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Workflow and Performance of the TaqPath COVID-19 Fast PCR Combo Kit 2.0

Enabling fast, trusted COVID-19 test results from raw saliva - an ideal choice for high-frequency testing

Introduction

The Applied Biosystems™ TaqPath™ COVID-19 Fast PCR Combo Kit 2.0 is an Emergency Use Authorized (EUA), real-time reverse transcription polymerase chain reaction (RT PCR) test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in saliva collected without preservatives in a sterile container under the supervision of a healthcare provider in a healthcare setting from individuals suspected of COVID-19 by their healthcare provider (please refer to the Instructions for Use for applicable intended use).

The TaqPath COVID-19 Fast PCR Combo Kit 2.0 utilizes an advanced assay design to compensate for SARS-CoV-2 mutations and to ensure accurate results even as the virus that causes COVID-19 continues to mutate.

The TaqPath COVID-19 Fast PCR Combo Kit 2.0 utilizes raw saliva treated with SalivaReady™ solution, omitting the need for sample extraction and offering a sample-to-result turnaround time of approximately 2 hours. The use of saliva as the sample matrix not only simplifies sample collection, but it also reduces costs when compared to using nasopharyngeal swab for SARS-CoV-2 detection.[1]

The TaqPath COVID-19 Fast PCR Combo Kit 2.0 delivers fast, trusted COVID-19 test results from raw saliva ideal for widespread, high frequency testing.

Component	Amount / (Storage)
TaqPath™ COVID-19 Fast PCR Assay 2.0	1 × 1 mL (–30° C to –10° C)
TaqPath™ COVID-19 Plus Control	10 × 10 µL (≤ –20° C)
TaqPath™ COVID-19 Control Dilution Buffer	10 × 250 µL (–30° C to –10° C)
SalivaReady™ Solution	1 × 20 mL (–30° C to –10° C)

Figure 1. TaqPath™ COVID-19 Fast PCR Combo Kit 2.0, 1,000 reactions (Cat. No. A51606) Components

- All kit components can be stored in the same –20° C freezer
- TaqPath™ 1-Step Multiplex Master Mix (No ROX™) sold separately

Advanced assay design compensates for current and future SARS-CoV-2 mutations

- Unique fluorescence channel for each genomic region (*orf1a*, *orf1b*, and N genes)
- Redundancy with multiple targets (amplicons) per genomic region
- 8 targets spanning 3 genomic regions compensates for emerging mutations
- Excludes the S-gene, which has a high risk of mutation
- Human RNase P gene serves as an endogenous specimen control

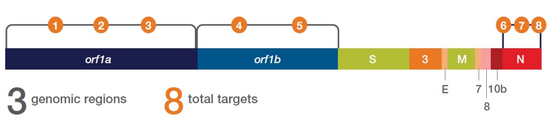

Figure 2. Schematic overview of the multi-target assay design

Simplified workflow enables high-frequency testing

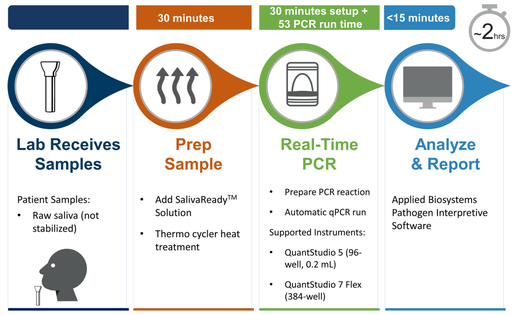

Figure 3. Schematic Overview of the TaqPath COVID-19 Fast PCR Combo Kit 2.0 workflow

- The turnaround time of the TaqPath COVID-19 Fast PCR Combo Kit 2.0 from sample to result is approximately 2 hours.
- Pathogen Interpretive Software automatically performs QC and generates interpretative report

Performance

Limit of detection (LoD)

The LoD study established the lowest SARS-CoV-2 viral concentrations (Genomic Copy Equivalents or GCE/mL) that can be detected at least 95% of the time.

- Pooled, contrived raw saliva samples were spiked with gamma-irradiated SARS-CoV-2 virus at various concentrations.
- The LoDs in Table 1 were confirmed with 20 replicates.

Table 1. Limit of detection

Real-Time PCR Instrument	Limit of Detection
QuantStudio™ 5 (QS5) 96-well, 0.2-mL	**1,000 GCE/mL**
QuantStudio™ 7 Flex (QS7 Flex) 384-well	**750 GCE/mL**

Cross-reactivity

Cross reactivity was assessed *in silico* with BLAST-based sequence homology alignment to fifty-five (55) known microbial sequences (Table 2).

Table 2. Summary of cross reactivity testing and analysis

***In silico* (sequence homology)**
BLAST sequence homology to 55 organisms: (2 fungi, 27 viruses, and 26 bacteria)
No cross-reactivity predicted*

*SARS-CoV showed a higher level of identity with the N gene and ORF1b assays but is not predicted to interfere with SARS-CoV-2 detection.
Note: SARS-CoV has not been in circulation since the 2003 outbreak.

Reactivity (Inclusivity)

In silico analysis executed using 1,802,689 complete SARS-CoV-2 genomes from the GISAID database (June 09, 2021).

- Positive match if amplification expected for at least one assay per target for at last two targets.

Based upon BLAST analysis, **the TaqPath COVID-19 Fast PCR Assay 2.0 maps with 100% homology to 100% of SARS-CoV-2 genome sequences.**

Interfering substances

The impact of potential interfering substances was tested by adding substances to saliva specimens spiked with SARS-CoV-2 virus at 3X the limit of detection and ran in six replicates for each substance and one no-interferent control on both the QuantStudio5 and QuantStudio 7 Flex.

No false-negative or false-positive interference was observed for any interferant

Table 3. Summary of interfering substances testing

Interferent	Final conc. in sample	Agreement with expected results			
		Positive for SARS-CoV-2		Negative for SARS-CoV-2	
		Positive agreement	Number of positive / Number tested	Negative agreement	Number of negative / Number tested
Mucin bovine[1]	2.5 mg/mL	100%	6/6	100%	6/6
Whole blood	1% v/v	100%	6/6	100%	6/6
Nasal spray[2]	15% v/v	100%	6/6	100%	6/6
Nasal gel[3]	1.25%	100%	6/6	100%	6/6
Lozenge[4]	3 mg/mL	100%	6/6	100%	6/6
Throat spray[5]	5% v/v	100%	6/6	100%	6/6
Toothpaste[6]	0.5% v/v	100%	6/6	100%	6/6
Mouthwash[7]	5% v/v	100%	6/6	100%	6/6
Nicotine	0.03 mg/mL	100%	6/6	100%	6/6
hgDNA	10 ng/µL	100%	6/6	100%	6/6
No Interferent	N/A	100%	6/6	100%	6/6

[1]Mucin bovine = Mucin: bovine submaxillary gland, type I-S; [2]Afrin™ Original nasal spray; [3]NeilMed™ NasoGel™; [4]Cepacol®(benzocaine/menthol lozenges); [5]Chloraseptic® Sore Throat spray/solution; [6]Colgate™ toothpaste; [7]Crest™ mouthwash; [8]hgDNA = Human genomic DNA

Clinical evaluation

A clinical evaluation study was performed to evaluate the performance of the TaqPath COVID-19 Fast PCR Combo Kit 2.0 using archived paired raw saliva and nasopharyngeal (NP) swab specimens from individuals with COVID-19 symptoms.

- The raw saliva specimens were tested using the TaqPath COVID-19 Fast PCR Combo Kit 2.0.
- The NP specimens were tested using an FDA EUA-Authorized comparator assay.

Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) were calculated relative to the comparator method, and results are shown in Table 4.

Clinical evaluation - continued

TaqPath™ COVID-19 Fast PCR Combo Kit 2.0 has a positive percent agreement (PPA) and negative percent agreement (NPA) of ≥97%.

Table 4. Summary of clinical evaluation

	Clinical evaluation study agreements			
	PPA (%)	95%CI	NPA (%)	95%CI
QuantStudio™ 5	97.1%	84.6% to 99.9%	97.6%	87.4% to 99.9%
QuantStudio™ 7 Flex	97.1%	84.6% to 99.9%	100.0%	91.5% to 100%

Conclusions

The TaqPath COVID-19 Fast PCR Combo Kit 2.0 is your choice for COVID-19 testing using raw saliva as a sample matrix:

- Raw saliva direct-to-PCR workflow (no RNA extraction required)
  - Simplifies sample collection: saliva is easily self-collected, reducing both the exposure to health care providers and the need for personal protective equipment (PPE)
  - Saliva collection can save significant amounts of money compared to using nasopharyngeal swabs for SARS-CoV-2 detection[1]
- Simple, convenient and efficient workflow to deliver trusted results quickly
  - Turnaround time from sample to result in only 2 hours
  - Enables widespread, high-frequency testing
- Innovative, multi-target assay design compensates for emerging SARS-CoV-2 mutations
- Accurate detection provides increased confidence in results.
  - Outstanding performance (LoD of 750–1,000 GCE/mL; PPA and NPA > 97%)
- Applied Biosystems Pathogen Interpretive Software:
  - Helps decrease analysis and interpretation time and risk of user interpretation error

REFERENCES

1. Bastos, M. et al. Annals of Internal Medicine (2021): doi:10.7326/M20-6569.

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Please refer to the Instructions for Use for more details and for the supported protocol. Deviations from the protocol are not permitted.