A Real-Time PCR (qPCR) Assay for Detection of 2019 Novel Coronavirus (SARS-CoV-2)

Instruction for Use
***ab*TES™ COVID-19 qPCR I Kit**
Kit Version: 1.1

REF 300142 (100 Reactions)

Store at -25 °C to -15 °C

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For use on Bio-Rad CFX96, ABI7500/7500 FAST, *ab*CyclerQ and abCyclerQ mini 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].

2. Test Description

The *ab*TES™ COVID-19 qPCR I Kit is a qualitative real-time polymerase chain reaction (qPCR) kit which enables simultaneous detection of two COVID-19- specific signature regions from its non-structure polypeptide (*orf1a*) in a single reaction. It also includes detection of human housekeeping gene, GAPDH, as an **Internal Control (IC)** to identify possible PCR inhibitions from sample processing.

The kit contains all the necessary PCR reagents for rapid, sensitive, and specific detection using target-specific primers and double-labelled hydrolysis probes. This kit has been validated on samples extracted from sputum, nasopharyngeal 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™ 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 aliquots.

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4. Kit Components

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

Tubes	Components	Volume
1	2x RT-PCR Reaction Mix	1000 µL
2	RT/ *Taq* Enzyme Mix	100 µL
3	Primer/Probe Mix	200 µL
4	COVID-19 Positive Control	100 µL
5	Nuclease-free Water	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.

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, positive 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.

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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.**

This kit has been validated on samples extracted from sputum, nasopharyngeal and throat swabs only using NucliSENS® easyMAG® Total Nucleic Acid Extraction Kit, EZ1 Virus Kit and Liferiver RNA Isolation Kit.

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™ 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.**

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 COVID-19 qPCR I Kit.

Reporter dye	Detector
FAM	NS1
Texas Red/ ROX	NS2
HEX/ VIC	GAPDH

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

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8.3.1. Bio-Rad CFX96™ Real-Time PCR Detection System

⚠ **IMPORTANT!**

- **Supported consumables: follow manufacturer's instructions**
- **PCR consumables must be certified free from DNA, DNase, RNase and PCR inhibitors**

Table 4. PCR cycling condition for Bio-Rad CFX96™ Real-Time PCR Detection System.

Phase	Description	No. of Cycles	Temperature	Duration
1	cDNA synthesis	1	59 °C	10 min
2	Initial Denaturation	1	95 °C	2 min
3	Amplification	45	95 °C	10 sec
			*57.5 °C	15 sec

**Data acquisition at annealing phase*

8.3.2. Applied Biosystems 7500/7500 FAST Real-Time PCR Systems

⚠ **IMPORTANT!**

- **Suppoted consumables: follow manufacturer's instructions**
- **PCR consumables must be certified free from DNA, DNase, RNase and PCR inhibitors**
- **The passive reference dye must be set <u>None.</u>**

Table 5. PCR cycling condition for Applied Biosystems 7500/7500 FAST Real-Time PCR Systems.

Phase	Description	No. of Cycles	Temperature	Duration
1	cDNA synthesis	1	59 °C	10 min
2	Initial Denaturation	1	95 °C	2 min
3	Amplification	45	95 °C	10 sec
			*57.5 °C	30 sec

**Data acquisition at annealing phase*

8.3.3. *ab*CyclerQ Real-Time PCR System (Part number: 800700)

⚠ **IMPORTANT!**

- **Supported consumables: <u>0.2 mL</u> 96-well qPCR plates, no skirt, clear OR <u>0.2 mL</u> qPCR strip tubes, clear**
- **qPCR consumables must be certified free from DNA, DNase, RNase and PCR inhibitors**

Table 6. PCR cycling condition for *ab*CyclerQ Real-Time PCR System.

Phase	Description	No. of Cycles	Temperature	Duration
1	cDNA synthesis	1	59 °C	10 min
2	Initial Denaturation	1	95 °C	2 min
3	Amplification	45	95 °C	10 sec
			*59 °C	15 sec

**Data acquisition at annealing phase*

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8.3.4. *ab*CyclerQ mini Real-Time PCR System (Part number: 800702)

⚠ **IMPORTANT!**

- **Supported consumables: 0.2 mL qPCR strip tubes, clear**
- **qPCR consumables must be certified free from DNA, DNase, RNase and PCR inhibitors**

Table 7. PCR cycling condition for *ab*CyclerQ Real-Time PCR System.

Phase	Description	No. of Cycles	Temperature	Duration
1	cDNA synthesis	1	59 °C	10 min
2	Initial Denaturation	1	95 °C	2 min
3	Amplification	45	95 °C	10 sec
			*59 °C	15 sec

**Data acquisition at annealing phase*

8.4. Data Analysis and Interpretation

⚠ **IMPORTANT!**

- ***ab*TES™ COVID-19 qPCR I Kit is function well if positive control generates sigmoid curve in both FAM and Texas Red 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 C_T data.

Assessment of clinical sample result must perform after positive and negative control are valid. The C_T cut-off value is 40, user must review the amplification curve before final assessment. The interpretation of result is shown in Table 8, 9 and Figure 2 to 5.

Table 8. Result interpretation for quality controls.

Quality Control	Observation		Interpretation
	NS1 (FAM)	NS2 (Texas Red)	
No template control	No amplification	No amplification	**Pass**; proceed to test sample analysis
Positive control	Presence of amplification	Presence of amplification	
No template control	Presence of amplification	Presence of amplification	**Fail**; rerun test sample with positive and negative controls
Positive control	No amplification	No amplification	
Positive control	**Either** NS1 **or** NS2 generates no amplification		

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Figure 1. Amplification Plot.

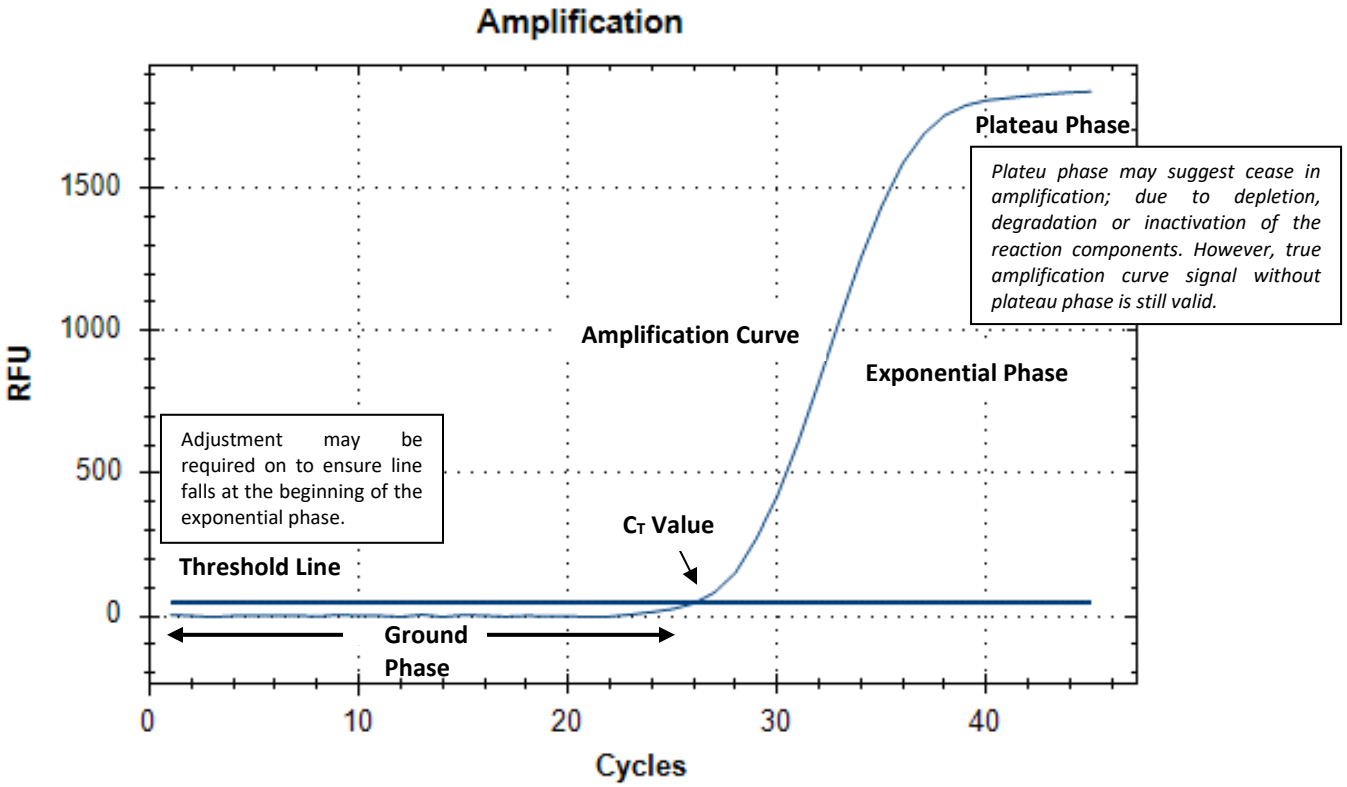

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Figure 2. Illustration of Positive Amplification Curve (Log and Linear View)

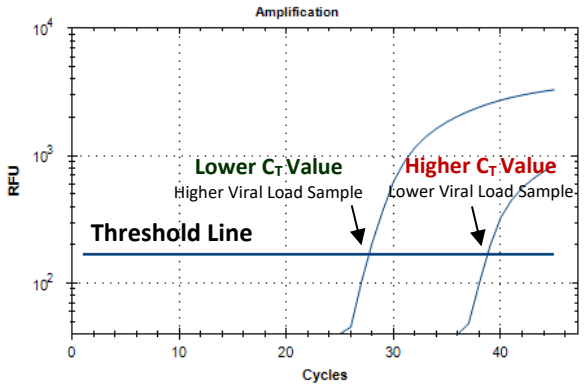
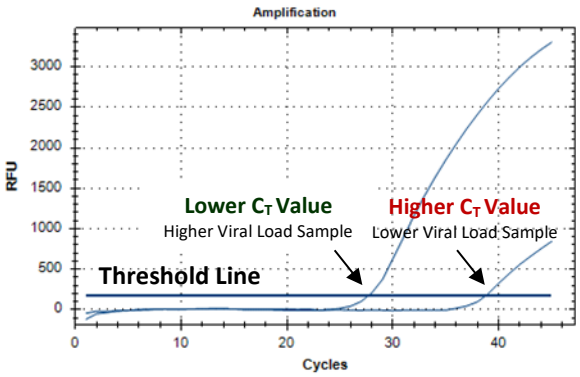
Log View	Linear View
	

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

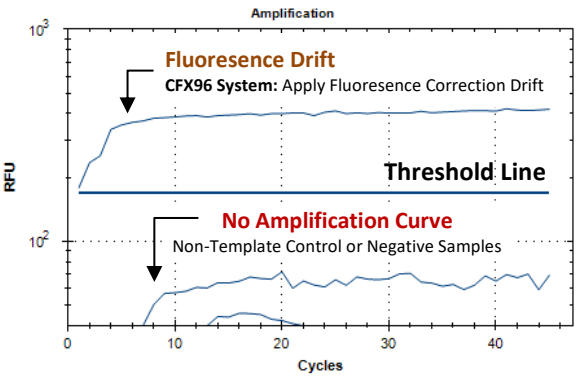
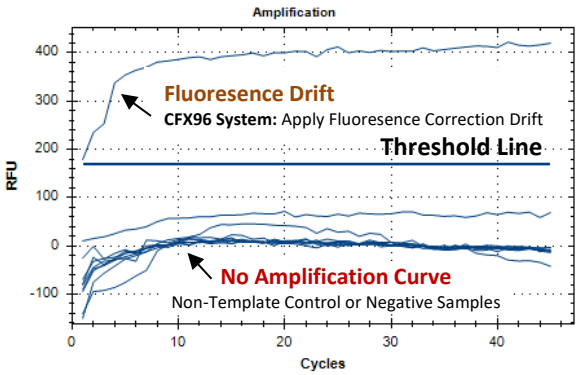
Log View	Linear View
	

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Table 9. Result interpretation for test samples.

Observation			Interpretation
NS1 (FAM)	**NS2 (Texas Red)**	**GAPDH (HEX/VIC)**	
Amplification Signal, **AND** C_T value <40	Amplification Signal, **AND** C_T value <40	Amplification Signal, **AND** C_T value <40	**POSITIVE**
Amplification Signal, **AND** C_T value <40	No Amplification, **OR** C_T value >40	Amplification Signal, **AND** C_T value <40	**POSITIVE**
No Amplification, **OR** C_T value >40	Amplification Signal, **AND** C_T value <40	Amplification Signal, **AND** C_T value <40	**POSITIVE**
Amplification Signal, **AND** C_T value <40	Amplification Signal, **AND** C_T value <40	No Amplification, **OR** C_T value >40	**POSITIVE**
Amplification Signal, **AND** C_T value <40	No Amplification, **OR** C_T value >40	No Amplification, **OR** C_T value >40	**POSITIVE**
No Amplification, **OR** C_T value >40	Amplification Signal, **AND** C_T value <40	No Amplification, **OR** C_T value >40	**POSITIVE**
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	**INVALID Retest***

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.*

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9. Troubleshooting

Table 10. Example of unexpected observations on positive control, non template control and test sample.

Sample	Observation	Potential Cause	Solution
Positive control	1. Only NS1 or NS2 is detected with normal amplification curve (Figure 2). 2. No amplification. 3. GAPDH is detected with normal amplification curve (Figure 2).	**Observation no.: 1 amd 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	NS1, NS2 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 (Refer section 7). • Ensure sample is assigned accordingly
Continue on next page			

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Table 10 (continued)

Sample	Observation	Potential Cause	Solution
Test sample	1. Both NS1 and NS2 are detected with normal amplification curve (Figure 2) but but CT values are vastly different. 2. Either NS1, NS2 or GAPDH is detected at early cycles with abnormal amplification curve (Figure 3). 3. No amplification. 4. Result is consistenly inconclusive (no amplifiifation).	**Observation no.: 1, 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 Fluoresence 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 • 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 to 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 0.002 to 2000 copies/µl for NS1 and NS2. The testing was carried out in either five-replicates (for concentrations ≥10 copies/µl) or seven-replicates (for concentrations <10 copies/µl). The analytical sensitivity was estimated by Probit analysis using SPSS release 16.0.0.

The analytical sensitivities with 95% confidence for both NS1 and NS2 are shown in Tables 11-15.

Table 11. LoD confirmation on the Bio-Rad CFX96™ Real-Time PCR Detection System.

Target	**NS1**		**NS2**	
SARS-CoV-2 IVT RNA concentration	**2.2 copies/µl**		**1.8 copies/µl**	
Sample	**Pos/Neg**	**C_T (FAM)**	**Pos/Neg**	**C_T (Texas Red)**
1	Pos	36.83	Pos	36.30
2	Pos	36.42	Pos	36.86
3	Pos	35.38	Pos	37.55
4	Pos	35.61	Pos	36.52
5	Pos	37.46	Pos	36.19
6	Pos	35.40	Pos	36.13
7	Pos	34.40	Pos	35.49
8	Pos	36.48	Pos	37.35
9	Pos	37.54	Pos	37.32
10	Pos	35.57	Pos	36.24
11	Pos	35.79	Pos	35.74
12	Pos	34.49	Pos	37.05
13	Pos	37.80	Pos	36.06
14	Pos	35.85	Pos	36.81
15	Pos	36.41	Pos	35.29
16	Pos	35.68	Pos	35.42
17	Pos	36.51	Pos	35.28
18	Pos	36.50	Pos	37.28
19	Pos	34.67	Pos	35.26
20	Pos	35.34	Pos	35.02
Statistics	**Mean C_T**	**36.01**	**Mean C_T**	**36.26**
	SD	**0.96**	**SD**	**0.81**
	CV%	**2.69%**	**CV%**	**2.23%**
	Positive/Total	**20/20**	**Positive/Total**	**20/20**

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Table 12. LoD confirmation on the Applied Biosystems 7500/7500 FAST Real-Time PCR Systems.

Target	**NS1**		**NS2**	
SARS-CoV-2 IVT RNA concentration	**2.2 copies/µl**		**2.2 copies/µl**	
Sample	**Pos/Neg**	**C_T (FAM)**	**Pos/Neg**	**C_T (Texas Red)**
1	Pos	34.16	Pos	34.92
2	Pos	36.59	Pos	37.69
3	Pos	36.40	Pos	36.00
4	Pos	35.45	Pos	36.98
5	Pos	35.14	Pos	35.85
6	Pos	34.56	Pos	36.45
7	Pos	35.70	Pos	35.91
8	Pos	37.90	Pos	38.14
9	Pos	35.76	Pos	37.14
10	Pos	36.03	Pos	36.41
11	Pos	36.15	Pos	37.83
12	Pos	36.01	Pos	35.99
13	Pos	38.36	Neg	-
14	Pos	36.17	Pos	37.28
15	Pos	36.29	Pos	37.83
16	Pos	35.55	Pos	35.62
17	Pos	36.23	Pos	36.23
18	Pos	36.47	Pos	36.52
19	Pos	36.34	Pos	35.39
20	Pos	34.86	Pos	36.5
Statistics	**Mean C_T**	**36.01**	**Mean C_T**	**36.56**
	SD	**0.98**	**SD**	**0.90**
	CV%	**2.73%**	**CV%**	**2.47%**
	Positive/Total	**20/20**	**Positive/Total**	**19/20**

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Table 13. LoD confirmation on *ab*CyclerQ Real-Time PCR System.

Target	**NS1**		**NS2**	
SARS-CoV-2 IVT RNA concentration	**2.6 copies/µl**		**3.0 copies/µl**	
Sample	**Pos/Neg**	**C_T (FAM)**	**Pos/Neg**	**C_T (Texas Red)**
1	Pos	34.57	Pos	31.53
2	Pos	33.97	Pos	31.26
3	Pos	34.56	Pos	30.36
4	Pos	34.26	Pos	31.81
5	Pos	34.64	Pos	31.63
6	Pos	34.62	Pos	30.79
7	Pos	34.62	Pos	31.09
8	Pos	34.28	Pos	31.26
9	Pos	36.15	Pos	32.48
10	Neg	-	Pos	32.57
11	Pos	34.91	Pos	31.86
12	Pos	35.11	Pos	31.43
13	Pos	32.97	Pos	31.65
14	Pos	34.24	Pos	31.35
15	Pos	34.67	Neg	-
16	Pos	35.83	Pos	31.87
17	Pos	34.80	Pos	32.31
18	Pos	34.46	Pos	31.28
19	Pos	35.09	Pos	31.84
20	Pos	34.45	Pos	31.82
Statistics	**Mean C_T**	**34.64**	**Mean C_T**	**31.45**
	SD	**0.67**	**SD**	**0.55**
	CV%	**1.92%**	**CV%**	**1.73%**
	Positive/Total	**19/20**	**Positive/Total**	**19/20**

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Table 13. LoD confirmation on *ab*CyclerQ mini Real-Time PCR System.

Target	**NS1**		**NS2**	
SARS-CoV-2 IVT RNA concentration	**3.0 copies/µl**		**3.0 copies/µl**	
Sample	**Pos/Neg**	**C_T (FAM)**	**Pos/Neg**	**C_T (Texas Red)**
1	Pos	34.51	Pos	33.98
2	Pos	34.07	Pos	33.42
3	Pos	34.92	Pos	35.17
4	Pos	34.25	Pos	34.44
5	Pos	34.07	Pos	34.07
6	Pos	34.35	Pos	37.81
7	Pos	33.65	Pos	34.02
8	Pos	33.18	Pos	34.98
9	Pos	35.05	Pos	33.30
10	Pos	33.81	Pos	33.95
11	Pos	34.48	Pos	34.10
12	Pos	33.36	Pos	34.06
13	Pos	34.75	Pos	34.38
14	Pos	34.95	Pos	34.06
15	Pos	34.35	Pos	34.23
16	Pos	34.79	Pos	35.21
17	Pos	34.22	Pos	35.09
18	Pos	34.06	Pos	33.84
19	Pos	35.13	Pos	34.88
20	Pos	35.20	Pos	35.47
21	Pos	34.39	Pos	34.39
22	Pos	33.20	Pos	33.01
23	Pos	36.58	Neg	-
24	Pos	35.21	Pos	34.70
Statistics	**Mean C_T**	**34.44**	**Mean C_T**	**34.46**
	SD	**0.76**	**SD**	**0.96**
	CV%	**2.2%**	**CV%**	**2.8%**
	Positive/Total	**24/24**	**Positive/Total**	**23/24**

Table 15. Summary of LoD results.

Target	**Detection Channel**	**Real-Time PCR System**	**Analytical Sensitivity (95% confidence)**
NS1	FAM	Bio-Rad CFX96™	2.2 copies/µL
		Applied Biosystems 7500/7500 FAST	2.2 copies/µL
		*ab*CyclerQ	2.6 copies/µL
		*ab*CyclerQ mini	3.0 copies/µL
NS2	Texas Red	Bio-Rad CFX96™	1.8 copies/µL
		Applied Biosystems 7500/7500 FAST	2.2 copies/µL
		*ab*CyclerQ	3.0 copies/µL
		*ab*CyclerQ mini	3.0 copies/µL

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10.2. Analytical Specificity

10.2.1. Inclusivity

The inclusivity of *ab*TES™ COVID-19 qPCR I Kit was evaluated by in silico analysis against 125,327 high-quality complete genome sequences of SARS-CoV-2 published via GISAID (www.gisaid.org) as of November 13, 2020. It turned out that the primers/probes of the kit show homology from 99.88-99.99% as shown in Table 16.

Table 16. Results of the in silico inclusivity analysis.

Primers/probes	Homology
NS1 forward primer	125,247 / 125,327 = 99.94%
NS1 reverse primer	125,264 / 125,327 = 99.95%
NS1 probe (FAM)	125,219 / 125,327 = 99.91%
NS2 forward primer	125,261 / 125,269 = 99.99%
NS2 reverse primer	125,229 / 125,269 = 99.97%
NS2 probe (TxR)	125,122 / 125,269 = 99.88%

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10.2.2. Cross-reactivity

The assay was tested for potential cross-reactivity against the following) panel of 28 organisms (Table 17. No cross-reactivity was observed.

Table 17. Organisms that were tested for cross-reactivity.

Organism	Result
MERS Coronavirus	Not Detected
Coronavirus 229E	Not Detected
Coronavirus NL63	Not Detected
Coronavirus OC43	Not Detected
Influenza A	Not Detected
Influenza B	Not Detected
Parainfluenza 1	Not Detected
Parainfluenza 2	Not Detected
Parainfluenza 3	Not Detected
Parainfluenza 4	Not Detected
Escherichia coli (vtec)	Not Detected
Candida albicans	Not Detected
Neisseria meningitidis	Not Detected
Pseudomonas aeruginosa	Not Detected
Streptococcus pneumoniae	Not Detected
Legionella pneumophila	Not Detected
Streptococcus pyogenes	Not Detected
Klebsiella pneumoniae	Not Detected
Staphylococcus aureus	Not Detected
Haemophilus influenza	Not Detected
Coxsackievirus A6	Not Detected
Coxsackievirus B1	Not Detected
Coxsackievirus B5	Not Detected
Respiratory Syncytial A	Not Detected
Respiratory Syncytial B	Not Detected
Rhinovirus	Not Detected
Adenovirus	Not Detected
Enterovirus	Not Detected

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10.3. Precision/Reproducibility

The inter-assay (variability between different runs) and intra-assay (variability within one run) precision was determined by performing the assay once per day in five-replicates over a period of two days for one sample (two targets: NS1 and NS2) of different concentrations (total ≥10 reactions per target).

For both targets, the qualitative results of all ten 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 18 and 19.

Table 18. 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	1.6 copies/µl
NS1	**SD**	0.12	0.20	0.16	0.32	0.42	0.55
	CV%	0.5%	0.7%	0.5%	1.0%	1.2%	1.6%
NS2	**SD**	0.08	0.18	0.27	0.37	0.53	0.82
	CV%	0.3%	0.6%	0.9%	1.1%	1.6%	2.3%

Table 19. 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	1.6 copies/µl
NS1	**SD**	0.12	0.17	0.13	0.17	0.42	0.66
	CV%	0.5%	0.7%	0.5%	0.4%	1.3%	1.5%
NS2	**SD**	0.08	0.20	0.27	0.42	0.40	0.77
	CV%	0.3%	0.7%	0.9%	1.1%	1.6%	2.2%

10.4. Diagnostic Evaluation

To predict the diagnostic evaluation of *ab*TES™ COVID-19 qPCR I Kit at the 95% confidence interval, a total of 112 clinically extracted samples used. The positive samples used in the study are from confirmed COVID-19 cases. This kit has been tested on samples extracted from sputum, nasopharyngeal and oropharyngeal swabs.

The nucleic acids (RNA) were extracted using a commercially available kit such as NucliSENS® easyMAG® Total Nucleic Acid Extraction Kit, EZ1 Virus Kit and Liferiver RNA Isolation Kit. The extracted samples were tested by the WHO recommended protocol as a reference test.

Table 20. Summary of the result of diagnostic sensitivity and specificity for COVID-19 as below.

Description	***ab*TES™ COVID-19 qPCR I Kit (*n*=112)**		**Sensitivity/ Specificity**
	COVID-19 Positive	**COVID-19 Negative**	**% (95% Confidence Internal)**
COVID-19 Positive	65	2*	97 % sensitivity
COVID-19 Negative	0	45	100 % specificity
Total	65	47	

**The C_T values of these two samples in its original runs was very high, implying the virus load was very low and reported as negative in this evaluation. Moreover, the RNA samples have since then been freeze/thawed repeatedly, possibly causing degradation.*

In summary, abTES™ COVID-19 qPCR I Kit showed 97 % sensitivity and 100% specificity.

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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.
- As SARS-CoV-2 will mutate over time, and potential mutations will likely occur within the primer/probe regions. These regions are actively monitored against the sequence database available at NCBI and GISAID.

12. Explanation of Symbols

Symbol	Explanation
IVD	*In vitro* diagnostic medical device. *abTES™ COVID-19 qPCR I Kit has received Provisional Authorization from the Health Sciences Authority in Singapore.*
REF	Catalogue number
-25°C -15°C	Store at -25°C to -15°C
	Manufacturer
EC REP	Authorized Representative in the European community
LOT	Lot number
	Use by
Σ	Contains sufficient for <n> tests
	Important

13. References

1. (2021) Archived: WHO Timeline - COVID-19. [Online] WHO. [Accessed 20 Jan 2021].
2. (2020) 2019 Novel Coronavirus (2019-nCoV), Wuhan, China. [Online] US Centers for Disease Control and Prevention. [Accessed 27 Jan 2020].
3. Zhu N, Zhang D, Wang W, Li X, Yang B et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. N Engl J Med. 2020; 1-7.
4. (2020) Naming the coronavirus disease (COVID-19) and the virus that causes it [Online] WHO. [Accessed 8 May 2020].

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

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