EMERGENCY USE AUTHORIZATION (EUA) OF THE AMAZON MULTI-TARGET SARS-CoV-2 REAL-TIME RT-PCR DTC TEST

Amazon Multi-Target SARS-CoV-2 Real-Time RT-PCR DTC Test

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

The Amazon Multi-Target SARS-CoV-2 Real-Time RT-PCR DTC Test ("Amazon Multi-Target 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).

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INTENDED USE

The Amazon Multi-Target SARS-CoV-2 Real-Time RT-PCR DTC Test ("Amazon Multi-Target 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 Multi-Target 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 anterior 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.

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 Multi-Target 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 Multi-Target 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.

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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 an outer shipping box with product labeling, 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 Multi-Target SARS-CoV-2 Real-Time RT-PCR DTC Test (the "Amazon Multi-Target DTC Test") which is the same as the Amazon Multi-Target SARS-CoV-2 Real-Time RT-PCR Test ("Amazon Multi-Target Test") authorized under EUA210481. The Standard Operating Procedure (SOP) for sample accessioning and performance of the test applies to both the Amazon Multi-Target Test and associated collection kits and to the Amazon Multi-Target DTC Test and Amazon COVID-19 Test Collection Kit DTC.

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 Multi-Target 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 it 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 anterior 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.

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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 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 Multi-Target 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.

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Table 1. Accessioning criteria applied to specimens received for analysis with the Amazon Multi-Target 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

1) *Specimen Testing*
The Amazon Multi-Target DTC Test includes primers and probes for the detection of the ORF1ab and N gene regions of the SARS-CoV-2 genome, in addition to human RNase P RNA as an endogenous internal control. Nucleic acid extraction is performed using the MGI Easy Nucleic Acid Extraction Kit (Cat. # 1000020261 or 1000020471) or Thermo Fisher MagMAX Viral/Pathogen Nucleic Acid Isolation Kit (Cat. # A48310).

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 Multi-Target Test has been validated for pooling of up to 5 samples in PBS. 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.

2) *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,

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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 assist with interpretation or explanation of the user's test result and will 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, Amazon On-site COVID-19 Test 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.

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[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 Multi-Target Test/Amazon Multi-Target DTC Test

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.
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

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Reported Result	Explanation
	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.
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.
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.
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.

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INSTRUMENTS USED WITH THE TEST

Table 3. Instruments and software for use with the Amazon Multi-Target 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

Instrument	Manufacturer	Model Number	Software Version
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
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

N/A: Not Applicable

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REAGENTS AND MATERIALS

Table 4. Reagents used to perform the Amazon Multi-Target DTC Test

Reagent Kit	Manufacturer	Catalog Number	Kit Components	Storage Temperature
MGIEasy Nucleic Acid Extraction Kit	MGI Tech Co., Ltd.	1000020261	Buffer MW1	0 to 30 °C
			Buffer MW2	0 to 30 °C
			RNase Free water	0 to 30 °C
			Enhancer Buffer	-25 to -15 °C
			Magnetic Beads	2 to 8 °C
			Proteinase K	2 to 8 °C
Luna Probe One-Step RT-qPCR 4X Mix with UDG.	New England Biolabs	Cat. # M3019	Luna Probe One-Step RT-qPCR 4X Mix with UDG	-15 to -25 °C
			Nuclease-free Water	
Twist Synthetic SARS-CoV-2 RNA Control	Twist Bioscience	102024	Control 2 (MN908947.3)	-70 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 to 8 °C
Heat-inactivated SARS-CoV-2	ATCC	VR-1986HK	Control	-70 °C

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Table 5. Primers and probes used to perform the Amazon Multi-Target DTC Test

Supplier	Oligonucleotide Name	Function	Modification		Storage
			5'	3'	
Eurogentec	ORF1ab-F	Primer			Dry: 18 months at ambient temperature Suspended in TE Buffer: 24 months at -20 °C
	ORF1ab-R	Primer			
	ORF1ab-Probe	Probe	ATTO488	BHQ-1	
	NIID_2019-nCOV_N_F2	Primer			
	NIID_2019-nCOV_N_R2	Primer			
	NIID_2019-nCOV_N_Probe	Probe	CY5	BHQ-3	
	RNAseP F	Primer			
	RNAseP R	Primer			
	RNAseP Probe	Probe	HEX	BHQ-1	
Millipore Sigma	ORF1ab-F	Primer			Suspended in TE Buffer: 2 years at -20 °C or 1 year at 4 °C or 3 months at ambient temperature
	ORF1ab-R	Primer			
	ORF1ab-Probe	Probe	ATTO488	BHQ-1	
	NIID_2019-nCOV_N_F2	Primer			
	NIID_2019-nCOV_N_R2	Primer			
	NIID_2019-nCOV_N_Probe	Probe	CY5	BHQ-3	
	RNAseP F	Primer			
	RNAseP R	Primer			
	RNAseP Probe	Probe	HEX	BHQ-1	

TE: Tris-EDTA buffer

Table 6. Consumables used to perform the Amazon Multi-Target 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 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

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Consumables	Manufacturer	Catalog Number
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
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

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Consumables	Manufacturer	Catalog Number
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 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

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Table 7. 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.

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CONTROLS

The assay controls used with the Amazon Multi-Target 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 Multi-Target DTC Test

Control Type	Material	Description
Positive [1]	Twist nCoV2 Synthetic Viral RNA (Cat. # 102024)	Diluted to 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 NATrol SARS-Related Coronavirus 2 (SARS-CoV-2) (Cat. # NATSARS(COV2)-ERC)	Diluted 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.
	ATCC Heat-inactivated SARS-CoV-2 (Cat. # VR-1986HK)	Diluted 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]	RNase-free water	Prepared in aliquots from bulk. Stored at -80 °C.
Internal	RNase P gene	Endogenous Internal Control for the presence of human RNA in patient samples and Positive Controls. [2]

[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.
[2] Positive controls are diluted in Applied Biosystems Human total RNA (Cat. # 4307281)

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Internal Control

Under EUA202760/S002 for the Amazon Test, 1687 anterior nasal swab specimens that were collected without supervision using the Amazon COVID-19 Test Collection Kit were tested for the presence of endogenous human β-actin RNA. Of these, 1686 (99.94%) produced positive results, demonstrating that individuals can follow the instructions that are included in the Amazon COVID-19 Test Collection Kit to collect an adequate anterior nasal swab sample for testing. Pooling negates the utility of an endogenous internal control in monitoring the adequacy of individual specimens; however, the results of this study demonstrated that unsupervised self-collection of anterior nasal swabs using the Amazon COVID-19 Test Collection Kit, as well as related collection kits with similar instructions, is sufficiently reliable and robust not to require verification of specimen adequacy through use of such a control. Pooling of anterior nasal swab specimens that are self-collected without supervision for use with the Amazon DTC Test was therefore determined to be acceptable.

Because there are no changes to the Instructions For Use of the Amazon COVID-19 Test Collection Kit DTC under the current submission for the Amazon Multi-Target DTC Test, the above study was not repeated. Pooling of anterior nasal swabs for use with the Amazon Multi-Target DTC Test may be performed with specimens collected with or without healthcare provider supervision.

As a Condition of Authorization, Amazon will provide a summary report of the results obtained from the first 1000 anterior nasal swab specimens collected with the Amazon COVID-19 Test Collection Kit that are tested individually using the Amazon Multi-Target Test.

Passive Reference Dye
Amazon has validated use of a passive reference dye (ROX) that is included in each amplification reaction to normalize fluorescent signals and thereby reduce variability instrument-to-instrument, run-to-run and well-to-well. The dye is a component of the Luna Probe One-Step RT-qPCR 4X Mix with UDG (New England Biolabs, Cat. #M3019) that is used with both the Applied Biosystems 7500 Fast Dx and QuantStudio 5 real-time PCR instruments.

INTERPRETATION OF RESULTS

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 to enable interpretation of the results from testing of patient samples. If one or both 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)	N Gene (Cy5)	RNase P (HEX)	
Positive Control	≤ 32	≤ 32	≤ 35	Pass [1]
	> 32	Any	Any	Fail [2]
	Any	> 32	Any	Fail
	Any	Any	> 35	Fail
Negative Control	Undetermined	Undetermined	> 35 or Undetermined	Pass
	Any	Any	> 35 or Undetermined	Fail

[1] *Both* Positive Controls within a run must have Ct ≤ 32 and ΔCt < 3 for the SARS-CoV-2 targets (i.e., $Ct_{ORF1ab-1} = Ct_{ORF1ab-2} \pm 3$ and $Ct_{N-1} = Ct_{N-2} \pm 3$) and Ct < 35 for RNase P; all curves should be sigmoidal

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

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Clinical Specimens

Pooled Specimens

The results from pooled specimens are interpreted according to the criteria described in **Table 10**.

- If a pool returns a non-negative test result (positive or failed), each individual sample in the pool must be tested separately ("hit-picked").
- If a pooled test result is negative then all samples within the pool are determined to have undetectable levels of SARS-CoV-2 and are reported as "Negative." Negative results from pooled specimens should be treated as presumptive.

Table 10. Interpretation of results from pooled specimens

Ct Value [1]			Interpretation for the Pool	Action
ORF1ab (FAM)	**N Gene (Cy5)**	**RNase P (HEX)**		
> 38 or Undetermined	> 38 or Undetermined	> 35 or Undetermined	Failed	Repeat testing of each constituent specimen in the pool as a separate "hit-pick" extraction.
Any or Undetermined	≤ 38	Any or Undetermined	Positive	
≤ 38	Any or Undetermined	Any or Undetermined	Positive	
≤ 38	≤ 38	≤ 35	Positive	
> 38 or Undetermined	> 38 or Undetermined	≤ 35	Negative	Report "Negative" [2]

[1] Only Ct values associated with sigmoidal amplification curves are considered valid
[2] Negative results from pooled specimens should be regarded as presumptive

Individual Specimens

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

- A positive result from an individual sample indicates the presence of detectable levels of viral RNA and is reported as "Positive."
- A negative result from an individual sample indicates the absence of detectable levels of SARS-CoV-2 RNA and the sample is reported as "Negative."
- If an individual sample returns a failed result on initial testing (not a "hit-pick"), the sample is retested individually.

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

Ct Value [2]			Interpretation for the Specimen	Action
ORF1ab (FAM)	**N Gene (Cy5)**	**RNase P (HEX)**		
> 37 or Undetermined	> 37 or Undetermined	> 35 or Undetermined	Failed	Repeat test
≤ 37	Any or Undetermined	Any or Undetermined	Positive	Report "Positive"
Any or Undetermined	≤ 37	Any or Undetermined	Positive	Report "Positive"
≤ 37	≤ 37	Any or Undetermined	Positive	Report "Positive"
> 37 or Undetermined	> 37 or Undetermined	≤ 35	Negative	Report "Negative"

[1] Either as a primary, individual specimen or as a reflex to the result obtained from a pool
[2] Only Ct values associated with sigmoidal amplification curves are considered valid

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

Ct Value [1]			Interpretation for the Specimen	Action
ORF1ab (FAM)	**N Gene (Cy5)**	**RNase P (HEX)**		
> 37 or Undetermined	> 37 or Undetermined	> 35 or Undetermined	Failed	Report "Unable to be processed" (Recollect) [2]
≤ 37	Any or Undetermined	Any or Undetermined	Positive	Report "Positive"
Any or Undetermined	≤ 37	Any or Undetermined	Positive	Report "Positive"
≤ 37	≤ 37	Any or Undetermined	Positive	Report "Positive"
> 37 or Undetermined	> 37 or Undetermined	≤ 35	Negative	Report "Negative"

[1] Only Ct values associated with sigmoidal amplification curves are considered valid
[2] 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

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If a pool is reported as positive but all five samples from the pool return negative test results when tested individually, 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.

PERFORMANCE EVALUATION

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

Except where noted, all the studies to characterize the performance of the Amazon Multi-Target Test were performed using the ABI 7500 Fast Dx Real-Time PCR Instrument and MGI Easy Nucleic Acid Extraction Kit.

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

LoD Determination

The LoD of the Amazon Multi-Target Test was determined using contrived samples comprised of inactivated SARS-CoV-2 (Zeptometrix NATtrol SARS-CoV-2 External Run Control; Cat. # NATSARS(COV2)-ERC; SARS-CoV-2 isolate USA-WA1/2020) in PBS containing nasal swab matrix. The study was performed using the MGIEasy Nucleic Acid Extraction Kit and ABI 7500 Fast Dx Real-Time PCR Instrument.

To estimate the LoD, initial testing was performed at concentrations ranging from 37.5 to 4,800 copies/mL (**Table 13**). The estimated LoD was determined to be 150 copies/mL for both the ORF1ab and 75 copies/mL for the N gene target. Additional testing was then performed to confirm the estimated LoD with target levels ranging from 37.5 to 600 copies/mL, and 20 individual extraction and PCR replicates at each target level (**Table 14**). The confirmed LoD of the Amazon Multi-Target Test, defined as the lowest level at which at least one of the two targets was positive in ≥ 95% of replicates, was 50 copies/mL.

Table 13. Estimation of the LoD of the Amazon Multi-Target Test when performed on the ABI 7500 Fast Dx Real-Time PCR Instrument

Level (copies/mL)	ORF1ab				N Gene				Test Result	
	Positive	% (n = 32)	Ct Value		Positive	% (n = 32)	Ct Value		Positive	% (n = 32)
			Mean	SD			Mean	SD		
4,800	32	100	29.71	0.20	32	100	29.45	0.18	32	100
2,400	32	100	30.80	0.24	32	100	30.36	0.21	32	100
1,200	32	100	31.78	0.22	32	100	31.31	0.23	32	100
600	32	100	32.94	0.38	32	100	32.26	0.26	32	100
300	32	100	34.00	0.43	32	100	33.28	0.47	32	100
150	***32***	***100***	***34.88***	***0.65***	32	100	34.35	0.69	32	100
75	28	87.5	35.99	0.67	***31***	***96.9***	***35.31***	***0.56***	***32***	***100***
37.5	15	46.9	36.40	0.44	23	71.9	35.96	0.69	27	84.3

SD: Standard Deviation
Only Ct values ≤ 37 were included in calculation of mean and SD
The estimated LoD for each target is shown in ***bold face, italicized text***.
The estimated LoD for the assay based on the authorized method of result interpretation is highlighted in yellow.

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Table 14. Confirmation of the LoD of the Amazon Multi-Target Test when performed on the ABI 7500 Fast Dx Real-Time PCR Instrument

Level (copies/mL)	ORF1ab				N Gene				Test Result	
	Positive	% (n = 20)	Ct Value		Positive	% (n = 20)	Ct Value		Positive	% (n = 20)
			Mean	SD			Mean	SD		
600	20	100	32.70	0.36	20	100	32.24	0.24	20	100
300	20	100	33.95	0.52	20	100	33.20	0.42	20	100
150	20	100	35.08	0.66	20	100	34.27	0.58	20	100
125	***19***	***95***	***35.55***	***0.58***	20	100	34.63	0.65	20	100
100	18	90	35.48	0.76	20	100	34.59	0.71	20	100
75	18	90	35.85	0.52	19	95	34.99	0.70	19	95
50	11	55	36.02	0.63	***19***	***95***	***35.69***	***0.57***	***19***	***95***
37.5	11	55	36.37	0.41	15	75	35.80	0.78	17	85

SD: Standard Deviation
Only Ct values ≤ 37 were included in calculation of mean and SD
The confirmed LoD for each target is shown in ***bold face, italicized text***.
The confirmed LoD for the assay based on the authorized method of result interpretation is highlighted in yellow.

PCR Instrument Bridging Study

To validate use of the Applied Biosystems QuantStudio 5 Real-Time PCR System as an alternative to the ABI 7500 Fast Dx, the extracted nucleic acids from the LoD Study described above were also tested using the QuantStudio 5 instrument (**Tables 15** and **16**). The confirmed LoD for the Amazon Multi-Target Test using the QuantStudio 5 instrument was 50 copies/mL which is the same as that obtained with the ABI 7500 Fast Dx. These results demonstrated that the Amazon Multi-Target Test exhibits similar analytical sensitivity on both PCR instruments and supports their use interchangeably.

Table 15. Estimation of the LoD of the Amazon Multi-Target Test when performed on the QuantStudio 5 Real-Time PCR System

Level (copies/mL)	ORF1ab				N Gene				Test Result	
	Positive	% (n = 32)	Ct Value		Positive	% (n = 32)	Ct Value		Positive	% (n = 32)
			Mean	SD			Mean	SD		
4,800	32	100	29.91	0.59	32	100	29.61	0.55	32	100
2,400 [1]	31	100	30.83	0.39	31	100	30.38	0.21	31	100
1,200 [1]	31	100	31.89	0.36	31	100	31.33	0.30	31	100
600 [1]	31	100	32.92	0.49	31	100	32.31	0.42	31	100
300 [1]	31	100	33.98	0.58	31	100	33.39	0.54	31	100
150 [1]	***31***	***100***	***34.85***	***0.49***	***31***	***100***	***34.21***	***0.61***	31	100
75	28	87.5	35.85	0.53	30	93.8	35.20	0.81	***32***	***100***
37.5	19	59.4	36.23	0.54	25	78.1	35.75	0.68	29	90.6

SD: Standard Deviation
[1] Due to a pipetting error, 5 reactions (1 from each of the indicated target levels) received no sample and were omitted from the analysis
Only Ct values ≤ 37 were included in calculation of mean and SD
The estimated LoD for each target is shown in ***bold face, italicized text***.
The estimated LoD for the assay based on the authorized method of result interpretation is highlighted in yellow.

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Table 16. Confirmation of the LoD of the Amazon Multi-Target Test when performed on the QuantStudio 5 Real-Time PCR System

Level (copies/mL)	ORF1ab				N Gene				Test Result	
	Positive	% (n = 20)	Ct Value		Positive	% (n = 20)	Ct Value		Positive	% (n = 20)
			Mean	SD			Mean	SD		
600	20	100	32.52	0.27	20	100	32.29	0.26	20	100
300	20	100	33.83	0.37	20	100	33.16	0.56	20	100
150	19	95	34.82	0.58	20	100	34.25	0.88	20	100
125	19	95	35.52	0.66	20	100	34.40	0.83	20	100
100	19	95	35.35	0.65	19	95	34.53	0.65	20	100
75	***19***	***95***	***35.74***	***0.64***	***20***	***100***	***34.90***	***0.72***	20	100
50	17	85	36.26	0.56	17	85	35.58	0.58	***20***	***100***
37.5	12	60	36.24	0.47	11	55	35.46	0.87	17	85

SD: Standard Deviation
Only Ct values ≤ 37 were included in calculation of mean and SD
The confirmed LoD for each target is shown in ***bold face, italicized text***.
The confirmed LoD for the assay based on the authorized method of result interpretation is highlighted in yellow.

Comparison of Performance with Alternative Nucleic Acid Extraction Kits

The LoD Study for the Amazon Multi-Target Test described above was performed with samples that were processed using the MGIEasy Nucleic Acid Extraction Kit. A separate study was therefore performed to evaluate use of the MagMAX Viral/Pathogen Nucleic Acid Isolation Kit as an alternative method of nucleic acid extraction. The results of this study are presented in **Tables 17** and **18** and show that the MGIEasy Nucleic Acid Extraction Kit and MagMAX Viral/Pathogen Nucleic Acid Isolation Kit yielded similar analytical sensitivity in side-by-side comparison (< 3-fold difference). The two methods of nucleic acid extraction may therefore be used interchangeably for processing of samples for analysis with the Amazon Multi-Target Test.

REVOKED

Table 17. Estimation of the LoD of the Amazon Multi-Target Test with alternative methods of nucleic acid extraction

MGIEasy Nucleic Acid Extraction Kit										
Level (copies/mL)	**ORF1ab**				**N Gene**				**Test Result**	
	Positive	**% (n = 32)**	**Ct Value**		**Positive**	**% (n = 32)**	**Ct Value**		**Positive**	**% (n = 32)**
			Mean	**SD**			**Mean**	**SD**		
4,800	32	100	29.57	0.14	32	100	29.67	0.10	32	100
2,400	32	100	30.62	0.15	32	100	30.54	0.17	32	100
1,200	32	100	31.67	0.22	32	100	31.47	0.22	32	100
600	32	100	32.78	0.34	32	100	32.42	0.33	32	100
300	32	100	33.86	0.44	32	100	33.37	0.53	32	100
150	***31***	***96.9***	***35.00***	***0.82***	***32***	***100***	***34.33***	***0.59***	***32***	***100***
75	24	75.0	36.01	0.51	28	87.5	35.62	0.72	30	93.8
37.5	11	34.4	36.28	0.40	22	68.8	36.00	0.62	26	81.3
MaxMAX Viral/Pathogen Nucleic Acid Isolation Kit										
Level (copies/mL)	**ORF1ab**				**N Gene**				**Test Result**	
	Positive	**% (n = 32)**	**Ct Value**		**Positive**	**% (n = 32)**	**Ct Value**		**Positive**	**% (n = 32)**
			Mean	**SD**			**Mean**	**SD**		
4,800	32	100	30.19	0.21	32	100	29.95	0.13	32	100
2,400	32	100	31.31	0.18	32	100	30.92	0.20	32	100
1,200	32	100	32.34	0.30	32	100	31.84	0.28	32	100
600	32	100	33.44	0.36	32	100	32.96	0.39	32	100
300	32	100	34.45	0.45	32	100	33.96	0.43	32	100
150	***32***	***100***	***35.42***	***0.62***	***31***	***96.9***	***34.89***	***0.57***	***32***	***100***
75	25	78.1	36.06	0.69	27	84.4	35.85	0.64	30	93.8
37.5	11	34.4	36.59	0.39	13	40.6	36.35	0.52	19	59.4

SD: Standard Deviation
Only Ct values ≤ 37 were included in calculation of mean and SD.
The estimated LoD for each target is shown in ***bold face, italicized text***.
The estimated LoD for the assay based on the authorized method of result interpretation is highlighted in yellow.

REVOKED

Table 18. Verification of the LoD of the Amazon Multi-Target Test with alternative methods of nucleic acid extraction

MGIEasy Nucleic Acid Extraction Kit										
Level (copies/mL)	ORF1ab				N Gene				Test Result	
	Positive	% (n = 20)	Ct Value		Positive	% (n = 20)	Ct Value		Positive	% (n = 20)
			Mean	SD			Mean	SD		
600	20	100	33.01	0.29	20	100	32.49	0.35	20	100
300	20	100	33.84	0.34	20	100	33.37	0.34	20	100
150	20	100	34.86	0.42	20	100	34.51	0.83	20	100
125	***20***	***100***	***35.14***	***0.53***	20	100	34.54	0.55	20	100
100	18	90	35.62	0.60	***20***	***100***	***35.10***	***0.73***	***20***	***100***
75	16	80	35.83	0.64	18	90	35.60	0.59	18	90
50	12	60	35.97	0.55	16	80	35.66	0.66	18	90
37.5	6	30	36.36	0.44	10	50	36.42	0.32	12	60
MaxMAX Viral/Pathogen Nucleic Acid Isolation Kit										
Level (copies/mL)	ORF1ab				N Gene				Test Result	
	Positive	% (n = 20)	Ct Value		Positive	% (n = 20)	Ct Value		Positive	% (n = 20)
			Mean	SD			Mean	SD		
600	20	100	33.30	0.38	20	100	33.01	0.30	20	100
300	20	100	34.25	0.36	20	100	33.78	0.45	20	100
150	20	100	35.08	0.39	20	100	34.85	0.87	20	100
125	***20***	***100***	***35.54***	***0.50***	***20***	***100***	***35.01***	***0.66***	20	100
100	18	90	35.90	0.67	17	85.0	35.21	0.78	20	100
75	16	80	35.86	0.75	16	80.0	35.52	0.66	***20***	***100***
50	9	45	36.16	0.41	12	60.0	36.22	0.58	16	80
37.5	11	55	36.36	0.53	12	60.0	36.15	0.57	15	75

SD: Standard Deviation
Only Ct values ≤ 37 were included in calculation of mean and SD.
The confirmed LoD for each target is shown in ***bold face, italicized text***.
The confirmed LoD for the assay based on the authorized method of result interpretation is highlighted in yellow.

Limit of Detection with Pooled Specimens

A study was conducted to verify the LoD of the Amazon Multi-Target Test with pooled samples comprised of one positive sample and four negative samples. Contrived positive anterior nasal swab samples were prepared at various concentrations using inactivated SARS-CoV-2 (Zeptometrix Cat. # NATSARS(COV2)-ERC; SARS-CoV-2 isolate USA-WA1/2020). Each positive sample was then used to prepare a 5-sample pool by mixing with 4 negative samples in equal volumes. The study was performed in two parts, with an initial estimation of the LoD (**Table 19**), followed by confirmation of the estimated LoD by testing a larger number of replicates around the estimated LoD concentration (**Table 20**). Samples were tested using the MGIEasy Nucleic Acid Extraction Kit and ABI 7500 Fast Dx Real-Time PCR Instrument. All results were interpreted as described in **Table 10** for pooled specimens (i.e., Ct ≤ 38 = "positive" for both the ORF1ab and N gene targets). The lowest concentration of SARS-CoV-2 in an individual sample that produced ≥ 95% positive results when tested in a 5-sample pool was 200 copies/mL. Accounting for the dilution factor from 5-sample pooling, this value agrees approximately with the confirmed LoD of 50 copies/mL from individual testing described in **Table 14**.

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Table 19. Estimation of the LoD of the Amazon Multi-Target Test for pooled specimens

Level [1] (copies/mL)	ORF1ab				N Gene				Test Result	
	Positive	% (n = 16)	Ct Value		Positive	% (n = 16)	Ct Value		Positive	% (n = 16)
			Mean	SD			Mean	SD		
4,800	15	100 [2]	32.42	0.25	15	100 [2]	31.68	0.21	15	100
2,400	16	100	33.48	0.34	16	100	32.70	0.42	16	100
1,200	16	100	34.36	0.36	16	100	33.63	0.48	16	100
600	***16***	***100***	***35.76***	***0.77***	***16***	***100***	***35.23***	***0.45***	***16***	***100***
300	14	87.5	36.13	0.85	15	93.8	35.59	0.84	15	93.8
150	13	81.3	36.94	0.70	13	81.3	36.52	0.77	15	93.8
75	9	56.3	37.40	0.62	12	75.0	36.58	0.72	12	75
37.5	3	18.8	37.92	0.01	5	31.3	37.30	0.33	7	43.8

SD: Standard Deviation
[1] Concentration of SARS-CoV-2 in the individual positive sample used for pooling with 4 negative samples; the final concentration in the pool was 1/5th the value shown
[2] Only 15 samples were tested at the highest concentration
Only Ct values ≤ 38 were included in calculation of mean and SD
The estimated LoD for each target is shown in ***bold face, italicized text***.
The estimated LoD for the assay based on the authorized method of result interpretation is highlighted in yellow.

Table 20. Confirmation of the LoD of the Amazon Multi-Target Test for pooled specimens

Level [1] (copies/mL)	ORF1ab				N Gene				Test Result	
	Positive	% (n = 20)	Ct Value		Positive	% (n = 20)	Ct Value		Positive	% (n = 20)
			Mean	SD			Mean	SD		
1200	20	100	34.37	0.43	20	100	33.81	0.42	20	100
600	20	100	35.47	0.78	20	100	35.06	0.63	20	100
500	20	100	35.84	0.73	20	100	35.43	0.83	20	100
400	***20***	***100***	***36.11***	***0.75***	***19***	***95***	***35.65***	***0.95***	20	100
300	16	80	36.06	0.78	17	85	35.72	0.93	19	95
200	17	85	36.82	0.56	18	90	36.54	1.10	***20***	***100***
150	6	30	36.87	0.60	14	70	36.84	0.62	15	75
75	8	40	36.96	0.46	11	55	37.22	0.71	14	70

SD: Standard Deviation
[1] Concentration of SARS-CoV-2 in the individual positive sample used for pooling with 4 negative samples; the final concentration in the pool was 1/5th the value shown
Only Ct values ≤ 38 were included in calculation of mean and SD
The confirmed LoD for each target is shown in ***bold face, italicized text***.
The confirmed LoD for the assay based on the authorized method of result interpretation is highlighted in yellow.

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2) Inclusivity (Analytical Sensitivity)

The Amazon Multi-Target Test uses primers and probes for the SARS-CoV-2 ORF1ab and nucleocapsid (N) genes that were originally developed by the Chinese Center for Disease Control and Prevention (CCDC) and Japanese National Institute or Infectious Disease (JNIID), respectively. The inclusivity of the primers and probes was evaluated *in silico* using 2,466,032 SARS-CoV-2 genome sequences available in the National Center for Biotechnology Information (NCBI) and Global Initiative on Sharing All Influenza Data (GISAID) databases as of June 27, 2021. Incomplete genomes, genomes from animal hosts and those with ambiguous bases in the target regions were excluded from the analysis. Sequence homology was compared using the blastn algorithm from the Basic Local Alignment Search Tool (BLAST, NCBI). A summary of the results is shown in **Table 21**. Most sequences exhibited 100% homology/complementarity with the ORF1ab primers and

probes. For the N gene, almost all sequences had a single base mismatch towards the 5' end of the reverse primer but exhibited 100% homology to the forward primer and probe. Due to its location and the PCR conditions, the mismatch with the reverse primer is not predicted to have an adverse effect on assay performance. This was verified in the LoD Study described above in which the strain of SARS-CoV-2 used (USA-WA1/2020) is known to exhibit this mismatch with the reverse N gene primer.

Table 21. Summary of *in silico* analysis of the inclusivity of the Amazon Multi-Target Test primers and probes

Target (Number of Sequences)	Oligonucleotide	Number of Mismatches			
		0	1	2	≥ 3
N Gene (2,366,291)	Forward	2,353,737 (99.47%)	12,511 (0.5287%)	41 (0.0017%)	2 (0.0001%)
	Reverse	2 (0.0001%)	2,335,109 [1] (98.68%)	31,02 (1.3110%)	159 (0.0067%)
	Probe	2,349,617 (99.30%)	16,639 (0.7032%)	27 (0.0011%)	8 (0.0003%)
ORF1ab (2,340,875)	Forward	2,334,631 (99.73%)	6,217 (0.2655%)	15 (0.0006%)	12 (0.0005%)
	Reverse	2,330,655 (99.56%)	10,167 (0.4343%)	20 (0.0009%)	33 (0.0014%)
	Probe	2,333,405 (99.68%)	7,444 (0.3180%)	21 (0.0009%)	5 (0.0002%)

Most strains exhibited a mismatch 6 bases from the 5' end of the N gene reverse

Independent *in silico* inclusivity analysis performed in July, 2021 predicted no significant impact from known SARS-CoV-2 mutations and/or variants of concern on the inclusivity of the primers and probes used in the Amazon Multi-Target Test.

3) Cross-reactivity (Analytical Specificity)

An *in silico* analysis was performed to evaluate the potential for cross-reaction of the Amazon Multi-Target Test with the organisms and viruses listed in **Table 22**. Although several sequences exhibited homology to the primers and probes for the Amazon Multi-Target Test, none of these potential interactions were predicted to result in exponential amplification and/or detection.

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Table 22. Organisms and viruses evaluated *in silico* for potential cross-reactivity with the Amazon Multi-Target Test

Viruses	**Bacteria**
Adenovirus	*Chlamydia pneumoniae*
Enterovirus	*Haemophilus influenzae*
Human coronavirus 229E	*Legionella pneumophila*
Human coronavirus OC43	*Mycobacterium tuberculosis*
Human coronavirus HKU1	*Streptococcus pneumoniae*
Human coronavirus NL63	*Streptococcus pyogenes*
Human Metapneumovirus (hMPV)	*Bordetella pertussis*
Influenza A & B	*Mycoplasma pneumoniae*
MERS-coronavirus	*Pseudomonas aeruginosa*
SARS-coronavirus	*Staphylococcus epidermis*
Parainfluenza virus 1-4	*Streptococcus salivarius*
Respiratory syncytial virus	**Fungi/Yeast**
Rhinovirus	*Pneumocystis jirovecii*
	Candida albicans

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 ≤ 38 vs Ct ≤ 37), which in theory could lead to a reduction in specificity when testing pooled samples. However, all sample pools with non-negative test results are reflexed ("hit-picked") for individual testing prior to reporting of results. Because the cut-off for reflex testing is the same as that for testing of individual samples, no adverse effect on analytical or clinical specificity is anticipated from use of a higher Ct cut-off when testing pooled samples.

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4) Collection Kits

Specimen Stability
The stability of anterior nasal swab specimens in PBS was evaluated under EUA202760 for the Amazon Test. 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 and User Comprehension
Usability and User Comprehension Studies for the Amazon COVID-19 Test Collection Kit DTC were conducted under EUA210308 for the Amazon DTC Test and are documented in the EUA Summary for that device. The studies included evaluation of the kit for collection of specimens from minors aged 14 years and older under adult supervision or aged 2 years and older with adult assistance. The results were acceptable and support direct-to-consumer distribution of this kit for unsupervised collection of anterior nasal swabs.

5) Clinical Evaluation:

Comparison to FDA-authorized RT-PCR Assay
The clinical performance of the Amazon Multi-Target Test was evaluated using archived specimens from the intended use asymptomatic population of Amazon employees that had previously been characterized as SARS-CoV-2 positive or negative using a previously authorized RT-PCR assay. A total of 99 SARS-CoV-2 positive and 168 and SARS-CoV-2

negative specimens were included in the study, as determined by the comparator method. All 267 specimens were tested individually using the Amazon Multi-Target Test (**Table 23**). Positive and negative agreement with the comparator were 99.0% and 99.4%, respectively. Among the positive specimens included in the study, 23% 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 Multi-Target Test on individual samples obtained from the intended use asymptomatic population.

Table 23. Performance of the Amazon Multi-Target Test with individual anterior nasal swab specimens in comparison to an FDA-authorized method

		FDA-Authorized Comparator		
		Positive	**Negative**	**Total**
Amazon Multi-Target Test [1]	**Positive**	98	1	**99**
	Negative	1	167	**168**
	Total	**99 [2]**	**168**	**267**
Positive Agreement		**99.0% (98/99); 94.5-99.8% [3]**		
Negative Agreement		**99.4% (167/168); 96.7-99.9%**		

[1] As determined using the Ct cut-off for individual samples (Ct ≤ 37 = Positive)
[2] 23/99 specimens (23.2%) were considered "weak positive" based on analysis of the Ct values for the comparator assay
[3] Two-sided 95% score confidence interval

Validation of 5-Sample Pooling
To validate pooling of anterior nasal swab specimens for use with the Amazon Multi-Target Test, a study was performed using 56 pools of comprised of 1 positive sample and 4 negative samples, as determined by a highly sensitive FDA-authorized comparator method. In addition, 36 negative pools, each comprised of 5 negative samples as determined by the comparator method, were also included in the study. The positive samples for the study included 37.5% (21/56) that were considered "weak positives" based on the Ct values of the comparator assay.

Of the 56 positive pools, 54 (96.4%) were reported positive by the Amazon Multi-Target Test (**Table 24**). Of the 36 negative pools, 34 (94.4%) were reported negative by the Amazon Multi-Target Test. Individual testing with the Amazon Multi-Target Test confirmed the presence of a positive sample in each of the 56 positive pools that were identified by the assay (100% agreement between pooled and individual test results).

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Table 24. Comparison of Amazon Multi-Target Test results with pooled samples to expected results based on individual test results from an FDA-authorized comparator

		Expected Result (based on FDA-Authorized Comparator) [1]		
		Positive	**Negative**	**Total**
Amazon Multi-Target Test (pooled)	**Positive**	54	2 [2]	**58**
	Negative	2 [3]	34	**34**
	Total	**56**	**6**	**92**
Positive Agreement		**96.4% (54/56); 87.9-99.0% [4]**		
Negative Agreement		**94.4% (34/36); 81.9-98.5%**		

[1] From testing of individual samples
[2] Both samples were positive by the Amazon Multi-Target Test for the N gene target and negative for the ORF1ab target; both samples were also reported positive for the N gene target by the Amazon Multi-Target Test upon individual testing
[3] Both samples were considered 'weak positive" based on analysis of the Ct values for the comparator assay
[4] Two-sided 95% score confidence interval

To evaluate the potential effect of 5-sample pooling on clinical performance with the Amazon Multi-Target Test, Passing-Bablock regression analysis was performed on the Ct values obtained in the above study (**Figure 1**). The predicted shifts in Ct value for the ORF1ab and N gene targets caused by 5-sample pooling at the individual sample cut-off of 37 were 2.59 and 2.51, which are close to the theoretical shift in Ct values attributable to the dilution factor due to 5-sample pooling ($\log_2 5 = 2.32$).

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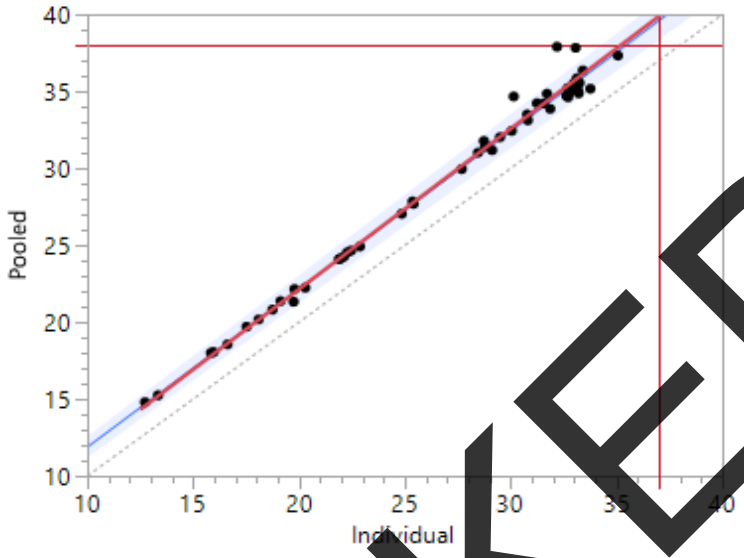
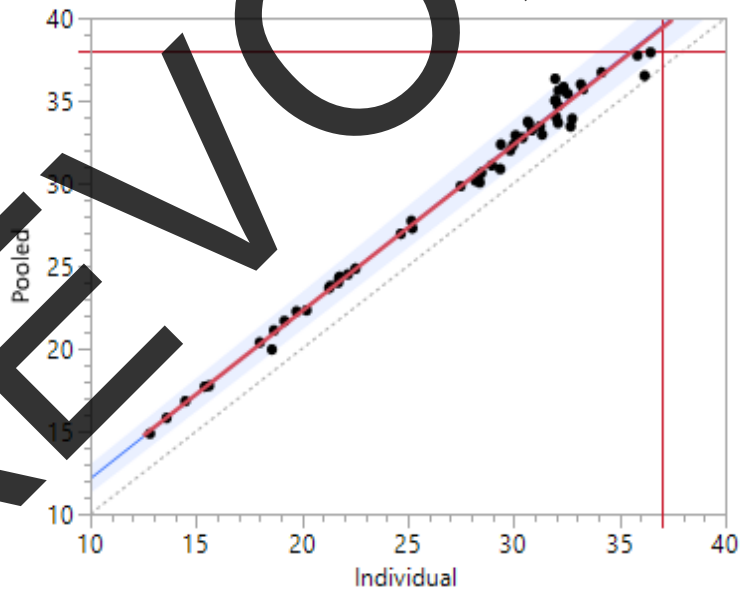
Figure 1. Passing -Bablock regression analysis of Ct values from pooled vs individual sample testing with the Amazon Multi-Target Test

ORF1ab



Intercept: 1.61264
Slope: 1.02655
Ct $_{Pooled}$ = 1.02655 * Ct $_{Individual}$ + 1.61264

N Gene



Intercept: 2.00045
Slope: 1.0137
Ct $_{Pooled}$ = 1.0137 * Ct $_{Individual}$ + 2.00045

Ct cut-offs indicated by red lines: individual ≤ 37; pooled ≤ 38

Application of the predicted Ct shifts from 5-sample pooling to the results from the Clinical Evaluation described above in **Table 23** demonstrated 97.0% positive agreement (96/99)

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between individual test results and the predicted results from 5-sample pooling (**Table 25**).

Table 25. Summary of predicted effect of 5-sample pooling on results obtained with the Amazon Multi-Target Test

	Positive (%)	
	Individual [1]	**Pooled (Predicted)**
ORF1ab	98 (99.0)	93 (93.9)
N Gene	91 (91.9)	87 (87.9)
Final Interpretation [2]	99 (100)	96 (97.0)

[1] The individual samples tested included 23/99 (23.2%) that were considered "weak positive" as determined by analysis of the Ct values obtained with an FDA-authorized comparator method

[2] Based on the result algorithm described in **Table 10** whereby a positive result is determined by a Ct value ≤ 38 for *either* the ORF1ab or N gene targets

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 26**).

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Table 26. 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

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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 26** to choose an appropriate validated pool size. **Table 26** 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 26**, choose n from **Table 26** which corresponds to the maximum efficiency (F).

- If $P_{individual}$ in your laboratory does not correspond to the largest validated pool size in **Table 26**, 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 ($P_{pool\text{-}x}$) for subsequent 7-10 day* period based on n-sample pool testing. ($P_{pool\text{-}x}$) should be updated daily using a moving average.

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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

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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

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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 Multi-Target SARS-CoV-2 Real-Time RT-PCR DTC Test 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 RNase P 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.

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