A Real-Time PCR (qPCR) Assay for Differential Detection of 2019 Novel Coronavirus (SARS-CoV-2), Influenza A and Influenza B

Instruction for Use

*ab*TES™ Flu/COVID-19 qPCR I Kit
Kit Version: 1.1

REF 300143 (50 Reactions)
300144 (100 Reactions)

Store at -25 °C to -15 °C

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For use on Bio-Rad CFX96, ABI7500/7500 FAST and *ab*CyclerQ Only

1. Pathogen Information

Coronavirus disease 2019 (COVID-19) is a disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)[4]. SARS-CoV-2 is a single-stranded, positive-sense RNA virus which is capable of person-to-person transmission. COVID-19 has spread to more than 20 countries within a month it was declared a pandemic on March 11, 2020[1]. It is genetically highly related to SARS and MERS coronaviruses[2].

So far, seven coronavirus species including COVID-19 are known to infect human. Four viruses, including 229E, OC43, NL63 and HKU1, typically cause a mild cold. However, people infected with the other three, including SARS, MERS and COVID-19, may develop acute and severe respiratory diseases, fever, cough and even death[3].

Influenza virus is a single stranded negative RNA virus which belong to the family of Orthomyxovirade. There are four known genera: Influenza A (FluA), Influenza B (FluB), Influenza C and Thogorovirus in which only FluA and FluB cause illness in human and remains one of the most crucial health problems throughout the world[5]. An infection with FluA and FluB can sometimes cause pneumonia, which can be fatal particularly for the young and the elderly.

Sharing very similar respiratory symptoms to that of Influenza Virus, it has become apparent that a differential detection and diagnosis of the virus is crucial to control the pandemic, and mediate the right course of treatment for affected individuals.

2. Test Description

The *ab*TES™ Flu/COVID-19 qPCR I Kit is a qualitative real-time polymerase chain reaction (qPCR) kit which enables simultaneous detection of **SARS-CoV-2** (*orf1a*)**, Flu A** and **Flu B** in a single reaction. It also includes detection of human housekeeping gene, GAPDH, as an **Internal Control (IC)** to monitor RNA extraction efficiency and potential PCR inhibitions from sample processing.

The kit contains all the necessary PCR reagents for rapid, sensitive and reproducible real-time detection of four Influenza types using highly specific primer pairs and double-dye hydrolysis probes. The recommended human sample types are nasal and throat swabs.

3. Storage Conditions

> ⚠ **IMPORTANT!**
> - **Improper storage conditions may compromise product performance.**
> - **Do not exceed three freeze-thaw cycles.**

The components of *ab*TES™ Flu/COVID-19 qPCR I Kit should be stored in the dark, between -25°C and -15°C in a **NON**-frost-free freezer. Frost-free freezers go through freeze-thaw cycles to remain frost-free and may cause accelerated degradation of enzymes and nucleic acids. Avoid repeated thawing and freezing (max 3 times) as this may lower the sensitivity. If reagents will be used intermittently, it is suggested to keep the reagents frozen in aliquots3

4. Kit Components

Table 1. *ab*TES™ Flu/COVID-19 qPCR I Kit components

Tubes	Components	Volume	
		300143 (50 rxn)	300144 (100 rxn)
1	2x RT-PCR Reaction Mix	1x 500 µL	2x 500 µL
2	RT/ *Taq* Enzyme Mix	1x 50 µL	2x 50 µL
3	Primer/Probe Mix	1x 100 µL	2x 100 µL
4	Positive Control	1x 100 µL	1x 100 µL
5	Nuclease-free Water	1x 450 µL	1x 450 µL

5. Materials and Devices Required but not Provided

- Appropriate Real-time thermal cycler
- Benchtop centrifuge with a rotor for 2 ml tubes
- Plate centrifuge, if using a 96-well plate
- Vortex mixer
- Disposable powder-free gloves
- Nucleic acid extraction kit
- Pipettes (adjustable) and pipette tips with filter (disposable)
- Desktop centrifuge with rotor
- 96-well PCR plate/ PCR strip tubes:
  - ABI7500/7500 FAST: follow manufacturer's instruction
  - Bio-Rad CFX96: follow manufacturer's instruction
  - *ab*CyclerQ: 0.2 mL 96-well qPCR plates, no skirt, clear OR 0.2 mL qPCR strip tubes, clear
  - *ab*CyclerQ mini: 0.2 mL qPCR strip tubes, clear
- 96-well PCR plate clear sealing film/ clear qPCR cap
  NOTE: qPCR consumables must be certified free from DNA, DNase, RNase and PCR inhibitors
- Ice box/cooling block

6. General Precautions

- Proper aseptic technique should always be used.
- Always work in RNase-free environment.
- Do not exceed three freeze-thaw cycles.
- Do not use the kit after its expiration date.
- Improper storage conditions may compromise product performance.
- Wear disposable gloves, laboratory coats and eye protection when handling samples and reagents. Wash hands thoroughly thereafter.
- Highly recommended to use disposable pipette tips with filter.
- Always select the pipette with the lowest volume possible and the matching filter tip
- Always treat samples as biohazardous and infectious.
- Decontaminate and dispose of all potentially infectious materials in accordance with local and national regulation.

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7. Contamination and Inhibition

- The presence of PCR inhibitors may lead to invalid or false-negative results
- If sample preparation system containing ethanol, make sure any traces of ethanol is eliminated. Ethanol is a strong qPCR inhibitor.
- Do not open reaction tubes/plates after amplification to avoid amplicon contamination.
- Store positive materials (test samples, controls, and amplicons) separately from all other reagents and add to the reaction mix in a separate facility.
- Use sterile pipette tips with filters and replace the tip for every procedure.
- Do not interchange tube as this may lead to cross-contamination.
- Laboratory area can be contaminated with amplicon or test sample if the waste materials are not carefully handled and disposed.

8. Procedures

⚠ **IMPORTANT!**
- **Be sure read section 6 and 7 before use.**

8.1. Nucleic Acids (NA) Extraction

⚠ **IMPORTANT!**
- **If sample preparation system containing ethanol, make sure any traces of ethanol is eliminated. Ethanol is a strong qPCR inhibitor.**

Standard NA extraction kits are compatible with this assay but must be validated by the user. Please carry out NA extraction as per instructed in the manufacturer's extraction kit manual.

8.2. PCR Reaction Setup

⚠ **IMPORTANT!**
- **Only use extracted and purified sample.**
- **Proper aseptic technique should always be used.**
- **Always work in RNase-free environment.**
- **Do not exceed three freeze-thaw cycles.**
- **Do not use the kit after its expiration date.**
- **Before starting an assay, thaw all the components thoroughly at room temperature except for RT/ *Taq* Enzyme Mix as it does not freeze at storage temperature.**
- **Always keep samples and components on the ice during use.**
- **When the components are thawed, mix the components and centrifuge briefly.**
- **Protect Primer/Probe Mix from light.**
- **Always include at least one positive and one negative controls on each run.**
- **Run sample immediately after PCR reaction setup to prevent degradation of RNA samples.**
- **Always select the pipette with the lowest volume possible and the matching filter tip**
- **Draw up and dispense RT/ *Taq* Enzyme Mix slowly to avoid air bubbles.**
- **Remove excess RT/ *Taq* Enzyme Mix coated on the filter tip.**
- **Do not write on the caps or sealers as this interferes with qPCR detection.**

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1. Thoroughly thaw all components, mix, and spin briefly. Keep all thawed components and samples on ice protected from light.
2. Prepare your PCR reaction based on the following pipetting scheme:

Table 2. Sample reagent preparation calculation for one reaction.

Reagent	Volume per reaction		
	Test sample reaction	Positive control reaction	Non template control reaction
2x RT-PCR Mix	10 µL	10 µL	10 µL
RT/ *Taq* Enzyme Mix	1 µL	1 µL	1 µL
Primer/Probe Mix	2 µL	2 µL	2 µL
Nuclease-Free Water	2 µL	2 µL	7 µL
Positive Control	-	5 µL	-
Extracted Test Sample	5 µL	-	-
Total Volume	**20 µL**	**20 µL**	**20 µL**

3. Mix the reaction mix thoroughly and spin briefly. Dispense 15µl of the reaction mix into each required well of a 96- well plate or each required PCR tube.
4. Add 5µl of test sample or positive control into each required well of a 96-well plate or each required PCR tube. Always include at least one positive and one negative controls on each run.
5. Seal all wells or tubes tightly. Mix the reaction mix by flipping or gently vortex. Centrifuge briefly to settle tube contents and eliminate large bubble.
6. Transfer the 96-well plate or PCR tubes to the real-time PCR system. Program thermal cycling protocol according to section 8.3.

8.3. Programming the Real-Time PCR System

*ab*TES™ Flu/COVID-19 qPCR I Kit was validated to be used with the following Real-time PCR system. You may need to adjust these conditions for other real-time platforms.

- Bio-Rad CFX96™ Real-Time PCR Detection System
- Applied Biosystems 7500/7500 FAST Real-Time PCR Systems
- *ab*CyclerQ Real-Time PCR System (Part number: 800700)
- *ab*CyclerQ mini Real-Time PCR System (Part number: 800702)

⚠ **IMPORTANT!**

- **Real-time PCR system must be calibrated before used**
- **Ensure the reporter dye and detector pairs are correct**
- **Supported consumable:**
  - **ABI7500/7500 FAST: follow manufacturer's instruction**
  - **Bio-Rad CFX96: follow manufacturer's instruction**
  - ***ab*CyclerQ: 0.2 mL 96-well qPCR plates, no skirt, clear OR 0.2 mL qPCR strip tubes, clear**
  - ***ab*CyclerQ mini: clear OR 0.2 mL qPCR strip tubes, clear**
- **96-well PCR plate clear sealing film/ clear qPCR cap**
- **qPCR consumables must be certified free from DNA, DNase, RNase and PCR inhibitors**
- ***ab*CyclerQ ramp rate should set at 3.5°C/sec**

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For general setup and programming of the real-time PCR system, please refer to the respective user manual.
Choose the reporter dye and detector pairs based on Table 3.

Table 3. Reporter dye and detector pairs for Flu/COVID-19 qPCR I Kit

Reporter dye	Detector
FAM	Flu A
Texas Red	Flu B
Cy5	SARS-CoV-2
HEX/VIC	GAPDH

Set the appropriate PCR cycling condition and the fluorescence is measured at the annealing phase of each cycle.

Table 4. PCR cycling condition for Bio-Rad CFX96TM Real-Time PCR Detection System, Applied Biosystems 7500/7500 FAST Real-Time PCR Systems and abCyclerQ

Phase	Description	No. of Cycles	Temperature	Duration
1	cDNA synthesis	1	55 °C	10 min
2	Initial Denaturation	1	95 °C	2 min 30 sec
3	Amplification	42	95 °C	17 sec
			*55 °C	31 sec
			68 °C	32 sec

**Data acquisition at annealing phase*

8.4. Data Analysis and Interpretation

⚠ **IMPORTANT!**

- ***ab*TES™ Flu/COVID-19 qPCR I Kit is function well if amplification is observed in positive control in FAM, Texas Red and Cy5 channels.**

For general information regarding the data analysis of the real-time PCR system, please refer to the respective user manual. It is advisable to analyze the real time PCR graph at the end of the run to determine the validity of the Ct data.

Assessment of clinical sample result must perform after positive and negative control are valid. The Ct cut-off value is 40, user must review the amplification curve before final assessment. The interpretation of result is shown in Table 5 and 6.

Table 5. Result interpretation for quality controls

Quality Control	Observation			Interpretation
	Flu A (FAM)	Flu B (Texas Red)	SARS-CoV-2 (Cy5)	
No template control	No amplification	No amplification	No amplification	**Pass**; proceed to test sample analysis
Positive control	Presence of amplification	Presence of amplification	Presence of amplification	
No template control	Presence of amplification	Presence of amplification	Presence of amplification	**Fail**; rerun test sample with positive and negative controls
Positive control	No amplification	No amplification	No amplification	
Positive control	**Either** Flu A, Flu B **or** SARS-CoV-2 generate no amplification			

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Figure 1. Amplification Plot

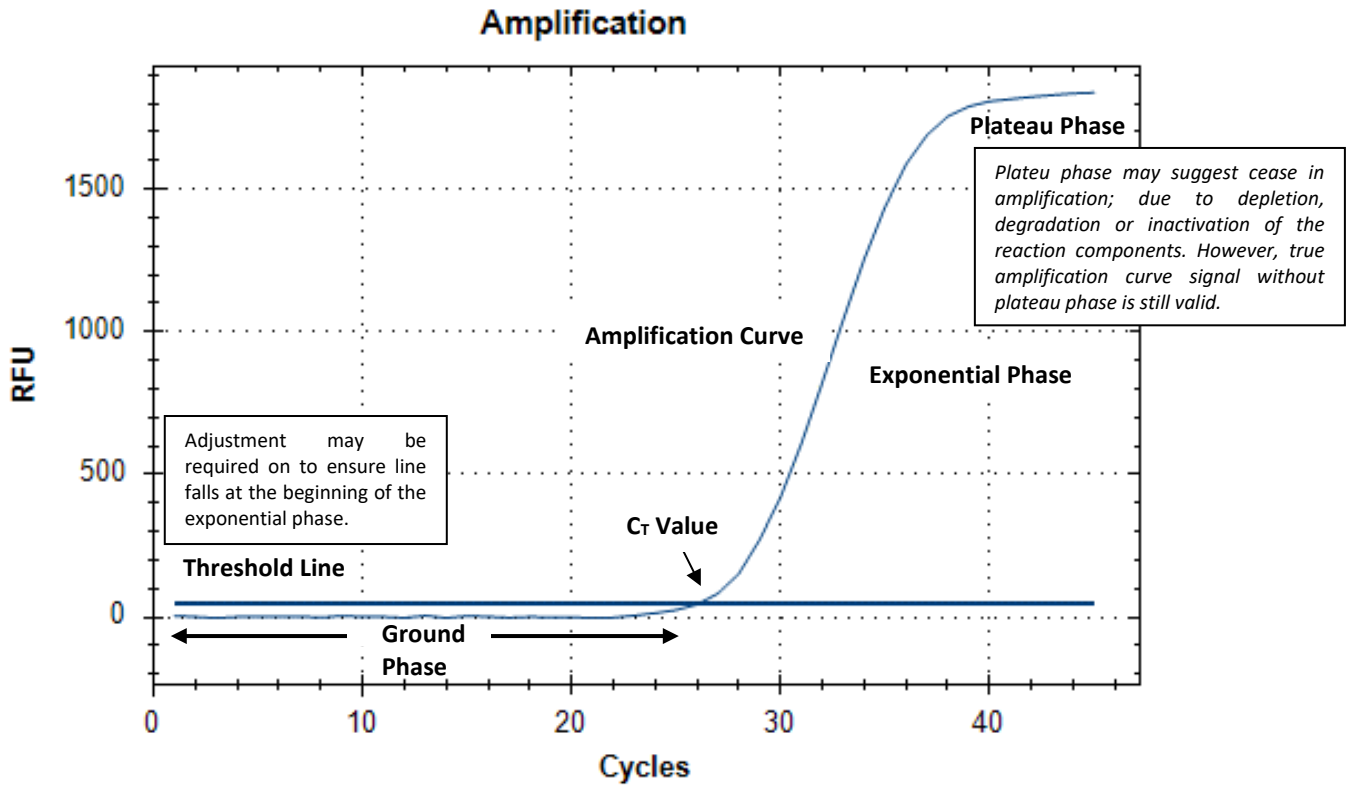


Figure 2. Illustration of Positive Amplification Curve (Log and Linear View)

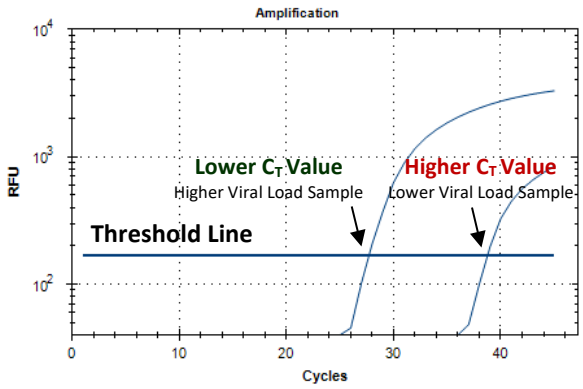
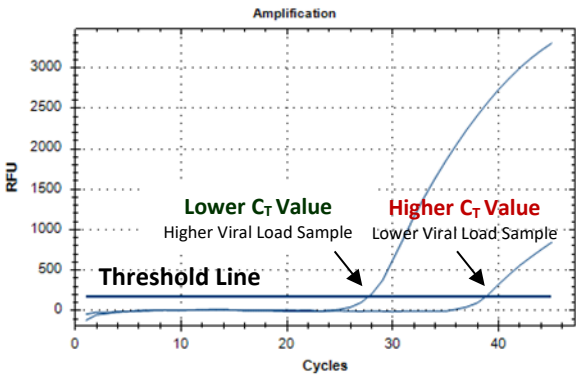
Log View	Linear View
	

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Figure 3. Illustration of Negative Amplification Curve (Log and Linear View)

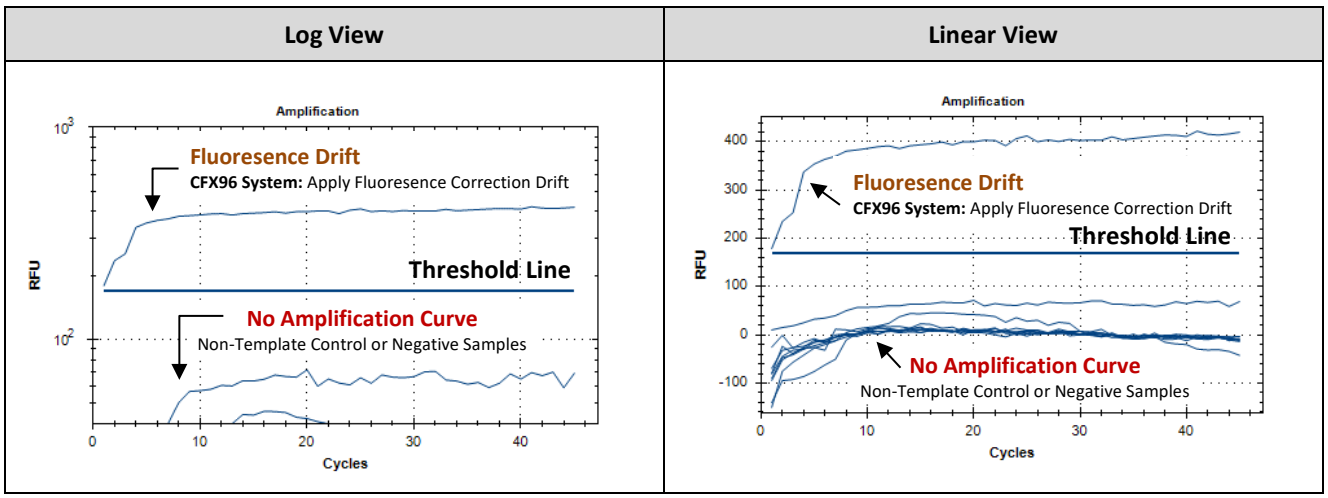

Table 6. Result interpretation for test samples

Observation				Interpretation
Flu A (FAM)	**Flu B (Texas Red)**	**SARS-CoV-2 (Cy5)**	**GAPDH (HEX)**	
Amplification Signal, **AND** C_T value <40	No Amplification, **OR** C_T value >40	No Amplification, **OR** C_T value >40	Amplification Signal, **AND** C_T value <40	**Flu A Positive**
No Amplification, **OR** C_T value >40	Amplification Signal, **AND** C_T value <40	No Amplification, **OR** C_T value >40	Amplification Signal, **AND** C_T value <40	**Flu B Positive**
No Amplification, **OR** C_T value >40	No Amplification, **OR** C_T value >40	Amplification Signal, **AND** C_T value <40	Amplification Signal, **AND** C_T value <40	**SARS-CoV-2 Positive**
Amplification Signal, **AND** C_T value <40	No Amplification, **OR** C_T value >40	No Amplification, **OR** C_T value >40	No Amplification, **OR** C_T value >40	**Flu A Positive**
No Amplification, **OR** C_T value >40	Amplification Signal, **AND** C_T value <40	No Amplification, **OR** C_T value >40	No Amplification, **OR** C_T value >40	**Flu B Positive**
No Amplification, **OR** C_T value >40	No Amplification, **OR** C_T value >40	Amplification Signal, **AND** C_T value <40	No Amplification, **OR** C_T value >40	**SARS-CoV-2 Positive**
Amplification Signal, **AND** C_T value <40	No Amplification, **OR** C_T value >40	Amplification Signal, **AND** C_T value <40	Amplification Signal, **AND** C_T value <40	**Flu A and SARS-CoV-2 Positive**
No Amplification, **OR** C_T value >40	Amplification Signal, **AND** C_T value <40	Amplification Signal, **AND** C_T value <40	Amplification Signal, **AND** C_T value <40	**Flu B and SARS-CoV-2 Positive**
Amplification Signal, **AND** C_T value <40	No Amplification, **OR** C_T value >40	Amplification Signal, **AND** C_T value <40	No Amplification, **OR** C_T value >40	**Flu A and SARS-CoV-2 Positive**
No Amplification, **OR** C_T value >40	Amplification Signal, **AND** C_T value <40	Amplification Signal, **AND** C_T value <40	No Amplification, **OR** C_T value >40	**Flu B and SARS-CoV-2 Positive**
No Amplification, **OR** C_T value >40	No Amplification, **OR** C_T value >40	No Amplification, **OR** C_T value >40	Amplification Signal, **AND** C_T value <40	**Negative**
No Amplification, **OR** C_T value >40	No Amplification, **OR** C_T value >40	No Amplification, **OR** C_T value >40	No Amplification, **OR** C_T value >40	**INVALID Retest***

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NOTE: Detected signal should be normal amplification curve (Figure 2)

** Initial Retest should be performed using the same collected sample. If result is consistently inconclusive, recollect new sample, ensure proper storage and handling, and repeat the qPCR test.*

9. Troubleshooting

Table 7. Example of unexpected observations on positive control, non template control and test sample

Sample	Observation	Potential Cause	Solution
Positive control	1. Only one or two out of three target is/are detected with normal amplification curve (Figure 2). 2. No amplification. 3. GAPDH is detected with normal amplification curve (Figure 2).	**Observation no.: 1 and 2** • Expired kit • Improper storage condition • Incorrect cycling condition • Improper PCR reaction setup • Faulty real-time PCR system • Sample assigned incorrectly **Observation no.: 2** • Positive control is not added • Positive control is added into the wrong tube **Observation no.: 3** • Contamination from extracted sample	**Observation no.: 1 and 2** • Do not use expired kit • Store kit at -25°C and -15°C • Check and rerun with correct cycling condition • Refer section 8.2 for PCR reaction setup • Calibration of real-time PCR system • Ensure sample is assigned accordingly **Observation no.: 2** • Re-run the entire sample or plate and always include at least one positive and one negative control. **Observation no.: 3** • Repeat qPCR run and investigate the source of contamination (Section 7).
Non template control	Flu A, Flu B, SARS-CoV-2 and/or GAPDH is/are detected with normal amplification curve (Figure 2).	• Contamination from either extracted sample and/or positive control • Sample assigned incorrectly	• Repeat qPCR run and investigate the source of contamination (Section 7). • Ensure sample is assigned accordingly
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Table 7 (continued)

Sample	Observation	Potential Cause	Solution
Test sample	1. More than one target is detected with normal amplification curve (Figure 2). **NOTE:** (Detection of GAPDH is not included). 2. Either Flu A, Flu B, SARS-CoV-2 or GAPDH is detected at early cycles with abnormal amplification curve (Figure 3). 3. No amplification. 4. Result is consistenly inconclusive (no amplification).	**Observation no.: 1** • Contamination from either extracted sample and/or positive control • Co-infection • More than one test sample is added into a single reaction tube **Observation no.: 2, 3 and 4** • Inhibitors present in test sample • Interference from test sample containing some impurities • Interference from substances present inside tubes/strips • Faulty real-time PCR system **Observation no.: 2** • Presence of Fluorescence Drift **Observation no.: 3 and 4** • Test sample is not added • Test sample is added into the wrong tube • Improper storage condition • Incorrect cycling condition • Improper PCR reaction setup • Improper collection and handling of sample • Improper nucleic acid extraction	**Observation no.: 1, 2, 3 and 4** • Re-test the test sample. • Re-extract RNA to repeat test. • Investigate the source of contamination (section 7). • If possible, re-collect sample to extract fresh RNA to repeat test. Be cautious with proper sample collection, transportation, storage, and handling • Try alternative kit to confirm result. • Calibration of real-time PCR system. **Observation no.: 2** • Apply Fluoresence Correction Drift. Applicable at CFX96 System only.

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10. Performance Characteristics

10.1 Analytical Sensitivity

Analytical sensitivity (limit of detection) is defined as the lowest concentration at which the assay can detect with a positivity rate of at least 95%.

The analytical sensitivity of the assay was determined by analyzing serial dilutions of *in vitro transcribed* (IVT*)* RNA from 0.01 to 2000 copies/µl for Flu A, Flu B and SARS-CoV-2. The testing was carried out in either five-replicates (for concentration ≥ 20 copies/µl) or seven-replicates (for concentrations <20 copies/µl The analytical sensitivity was estimated by Probit analysis using SPSS release 16.0.0.

The analytical sensitivities with 95% confidence for Flu A, Flu B and SARS-CoV-2 are shown in Tables 8-11.

Table 8. LoD confirmation on the Bio-Rad CFX96™ Real-Time PCR Detection System

Target	**Flu A**		**Flu B**		**SARS-CoV-2**	
IVT RNA concentration	**4.0 copies/µL**		**25.0 copies/µL**		**4.0 copies/µL**	
Sample	**Pos/Neg**	**C_T (FAM)**	**Pos/Neg**	**C_T (Texas Red)**	**Pos/Neg**	**C_T (Cy5)**
1	Pos	34.27	Pos	33.91	Pos	35.80
2	Pos	34.57	Pos	36.24	Pos	35.05
3	Pos	35.44	Pos	33.48	Pos	35.33
4	Pos	34.64	Pos	33.76	Pos	35.34
5	Pos	34.64	Pos	33.02	Pos	35.49
6	Pos	35.63	Pos	35.63	Pos	34.65
7	Pos	34.36	Pos	34.29	Pos	35.42
8	Pos	35.29	Pos	33.33	Pos	35.85
9	Pos	35.07	Pos	33.27	Pos	34.8
10	Pos	34.96	Pos	35.46	Pos	35.71
11	Pos	34.87	Pos	37.39	Pos	34.73
12	Pos	35.29	Pos	34.74	Pos	36.01
13	Pos	35.79	Pos	32.45	Pos	35.32
14	Pos	35.27	Pos	33.47	Pos	35.78
15	Pos	34.74	Pos	35.69	Pos	35.7
16	Pos	35.33	Pos	37.75	Pos	34.68
17	Pos	35.88	Pos	33.21	Pos	35.84
18	Pos	34.18	Pos	34.23	Pos	35.32
19	Pos	34.53	Neg	-	Pos	35.00
20	Pos	35.23	Pos	33.69	Pos	35.74
Statistics	**Mean C_T**	**35.00**	**Mean C_T**	**34.47**	**Mean C_T**	**35.40**
	SD	**0.50**	**SD**	**1.50**	**SD**	**0.43**
	CV%	**1.43%**	**CV%**	**4.34%**	**CV%**	**1.22%**
	Positive/Total	**20/20**	**Positive/Total**	**19/20**	**Positive/Total**	**20/20**

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Table 9. LoD confirmation on the Applied Biosystems 7500/7500 FAST Real-Time PCR Systems

Target	**Flu A**		**Flu B**		**SARS-CoV-2**	
IVT RNA concentration	**2.4 copies/µL**		**25.0 copies/µL**		**2.2 copies/µL**	
Sample	**Pos/Neg**	**C_T (FAM)**	**Pos/Neg**	**C_T (Texas Red)**	**Pos/Neg**	**C_T (Cy5)**
1	Pos	36.15	Pos	33.96	Pos	37.14
2	Pos	37.11	Pos	36.14	Pos	37.61
3	Pos	35.81	Pos	34.53	Pos	36.84
4	Pos	36.73	Pos	39.22	Neg	-
5	Pos	35.10	Pos	41.80	Pos	37.57
6	Pos	38.02	Pos	37.29	Pos	35.90
7	Pos	37.77	Neg	-	Pos	37.16
8	Pos	34.19	Pos	33.80	Pos	33.40
9	Pos	35.68	Pos	38.41	Pos	35.45
10	Neg	-	Pos	35.40	Pos	36.12
11	Pos	37.02	Pos	33.24	Pos	35.63
12	Pos	35.30	Pos	34.93	Pos	37.04
13	Pos	38.09	Pos	35.54	Pos	36.54
14	Pos	40.90	Pos	33.14	Pos	35.50
15	Pos	37.26	Pos	34.34	Pos	37.01
16	Pos	34.76	Pos	34.50	Pos	36.33
17	Pos	36.81	Pos	33.92	Pos	35.43
18	Pos	34.89	Pos	33.98	Pos	35.96
19	Pos	35.31	Pos	33.50	Pos	36.21
20	Pos	37.95	Pos	34.74	Pos	36.74
Statistics	**Mean C_T**	**36.60**	**Mean C_T**	**35.39**	**Mean C_T**	**36.30**
	SD	**1.60**	**SD**	**2.29**	**SD**	**0.99**
	CV%	**4.38%**	**CV%**	**6.47%**	**CV%**	**2.73%**
	Positive/Total	**19/20**	**Positive/Total**	**19/20**	**Positive/Total**	**19/20**

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Table 10. LoD confirmation on *ab*CyclerQ Real-Time PCR System

Target	**Flu A**		**Flu B**		**SARS-CoV-2**	
IVT RNA concentration	**4.0 copies/µL**		**55.0 copies/µL**		**4.0 copies/µL**	
Sample	**Pos/Neg**	**C_T (FAM)**	**Pos/Neg**	**C_T (Texas Red)**	**Pos/Neg**	**C_T (Cy5)**
1	Pos	36.51	Pos	30.80	Pos	36.62
2	Pos	35.39	Pos	30.16	Pos	35.94
3	Pos	35.52	Neg	-	Pos	34.41
4	Pos	35.36	Pos	31.93	Pos	34.03
5	Pos	34.93	Pos	32.04	Pos	35.00
6	Pos	35.29	Pos	32.38	Pos	33.91
7	Pos	35.81	Pos	32.33	Pos	36.11
8	Pos	34.76	Pos	31.35	Pos	34.77
9	Pos	35.35	Pos	31.65	Pos	34.25
10	Pos	34.97	Pos	31.45	Pos	33.90
11	Pos	35.96	Pos	31.15	Pos	36.87
12	Pos	35.32	Pos	30.29	Pos	35.40
13	Pos	35.90	Pos	31.65	Pos	35.37
14	Pos	35.31	Pos	32.31	Pos	33.15
15	Pos	35.73	Pos	32.46	Pos	35.48
16	Pos	36.82	Pos	31.15	Pos	37.49
17	Pos	35.57	Pos	31.45	Pos	35.68
18	Pos	36.36	Pos	32.45	Pos	35.20
19	Pos	35.26	Pos	32.87	Pos	33.57
20	Pos	36.00	Pos	32.51	Pos	34.75
Statistics	**Mean C_T**	**35.60**	**Mean C_T**	**31.70**	**Mean C_T**	**35.09**
	SD	**0.54**	**SD**	**0.77**	**SD**	**1.15**
	CV%	**1.50%**	**CV%**	**2.42%**	**CV%**	**3.28%**
	Positive/Total	**20/20**	**Positive/Total**	**19/20**	**Positive/Total**	**??/20**

Table 11. Summary of LoD results

Target	**Detection Channel**	**Real-Time PCR System**	**Analytical Sensitivity (95% confidence)**
Flu A	FAM	Bio-Rad CFX96™	4.0 copies/µL
		Applied Biosystems 7500/7500 FAST	2.4 copies/µL
		*ab*CyclerQ	4.0 copies/µL
Flu B	Texas Red	Bio-Rad CFX96™	25.0 copies/µL
		Applied Biosystems 7500/7500 FAST	25.0 copies/µL
		*ab*CyclerQ	55.0 copies/µL
SARS-CoV-2	Cy5	Bio-Rad CFX96™	4.0 copies/µL
		Applied Biosystems 7500/7500 FAST	2.2 copies/µL
		*ab*CyclerQ	4.0 copies/µL

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10.2. Precision/Reproducibility

The inter-assay (the variability between different runs) and intra-assay (the variability within one run) precision were determined by performing the assay once per day in five- and seven-replicates for 2000 to 200 copies/µl and 20 to 2 copies/µl, respectively, over a period of two days. Each target (the total of three targets: Flu A, Flu B and orf1a) were analyzed with six different concentrations.

For all targets, the qualitative results of all ten (2000 to 200 copies/µl) and 14 (20 to 2 copies/µl) reactions were 100% reproducible. The coefficient of variation (CV) of the cycle threshold (C_T) for the intra- and inter-assay precision are shown in Table 8 and 9.

Table 12. Inter-assay precision data showing standard deviation and CV% (calculated from C_T values) at each concentration

Target	IVT RNA Concentration	2000 copies/µl	200 copies/µl	20 copies/µl	10 copies/µl	5 copies/µl	2 copies/µl
Flu A	**SD**	0.40	0.35	0.40	0.35	0.64	1.02
	CV%	1.6%	1.2%	1.2%	1.1%	1.8%	2.8%
Flu B	**SD**	0.26	0.34	0.55	0.78	1.32	0.99
	CV%	1.0%	1.15	1.7%	2.3%	3.7%	2.7%
SARS-CoV-2	**SD**	0.33	0.30	0.39	0.37	0.56	0.66
	CV%	1.2%	1.0%	1.1%	1.6%	1.8%	4.2%

Table 13. Intra-assay precision data showing standard deviation and CV% (calculated from C_T values) at each concentration

Target	IVT RNA Concentration	2000 copies/µl	200 copies/µl	20 copies/µl	10 copies/µl	5 copies/µl	2 copies/µl
Flu A	**SD**	0.07	0.14	0.43	0.31	0.51	0.93
	CV%	1.1%	0.7%	1.3%	0.8%	1.7%	2.2%
Flu B	**SD**	0.10	0.19	0.75	0.67	0.98	1.12
	CV%	0.5%	0.7%	1.7%	2.3%	3.6%	2.6%
SARS-CoV-2	**SD**	0.17	0.25	0.28	0.40	0.28	0.65
	CV%	0.6%	0.7%	0.8%	1.1%	1.5%	1.5%

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10.3. Diagnostic Evaluation

To predict the diagnostic evaluation of *ab*TES™ COVID-19 qPCR I Kit at the 95% confidence interval, a total of 20 samples from Quality Control for Molecular Diagnostics (QCMD) were used.

The nucleic acids (RNA) were extracted using a commercially available kit such as QIAamp® Viral RNA Mini Kit (Cat. No.: 52904 and 52906) and *ab*Genix™ Viral DAN and RNA Extraction Kit (Cat No.: 800811_C).

Table 14. Summary of the result of diagnostic sensitivity and specificity for Flu A as below

Sample Description	Sensitivity/ Specificity			
	Total No. of sample	Flu A Positive	Flu A Negative	% (95% Confidence Internal)
Flu A Positive	20	6	0	100 % sensitivity
Flu A Negative		0	14	100 % specificity

Table 15. Summary of the result of diagnostic sensitivity and specificity for Flu B as below

Sample Description	Sensitivity/ Specificity			
	Total No. of sample	Flu B Positive	Flu B Negative	% (95% Confidence Internal)
Flu B Positive	20	4	0	100 % sensitivity
Flu B Negative		0	16	100 % specificity

Table 16. Summary of the result of diagnostic sensitivity and specificity for Flu SARS-CoV-2 as below

Sample Description	Sensitivity/ Specificity			
	Total No. of sample	SARS-CoV-2 Positive	SARS-CoV-2 Negative	% (95% Confidence Internal)
SARS-CoV-2 Positive	20	7	0	100 % sensitivity
SARS-CoV-2 Negative		0	13	100 % specificity

11. Limitations

- The use of this product and its data interpretation is intended for personnel trained in real-time PCR techniques and *in vitro* diagnostics procedures only.
- Appropriate sample collection, transport, storage, and nucleic acid extraction procedures are required for reliable results.

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*ab*TES™ Flu/COVID- qPCR I Kit

12. Explanation of Symbols

Symbol	Explanation
IVD	*In vitro* diagnostic medical device.
REF	Catalogue number
	Store at -25°C to -15°C
	Manufacturer
EC REP	Authorized Representative in the European community
LOT	Lot number
	Use by
	Contains sufficient for <n> tests
	Important

13. References

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5. Arbeitskreis Blut, Untergruppe «Bewertung Blutassoziierter Krankheitserreger». Influenza Virus, 2009. Transfus Med Hemother.36(1):32-39.

Electronic copy of the product insert can be downloaded at http://aitbiotech.com/covid-19/

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