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Workflow and Performance of the TaqPath™ COVID-19, FluA, FluB Combo Kit

Multiplex real-time RT-PCR test for the detection and differentiation of SARS-CoV-2, influenza A, and influenza B RNA

Introduction

The Applied Biosystems™ TaqPath™ COVID-19, Flu A, Flu B Combo Kit is an Emergency Use Authorized (EUA) multiplex real-time reverse transcription polymerase chain reaction (RT-PCR) test intended for the simultaneous qualitative detection and differentiation of RNA from the **SARS-CoV-2**, **influenza A**, and/or **influenza B** viruses in nasopharyngeal swab and anterior nasal swab specimens collected from individuals suspected of respiratory viral infection consistent with COVID-19 symptoms.

Product	Description	Contents
TaqPath RT-PCR COVID-19, Flu A, Flu B Assay Kit	Multiplex real-time PCR assay for: • SARS-CoV-2 (S & N genes) • Flu A (matrix gene – detects flu A subtypes including H1N1, H3N2, H5N1, H7N9) • Flu B (matrix gene – detects flu B strains in both major lineages (B/Yamagata and B/Victoria))	1 tube (1,500 µL)
	MS2 Phage Control	10 tubes (1 mL)
TaqPath COVID-19, Flu A, Flu B Control Kit	COVID-19, influenza A, influenza B controls	10 tubes (10 µL)
TaqPath Control Dilution Buffer	Buffer for the IVT RNA Control	10 tubes (250 µL)

Table 1 and Figure 1. Kit contents (1000 reactions, SKU A49868)

Workflow and turnaround time

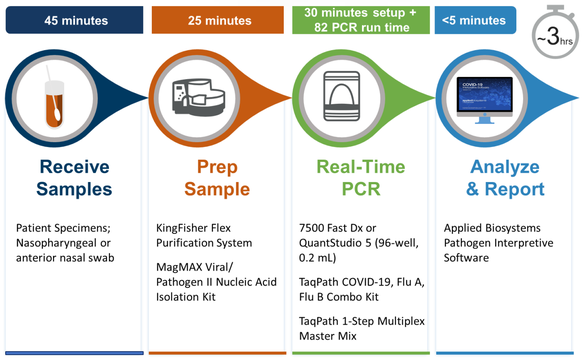


Figure 2. Schematic Overview of the TaqPath COVID-19, Flu A, Flu B Combo Kit workflow and turnaround time.

- Up to 94 specimens can be run simultaneously on a 96-well plate with a turnaround time around 3 hours

Performance of the TaqPath COVID-19, Flu A, Flu B Combo Kit

Limit of detection (LoD)

The LoD study established the lowest SARS-CoV-2, influenza A, and influenza B viral concentrations (Genomic Copy Equivalents or GCE, or TCID50/mL, as indicated) that can be detected at least 95% of the time.

- Negative nasopharyngeal swab (NP) specimens were pooled and spiked with the SARS-CoV-2 virus, influenza A virus, or influenza B virus, at different concentrations.
- The LoD was established using a 3-phase approach, 20 replicates were used for the third confirmatory phase.

Note: The LoD for influenza A and influenza B varies between strains.

Table 2. Limit of detection

	Limit of Detection (LOD)	
Target	$TCID_{50}$/mL	GCE/mL
SARS-CoV-2	1.6×10^{-1}	100
Influenza A	1.6×10^{-3} to 2.5×10^{-3}	200–500
Influenza B	5.6×10^{-3} – 5.9×10^{-2}	500–1,000

Reactivity (Inclusivity)

In Silico Analysis

Sequences for the probes of each target were compared to published full-length viral genomes from two different databases, and the percent of sequences that resulted in 100% alignment were reported for each target.

Table 3. *In Silico* Analysis of Probe Alignment to Published Database Sequences

Probe/Target	Genomes for BLAST Analysis	100% Probe Alignment
SARS-Cov-2	>309,579 complete SARS-CoV-2 genomes from 2 databases Mapping deemed successful if at least 1 target showed 100% identity	99.67% Homology (Genbank) 96.61% Homology (GISAID) January 2021
Influenza A	Full-Length Segment 7 sequences: 32,460 (NCBI) 30,858 (GSAID)	88% Homology August 2020
Influenza B	Full-Length Segment 7 sequences: 8,660 (NCBI) 12,577 (GSAID)	41% Homology August 2020

In vitro Analysis:

Functional testing was performed to determine the lowest concentration that would produce positive results in 3/3 replicates, starting at 3X LoD, for 10 influenza A and 5 influenza B strains (Table 4).

The TaqPath COVID-19, Flu A, Flu B Combo Kit detects the common influenza A subtypes H1N1 and H3N2, and less common subtypes including H5N1 and H7N9, and both major influenza B lineages: B/Yamagata and B/Victoria.

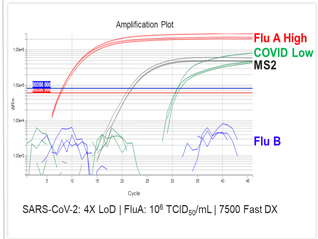
Table 4. Strains Detected in Reactivity Wet-Lab Testing.

Influenza A	Influenza B
H1N1/Georgia/M5081/2012	Unknow Lineage/Taiwan/2/62
H1N1/New Caledonia/20/99	Mixed Lineage/Malaysia/2506/2004
H1N1/Puerto Rico/08/1934	Victoria/Colorado/06/2017
H1N1/Solomon Islands/3/2006	Yamagata/Massachusetts/02/2012
H1N1/California/04/2009	Yamagata/Brisbane/03/2007
H3N2/Wisconsin/15/2009	
H3N2/Switzerland/9715293/2013	
H3N2/Wisconsin/67/2005	
H3N2/Aichi/2/68	
H3N2/Hong Kong/8/68	

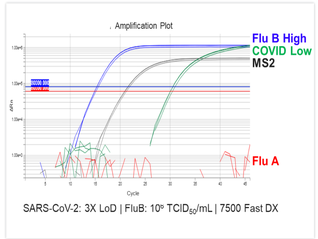
Competitive interference

Negative NP specimens were pooled and spiked with SARS-CoV-2 (USA-WA1/2020), influenza A (A/Brisbane/59/07), or influenza B (B/Florida/04/06) virus in combinations where at least one virus was present at a low concentration (3X LoD) and one of the other at a high concentration (10^5 $TCID_{50}$/mL).
The TaqPath COVID-19, Flu A, Flu B Combo Kit is highly accurate in the presence of co-infections.

Figure 3. Competitive Interference.



SARS-CoV-2: 4X LoD | FluA: 10^6 $TCID_{50}$/mL | 7500 Fast DX



SARS-CoV-2: 3X LoD | FluB: 10^5 $TCID_{50}$/mL | 7500 Fast DX

Cross-reactivity

No cross-reactivity was observed in vitro against microbic DNA/RNA from 39 organisms

Interfering substances

The impact of 10 potential interfering substances was assessed by adding substances to pooled negative NP specimens spiked with either SARS-Cov-2, influenza A, or influenza B viruses at 3X LoD. All conditions were tested in triplicate and results were compared to a no-interferent control.

No interference was observed for mucin, blood, corticosteroid nasal spray, nasal gel, homeopathic allergy relief nasal spray, throat lozenges, Oseltamivir, antibiotic ointment, and systemic antibiotics at any of the concentrations tested.
Afrin™ Original nasal spray showed interference at 10% v/v, but not at 0.6% or 1.3%.

Clinical Evaluation

A clinical evaluation study was carried out to evaluate the performance of the TaqPath™ COVID-19, Flu A, Flu B Combo Kit using archived nasopharyngeal specimens. The following specimens were tested:

51 NP positive/ 59 NP negative for SARS-CoV-2
56 NP positive/ 104 NP negative for influenza A virus
36 NP positive/ 124 NP negative for influenza B virus

Samples were tested with the TaqPath™ COVID-19, Flu A, Flu B Combo Kit as well as an FDA-EUA comparator test for SARS-CoV-2 and an FDA-cleared test for influenza A/B (listed below table 5). Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) were calculated relative to the comparator test. Results are shown in Table 5.

Table 5. Summary of clinical evaluation

Target	Positive Percent Agreement (PPA)	95%CI	Negative Percent Agreement (NPA)	95%CI
SARS-CoV-2*	96.1%	86.5% to 99.5%	100%	93.9% to 100%
Influenza A*	96.4%	87.7% to 99.6%	99.0%	94.8% to 100%
Influenza B*	91.7%	77.5% to 98.2%	96.8%	91.9% to 99.1%

*** TaqPath COVID-19, FluA, FluB Combo Kit run on the 7500 Fast Dx Real-Time PCR Instrument**

Conclusions

The TaqPath COVID-19, FluA, FluB Combo Kit is an all-in-one real-time PCR respiratory test that detects and differentiates SARS-CoV-2, influenza A, and influenza B.

- Multi-target assay design helps compensate for emerging SARS-CoV-2 mutations and variants.
- Pathogen Interpretive Software converts analysis data into a readable report.
- Offers a simple, convenient and efficient workflow to deliver trusted results quickly
- Turnaround time from sample to result in as little as 3 hours
- Enables widespread, high-frequency testing.

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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.

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