applied biosystems

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 a CE-IVD marked, real time reverse transcription polymerase chain reaction (RT PCR) test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in raw saliva in sterile containers 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 directly, omitting the need for sample extraction and offering a sample-to-result turnaround time of approximately 2 hours. The use of saliva as 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.

TaqPath™ COVID-19 Fast PCR Assay 2.0	(1 × 1 mL)
TaqPath™ COVID-19 Plus Control	(10 × 10 µL)
TaqPath™ COVID-19 Control Dilution Buffer	(10 × 250 µL)
SalivaReady™ Solution	(1 × 20 mL)
TaqPath™ 1-Step Multiplex Master Mix	(1 × 10 mL)

Figure 1. TaqPath COVID-19 Fast PCR Combo Kit 2.0 (A51605) Components for 1,000 reactions

Simplified workflow enables high-frequency testing

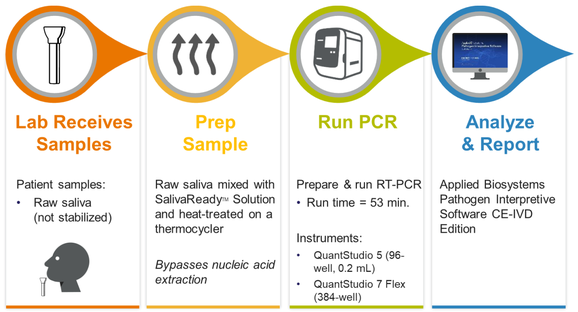

Figure 2. Schematic Overview of the TaqPath COVID-19 Fast PCR Combo Kit 2.0 workflow.

- 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 converts genetic analysis data into reporting, to reduce interpretation errors

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

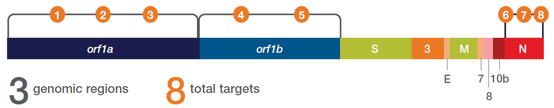

Figure 3. Schematic overview of the multi-target assay design

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 and 100% detection.

*Isolate USA-WA1/2020 (BEI Resources,PN NR-52287,LN 70033322)

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 vitro* using microbial genomic material and *in silico* with BLAST-based sequence homology alignment to known microbial sequences (Table 2).

Table 2. Summary of cross reactivity testing and analysis

***In vitro* (wet-lab testing)**	***In silico* (sequence homology)**
Tested RNA or DNA from 17 organisms (4 bacteria and 13 viruses)	**BLAST sequence homology to 55 organisms: (2 fungi, 27 viruses, and 26 bacteria)**
No cross reactivity detected	**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 gamma-irradiated SARS-CoV-2 virus* at 3X the limit of detection as compared to a no-interferent control.

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

Table 3. Summary of interfering substances testing

Interferent*	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**	100%	6/6	100%	6/6
Mucin porcine	100%	6/6	100%	6/6
Blood	100%	6/6	100%	6/6
Afrin Nasal Spray	100%	6/6	100%	6/6
NasoGel	100%	6/6	100%	6/6
Lozenge	100%	6/6	100%	6/6
Sore Throat Spray	100%	6/6	100%	6/6
Toothpaste	100%	6/6	100%	6/6
Mouthwash	100%	6/6	100%	6/6
Nicotine	100%	6/6	100%	6/6
hgDNA	100%	6/6	100%	6/6
No Interferent	100%	6/6	100%	6/6

*Isolate USA-WA1/2020 (BEI Resources, PN NR-52287,LN 70039067) was used for all interfering substances except for Mucin bovine, which was tested using PN NR-522287, LN 70033322
**Mucin bovine = Mucin: bovine submaxillary gland, type I-S; Mucin porcine = Mucin: porcine stomach – type II; Afrin Nasal Spray = Afrin® Original nasal spray; NasoGel = NeilMed® NasoGel®; Lozenge = Cepacol®(benzocaine/menthol lozenges); Sore Throat Spray = Chloraseptic® Sore Throat spray/solution; Toothpaste = Toothpaste (Colgate); Mouthwash = Crest mouthwash; 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.

Clinical Evaluation - continued

The results are shown in Table 4. **TaqPath™ COVID-19 Fast PCR Combo Kit 2.0 has a positive percent agreement (PPA) and negative percent agreement (NPA) of ≥95%.**

Table 4. Summary of clinical evaluation

	Comparator Method			
	PPA (%)	95%CI	NPA (%)	95%CI
TaqPath™ COVID-19 Fast PCR Combo Kit 2.0 (QuantStudio™ 5)	96.8%	83.3% to 99.9%	97.4%	86.5% to 99.9%
TaqPath™ COVID-19 Fast PCR Combo Kit 2.0 (QuantStudio™ 7 Flex)	96.8%	83.3% to 99.9%	100.0%	90.9% 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:

- From 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 swab for SARS-CoV-2 detection[1]
- Offers a 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 > 95%)
- Applied Biosystems Pathogen Interpretive Software CE-IVD edition:
  - 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.

TRADEMARKS/ LICENSING

ThermoFisher SCIENTIFIC

CE IVD
For *in vitro* diagnostic use.

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