EMERGENCY USE AUTHORIZATION (EUA) SUMMARY
SARS-CoV-2 ASSAY
(Infinity BiologiX Clinical Genomics Laboratory)

For in vitro diagnostic use
Rx only
For use under Emergency Use Authorization (EUA) Only

(The Infinity BiologiX TaqPath SARS-CoV-2 Assay will be performed at Infinity BiologiX LLC laboratories, located at Nelson Biological Sciences, C-Wing, 604 Allison Road, Piscataway, NJ 08854 and 3510 Hopkins Place, Bldg 4, Oakdale, MN 55128, which are certified under Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a and meet requirements to perform high-complexity tests, per the laboratory procedures that were reviewed by the FDA under this EUA).

INTENDED USE

The Infinity BiologiX TaqPath SARS-CoV-2 Assay is a real-time reverse transcription polymerase chain reaction (rRT-PCR) test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in oropharyngeal (throat) swab, nasopharyngeal swab, anterior nasal swab, mid-turbinate nasal swab, and bronchoalveolar lavage (BAL) fluid from individuals suspected of COVID-19 by their healthcare provider.

When determined to be appropriate by a healthcare provider, this test is also for use with saliva specimens that are self-collected at home using the IBX Saliva Collection Kit or that are collected in a healthcare setting by individuals using the Spectrum Solutions SDNA-1000 Saliva Collection Device.

This test is also for the qualitative detection of nucleic acid from SARS-CoV-2 in pooled saliva samples containing up to 5 individual saliva specimens that are self-collected at home using the IBX Saliva Collection Kit or that are collected in a healthcare setting by individuals using the Spectrum Solutions SDNA-1000 Saliva Collection Device, when determined to be appropriate by healthcare provider. Negative results from pooled testing should not be treated as definitive. If a patient's clinical signs and symptoms are inconsistent with a negative result or results are necessary for patient management, the patient should be considered for individual testing. Specimens included in pools with a positive and indeterminate result must be tested individually prior to reporting a result. Specimens with low viral loads may not be detected in sample pools due to decreased sensitivity in pooled testing.

Testing is limited to Infinity BiologiX LLC laboratories, located at Nelson Biological Sciences, C-Wing, 604 Allison Road, Piscataway, NJ 08854 and 3510 Hopkins Place, Bldg 4, Oakdale, MN 55128, which are certified under Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a and meet requirements to perform high- complexity tests.

Results are for the detection and identification of SARS-CoV-2 RNA. The SARS- CoV-2 RNA is generally detectable in respiratory 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 for SARS-CoV-2 RNA from saliva should be confirmed by testing of an alternative specimen type if clinically indicated.

The Infinity BiologiX TaqPath SARS-CoV-2 Assay 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 assay is intended for use under the Food and Drug Administration's Emergency Use Authorization.

DEVICE DESCRIPTION AND TEST PRINCIPLE

The Infinity BiologiX TaqPath SARS-CoV-2 Assay is a real-time reverse transcription polymerase chain reaction (rRT-PCR) test. The assay uses primers and probes that were developed and validated under the Emergency Use Authorization (EUA) for the TaqPath COVID-19 Combo Kit and are designed to detect RNA from SARS-CoV-2 in respiratory specimens from patients as recommended for testing by public health authority guidelines. This EUA authorizes testing of additional specimen types, including saliva, and use of alternative nucleic acid extraction and amplification systems available to Infinity BiologiX.

Anterior nasal swabs, mid-turbinate nasal swabs, oropharyngeal (throat) swabs and nasopharyngeal swabs and bronchoalveolar lavage fluid should be collected, transported and stored according to standard procedures. Saliva specimens may be collected in a healthcare setting using the Spectrum Solutions SDNA-1000 Saliva Collection Device or be self-collected at home using the IBX Saliva Collection Kit (comprised of the Spectrum Solutions SDNA-1000 Saliva Collection Device and instructions for sample collection and shipment to the testing laboratory). Saliva specimens must be transported and stored at ambient temperature and tested within 56 hours of collection when stored at ambient temperature.

RNA extraction for all specimen types is performed using the PerkinElmer Chemagic 360 automated specimen processing system with the Chemagic Viral DNA/RNA 300 Kit H96.

Reverse transcriptase-PCR (RT-PCR) is performed using the Applied Biosystems TaqPath COVID-19 Combo Kit.

In addition to testing of individual specimens, the Infinity BiologiX TaqPath SARS-CoV-2 Assay is validated for use with pooled saliva samples that are collected with the IBA Saliva Collection Kit or Spectrum Solution SDNA-1000 Collection Device, with up to 5 individual saliva specimens per pool. To mitigate the potential for false negative results due to pooling of samples that contain low levels of RNA, modified nucleic acid extraction and RT-PCR procedures are used with pooled saliva specimens whereby the volume of extracted sample and volume of extracted eluate per amplification reaction are both increased compared to that used for testing of individual samples.

INSTRUMENTS USED WITH THE TEST

The Infinity BiologiX TaqPath SARS-CoV-2 Assay is for use with the ThermoFisher Applied Biosystems QuantStudio 5 Real-Time PCR System equipped with software v1.3, or the Applied Biosystems ViiA7 Real-Time PCR System with the Applied Biosystems QuantStudio 5 software v1.3 for data analysis, and Perkin Elmer Chemagic 360 extraction instrument (software v6.3.0.3).

REAGENTS AND MATERIALS

Table 1. Reagents and materials required for use of the Infinity BiologiX TaqPath SARS-CoV-2 Assay

Reagent	Manufacturer	Catalogue #
Chemagic Viral DNA/RNA 300 Kit H96	PerkinElmer	CMG-1033-S
96 well Deep Well Plates	PerkinElmer	43001-0120
TaqPath COVID-19 Combo Kit	ThermoFisher Scientific	A147814
384 well PCR plate	ThermoFisher Scientific	4483273
Optical adhesive PCR plate cover	ThermoFisher Scientific	4311971
Nuclease-free water	--	--
Ethanol (96-100%)	--	--

CONTROLS

The controls supplied with the ThermoFisher - Applied Biosystems TaqPath COVID- 19 Combo Kit are described in **Table 2**.

Table 2. Controls supplied with the Applied Biosystems TaqPath COVID-19 Combo Kit

Control Type	Purpose	Frequency of Testing
Negative	To monitor for cross-contamination during RNA extraction and RT-PCR	Once per batch of specimens
Positive	To monitor the integrity of the RT-PCR reagents and process	Once per run of RT-PCR
Internal (MS2 Phage)	To monitor the integrity of nucleic acid extraction and RT-PCR for each specimen	Added to each specimen and the Negative Control prior to extraction

In addition to these controls, a No Template Control containing none of the SARS-CoV-2 targets or the Internal Control is included in every PCR run. The results from the controls are interpreted according to the criteria shown in **Table 3**. If the results obtained with the Positive, Negative and No Template Controls do not meet the criteria shown, the results from the entire batch of samples are considered invalid and repeat testing must be performed.

Table 3. Ct values for controls that must be observed to obtain valid results

Control	Ct Value (Optical Channel)			
	N Gene (VIC)	S Gene (ABY)	ORF1ab (FAM)	MS2 Phage (JUN)
Negative	> 40	> 40	> 40	≤ 37
Positive	< 37	< 37	< 37	Undetermined [1]
No Template	Undetermined	Undetermined	Undetermined	Undetermined [1]
Internal	Any	Any	Any	< 37

[1] The MS2 Phage Internal Control is not added to the Positive Control or No Template Control and no signal should be obtained

INTERPRETATION OF RESULTS

The results from testing of individual patient samples are interpreted according to the criteria described in **Table 4**.

Table 4. Result interpretation for individual patient samples

Ct Value (Optical Channel)				Result Interpretation
N Gene (VIC)	S Gene (ABY)	ORF1ab (FAM)	MS2 Phage (JUN)	
Undetermined	Undetermined	Undetermined	< 37	Negative
Two of three < 37			< 37	Positive
One of three < 37			< 37	Re-test [1]
Undetermined	Undetermined	Undetermined	Undetermined	Re-test [1]

[1] Re-test required from the residual extracted sample and by processing a new aliquot of the original sample if volume permits; if the re-test result is the same as the original then report result as "inconclusive"

The results from testing pooled saliva samples are interpreted according to the criteria in **Table 5**. As appropriate, individual samples within a pool may be retested and dispositioned according to the criteria for individual patient samples described above.

Table 5. Result interpretation for pooled saliva samples

Ct Value (Optical Channel)				Result Interpretation/ Action
N Gene (VIC)	**S Gene (ABY)**	**ORF1ab (FAM)**	**MS2 Phage (JUN)**	
Undetermined or > 37	Undetermined or > 37	Undetermined or > 37	< 37	Negative - report
Two of three < 37			< 37	Positive - reflex to individual testing
One of three < 37			< 37	Indeterminate - reflex to individual testing
Undetermined or > 37	Undetermined or > 37	Undetermined or > 37	Undetermined or > 37	Indeterminate - re-test pool [1]

[1] Failure of the MS2 control requires retesting of the pool starting with re-extraction. If the same result is obtained a second time, samples within the pool will be reflexed for individual testing.

PERFORMANCE EVALUATION

1) Analytical Sensitivity

The LoD was determined using *in vitro* transcripts from Exact Diagnostics (SARS-CoV-2 Standard) that were diluted in SARS-CoV-2 negative nasopharyngeal swab matrix. An initial estimate of the LoD with the Applied Biosystems QuantStudio 5 Real-Time PCR System was obtained by testing three replicates at each of four different target levels: 1000, 500, 200 and 100 copies/mL. The lowest level at which all three replicates were positive for all three SARS-CoV-2 targets was 200 copies/mL. The estimated LoD was confirmed by testing an additional 20 replicates at the same target level. All 20 replicates produced the expected results for each SARS-CoV-2 target, and the LoD was therefore confirmed to be 200 copies/mL.

To validate use of the Applied Biosystems ViiA7 Real-Time PCR System for PCR amplification, an additional study was performed by testing 20 nasopharyngeal and 10 saliva samples that were each spiked with 400 copies/mL of the Exact Diagnostics SARS-CoV-2 transcripts. Positive results were obtained for each of the samples for all three target genes and the MS2 internal control, demonstrating that the ViiA7 Real-Time PCR system performed similarly to the QuantStudio 5. These results are acceptable.

2) Analytical Specificity

Inclusivity

The Infinity BiologiX TaqPath SARS-CoV-2 Assay is a modification of the previously authorized ThermoFisher Applied Biosystems TaqPath COVID-19 Combo Kit. The assay targets specific genomic regions of the SARS-CoV-2 nucleocapsid (N) gene, spike (S) gene, and ORF1ab region. Inclusivity was demonstrated under the original EUA by mapping the

primers and probes to 185 complete SARS-CoV-2 genomes that were available in the GenBank and GISAID (Global Initiative on Sharing All Influenza Data) databases as of March 5, 2020. For all primers and probes, there was 100% homology to each of the SARS-CoV-2 sequences analyzed, with one exception; a single base mismatch (95.6% homology) with the reverse primer for ORF1ab in sequence EPI_ISL_407084 (BetaCoronavirus/Japan/AI/I-004/2020). The mismatch is located at the 5' end of the primer and is not expected to affect test performance.

In January, 2021 FDA published a bulletin regarding the potential for mutations associated with specific variants of SARS-CoV-2 to have an adverse effect on specific assays, including those such as the Infinity BiologiX assay, that are based on the TaqPath COVID-19 Combo Kit (https://www.fda.gov/medical-devices/letters-health-care-providers/genetic-variants-sars-cov-2-may-lead-false-negative-results-molecular-tests-detection-sars-cov-2).

Cross-reactivity

The analytical specificity of the Infinity BiologiX TaqPath SARS-CoV-2 Assay was demonstrated *in silico* under the original EUA for the ThermoFisher Applied Biosystems TaqPath COVID-19 Combo Kit. The analysis included evaluation of the primer and probe homology with the 43 organisms and viruses listed in **Table 6**. Based on this analysis, significant amplification of non-target sequences that could result in cross-reaction (false-positive results) or interference (false-negative results) was considered unlikely to occur.

Table 6. Organisms and viruses evaluated for potential cross-reaction and/or interference with the Applied Biosystems TaqPath COVID-19 Combo Kit

Viruses	**Bacteria**
Adenovirus	*Bacillus anthracis*
Enterovirus	*Bordetella pertussis*
Human coronavirus 229E	*Chlamydophila pneumoniae*
Human coronavirus HKU1	*Chlamydophila psittaci*
Human coronavirus NL63	*Corynebacterium diphtheriae*
Human coronavirus OC43	*Coxiella burnetii*
Human Metapneumovirus (hMPV)	*Haemophilus influenzae*
Influenza A, B and C	*Legionella* (non-pneumophila)
MERS-coronavirus	*Legionella pneumophila*
Parainfluenza 1-4	*Leptospira* sp.
Parechovirus	*Moraxella catarrhalis*
Respiratory Syncytial Virus A and B	*Mycobacterium tuberculosis*
Rhinovirus/Enterovirus	*Mycoplasma pneumoniae*
SARS-coronavirus	*Neisseria elongata* and *Neisseria meningitidis*
Yeast/Fungus	*Pseudomonas aeruginosa*
Candida albicans	*Staphylococcus aureus*
Pneumocystis jirovecii	*Staphylococcus epidermidis*
	Streptococcus pneumoniae
	Streptococcus pyogenes
	Streptococcus salivarius

3) Clinical Evaluation

Nasopharyngeal Swabs

The performance of the Infinity BiologiX TaqPath SARS-CoV-2 Assay with nasopharyngeal swabs was evaluated using contrived specimens composed of leftover nasopharyngeal swab samples that were spiked with SARS-CoV-2 *in vitro* transcripts or human DNA (both Exact Diagnostics). A total of 30 contrived positive and contrived negative samples were tested. A summary of the results of the study is provided in **Tables 7** and **8**. All 30 (100%) contrived negative samples produced the expected results. Of the 30 contrived positive samples, all 30 (100%) produced positive results for the N and S genes, whereas the ORF1ab target was positive for 25/30 samples (83.3%). No amplification of the ORF1ab target was observed with 1/10 samples (10.0%) at 200 copies/mL and 4/10 samples (40.0%) at 400 copies/mL. According to the result algorithm described in **Table 4**, above, a sample is considered positive for SARS-CoV-2 RNA if amplification is detected with at least two of the three SARS-CoV-2-specific target sequences. The results of the Clinical Evaluation with contrived nasopharyngeal swabs were therefore considered acceptable.

Table 7. Summary of results from the contrived specimen study with nasopharyngeal swabs, stratified by target level and measurand

Transcript Copies/mL	Number Tested	Analysis	Target (Optical Channel)			
			N Gene (VIC)	S Gene (ABY)	ORF1ab (FAM)	MS2 (JUN)
0	30	Positive (%)	0 (0)	0 (0)	0 (0)	0 (0)
		Mean Ct (SD)	N/A	N/A	N/A	24.4 (0.4)
200	10	Positive (%)	10 (100)	10 (100)	9 (100)	10 (100)
		Mean Ct (SD)	21.7 (4.3)	22.1 (6.0)	19.7 (1.6)	27.1 (1.2)
400	10	Positive (%)	10 (100)	10 (100)	6 (60.0)	10 (100)
		Mean Ct (SD)	27.0 (6.7)	26.6 (6.8)	21.1 (2.3)	26.1 (1.2)
600	4	Positive (%)	4 (100)	4 (100)	4 (100)	4 (100)
		Mean Ct (SD)	28.5 (5.2)	27.2 (4.5)	27.4 (6.2)	25.7 (0.9)
800	3	Positive (%)	3 (100)	3 (100)	3 (100)	3 (100)
		Mean Ct (SD)	33.0 (1.6)	30.5 (0.4)	35.0 (3.9)	25.0 (0.9)
1000	3	Positive (%)	3 (100)	3 (100)	3 (100)	3 (100)
		Mean Ct (SD)	28.8 (6.6)	27.7 (5.6)	29.0 (7.3)	25.8 (1.2)
All Positives	30	Positive (%)	30 (100)	30 (100)	25 (83.3)	30 (100)
		Mean Ct (SD)	26.2 (6.3)	25.7 (5.1)	24.2 (6.4)	26.2 (1.3)

N/A: Not applicable; SD: Standard Deviation

Table 8. Summary of positive and negative agreement with contrived nasopharyngeal swab specimens

		Contrived Specimen Type		
		Positive	**Negative**	**Total**
TaqPath SARS-CoV-2 Assay	**Positive**	30	0	**30**
	Negative	0	30	**30**
	Total	**30**	**30**	**60**
Positive Agreement		100% (30/30); 88.7-100% [1]		
Negative Agreement		100% (30/30); 88.7-100%		

[1] Two-sided 95% score confidence interval

Saliva

A study was performed to evaluate the use of saliva as a specimen type for detection of SARS-CoV-2 in patients who are suspected of COVID-19. The study was conducted with symptomatic patients from three ambulatory care centers who were each provided with instructions for self-collection of saliva using the Spectrum Solutions SDNA-1000 Saliva Collection Device. Self-collection of saliva samples was performed under the observation of a healthcare provider who subsequently (within 10 minutes) also collected either a nasopharyngeal or oropharyngeal swab from each patient for parallel testing for SARS-CoV-2. The swabs were placed in viral transport medium for shipment to the testing laboratory. Both the saliva and swabs were transported at ambient temperature and tested using the Infinity BiologiX Clinical Genomics Laboratory TaqPath SARS- CoV-2 Assay within 48 hours of collection. A summary of the results of the study is presented in **Tables 9** and **10**.

There was 100% positive and negative agreement between the results obtained from testing of saliva and those obtained from nasopharyngeal and oropharyngeal swabs. Overall mean Ct values were similar for saliva and either nasopharyngeal or oropharyngeal swabs, there was

no correlation between Ct values from different samples from the same patient. Nevertheless, the results support the use of saliva as a specimen type for use with the Infinity BiologiX TaqPath SARS-CoV-2 Assay.

Table 9. Summary of qualitative results obtained from parallel testing of nasopharyngeal and oropharyngeal swab samples and saliva from patients suspected of COVID-19

		Nasopharyngeal Swab		
		Positive	**Negative**	**Total**
Saliva	**Positive**	26	0	**26**
	Negative	0	27	**27**
	Total	**26**	**27**	**53**
Positive Agreement		100% (26/26); 87.1-100% [1]		
Negative Agreement		100% (27/27); 87.5-100%		
		Oropharyngeal Swab		
		Positive	**Negative**	**Total**
Saliva	**Positive**	4	0	**4**
	Negative	0	3	**3**
	Total	**4**	**3**	**7**
Positive Agreement		100% (4/4); 51.0-100% [1]		
Negative Agreement		100% (3/3); 43.9-100%		
		Nasopharyngeal or Oropharyngeal Swab		
		Positive	**Negative**	**Total**
Saliva	**Positive**	30	0	**30**
	Negative	0	30	**30**
	Total	**30**	**30**	**60**
Positive Agreement		100% (30/30); 88.7-100% [1]		
Negative Agreement		100% (30/30); 88.7-100%		

[1] Two-sided 95% score confidence interval

Table 10. Summary of results obtained from parallel testing of nasopharyngeal and oropharyngeal swab samples and saliva from patients suspected of COVID-19, stratified by measurand

Number of Patients	Sample Type	Analysis	Target (Optical Channel)			
			N Gene (VIC)	S Gene (ABY)	ORF1ab (FAM)	MS2 (JUN)
26 NP positive	NP swab	Positive (%)	26 (100)	26 (100)	26 (100)	26 (100)
		Mean Ct (SD)	24.4 (4.0)	24.5 (3.9)	23.6 (3.7)	24.3(2.6)
	Saliva	Positive (%)	26 (100)	26 (100)	26 (100)	26 (100)
		Mean Ct (SD)	23.5 (6.2)	24.6 (6.0)	23.6 (5.7)	26.0 (4.1)
27 NP negative	NP swab	Positive (%)	0 (0)	0 (0)	0 (0)	27 (100)
		Mean Ct (SD)	N/A	N/A	N/A	24.4 (1.2)
	Saliva	Positive (%)	0 (0)	0 (0)	0 (0)	27 (100)
		Mean Ct (SD)	N/A	N/A	N/A	25.0 (1.9)
4 OP positive	OP swab	Positive (%)	4 (100)	4 (100)	4 (100)	4 (100)
		Mean Ct (SD)	24.7 (4.0)	24.3 (3.9)	23.5 (4.4)	25.4 (1.8)
	Saliva	Positive (%)	4 (100)	4 (100)	4 (100)	4 (100)
		Mean Ct (SD)	22.0 (7.1)	22.3 (7.2)	21.4 (7.1)	29.6 (5.6)
3 OP negative	OP Swab	Positive (%)	0 (0)	0 (0)	0 (0)	23.5 (1.5)
		Mean Ct (SD)	N/A	N/A	N/A	3 (100)
	Saliva	Positive (%)	0 (0)	0 (0)	0 (0)	3 (100)
		Mean Ct (SD)	N/A	N/A	N/A	23.1 (1.4)

NP: Nasopharyngeal; OP: Oropharyngeal; N/A: Not applicable; SD: Standard Deviation

Clinical Confirmation

The first 5 positive and first 5 negative nasopharyngeal specimens as using the Infinity BiologiX TaqPath SARS-CoV-2 Assay were also tested by the New Jersey State Health Department using the previously authorized CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel. There was 100% (5/5) positive and negative agreement for the specimens tested. These results are acceptable and support use of the Infinity BiologiX TaqPath SARS-CoV-2 Assay for testing clinical specimens.

4) Simulated Shipping Study with the SDNA-1000 Saliva Collection Device

To support home use of the Spectrum Solutions SDNA-1000 Saliva Collection Device, used as part of the IBX Saliva Collection Kit, a Simulated Shipping Study was performed that was designed to evaluate the effect of temperature variation on the stability of SARS- CoV-2 RNA during transport of saliva specimens. The study was conducted using residual clinical specimens that had previously been reported as SARS-CoV-2 positive or negative using the Infinity BiologiX TaqPath SARS-CoV-2 Assay, and which were stored at -80°C until the start of the study. The SARS-CoV-2 positive specimens were selected based on the Ct values obtained upon initial testing and covered the spectrum of Ct values observed with the assay.

To perform the study, the specimens were thawed and then subjected to the thermal profiles outlined in **Tables 11** and **12** which were intended to simulate the extreme temperature conditions that may be experienced in shipment of specimens during the summer and winter, respectively. At the conclusion of each thermal profile, the samples were retested with the Infinity BiologiX TaqPath SARS-CoV-2 Assay and the results obtained were compared to those reported upon initial testing at the time the specimens were received. A summary of the mean Ct values observed for each SARS-CoV-2 specific target gene is provided in **Table 13**. The Ct values for each individual sample are presented graphically in **Figure 1**.

Nineteen out of 20 Low Positive samples (95%) and 10/10 High Positive samples were reported as positive after exposure to the summer and winter temperature excursions. The mean and standard deviation of the Ct values for each gene target were similar before and after simulated shipping, with no evidence of significant degradation of the SARS-CoV-2 RNA. All SARS-CoV-2 negative specimens were reported as "negative."

These results demonstrate that SARS-CoV-2 RNA positive saliva specimens are stable in the SDNA-1000 Saliva Collection Device when exposed to a broad range of temperature conditions. These data support the use of the SDNA-1000 Saliva Collection Device for transport and storage of specimens following home collection of saliva.

Table 11. Summer temperature excursion

Temperature (°C)	Cycle Period	Time (hours)	
		Cycle Period	Total Time [1]
40	1	8	8
22	2	4	12
30	3	2	14
22	4	36	50
40	5	6	56

[1] Sum of Cycle Periods

Table 12. Winter temperature excursion

Temperature (°C)	Cycle Period	Time (hours)	
		Cycle Period	Total Time [1]
-80	1	8	8
18	2	4	12
-10	3	4	16
4	4	38	56

[1] Sum of Cycle Periods

Table 13. Summary of results from the Simulated Shipping Study with the SDNA-1000 Saliva Collection Device

Sample Group	Test Point	N	Mean Ct (Standard Deviation)			Positive (%)
			N Gene	ORF1ab	S Gene	
Negative	T = 0	10	N/A	N/A	N/A	0 (0)
	Summer [1]	10	N/A	N/A	N/A	0 (0)
	Winter [2]	10	N/A	38.6 (--) [3]	N/A	0 (0)
Low Positive	T = 0	20	29.0 (1.9)	29.3 (2.1)	29.4 (3.1)	20 (100)
	Summer	20	29.9 (2.4)	29.3 (2.9)	29.0 (2.5)	19 (95)
	Winter	20	30.0 (2.4)	29.1 (2.7)	28.8 (2.0)	19 (95)
High Positive	T = 0	10	20.8 (2.2)	21.3 (1.9)	20.7 (2.7)	10 (100)
	Summer	10	23.5 (3.5)	22.3 (3.8)	22.5 (4.0)	10 (100)
	Winter	10	23.4 (3.3)	22.2 (3.4)	22.1 (3.2)	10 (100)

N/A: Not Applicable

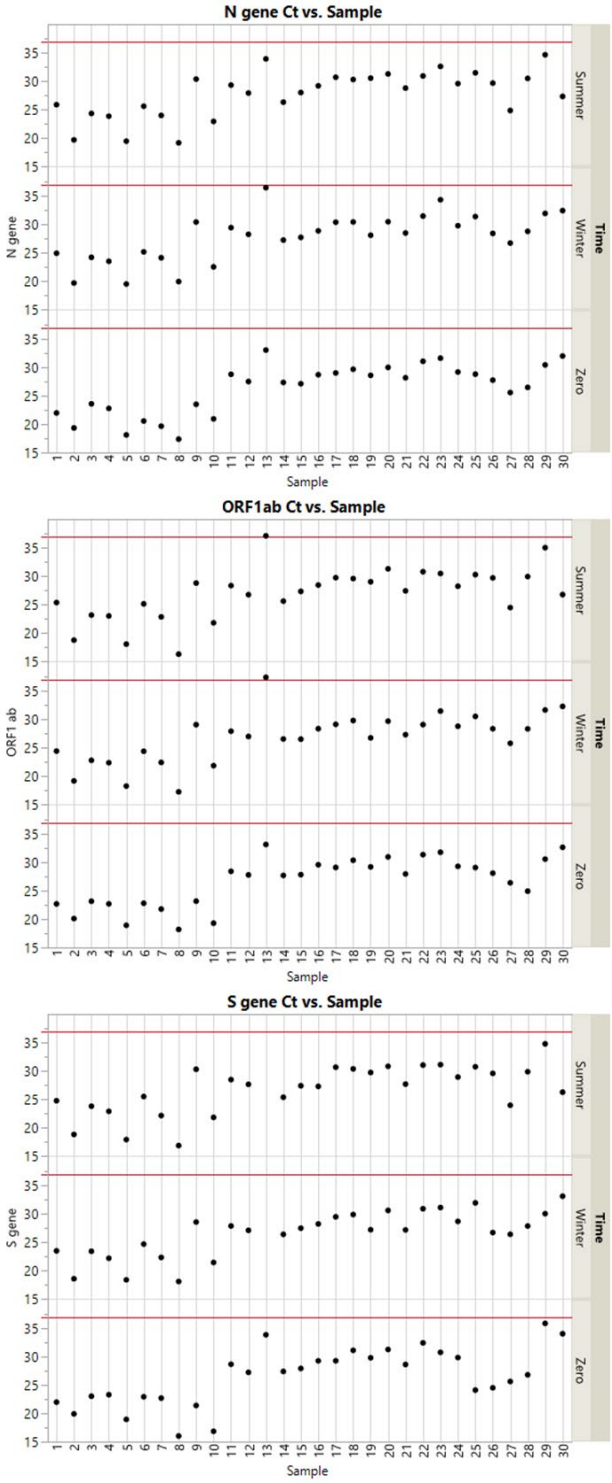
[1] Testing performed at the conclusion of the thermal excursions described in **Table 11**

[2] Testing performed at the conclusion of the thermal excursions described in **Table 12**

[3] 1 sample gave a Ct value for ORF1ab but no amplification was observed for the other two SARS-CoV-2 targets. Based on the algorithm used for the Infinity BiologiX TaqPath SARS CoV-2 Assay (**Table 4**), at least two targets must have Ct values <37 for a specimen to be called positive for SARS-CoV-2 RNA. Therefore, this sample was recorded as "SARS-CoV-2 RNA Negative."

[4] Low Positive: Ct >25 at T= 0 for all targets; High Positive: Ct <25 at T = 0 for all targets

Figure 1. Ct values for each SARS-CoV-2 target gene by sample



Samples 1-10: High Positive (Ct value <25 for each target at T = 0) Samples 11-30: Low Positive (Ct value >25 for each target at T = 0)

5) Validation of 5-sample Pooling of Saliva Specimens

Clinical Evaluation of Pooling

To validate pooling of saliva specimens, a Clinical Evaluation was performed with 20 positive samples that were tested individually using the authorized Infinity BiologiX TaqPath SARS-CoV-2 Assay and again using the modified assay when combined into 5-sample pools comprised of 1 positive sample and 4 negative samples in equal volume. In addition to the positive pools, 20 negative pools comprised of 5 SARS-CoV-2 negative samples as determined using the candidate assay were also tested. Because any pool with a with an Indeterminate result caused by detection of just 1/3 SARS-CoV-2 targets is automatically reflexed for individual testing, such results were counted as "SARS-CoV-2 positive" for the purposes of determining Positive and Negative Agreement (PPA/NPA).

Eighteen of the 20 pools containing positive individual samples produced positive results and one additional pool was reported as Indeterminate due to detection of single SARS-CoV-2-specific target (**Table 14**). Nineteen of the 20 pools comprised of negative individual samples produced negative results and one pool was reported as Indeterminate due to detection of ORF1ab (Ct = 36.5). Positive and Negative Percent Agreement (PPA and NPA) were therefore both 95.0% (19/20).

Table 14. Summary of qualitative test results from the validation of 5-sample pooling with the Infinity BiologiX TaqPath SARS-CoV-2 Assay

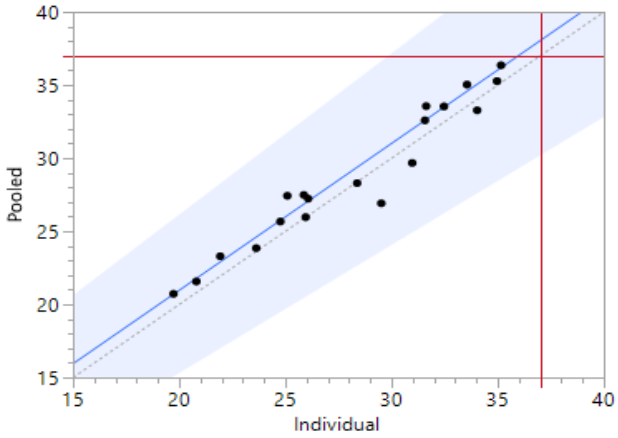
		Individual			
		Positive	**Indeterminate**	**Negative**	**Total**
Pooled	**Positive**	18	0	0	**18**
	Indeterminate	1 [1]	0	1 [1]	**2**
	Negative	1	0	19	**20**
	Total	**20**	**0**	**20**	**40**
Positive Agreement		**95.0% (19/20); 76.4 - 99.1%**			
Negative Agreement		**95.0% (19/20); 76.4 - 99.1%**			

[1] Because pooled samples with Indeterminate results caused by detection of 1/3 SARS-CoV-2 targets are automatically submitted for individual testing, such results were counted as "SARS-CoV-2 positive" for the purposes of determining agreement.

Figure 2 shows the results from Passing-Bablock regression analysis of the data from the Infinity BiologiX Clinical Evaluation of Pooling. The predicted Ct shift at the assay Ct cut-off for each target in the Infinity BiologiX TaqPath SARS-CoV-2 Assay is shown in **Table 15**. The highest predicted Ct shift was 2.14 for the S gene target which is approximately equal to the theoretical shift of 2.32 (i.e., $\log_2 5$) due to the dilution factor associated with 5-sample pooling. The predicted Ct shifts for the N gene and ORF targets at the cut-off were 1.04 and 0.61, respectively.

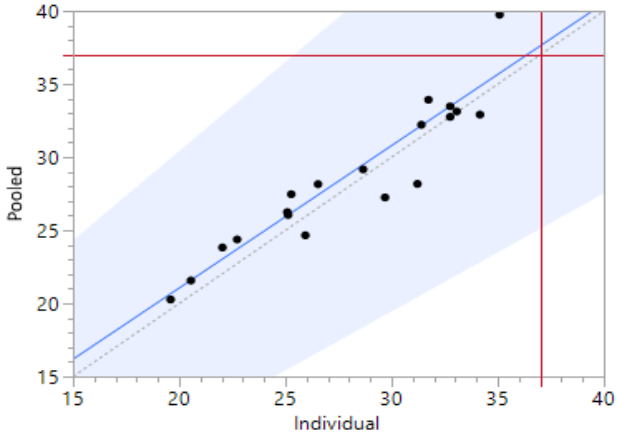
Figure 2. Passing-Bablock regression of Ct values for pooled vs individual samples

N Gene



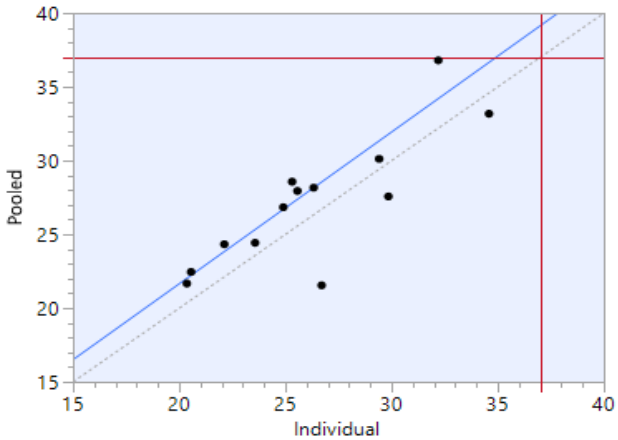
Slope: 1.00408; Intercept: 0.88874

ORF1ab



Slope: 0.97368; Intercept: 1.58336

S Gene



7 samples did not produce Ct values for the S Gene target and are excluded from the analysis
Slope: 1.02875; Intercept: 1.02875

Table 15. Summary of predicted Ct shifts from 5-sample pooling with the Infinity BiologiX TaqPath SARS-CoV-2 Assay

Target	Slope	Intercept	Predicted Ct Shift at Cut-off (Ct = 37)
N Gene	1.00408	0.88874	1.03970
ORF	0.97368	1.58336	0.60952
S Gene	1.02875	1.02875	2.14237

Evaluation of Real-World Data
To validate the proposed 5-sample pooling strategy, a retrospective analysis was performed on results from 100 consecutively tested positive individual samples that were received by the Infinity BiologiX laboratory between June 24 and 29, 2021. The expected Ct shift due to 5-sample pooling was calculated using the equations from the Passing-Bablok regression analysis above. The predicted effect on qualitative test results for each target in the Infinity BiologiX TaqPath SARS-COV-2 Assay is shown in **Table 16**. For the purposes of this analysis, pooled samples that were predicted to produce a positive result for at least one SARS-CoV-2 analyte (and which would therefore have undergone reflex testing as individual samples) were counted as "SARS-CoV-2 positive." On this basis, positive agreement between individual test results and predicted results from testing of 5-sample pools was 100% (95% score confidence interval 96.3-100%), with 97.0% (91.9-99.0%) of samples producing positive results for two or more SARS-CoV-2 analytes.

Table 16. Summary of observed individual vs predicted qualitative test results by analyte for 100 consecutively collected specimens

Result	Number/Percentage of Samples	
	Individual	**Pooled (predicted)**
N Gene Positive	100	98
ORF1ab Positive	100	99
S Gene Positive	50 [1]	50
SARS-CoV-2 Positive [2]	100	97
SARS-CoV-2 Positive or Inconclusive [3]	100	100

[1] The low proportion of S Gene positive results is attributed to the presence of the B1.1.7 variant that has a mutation in the region of the gene that is targeted by the Infinity BiologiX TaqPath SARS-CoV-2 Assay (and which is based on the Applied Biosystems TaqPath COVID-19 Combo Kit)
[2] ≥ 2/3 targets reported positive: agreement = 97.0%
[3] ≥ 1/3 targets reported positive: agreement = 100%

6) 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 17**).

Table 17. 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 17** to choose an appropriate validated pool size. **Table 17** 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 17**, choose n from **Table 17** which corresponds to the maximum efficiency (F).

- If $P_{individual}$ in your laboratory does not correspond to the largest validated pool size in **Table 17**, 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").

7) FDA SARS-CoV-2 Reference Panel Testing

The evaluation of sensitivity and MERS-CoV cross-reactivity was performed using reference material (T1), blinded samples and a standard protocol provided by the FDA. The study included a range finding study and a confirmatory study for LoD. Blinded sample testing was used to establish specificity and to corroborate the LoD. The extraction method used was the Chemagic Viral DNA/RNA 300 Kit H96 on the PerkinElmer Chemagic 360 automated specimen processing system. Amplification was carried out on the ThermoFisher Applied Biosystems QuantStudio 5 Real-Time PCR System. The results are summarized in the following Table.

Table 18. Summary of LoD Confirmation Results Using the FDA SARS-CoV-2 Reference Panel

Reference Materials Provided by FDA	Specimen Type	Product LoD	Cross-Reactivity
SARS-CoV-2	Saliva	$6.0x10^3$ NDU/mL	N/A
MERS-CoV		N/A	ND
SARS-CoV-2	Nasopharyngeal Swab	$6.0x10^4$ NDU/mL	N/A
MERS-CoV		N/A	ND

NDU/mL: RNA NAAT detectable units/mL N/A: Not Applicable
ND: Not Detected

LIMITATIONS

- Testing of saliva specimens is limited to patients with symptoms of COVID-19.
- 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.
- Certain mutations may affect detection of individual targets with the Infinity BiologiX TaqPath SARS-CoV-2 RT-PCR Assay, including the B.1.1.7 variant of SARS-CoV-2 which may exhibit positive results for the N and ORF1ab targets and negative results for the S-gene target. If such a pattern of detection is observed, further characterization of the specimen by sequence analysis should be considered. If such services are not readily available, local or state clinical laboratories should consider contacting the Centers for Disease Control and Prevention at EOCenter177@cdc.gov for additional information.

WARNINGS

- This product has not been FDA cleared or approved but has been authorized 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 authorization is revoked sooner.