Workflow and performance of the TaqPath COVID-19, Flu A, Flu B 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 a qualitative, emergency use–authorized (EUA), real-time reverse transcription polymerase chain reaction (RT-PCR) multiplex assay intended for the simultaneous detection and differentiation of SARS-CoV-2, influenza A, and influenza B viral RNA in nasopharyngeal (NP) swab and anterior nasal swab specimens collected from individuals with suspected respiratory virus infections and COVID-19 symptoms. The kit components and controls are listed in Table 1.

Table 1. Description and components of the TaqPath COVID-19, Flu A, Flu B Combo Kit (Cat. No. A49868).

Component	Description	Contents
TaqPath COVID-19, Flu A, Flu B Combo Kit (1,000 reactions)	Multiplex real-time RT-PCR assay for: SARS-CoV-2 (S and N genes) Flu A (matrix gene for detection of flu A subtypes, including H1N1, H3N2, H5N1, and H7N9) Flu B (matrix gene for detection of flu B strains in 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	COVID-19, influenza A, and influenza B controls	10 tubes (10 µL)
TaqPath Control Dilution Buffer	Viral RNA control buffer	10 tubes (250 µL)

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Method—workflow and turnaround time

The TaqPath COVID-19, Flu A, Flu B Combo Kit assay offers an end-to-end workflow for evaluating 94 specimens in approximately 3 hours (Figure 1). It can also be run on multiple instruments in parallel to increase sample throughput. Figure 2 is a schematic overview that shows how the assay workflow can be staggered over 24 hours using the Thermo Scientific™ KingFisher™ Flex Purification System and two Applied Biosystems™ 7500 Fast Dx Real-Time PCR Instruments or QuantStudio™ 5 Real-Time PCR Systems with 2 full-time employees per shift.

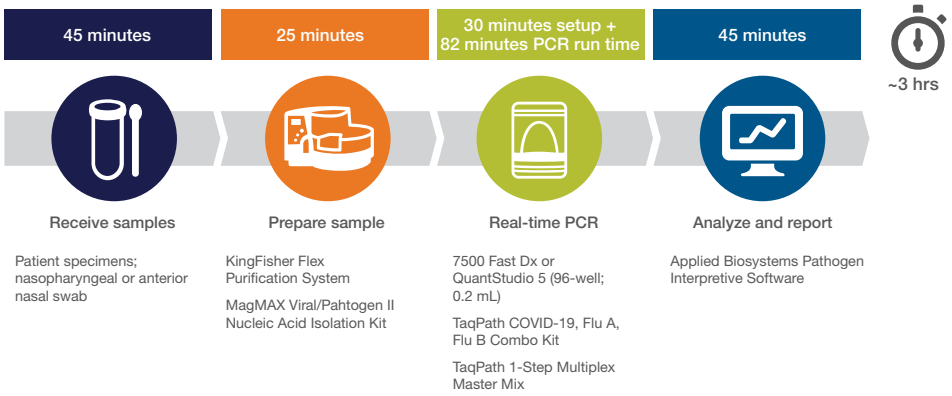


Figure 1. Schematic overview of the TaqPath COVID-19, Flu A, Flu B Combo Kit workflow and turnaround time. Up to 94 specimens can be run simultaneously on a 96-well plate with a turnaround time of approximately 3 hours.

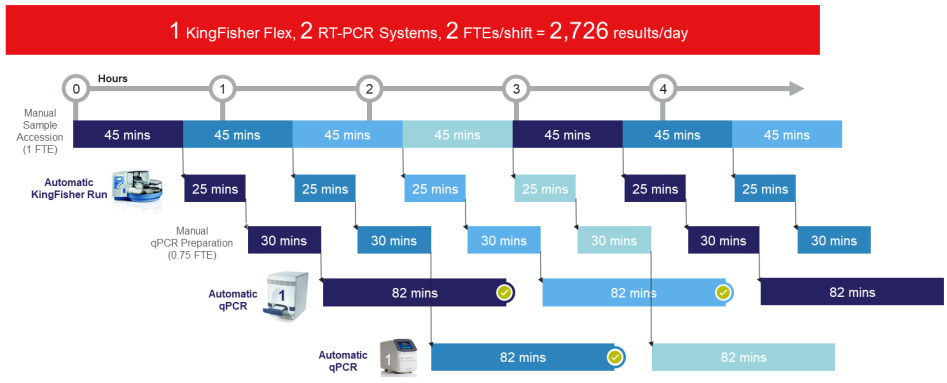


Figure 2. Increasing sample throughput using a staggered workflow.

Results and discussion

Limit of detection (LOD)

The LOD study established the lowest concentrations of SARS-CoV-2, influenza A, and influenza B that could be detected at least 95% of the time. LODs were reported in genomic copy equivalents (GCEs) per mL or median tissue culture infectious dose ($TCID_{50}$) per mL. Negative NP swab specimens were pooled and spiked with SARS-CoV-2, influenza A, or influenza B at different concentrations. The LODs were established using a 3-phase approach, and 20 replicates were used for the third confirmatory phase (Table 2).

Reactivity (inclusivity)

In-silico analysis

The sequences of the probes for the viral targets were compared to published, full-length viral genomes from two different databases. The percentage of sequences that were 100% aligned was reported for each target (Table 3).

Table 2. LODs for SARS-CoV-2, influenza A, and influenza B.

Note: The LODs for influenza A and influenza B varied between strains.

	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} to 5.9×10^{-2}	500–1,000

Table 3. *In-silico* analysis of probe alignment with published 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 sequences of segment 7: 32,460 (NCBI) 30,858 (GSAID)	88% homology August 2020
Influenza B	Full-length sequences of segment 7: 8,660 (NCBI) 12,577 (GSAID)	41% homology August 2020

In-vitro analysis

Functional testing was performed for 10 influenza A and 5 influenza B strains (Table 4) at a concentration of 3x LOD, which produced positive results with 3 out of 3 replicates.

The TaqPath COVID-19, Flu A, Flu B Combo Kit can be used to detect the common influenza A subtypes H1N1 and H3N2 in addition to less common subtypes, including H5N1 and H7N9. The major influenza B lineages B/Yamagata and B/Victoria can also be detected.

Competitive interference

Negative NP specimens were pooled and spiked with combinations of SARS-CoV-2 (USA-WA1/2020), influenza A (A/Brisbane/59/07), and influenza B (B/Florida/04/06). The concentration of at least one virus was low (3–4x LOD), while the concentration of one of the other viruses was ≥10^5 $TCID_{50}$/mL (Figure 3). The data indicate that the TaqPath COVID-19, Flu A, Flu B Combo Kit can provide highly accurate results in cases of co-infection.

Cross-reactivity

No cross-reactivity was observed with microbial DNA and RNA from 39 organisms *in vitro* (data not shown).

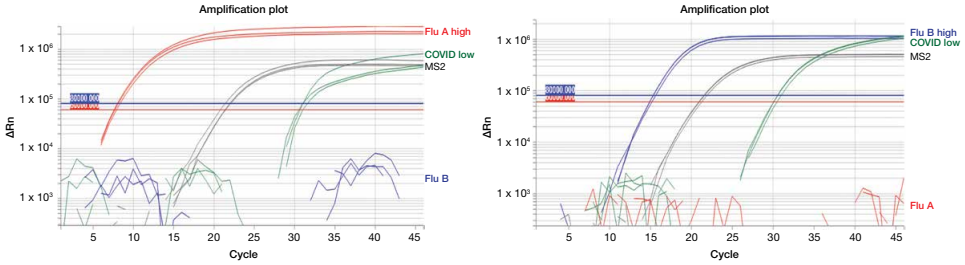
Interference

The impact of 10 potential interferents was assessed by adding them to pooled negative NP specimens spiked with either SARS-Cov-2, influenza A, or influenza B at 3x LOD. All tests were performed in triplicate, and the results were compared to results obtained with a control that contained no interferents. No interference was observed with mucin, blood, corticosteroid nasal spray, nasal gel, homeopathic allergy relief nasal spray, throat lozenges, oseltamivir, antibiotic ointment, or a systemic antibiotic at any concentration

Table 4. Strains detected in wet-lab reactivity tests.

Influenza A	Influenza B
H1N1/Georgia/M5081/2012	Unknown 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	

* Strain tested at 4,500 GCE/mL.



SARS-CoV-2: 4x LOD | Flu A: 10^6 $TCID_{50}$/mL | 7500 Fast Dx system

SARS-CoV-2: 3x LOD | Flu B: 10^6 $TCID_{50}$/mL | 7500 Fast Dx system

Figure 3. Competitive interference between SARS-CoV-2, influenza A, and influenza B.

Table 5. Effect of potential interferents in SARS-CoV-2, influenza A, and influenza B samples.

Interferent*	Final concentration in sample	Agreement with expected results		
		SARS-CoV-2	Flu A	Flu B
None	N/A	100%	100%	100%
Mucin (bovine submaxillary gland, type I-S)	0.1 mg/mL	100%	100%	100%
Blood (human)	1% v/v	100%	100%	100%
Nasal sprays or drops (Afrin Original)	0.6% v/v	100%*	100%*	100%**
Nasal corticosteroids (Flonase)	5 µg/mL	100%	100%	100%
Nasal gel (NeilMed Nasogel)	1% w/v	100%	100%	100%
Homeopathic allergy relief medicine (NutraBio Allergy Relief)	10% v/v	100%	100%	100%
Throat lozenges, oral anesthetic and analgesic (Chloraseptic)	1% w/v	100%	100%	100%
Oseltamivir phosphate	33 µg/mL	100%	100%	100%
Antibiotic nasal ointment (Bactroban)	5 µg/mL	100%	100%	100%
Antibacterial, systemic (tobramycin)	0.6 mg/mL	100%	100%	100%

* All replicates tested at 10%, 5%, and 2.5% were undetected on the 7500 Fast Dx Real-Time PCR Instrument and the QuantStudio 5 Real-Time PCR System (96-well, 0.2 mL block).

** All replicates tested at 10%, 5%, 2.5%, and 1.3% v/v were undetected on the 7500 Fast Dx Real-Time PCR Instrument.

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tested. Afrin™ Original nasal spray caused interference at 10% v/v but none at 0.6% on the 7500 Fast Dx Real-Time PCR Instrument or 1.3% on the QuantStudio 5 Real-Time PCR System (Table 5).

Clinical evaluation

A clinical evaluation was performed to assess the performance of the TaqPath COVID-19, Flu A, Flu B Combo Kit using archived NP specimens. The following specimens were tested:

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

Samples were tested using the TaqPath COVID-19, Flu A, Flu B Combo Kit. Samples were also tested using an FDA- and EUA-authorized comparator assay for SARS-CoV-2 with the Applied Biosystems™ TaqPath™ COVID-19 Combo Kit Advanced against an FDA-cleared assay for influenza A and B with the Quidel™ Lyra™ Influenza A + B Assay. Positive percent agreement (PPA) and negative percent agreement (NPA) were then calculated relative to the comparator test. The results are shown in Table 6.

Conclusions

The TaqPath COVID-19, Flu A, Flu B Combo Kit is an all-in-one real-time PCR test for detecting and differentiating SARS-CoV-2, influenza A, and influenza B. Applied Biosystems™ Pathogen Interpretive Software automatically generates a readable genetic analysis report to reduce the risk of user error. The multi-target assay design helps compensate for emerging SARS-CoV-2 variants and mutations, which can provide confidence in your data. The simple, convenient, efficient workflow and 3-hour turnaround time can enable rapid delivery of trustworthy results to support widespread, high-frequency testing.

Table 6. Summary of the clinical evaluation.

Target	PPA	95% CI	NPA	95% CI
SARS-CoV-2*	96.1%	86.5–99.5%	100%	93.9–100%
Influenza A*	96.4%	87.7–99.6%	99.0%	94.8–100%
Influenza B*	91.7%	77.5–98.2%	96.8%	91.9–99.1%

* The TaqPath COVID-19 Combo Kit Advanced was run on the 7500 Fast Dx Real-Time PCR Instrument.

Find out more at **thermofisher.com/covidflu**

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