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

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

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

The Amazon Real-Time RT-PCR DTC Test for Detecting SARS-CoV-2 ("Amazon DTC 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 DTC Test for Detecting SARS-CoV-2 ("Amazon DTC Test") is a direct-to-consumer product for testing individual anterior nasal swab specimens collected at home using the Amazon COVID-19 Test Collection Kit DTC by any individual, 18 years and older (self-collected), 14 years and older (self-collected under adult supervision) or 2 years and older (collected with adult assistance), including individuals without symptoms or other reasons to suspect COVID-19. Testing of anterior nasal swab specimens is limited to 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 U.S.C. §263, and meet the requirements to perform high-complexity tests.

The Amazon DTC 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 collected in individual vials containing transport medium by any individual (18 years and older (self-collected), 14 years and older (self-collected under adult supervision) or 2 years and older (collected with adult assistance)), including individuals without symptoms or other reasons to suspect COVID-19.

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 medical history and other diagnostic information is necessary to determine 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 Negative results from pooled testing should not be treated as definitive and may need follow up testing if inconsistent with an individual's signs and symptoms. 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.

All test results are delivered to the user online (via AmazonDx.com), and users receive email and SMS text message notifications that their results are available. The direct-to-consumer home collection system is intended to enable users to access information about their COVID-19

infection status that could aid with determining if self-isolation or quarantine is appropriate and to assist with healthcare decisions after discussion with a healthcare provider.

The Amazon COVID-19 Test Collection Kit DTC is not a substitute for visits to a healthcare provider. The information provided by this product should not be used to start, stop, or change any course of treatment unless advised by the patient's healthcare provider.

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

DEVICE DESCRIPTION AND TEST PRINCIPLE

As applicable, individual users of the Amazon COVID-19 Test Collection Kit DTC described below include the parents or guardians of minor children 2 - 17 years of age.

Device Description
The Amazon COVID-19 Test Collection Kit DTC consists of a flocked nylon swab, a collection tube containing phosphate-buffered saline solution ("PBS"), a plastic biohazard bag with absorbent pad, Instructions For Use (IFU), Fact Sheet for Individuals, a return box with shipping label and collection tube holder insert, and tape to seal the shipping box.

The Amazon COVID-19 Test Collection Kit DTC is for use with the Amazon Real-Time RT-PCR DTC Test for Detecting SARS-CoV-2 ("Amazon DTC Test") which is the same as the Amazon Real-Time RT-PCR Test for Detecting SARS-CoV-2 ("Amazon Test") that was authorized under EUA202760. The Standard Operating Procedure (SOP) for sample accessioning and performance of the test has been revised to indicate that it applies to both the Amazon Test and associated collection kits authorized under EUA202760 and the Amazon DTC Test and Amazon COVID-19 Test Collection Kit DTC that are the subject of the current submission (EUA210308).

Amazon COVID-19 Test Collection Kit DTC Ordering and Processing
The Amazon COVID-19 Test Collection Kit DTC will be available to any individual 2 years of age and older direct-to-consumer ("DTC") without a prescription. The Amazon COVID-19 Test Collection Kit DTC is for the collection of anterior nasal swab specimens and stabilization of SARS-CoV-2 RNA for shipping to a clinical laboratory. Once the kit is received by the individual, they are required to register their kit on-line. For first time users, kit registration requires creation of an account.

Instructions are included in the kit to direct individuals on how to collect an appropriate nasal swab specimen and place it in the transport tube, how to package the specimen for shipment, and how to mail the specimen back to the laboratory using the pre-labeled United Parcel Service (UPS) return box. An instructional video and responses to Frequently Asked Questions (FAQs) are also available online, in addition to the same instructions that are included in the kit. After the anterior nasal swab specimen is collected, the swab is inserted into the transport medium (phosphate buffered saline; PBS), and the swab shaft is broken off at the score mark. The transport medium stabilizes the sample during transport and storage. The user caps the collection

tube, places the sealed tube in a zip-lock biohazard bag, and then into the provided return shipping box. After applying the pre-paid shipping label, the user then drops off the package at a UPS location for overnight shipment to a testing laboratory designated by Amazon.

The packaging used for shipment of specimens complies with standards put forth by the Centers for Disease Control and Prevention (CDC), World Health Organization (WHO), and the United States Department of Transportation (DOT) for the transport of suspected COVID-19 samples.

Specimens received at laboratories designated by Amazon for testing undergo accessioning prior to acceptance for testing. All acceptable specimens are processed by the laboratory. All rejected specimens are disposed of and the individual is contacted for potential recollection.

Users of the Amazon COVID-19 Test Collection Kit DTC receive email and Short Messaging Service (SMS) text notifications that their results are available, and all test results are delivered to the individual via the same website that is used to register the collection kit (AmazonDx.com). Most test results will be provided within 24 hours of receipt of the collection kit at the laboratory.

The Amazon DTC Test will be performed at laboratories designated by Amazon 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.

Specimen Collection
After ordering an Amazon COVID-19 Test Collection Kit DTC online, the individual receives the kit by mail and registers the kit using a designated website, AmazonDx.com. The web address is provided in the sample collection instructions that are included with the collection kit and on the Amazon.com ordering webpage. First-time users of AmazonDx.com will create an account and enter personal information required for communication of results and public health reporting.

After account creation, registration involves confirming personal information and entering the collection tube identification number listed on the label of the sample collection vial that came with the kit. The website also provides an instructional video as part of the sample collection kit registration workflow. The video also is available on the FAQs page after registration is completed.

The individual follows the provided sample collection instructions to collect the nasal swab specimen and prepare their sample for shipment. Collected specimens should be returned to a UPS drop-off location for shipment to the testing laboratory on the same day that they are collected. A test is automatically cancelled if the specimen is not received in the laboratory within 96 hours of collection.

Specimen Transport and Storage
Anterior nasal swabs collected in Phosphate Buffered Saline (PBS) using the Amazon COVID-19 Test Collection Kit DTC 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.

Specimen Accessioning
Specimens received at laboratories designated by Amazon for testing with the Amazon DTC Test will undergo inspection by laboratory personnel for the following criteria before acceptance (refer also to **Table 1**):

a) Proper return of sample packaging: confirm that the sample tube is in the biohazard bag, the biohazard bag is sealed, the swab is present in the tube, and the swab is tip down in the tube;

b) Sample acceptability: ensure sufficient sample volume is present and there is no evidence of leaking from the tube; ensure sample was received within the acceptable stability window after collection;

c) Kit registration: ensure proper registration of the sample collection kit; ensure the barcode on the tube is associated with a valid test order.

Table 1. Accessioning criteria applied to specimens received for analysis with the Amazon DTC 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

Specimen Testing
The Amazon DTC Test is a modification of the BGI Genomics Real-Time Fluorescent 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 Fluorescent 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 samples from the individual collection tubes to 96 well plates for automated sample processing. The Amazon DTC Test has been validated for pooling of up to 5 samples. 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.

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

Result Reporting
Individuals will receive email and SMS text message notification that their test results are available, and test results are communicated via the same website that is used to register the collection kit (AmazonDx.com). Customer service will not call users regarding their results, but users will have multiple customer service options to choose from if they want more information, as described below.

All users will have on-demand access to self-service resources through the AmazonDx.com website (IFU, instructional video(s), and FAQs), and 24/7 access to customer support in the form of live chat or telephone calls. The Customer Service Agents will be part of a specialized Amazon Customer Service team that undergoes training based on the Amazon COVID-19 Test Collection Kit DTC FAQs, instructional video(s), and IFU. Agents will not offer assistance with interpretation or explanation of the user's test result and only direct them to the Fact Sheet for Individuals that was included with the collection kit (and which is also available via the AmazonDx.com website on the page displaying the individual's test result). Training for Customer Service Agents includes:

a) step-by-step guidance on how to use the Amazon COVID-19 Test Collection Kit DTC
b) how to register a test kit and view results on AmazonDx.com
c) how to look up a test kit status on behalf of a user
d) how to direct users' questions about their test results
e) and data handling and privacy.

Test results are communicated regardless of whether the test is negative or positive, or if there was an error leading to an invalid result. For post-test support, trained Customer Service Agents will direct users to the Fact Sheet for Individuals for guidance on what they should do if they have received a positive or negative result, or if a result could not be obtained, including a recommendation to consult with their healthcare provider, as appropriate. Customer Service Agents may also refer individuals to publicly available CDC guidance regarding general medical questions, such as the symptoms of COVID-19, but will not answer any other clinical questions.

In sample pooling, nasal swab samples collected with either the Amazon COVID-19 Test Collection Kit DTC, Amazon COVID-19 Collection Kit or Amazon COVID-19 Test Collection Kit may be pooled with a maximum pooling ratio of 5:1 (5 samples for 1 test).[1] Individual samples are retested if results for pooled samples are positive for the presence of SARS-CoV-2 RNA or there is a test error (e.g., an invalid result).

An authorized Fact Sheet for Healthcare Providers is also available for download via the test report on the AmazonDx.com website and may be used by individuals to facilitate appropriate follow-up with their healthcare provider.

Table 2. Result reports displayed to individuals via the AmazonDx.com website

Reported Result	Explanation
SARS-CoV-2 Positive	Your test result was POSITIVE, which means that the virus that causes COVID-19 was found in your sample. If you have a positive result you should follow up with your healthcare provider who will work with you to determine how best to care for you based on the test results along with medical history and your symptoms. You should follow the latest CDC guidance to avoid spreading the virus to others, such as self-isolation, to reduce the potential transmission of disease. There is a small possibility that this test can give a positive result that is wrong (a false positive result). Please see the Fact Sheet for Individuals for more information about your test result. If needed you can provide the Fact Sheet for Healthcare Providers to your healthcare provider.

[1] Pooling may be performed with a mixture of samples collected using the prescription use and non-prescription (DTC) collection devices that are authorized for use with the Amazon Test/Amazon DTC Test

Reported Result	Explanation
SARS-CoV-2 Negative (Pooled Samples)	Your test result was NEGATIVE, which means that the virus that causes COVID-19 was not found in your sample. However, it is possible for this test to give a negative result that is incorrect (false negative) in some people with COVID-19. Pooling was used during testing of your sample, which means that the laboratory combined your sample with other individuals' samples prior to testing. Patient specimens with low viral loads may not be detected in sample pools due to the decreased sensitivity of pooled testing. You might test negative if the sample was collected early during your infection. You could also be exposed to COVID-19 after your sample was collected and then have become infected. In particular, people infected with COVID-19 but who have no symptoms may not shed enough virus to trigger a positive test. This means that you could possibly still have COVID-19 even though the test result is negative. If your test is negative, but you either develop symptoms or continue to have symptoms and/or they get worse, you should reach out to your healthcare provider who will work with you to determine the next steps you should take. Please see the Fact Sheet for Individuals for more information about your test result. If needed you can provide the Fact Sheet for Healthcare Providers to your healthcare provider.
SARS-CoV-2 Negative (Individual Samples)	Your test result was NEGATIVE, which means that the virus that causes COVID-19 was not found in your sample. However, it is possible for this test to give a negative result that is incorrect (false negative) in some people with COVID-19. You might test negative if the sample was collected early during your infection. You could also be exposed to COVID-19 after your sample was collected and then have become infected. In particular, people infected with COVID-19 but who have no symptoms may not shed enough virus to trigger a positive test. This means that you could possibly still have COVID-19 even though the test result is negative. If your test is negative, but you either develop symptoms or continue to have symptoms and/or they get worse, you should reach out to your healthcare provider who will work with you to determine the next steps you should take. Please see the Fact Sheet for Individuals for more information about your test result. If needed you can provide the Fact Sheet for Healthcare Providers to your healthcare provider.

Reported Result	Explanation
Lab could not process – Delayed return	The lab could not process your sample. The test collection kit did not arrive to the lab in a timely manner, and the results could not be interpreted. Re-collection and re-testing is recommended. For additional support, visit the Frequently Asked Questions and Support page.
Lab could not process – Sample collection Issue	The lab could not process your sample. The lab identified an issue with how your sample was collected or packaged for return, and the results could not be interpreted. Re-collection and re-testing is recommended. For additional support, visit the Frequently Asked Questions and Support page.
Lab could not process	The lab could not process your sample, and the results could not be interpreted. This can happen for a variety of reasons including the sample leaked or was damaged during transport back to the lab. Re-collection and re-testing is recommended. For additional support, visit the Frequently Asked Questions and Support page.

INSTRUMENTS USED WITH THE TEST

Table 3. Instruments and software for use with the Amazon DTC 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 660518-01	N/A
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

Instrument	Manufacturer	Model Number	Software Version
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 4. Reagents used to perform the Amazon DTC 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-2) External Run Controls	Zeptometrix Corporation	NATSARS (COV2)-ERC	Control	2°C to 8°C

Table 5. Consumables used to perform the Amazon DTC 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	Bio-Rad	HSP9601

Consumables	Manufacturer	Catalog Number
DNase Free, Box = 50 Plates		
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
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

Consumables	Manufacturer	Catalog Number
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
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	Toledo Mettler Rainin LTD	L-200XLS+

Consumables	Manufacturer	Catalog Number
tips		
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 6. Amazon COVID-19 Test Collection Kit DTC

Component [1]	Description	Supplier	Part Number
Outer Shipping Box	Outer shipping box with product labeling	Various	N/A
Carboard return shipping box with collection tube holder	Cardboard box with collection tube holder insert and pre-applied UPS return label	Various	N/A
Nasal swab	Individually wrapped sterile flocked nylon swab for nasal specimen collection	Puritan Medical Products Medico Technology	25-3306-U 96000BQ
Collection Tube	Sterile plastic collection tube aseptically pre-filled with 1 mL sterile phosphate-buffered saline (PBS) solution	*Tube:* Corning Inc. Tarsons Products PVT, Ltd. StandAlone Scientific *PBS:* Thermo Fisher Molecular Biologicals, Inc. (Growcells) VWR Chemicals, LLC	 430663 95589G 311M-CST5 BP2438 MRGF-6230-010L 97063-660
Biohazard bag	Clear, 2 mm plastic 2-wall zip top bag	Various	N/A
Tape	Adhesive tape to seal the return shipping box.	Various	N/A
Instructions for Use	Kit registration, sample collection, and drop-off instructions	Amazon.com Services LLC (printed by various suppliers)	N/A
Fact Sheet for Individuals	Authorized Fact Sheet for Individuals [2]	Amazon.com Services LLC (printed by various suppliers)	N/A

N/A: Not Applicable
[1] 1 of each component per collection kit
[2] Also available electronically
Note: An authorized Fact Sheet for Healthcare Providers is available electronically via the result report on the amzondx.com webpage.

CONTROLS

The assay controls used with the Amazon DTC Test are described in **Table 7**. 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 7. Assay controls used with the Amazon DTC 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 DTC 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 > 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 8**. All controls must produce the expected results in order to interpret the results from testing of patient samples. If either 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 8. 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

If a pool returns a positive or failed (invalid) test result, each sample within the pool must be retested individually (**Table 9**). If a pool returns a negative result, all samples within the pool are reported as "Negative" with the following additional comment on the test report:

> *Pooling was used during testing of your sample. Patient specimens with low viral loads may not be detected in sample pools due to the decreased sensitivity of pooled testing.*

Table 9. Interpretation of results from pooled specimens

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 each constituent member of the pool as Negative with explanation of the potential for decreased sensitivity of pooled testing. [1]

[1] Refer to the comment included on the test report shown in *italics* above

Note: The Fact Sheet for Individuals contains the following additional explanation of the results from pooled specimens:

> *Laboratories may use pooling when testing your specimen, which means they combine your sample with other individuals' samples prior to testing. If your test result indicates your specimen was pooled and you have a negative test result, there is a small chance that your result is incorrect. You should talk with your healthcare provider if you are concerned.*

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 10** and **11**.

Table 10. 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 11. 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 to re-submit samples for testing. Re-collected samples will be processed according to the standard pooling workflow.

PERFORMANCE EVALUATION

The Amazon Real-Time RT-PCR DTC Test for Detecting SARS-CoV-2 ("Amazon DTC Test") is the same real-time PCR test as the FDA authorized prescription use only Amazon Real-Time RT-PCR Test for Detecting SARS-CoV-2 ("Amazon Test"). Except for additional Usability Studies conducted to support authorization of the Amazon COVID-19 Test Collection Kit DTC, the performance data described below are the same as those used to support authorization of the prescription use only Amazon Test. For clarity, the Amazon Test name is maintained in the summary of the performance studies conducted to support its authorization.

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 12**. 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 12. 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 13**). 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 13. 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 14**). 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 15**).

Table 14. 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 15. 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):

The Amazon Test is a modification of the FDA-authorized BGI Genomics Real-Time Fluorescent RT-PCR Test for Detection SARS-CoV-2 (EUA200034) that amplifies a single target within the ORF1ab region of the SARS-CoV-2 genome, as well as human β-actin RNA as an endogenous control.

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.

Analysis of SARS-CoV-2 Variants
Independent *in silico* inclusivity analysis performed in May 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.

Ct Cut-off for Individual vs Pooled Testing
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.

3) Amazon COVID-19 Test Collection Kit DTC

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 performed a Usability Study to evaluate the ease of use of the Amazon COVID-19 Test Collection Kit DTC. The study was conducted in a simulated home environment at an Amazon facility where participants collected their own samples and returned their sample collection kits to a UPS drop-off location.

The entire workflow was performed by each individual participant as described in the

Instructions for Use, including:

a) Registration of the kit (using a prototype version of amazondx.com)
b) Sample collection
c) Packaging of the sample, and
d) Return of the packaged sample to a UPS drop-off location.

Draft FAQs and a prototype instructional video (accessible during the registration workflow and linked in the FAQs) were also available through amazondx.com during the Usability Study. Sample collection by each participant was observed by a remote passive observer, who competed a questionnaire regarding their observations. At the conclusion of the study, each individual participant was also asked to complete an online questionnaire to assess the ease of use of the kit and their understanding of the consequences if steps are not performed correctly.

Collected samples were shipped by the participants UPS to Amazon's laboratory in Hebron, KY for testing. Upon arrival at the laboratory, the packaging ad samples were inspected laboratory personnel according to the previously authorized laboratory accessioning SOP. The samples were tested for acceptability and RNA integrity by amplifying the housekeeping gene/internal control β-actin RNA using the authorized Amazon Test, with samples yielding a Ct value < 35 considered valid.

Ninety-two participants of different ages and levels of education who met the inclusion and exclusion criteria (**Table 16**) participated in the study. The demographics of the study population are summarized in **Table 17**.

Table 16. Inclusion and exclusion criteria for participation in the Amazon DTC Test Usability Study

Inclusion Criteria	**Exclusion Criteria**
≥ 18 years of age	Previous experience of self-collection at home
Willingness to receive the collection kit, perform specimen collection and complete the participant questionnaire	Previous or current medical or laboratory training

Table 17. Demographic characteristics of participants in the Amazon COVD-19 Test Collection Kit DTC

Age	**Participants**
18-29 years	28
30-39 years	35
40-49 years	21
50-59 years	7
60 years or older	1
Gender	**Participants**
Male	49
Female	43
Other	0
Highest Education	**Participants**
High School Diploma / Associate Degree	13
Bachelor's Degree	38
Master's Degree	36
Doctoral Degree / Post Graduate (Ph.D. or J.D.)	5

Of the samples from the 92 participants, 88 (95.7%) were received by the testing laboratory, of which 4 (4.3%) were expired upon receipt (received > 96 hours post collection) and 3 (3.3%) were improperly registered and could not be linked to a specific individual. All 88 samples (100%) that were evaluated with the Amazon Test (including those that were expired) produced a positive result for the presence of β-actin, with a mean Ct value of 21.3 (standard deviation 2.05), indicating the presence of human genetic material.

Analysis of the results of the Usability Study, including the participant and observer surveys, identified several opportunities to improve the user experience and reduce the potential for errors. As a result, various changes were made to the kit registration procedure, Instructions for Use, instructional video and FAQs, the effectiveness of which were validated through a second Usability Study. These changes included the following:

1) To remind customers to ship their specimens in a timely manner and/or prevent delays in shipment, Amazon will send each customer an email and an SMS text message within 2 hours of kit registration. These messages will include a link to the UPS website to help identify a nearby drop-off location and will remind users that their samples may not be processed if they are not dropped off within 24 hours.
2) The recommended depth of insertion of the collection swab into the nostril was decreased from 1 inch to ½ to ¾ of an inch to reduce discomfort and align with CDC recommendations.
3) Clarification of the appropriate technique by which to break the collection swab into the specimen transport tube.
4) Instructions added not to remove the absorbent pad from the biohazard bag prior to shipment and to place the collected sample in the bag "with the absorbent pad."
5) Emphasis added to instructions to avoid spilling of the contents of the collection tube

and the consequences of such occurrence.

6) Emphasis added not to touch the swab head to any surface other than the inside of the nostril and addition of instructions in case such an event occurs.

The second Usability Study included 37 participants (> 18 years of age), who were monitored by passive observers as they performed the specimen collection procedure using the modified instructions. Samples from 36/37 (97.3%) participants were received in the laboratory, and all 36 were reported as positive for β-actin RNA using the Amazon Test (mean Ct = 21.0; standard deviation = 2.89). One of 36 specimens (2.8%) was expired upon receipt (i.e., received > 96 hours post collection). Analysis of participant surveys indicated less discomfort with the collection procedure and improved user comprehension of the consequences of deviating from the instructions than in the original study.

Amazon's steps to improve user experience with the Amazon COVID-19 Test Collection Kit DTC were found to be acceptable to mitigate the risks associated with unobserved sample collection at home. Amazon has agreed to provide a report within 30 days post-authorization of the error rates associated with use of the Amazon COVID-19 Test Collection Kit DTC based on the accessioning criteria and results from testing of individual samples for β-actin. The report will also include an evaluation of customer complaints and calls to Customer Service.

User Comprehension Study
As a post authorization commitment, Amazon conducted a User Comprehension Study to evaluate whether users of the Amazon COVID-19 Test Collection Kit DTC and Amazon DTC Test would be able to understand the Test Reports and the appropriate course of action associated with each result. The study was conducted with 30 participants representing different age groups, levels of education and geographic locations. Each participant was provided with the authorized Fact Sheet for Individuals and authorized FAQs to assist with interpretation of results and was asked to review example test reports that were presented to them in random order. Following review of each report, the study proctor asked questions to probe the participant's understanding of the test result and appropriate next steps.

In general, participants were able understand what test result was associated with each report, found the information provided to be clear and understood what steps they should take under each circumstance. However, there was some confusion about what it means to pool specimens for testing and how this may affect test results. To address this, Amazon revised the FAQs that are posted on the AmazonDx.com website to include a specific question addressing what it means to pool samples and the potential consequences of pooling. Amazon also revised the Negative Test Report for pooled specimens to clarify the meaning of the term "pooling".

Usability Study to Support Specimen Collection from Minors (2 - 17 years of age)
To support the addition of testing samples collected from minors to the Intended Use for the Amazon DTC Test, a Usability Study was conducted with 30 pairs of adults and minors in a simulated home environment. Included in the study were equal numbers of children aged 14 to 17 years who self-collected their anterior nasal swab specimens under adult

supervision and 2 to 13 year-old children whose samples were collected by an accompanying parent. None of the participants had previous experience with at-home self-collection of specimens or current or previous laboratory or medical training.

A summary of the demographics of the participants is shown in **Table 18**. The participants in both the "parent-collected" and "self-collected" arms of the study exhibited a broad distribution of ages and different educational backgrounds and came from various racial and ethnic groups.

Table 18. Demographic characteristics of Usability Study participants

Group	Age (years)	*N*	Highest Education	*N*	Ethnicity	*N*	Race	*N*
Children 2-13 years (n =15)	2	3	Middle School	3	Hispanic/ Latino	4	White	6
	3	1	Elementary School	6	Not Hispanic/Latino	11	Asian	3
	4	1	Kindergarten	1			Other	6
	5	1	Not applicable	5				
	6	2						
	7	0						
	8	1						
	9	2						
	10	0						
	11	1						
	12	2						
	13	1						
Parents of children 2-13 years (n = 15)	20-30	4	Master's Degree	1	Hispanic/ Latino	4	White	7
	21-40	5	Bachelor's Degree	8	Not Hispanic/Latino	11	Asian	2
	41-50	4	Associate Degree	2			Other	6
	51+	2	Some College	2				
			Vocational Training	1				
			High School	1				
Children 14-17 years (n = 15) [1]	14	4	Middle	3	Hispanic/ Latino	4	White	11
	15	4	High	12	Not Hispanic/Latino	11	Asian	1
	16	3					Other	4
	17	4						

[1] The demographic characteristics of the parents of the children aged 14-17 years were not recorded

The study participants were not asked to complete the process of creating a user profile and registering their kit because these aspects of the workflow were previously validated under the original submission for the Amazon COVID-19 Test Collection Kit DTC and remain unchanged with the expansion of the intended use population (except for the added feature by which individual profiles for family or household members can be created under the same Amazon.com account).

Prior to conducting the study, the Instructions For Use (IFU) of the Amazon COVID-19 Test Collection Kit DTC were revised to include specific instructions for collection of swabs from minors and to clarify the instructions for breaking the swab shaft to reduce the likelihood of breakage below the indicator line, which adversely affects the workflow upon receipt of the samples in the laboratory.

Following specimen collection, participants in the Usability Study were asked to package their specimens for shipment. However, because specimen drop-off was evaluated previously as part of the original authorization of the collection kit, they were not required to drop-off their specimens at a UPS location and the specimens were therefore shipped to the laboratory overnight in bulk where they were evaluated using the Amazon DTC Test.

Twenty-nine of 30 (96.7%) specimens produced acceptable Ct values for β-actin (i.e., Ct < 35). The one exception was a sample from a 2 year-old child that was collected by an adult. This specimen had insufficient volume for testing, although there was no evidence of incorrect application of the cap of the transport tube, spillage or leakage. The root cause for this failure was not established. The overall mean Ct value for β-actin was 26.8 (standard deviation = 2.87).

At the end of the study, participants also completed both a User Satisfaction Survey and a brief User Comprehension Survey to verify their understanding of the instructions. Overall, users were satisfied that the instructions were easy to follow, that the collection kit was easy to use and understood the instructions for appropriate sample collection and sample drop-off. However, based on the feedback received, the following minor improvements were implemented:

- Emphasis was added to the Instructions For Use for users to follow the instructions for packaging the collected sample for shipment.
- For ease of use, the tape used to seal the box prior to shipment was modified by adding a slit at the point at which the user should peel the backing.
- The collection kit manufacturing procedure was also revised to specify that the sealing tape must be packaged with the label facing up so that it is easily identifiable within the kit box.

4) 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 19**). 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.

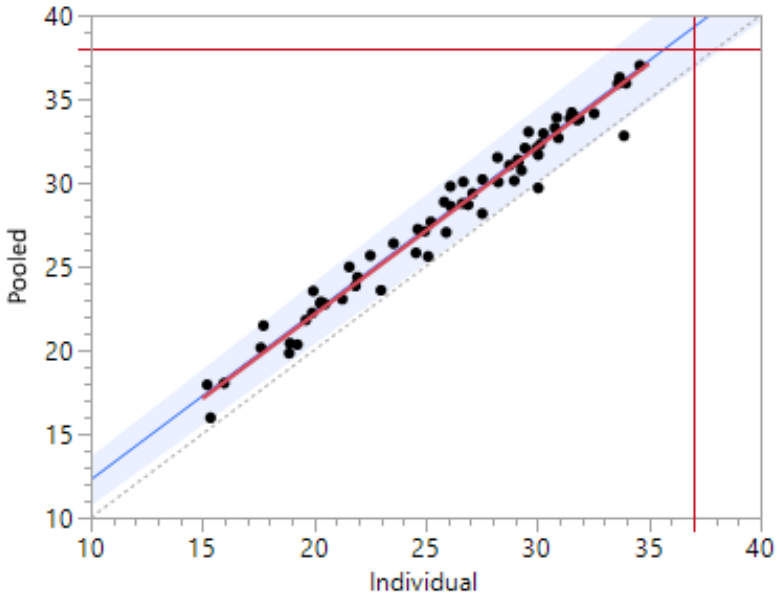
Table 19. Performance of the Amazon Test on individual nasal swab specimens in comparison to an FDA-authorized method

		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 19** demonstrated 89.9% positive agreement compared to the FDA-authorized comparator and 93.9% agreement with the individual Amazon Test results (**Table 20**).

Table 20. 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 20** 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 21**).

Table 21. 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 22**).

Table 22. 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 22** to choose an appropriate validated pool size. **Table 22** 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 22**, choose n from **Table 22** which corresponds to the maximum efficiency (F).
- If $P_{individual}$ in your laboratory does not correspond to the largest validated pool size in **Table 22**, 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

- For *in vitro* diagnostic use.
- 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.
- 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 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 Fluorescent RT-PCR Test for Detecting SARS-CoV-2 was validated with specimens from asymptomatic subjects, performance has not been established in patients with symptoms.
- 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.
- 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.
- Detection of β-actin indicates that human nucleic acid is present and implies that human biological material was collected and successfully extracted and amplified. It does not necessarily indicate that the specimen is of appropriate quality to enable detection of SARS-CoV-2.