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Northeastern University Life Science Testing Center ThermoFisher TaqPath COVID-19 Combo for Swab Pooling Test Performance Summary

1. EXECUTIVE SUMMARY

The Applied Biosystems (Thermo) TaqPath™ COVID-19 Combo Kit SARS-CoV-2 assay is a nucleic acid amplification in vitro diagnostic test intended for the qualitative detection of RNA from SARS-CoV-2 isolated and purified from nasal swab specimens obtained from individuals who meet COVID-19 clinical and/or epidemiological criteria. Testing is limited to laboratories - certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform high complexity tests, or by similarly qualified non-U.S. laboratories. Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in nasal swab specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA, clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. Laboratories within the United States and its territories are required to report all positive 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. The Applied Biosystems (Thermo) TaqPath™ COVID-19 Combo Kit SARS-CoV-2 assay on the Applied Biosystems 7500 Fast Dx qPCR system is intended for use by trained clinical laboratory personnel specifically instructed and trained in the operation of the Applied Biosystems 7500 Fast Dx qPCR system and in vitro diagnostic procedures.The SARS-CoV-2 assay is only for use under the Food and Drug Administration's Emergency Use Authorization.

2. PURPOSE OF THE VERIFICATION STUDY

The SARS-CoV-2 assay is commercially available for use under the Emergency Use Authorization (EUA). Laboratories are expected to confirm that the performance characteristics of the tests performed within their laboratories are comparable to the performance characteristics listed by the manufacturer.

3. ASSAY PROTOCOL OVERVIEW

The Northeastern University Life Science Testing Center (LSTC) SARS-CoV-2 assay is to be used with an RNA extraction procedure using the Agilent AssayMap Bravo NGS Liquid Handler System with Applied Biosystems MagMAX Viral/Pathogen Nucleic Acid Isolation Kit. RT-PCR is performed using an Applied Biosystem 7500 Fast DX clinical qPCR machine for thermocycling. The Northeastern LSTC uses the ThermoFisher TaqPath COVID-19 Combo Kit monitoring the presence of three SARS-CoV-19 RNA genes (ORF1ab, N, and S) and an internal control (MS2 bacteriophage).

The Northeastern LSTC is aware of the FDA Lab Alert for the Thermo Fisher Scientific TaqPath COVID-19 Combo Kit. The lab implemented both the recommended protocol change as well as the software update in August/September 2020.

See also section 8 regarding Assay Results and Interpretation.

4. REPORTABLE RANGE

The SARS-CoV-2 assay is a qualitative test and therefore a reportable range does not apply.

5. REFERENCE RANGE

The reference range will be “SARS-CoV-2 not detected” and will not be validated by a clinical study.

6. PERFORMANCE CHARACTERISTICS

6.1. Accuracy

Thirty positive and thirty negative patient swab samples were obtained from Precision for Medicine, which were analyzed using the DiaSorin Simplexa COVID-19 kit.

Table 1. Accuracy

		Comparator Assay: DiaSorin Simplexa COVID-19 kit		
Batch ID: FinalValidationRunPCR04		**Positive**	**Negative**	**Total**
Northeastern University ThermoFisher TaqPath COVID-19 Combo Assay	Positive	29	1	30
	Negative	0	26*	26*
	Total	29*	27*	56*
Positive Percent Agreement		100%; 95% CI [88.1%-100%]		
Negative Percent Agreement		96.3%; 95% CI [81.0%-99.9%]		
*INVALID results (N=4) represent quality control failures and are not included in the percent agreement calculations.				

6.2. Precision

6.2.1. Repeatability

Within run repeatability

3 positive samples and 3 negative samples will be run in triplicate in the same run. Results will be compared for each specimen. Within run repeatability will be calculated as follows:

Number of samples with reproducible results/9 x100 (%)

Table 2. Repeatability

Batch ID	True Positives	False Negatives	True Negatives	False Positives	Run Repeatability
RunPCR02	9	0	9	0	100%
Average	100%				

6.2.2. Reproducibility

Between-run repeatability

3 positive and 3 negative samples will be run in triplicate in two separate runs on two separate instruments.

Inter-run precision = Number of samples with reproducible results/9 x100 (%)

Combined reproducibility

(Number of samples with reproducible results within run + Number of samples with reproducible results in separate runs) / 18 x 100 (%)

Table 3. Reproducibility

Batch ID	True Positives	False Negatives	True Negatives	False Positives	Inconclusive
RunPCR02	9	0	9	0	0
RunPCR05	9	0	7	1	1
Inter-run precision	87.5%				

Combined reproducibility	94.4%

6.3. Analytical Sensitivity

6.3.1. Limit of Detection

(29) 50-mL conical tubes containing pools of 25 dry swabs with negative clinical matrix spiked with γ-irradiated inactivated SARS-CoV-2 (BEI Resources NR-52287; lot 70039067) were received from Concentric by Ginkgo. Swab pools were reconstituted at Northeastern LSTC using 6 mL of VTM resulting in pool concentrations of 0, 25, 50, 100, 250, 500, 1000 or 1500 GCEs / mL and vortexed/incubated per the swab pooling reconstitution protocol. After reconstitution and incubation, testing was performed following the SARS-CoV-2 assay.

Our SARS-CoV-2 diagnostic test requires 200 µL of sample. 10 RNA extraction replicates were run per 50-mL conical tube sample. Results were interpreted as described below. The minimum concentration at which at least 95% of replicates were POSITIVE is 250 copies/mL.

Table 4. LoD results (25-swab pools with negative clinical matrix with a single swab spiked with virus; pool is reconstituted in 6 mL VTM per swab pooling sample reconstitution protocol)

Virus (GCEs/mL)	Invalid	Inconclusive	Negative	Positive	Positive / Total valid result	Percent positive
0	0	1	39	0	0/40	0%
25	0	14	13	3	3/30	10%
50	0	5	4	21	21/30	70%

100	0	6	0	24	24/30	80%
250	**0**	**1**	**0**	**29**	**29/30**	**96.7%**
500	0	0	0	30	30/30	100%
1000	0	0	0	30	30/30	100%
1500	0	0	0	70	70/70	100%
*INVALID results represent quality control failures and are not included in the percent positive calculation.						

6.4. Swab pooling

Swab pooling is an approach that minimizes loss of sensitivity of the test during pooling by adding multiple swabs into a small amount of media (similar to that of an individual test). However, this approach substantially increases the concentration of swab specimens, which could possibly compromise the analytical sensitivity of the test. Thus, to ensure the sensitivity of the test, studies should be performed to demonstrate that the test's limit-of-detection is robust to concentrated amounts of clinical matrix (e.g., mucins or human RNA) and/or analyte (i.e., virus).

6.4.1. Comparison of SARS-CoV-2 assay on 1 swab samples versus 25-swab samples

To compare the detection of a swab by the same positive patient in isolation and in a pool, 21 swabs were collected from the same negative donor at Concentric by Ginkgo and each was spiked with 9,000 GCEs of γ-irradiated SARS-CoV-2 virus (BEI Resources NR-52287; lot 70039067). Of these swabs, 14 were individually placed in 50-mL conical tubes as singlet swabs and 7 were each added to 50-mL conical tubes containing 24 swabs with negative clinical matrix to simulate a single positive swab in a 25-swab pool. Pools were shipped overnight to the Northeastern LSTC where the 7 25-swab pools and 7 of the singlet swabs were each reconstituted in 6 mL VTM (final concentration of 1,500 GCEs / mL virus per tube) and 7 of the singlets were each reconstituted in 3 mL VTM (final concentration of 3,000 GCEs / mL virus per

tube). From each pool, 10 RNA extraction replicates were tested. The Ct of detection of 9,000 GCEs in each of the 3 conditions (25 swabs/6 mL, 1 swab/6 mL, 1 swab/3 mL) were compared.

Acceptance criteria: The percent positive detection of a patient with 9,000 GCEs should be > 95%. PASS

Table 5. Detection of single swab containing 9,000 GCEs in 1- and 25-dry swab pools

Performance metric	Description	Performance
Detection of weak positive swabs simulating individual test	Percent of positive samples when single spiked swab is reconstituted in 3 mL VTM per individual testing SOP (with clinical matrix[1])	70 / 70 = 100%; 95% CI [94.9%-100%]
Detection of weak positive swabs in a 1-swab pool	Percent of positive samples when single spiked swab is reconstituted in 6 mL VTM per swab pool testing SOP (with clinical matrix[1])	70 / 70 = 100%; 95% CI [94.9%-100%]
Detection of weak positive swabs in a 25-swab pool	Percent of positive samples when spiked swab is included in 25-swab pool and reconstituted in 6 mL VTM per swab pool testing SOP (with clinical matrix[1])	70 / 70 = 100%; 95% CI [94.9%-100%]

Table 6. Comparison of Ct values for single swab containing 9,000 GCEs in 1- and 25-dry swab pools

Condition	Average Ct		
	ORF1ab-gene Ct	N-gene Ct	S-gene Ct
Single swab in 3 mL	29.07 +/- 0.75	30.03 +/- 0.69	29.60 +/- 0.93
Single swab in 6 mL	28.62 +/- 0.57	29.43 +/- 0.53	28.98 +/- 0.68
Swab in 25-swab pool in 6 mL	29.99 +/- 0.93	30.60 +/- 0.79	30.72 +/- 1.21

6.4.2. Dry swab pools reconstituted in 6 mL VTM (dry swabs)

To confirm that the analytical sensitivity of pooled samples would not be compromised by high amounts of clinical matrix in a 25-swab pool, we performed a bridging study. In this study, 5 pools of 25 AN swabs (Miraclean 96000E) were collected dry by Concentric by Ginkgo. 1 of the 25 swabs in each pool was spiked with 9,000 GCEs of γ-irradiated SARS-CoV-2 virus (BEI Resources NR-52287; lot 70039067) to simulate a single positive swab among 24 negative swabs. Pools were shipped overnight to the Northeastern LSTC and each was subsequently reconstituted in a total of 6 mL VTM resulting in a final concentration of 1,500 GCEs / mL virus per pool. From each pool, 10 RNA extraction replicates were tested.

Acceptance criteria: The percent positive at 1,500 GCEs / mL virus should be > 95%. PASS

Table 7. 25-dry swab pool results with low positive swabs in negative clinical matrix

Performance metric	Description	Performance
Detection of weak positive swabs in a pool	Percent of swab pools positive at 1,500 copies/mL (with clinical matrix[1])	50 / 50 = 100%; 95% CI [92.9%-100%]

To ensure that the analytical sensitivity of pooled samples would not be compromised by high levels of virus (e.g., multiple positive swabs per pool), we tested 25-swab pools containing high concentrations of virus. In one study, 2 pools of 25 AN swabs (Miraclean 96000E) were collected dry by Concentric by Ginkgo. 1 of the 25 swabs in each pool was spiked with 6,000,000 GCEs of γ-irradiated SARS-CoV-2 virus (BEI Resources NR-52287; lot 70039067) to simulate a single positive swab among 24 negative swabs. Pools were shipped overnight to the Northeastern LSTC and each was subsequently reconstituted in a total of 6 mL VTM resulting in a final concentration of 1,000,000 GCEs / mL virus per pool (4,000X LoD of 250 GCEs / mL, see 6.3.1 Table 4). From each pool, 10 RNA extraction replicates were tested.

Acceptance criteria: The percent positive high virus swabs detected should be > 95%. PASS

Table 8. 25-swab pool results with high positive swabs in negative clinical matrix (4,000x LoD)

Performance metric	Description	Performance
Detection of strong positive swabs in a pool	Percent of swab pools positive at 1,000,000 copies/mL (with clinical matrix[1])	20 / 20 = 100%; 95% CI [83.2%-100%]

In a second experiment using high positive swabs, we followed the same procedure as above, except that we further increased the amount of virus by using a more concentrated stock of virus. Gamma-irradiated virus (BEI NR-52287; lot 70039067) was spiked into a 25-swab pooled negative clinical matrix at a final concentration of 5,000,000 GCEs / mL virus per pool, which is 20,000x LoD; results are shown below.

Table 9. 25-swab pool results with high positive swabs in negative clinical matrix (20,000x LoD)

Performance metric	Description	Performance
Detection of strong positive swabs in a pool	Percent of swab pools positive at 5,000,000 copies/mL (with clinical matrix[1])	10 / 10 = 100% 95% CI [69.1%-100%]

7. QUALITY CONTROL

The Northeastern LSTC strives to provide the highest quality service and results to its clients. It ensures test quality in a number of different ways including monitoring for contamination. Our QA/QC manager monitors the lab operations closely to ensure quality results are returned. We have extensive QA/QC procedures that staff review and sign off on in our document control system. The laboratory is designed with a unidirectional sample (work) flow to help reduce the

[1] Samples from the same individuals utilized to prepare the contrived samples were confirmed to be negative for the virus in the same experiment (results not shown).

chance of contamination. Once a PCR plate is set-up it is sealed and never unsealed after amplification, thus reducing the probability for contamination. Each plate includes both a positive control and a negative control to monitor for contamination. Results are not returned unless both the positive and negative control pass. Each and every result is reviewed by an analyst and general supervisor. If there are any questions on the quality of a given plate run we repeat the entire plate prior to reporting any results. We process pooled samples with the same rigor as associated with our MA license to process individual COVID tests; that is, all quality control processes applied to our individual testing program are applied to pooled testing.

8. ASSAY RESULTS AND INTERPRETATION

Each multiplex assay includes probes for SARS-CoV-2 N, S, and ORF1ab genes as well as an MS2 phage control. The MS2 phage control is a control for RNA extraction; thus if the MS2 control fails, the well is reported as 'INVALID'.

'NEGATIVE' means the interpretive software did not detect any signal for any of the three SARS-CoV-2 genes.

'INCONCLUSIVE' means the interpretive software detected signal for one SARS-CoV-2 gene.

'POSITIVE' means the interpretive software detected signal for at least two SARS-CoV-2 genes.

The highest Ct value at which the interpretive software detects a signal for a given gene is 37.

9. REFERENCES

9.1. FDA. Molecular Diagnostic Template for Laboratories, updated 28 July 2020, https://www.fda.gov/media/135658/download.

10. APPENDIX

Line data is on file.