparator method as described in **Table 18** were selected based on the available residual volume for use in validation of the Amazon 5-sample pooling strategy. Thirty SARS-CoV-2 positive pools were created by combining equal volumes of one known SARS-CoV-2 positive sample and four randomly selected SARS-CoV-2 negative samples to create pools of five samples. Thirty SARS-CoV-2 negative pools were also prepared by combining equal volumes of five randomly selected comparator negative samples.

All SARS-CoV-2 positive pools were reported as Positive by the Amazon Test and all SARS-CoV-2 negative pools were reported as Negative (**Table 20**).

Table 20. Agreement of results from 5 sample pools with expected results using the Amazon Test

		Expected Result [1]		
		Positive	**Negative**	**Total**
Pooled Result [3]	**Positive**	30 [2]	0	**30**
	Negative	0	30	**30**
	Total	**30**	**30**	**60**
Positive Agreement		**100% (30/30); 88.7-100%**		
Negative Agreement		**100% (30/30); 88.7-100%**		

[1] Based on individual Amazon Test results
[2] Of the 30 positive samples used for the pooling validation, when tested individually, 13 (43%) were determined to be "weak positive" based on the Ct values obtained with an FDA-authorized comparator method
[3] As determined using the Ct cut-off for pooled samples (Ct ≤ 38 = Positive)

Amazon began conducting individual testing with the BGI assay on August 12th, 2020. Between August 12th and August 31st, Amazon tested approximately 3,293 samples of which 4 were reported as positive for SARS-CoV-2, demonstrating a 0.1% positivity rate. Amazon began testing using the equipment and procedures for the Amazon Test as outlined in this EUA submission on August 28th. Between August 28th and September 8th, Amazon tested approximately 3,967 samples of which 5 were reported positive for SARS-CoV-2, again demonstrating a positive rate of 0.1%.

The prevalence of SARS-CoV-2 observed in the Amazon employee population through September of 2020 was therefore extremely low, although this does not necessarily reflect that in locally communities or nationally. Based on the above low prevalence in the intended use population, it was concluded that the pooling ratio of 5:1 (5 samples for 1 test) was efficient and appropriate.

Assessment of pooling efficiency and sensitivity will be conducted periodically as described below. The Amazon laboratory data management and analytic system has the functionality to monitor positive rates continuously for both pools and individual samples and post the data on an operational dashboard.

From September 27th, 2020 through December 19th, 2020, Amazon tested 561,170 samples in

pools of up to 5 samples, of which 12,534 (2.2%) returned a positive result triggering the need for individual testing. From the 62,670 samples that were "hitpicked" for individual testing, 13,112 (20.9%) were reported as positive for SARS-CoV-2.

Specimen Pooling Implementation and Monitoring Guidelines

Sample Pooling Implementation (Laboratory Monitoring Part A)

Before a sample pooling strategy is implemented, a laboratory should determine the appropriate pool size based on percent positivity rate in the testing population and pooling testing efficiency (**Table 21**).

Table 21. Efficiency of pooling based on the positivity of SARS-CoV-2 RNA in individual samples (as an example)

P, percent of positive subjects in the tested population	**$n_{maxefficiency}$ (n corresponding to the maximal efficiency)**	**Efficiency of n-sample pooling corresponding to $n_{maxefficiency}$ (a maximum increase in the number of tested patients when Dorfman n-pooling strategy used)**
5%	5	2.35
6%	5	2.15
7%	4	1.99
8%	4	1.87
9%	4	1.77
10%	4	1.68
11%	4	1.61
12%	4	1.54
13%	3	1.48
14%	3	1.43
15%	3	1.39
16%	3	1.35
17%	3	1.31
18%	3	1.28
19%	3	1.25
20%	3	1.22
21%	3	1.19
22%	3	1.16
23%	3	1.14
24%	3	1.12
25%	3	1.10

A.1 If Historical Data for Individual Specimens are Available

A.1.1 Positivity Rate of Individual Testing

- Estimate positivity rate ($P_{individual}$) in the laboratory based on individual sample testing. For this consider the 7-10 previous days and calculate the number of patients tested during those days. $P_{individual}$ is the number of positive results divided by the total number of tested patients during these 7-10 days.

A.1.2 Selection of test developer validated size of sample pools, n

- Use $P_{individual}$ and **Table 21** to choose an appropriate validated pool size. **Table 21** presents the pool size with the maximum efficiency for the validated pool sizes and positivity rates. If the positivity rate ($P_{individual}$) is in **Table 21**, choose n from **Table 21** which corresponds to the maximum efficiency (F).
- If $P_{individual}$ in your laboratory does not correspond to the largest validated pool size in **Table 21**, the pool size with maximum efficiency for this positivity rate was not validated and you should choose the maximum n which was validated. For example, for the calculation of efficiency of 5-sample pooling, using formula $F=1/(1+1/5-(1-P)^5)$, when $P_{individual}$ is 1%, the efficiency F is 3.46 for n=5. It means that 1,000 tests can cover testing of 3,460 patients on average.
- If $P_{individual}$ is greater than 25%, then pooling patient samples is not efficient and should not be implemented.

A.2 If Historical Individual Data for Individual Specimens are Unavailable

If historical data from the previous 7-10 days are unavailable, the maximum pool size validated in the EUA and any smaller pool sizes can still be implemented, because the EUA test has been validated for the maximum pool size-specimen pooling. However, note that without $P_{individual}$, the laboratory may choose a pooling size that does not maximize pooling efficiency.

Sample Pooling Monitoring (Laboratory Monitoring Part B)

After implementing a n-sample pooling strategy, calculate the percent positivity rate (P_{pool}) based on n sample pooling strategy periodically using the data from pooled samples from the previous 7-10 days. *

B.1 If Historical Data for Individual Specimens are Available

If historical data for individual specimens are available, compare P_{pool} to $P_{individual}$ periodically. If P_{pool} is less than 85% of $P_{individual}$ ($P_{pool} < 0.85 \times P_{individual}$), it is recommended that:

- The n-samples pooling should be re-assessed by conducting a re-assessment study as described in "Laboratory Monitoring Part C" below.
- If P_{pool} is greater than 25%, pooling of patient samples is not efficient and should be discontinued until the percent positivity rate decreases.

B.2 If Historical Data for Individual Specimens are Unavailable

- After implementing a n-sample pooling strategy, first calculate the positivity rate ($P_{pool\text{-}initial}$) based on n-sample pool size using the data from testing pooled samples from the first 7-10 days. *
  - If $P_{pool\text{-}initial}$ is greater than 25%, pooling of patient specimens is not efficient and should be discontinued until the percent positivity rate decreases.
  - If $P_{pool\text{-}initial}$ is less than or equal to 25%, pooling of patient specimens can be continued.
- Continue to monitor n-sample pooling strategy by calculating the positivity rate among patient samples during n-sample pooling ($P_{pools\text{-}x}$) for subsequent 7-10 day* period based on n-sample pool testing. ($P_{pool\text{-}x}$) should be updated daily using a moving average.

  Compare $P_{pool\text{-}initial}$ to $P_{pool\text{-}x}$ periodically. If $P_{pool\text{-}x}$ is less than 90% of $P_{pool\text{-}initial}$ ($P_{pool\text{-}x} < 0.90 \times P_{pool\text{-}initial}$), it is recommended that:
- The n-samples pooling should be re-assessed by conducting a re-assessment study as described in "Laboratory Monitoring Part C" below.
- If P_{pool} is greater than 25%, pooling of patient samples is not efficient and should be discontinued until the percent positivity rate decreases.

* It is recommended that $P_{individual}$ be calculated from the previous 7-10 days, while P_{pool} and $P_{pool\text{-}x}$ are calculated from data collected during a 7-10 day time frame. However, when determining if 7-10 days is appropriate, take into consideration the laboratory testing volume and percent positivity, among other factors. Note that if the number of individual or pooled positive results collected during a given time frame is less than 10, $P_{individual}$, P_{pools}, and $P_{pool\text{-}x}$ may not be representative of the percent positivity in the testing population and the laboratory may want to consider extending the testing time period to increase the chance of capturing positives.

Sample Pooling Re-assessment (Laboratory Monitoring Part C)

Option 1: Stop n-sample pooling and return to individual testing

- Patient samples should be tested individually until 10 consecutive positive samples have been collected. The total number of samples, tested individually, depends on the positivity rate.
- Using these samples, 10 pools should be created and tested with 1 positive and (n-1) negative samples and the PPA between testing sample pools and individual samples should be calculated.

Option 2: Continue n-sample pooling

- Re-assessment study should start from time T0 and should consist of individual sample testing in parallel with the pooled testing. However, since all non-negative sample pools require individual testing of all individual samples included

in the pool as a part of the n-sample pooling and deconvoluting workflow, the re-assessment study essentially consists of testing individual samples from the negative n-sample pools.

- Re-assessment study may pause at time T1 when a minimum of 10 consecutive positive individual results are obtained, including both positive individual results generated from individual testing of samples from the non-negative sample pools following the n-sample pooling and deconvoluting workflow, and positive individual results obtained from individual testing of samples from the negative sample pools for the time period from T0 to T1 [T0, T1].
- Considering that number of positive individual sample results among negative pools is K, PPA between testing n-sample pools and assaying single specimens using the candidate test should be calculated as PPA (EUA Test $_{pool}$ vs. EUA Test $_{individual}$) = 100% x (10-K)/10. It is critical that all consecutive positive samples from time period [T0, T1] are included in the PPA calculations. With regard to calculating the PPA, all non-negative results testing pooled samples should be counted as in agreement with positive individually tested results.

Re-assessment Acceptance Criteria for Option 1 and Option 2

- If the PPA (EUA Test $_{pool}$ vs. EUA Test $_{individual}$) is ≥ 90% (9 out of 10 or 10 out of 10), then implementation of testing using n-sample pooling is acceptable.
- If the PPA between pooled-testing results and individual-testing results is less than 90%:
  - If PPA ≤70% (7 out of 10), reduce the pool size (consider a new n as n-1)
  - If PPA is 80% (8 out of 10), collect an additional 10 consecutive individually positive samples. Then, calculate the PPA from the combined data of 20 samples, between pooled testing results and individual testing results. If the PPA is ≥ 85%, then implementation of testing using n-sample pooling is acceptable. Or, to compensate for lost sensitivity, reduce the pool size (consider a new n as n-1) and continue with the re-assessment testing until PPA of pooled compared to individual testing is ≥ 90%.
- If PPA of at least 85% cannot be reached for any pool size evaluated in the re-assessment, cease pooling patient specimens.

If n-sample pooling is acceptable based on re-assessment, re-establish P $_{individual}$ in your laboratory by estimating the positivity rate from individual testing in the population from which the 10 (or 20) consecutive individual positive samples were collected. If the total number of samples (N*) that needed to be tested to obtain the 10 (or 20) consecutive positive samples is stopped at the 10^{th} (or 20^{th}) positive sample, then the positivity rate of 10/N* (or 20/N*) is overestimated. The positivity rate should be corrected by the following corresponding multiplier:

- Positivity rate for 10 samples is (10/N*) × (10/11)
- Positivity rate for 20 samples is (20/N*) × (20/21).

This updated new positivity rate should be used as $P_{individual}$ in the future laboratory monitoring (return to section B.1 of the "Laboratory Monitoring Part B").

WARNINGS

- Caution: Federal Law restricts this device to sale by or on the order of a licensed practitioner.
- For *in vitro* diagnostic use.
- This product has not been FDA cleared or approved, but has been authorized by FDA under an EUA for use by laboratories designated by STS Lab Holdco (a subsidiary of Amazon.com Services LLC) which are certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 USC § 263a, and meet requirements to perform high-complexity tests.
- This product has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.
- 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 diagnostic tests for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug and Cosmetics Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated or the authorization is revoked sooner.

LIMITATIONS

- The Amazon Real-Time RT-PCR Test for Detecting SARS-CoV-2 was validated with specimens from asymptomatic subjects, performance has not been established in patients with symptoms.
- 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.
- Samples should only be pooled when testing demand exceeds laboratory capacity and/or when testing reagents are in short supply.
- Specimens with low viral loads may not be detected in sample pools due to the decreased sensitivity of pooled testing.
- Asymptomatic individuals infected with COVID-19 may not shed enough virus to reach the limit of detection of the test, giving a false negative result.