1. Background & Objective

BGI's *"Real-Time Fluorescent RT-PCR Kit for Detecting SARS-CoV-2"* is an *in vitro* diagnostic real-time reverse transcription-PCR assay for the qualitative detection of SARS-CoV-2 nucleic acids in throat (oropharyngeal) swabs, nasopharyngeal swabs, anterior nasal swabs, mid-turbinate nasal swabs, nasal washes, nasal aspirates, and bronchoalveolar lavage fluid (BALF). It has received an EUA by FDA and is recommended with RNA extraction.

Mawi DNA Technologies has developed a modified "extraction-less" version of the iSWAB-Microbiome technology. The ISWAB-Microbiome-EL is designed to eliminate the RNA extraction step in the COVID-19 Molecular testing workflow, allowing researchers to perform direct RT-PCR. This experiment was performed to test and optimize the compatibility of iSWAB – Microbiome – EL with BGI's "Real-Time Fluorescent RT-PCR Kit for Detecting SARS-CoV-2" without the extraction process.

2. Materials & Methods

2.1 Materials

- iSWAB-MB-EL (Mawi DNA Technology, Cat. ISM-T-EL)
- FLOQ Swabs (Copan, Cat. 519CS01)
- *"Real-Time Fluorescent RT-PCR Kit for Detecting SARS-CoV-2"* (BGI, Cat. # "assay_name")
- 1.5 ml microcentrifuge tubes (Eppendorf® Safe-Lock, Cat. # 0030120.086)
- 2 ml microcentrifuge tubes (Fisher Scientific, Cat. 05-408-141)
- Heat-inactivated SARS-CoV-2 (ATCC, Cat. VR-1986HK)
- Molecular Grade (Nuclease-free) water (e.g., IDT Cat. # 11-04-02-01)
- Bio-Rad CFX Opus Real-Time PCR System
- Bio-Rad CFX Maestro™ Software for Windows (Version 2.0)
- Pipettes p2, p10, p20, p200, p1000
- RNAse/DNase free filtered tips for p2, p10, p20, p200, and p1000 pipettes
- Microcentrifuge (Sorvall Legend Micro 21 REF: 75002436)
- Vortex (Genie 2) (VWR Scientific, Cat. # G-560)

2.2 Methods

BGI's *"Real-Time Fluorescent RT-PCR Kit for Detecting SARS-CoV-2"* Assay

The assay detects a specific target region in the ORF1ab region of SARS-CoV-2 genome (FAM channel) as well as the human housekeeping gene β-Actin (VIC/HEX channel) as an internal control. The final reaction volume is 30 µl and is achieved by adding 10 µl of extracted RNA

template into the primer/probe/master mix. However, since RNA is not extracted using the iSWAB-Microbiome-EL buffer and therefore, it is not purified and may contain RT-qPCR inhibitory impurities or/and chemical components, the unextracted RNA template was diluted in nuclease-free water at different ratios to achieve the final 10 µl template volume. Therefore, different unextracted RNA template volumes were tested.

Results Interpretation

Table 1. Guide to interpretation of patient specimen results according to BGI's "Real-Time Fluorescent RT-PCR Kit for Detecting SARS-CoV-2" assay manual.

	VIC/HEX Observation	FAM Observation	Interpretation
Sample 1	Sigmoidal amplification curve and Ct value is <35.	Sigmoidal amplification curve and Ct value is <37.	**Positive for SARS-COV-2 RNA**; Valid Internal Reference
Sample 2	Sigmoidal amplification curve and Ct value is <35.	± No Ct data or no sigmoidal amplification curve or Ct value is >37.	**Negative for SARS-COV-2 RNA;** Valid Internal Reference
Sample 3	No Ct data or no sigmoidal amplification curve or Ct value is >35.	Sigmoidal amplification curve and Ct value is <37.	**Invalid test, please retest***
Sample 4	No Ct data or no sigmoidal amplification curve or Ct value is >35.	No Ct data or no sigmoidal amplification curve or Ct value is >37.	**Invalid test, please retest***

*First retest by re-extracting RNA from the same specimen. If the test fails again, collect a new specimen from the patient and repeat the test.

Sampling

Samples were self-collected from the nasal mid-turbinate area with a standard flocked swab from 2 individuals and released in iSWAB-Microbiome-EL devices. Samples were then pooled

into a 2 mL Eppendorf Tube. The pooled sample was vortexed, homogenized, and aliquoted into three 1.5 mL Eppendorf tubes, each containing 300 µl of nasal background. Samples were then spiked with heat-inactivated SARS-CoV-2 at different concentrations as described below.

Spiking

ATCC Heat Inactivated 2019 Novel Coronavirus with initial concentration 390,000 copies/µl was 10-fold serially diluted with Microbiome-EL buffer to achieve three concentrations: (A) 390,000 (undiluted), (B) 39,000, and (C) 3,900 copies/ µl. Diluted virus was then used to spike the nasal sample with (A) 30,000, (B) 3,000, and (C) 300 copies/µl final virus concentration. The spiked nasal was rocked for two hours to one day in a Chemistry/Hematology Mixer at room temperature until further processing. Spiked nasal samples from each concentration were initially tested undiluted and diluted 1:5 with nuclease-free water (Table 2) to a final reaction template of 10 µl and in duplicates. A follow-up confirmation experiment tested additionally a 1:1 template dilution, only concentrations B and C, with all templates run in triplicate RT-qPCR reactions.

Table 2. Spiked nasal sample in iSWAB-Microbiome-EL and nuclease-free water ratio.

Sample	SARS-CoV-2 (copies/µl)	Sample: Water Ratio	Spiked Nasal Sample (µl)	Water (µl)	Reaction Volume (µl)	Copies/Rxn
A1	30000	Undiluted	10	0	30	10000
B1	3000	Undiluted	10	0	30	1000
C1	300	Undiluted	10	0	30	100
A5	30000	1:5	2	8	30	2000
B5	3000	1:5	2	8	30	200
C5	300	1:5	2	8	30	20
B2	3000	1:1	5	5	30	500
C2	300	1:1	5	5	30	50

3. Results

Experiment I

Table 3. A summarizing table of the collected data of spiked samples used either undiluted (A-C) or diluted 1:5 (A5-C5) as template in the final RT-qPCR reaction. Numbers correspond to the Ct of two replicates per sample for the amplification of the Orf1ab SARS-CoV-2 and the human β-Actin gene targets.

Samples	Volume Spiked Nasal Sample (µl)	Copies/Rxn	HEX	FAM
A	10	10000	34.19	26.3
			33.9	26.33
B	10	1000	32.91	31.16
			34.11	30.81
C	10	100	34.6	35.56
			34.58	35.07
A5	2	2000	32.4	27.8
			32.48	27.85
B5	2	200	32.08	32.16
			32	32.02
C5	2	20	33.27	35.99
			33	36.21

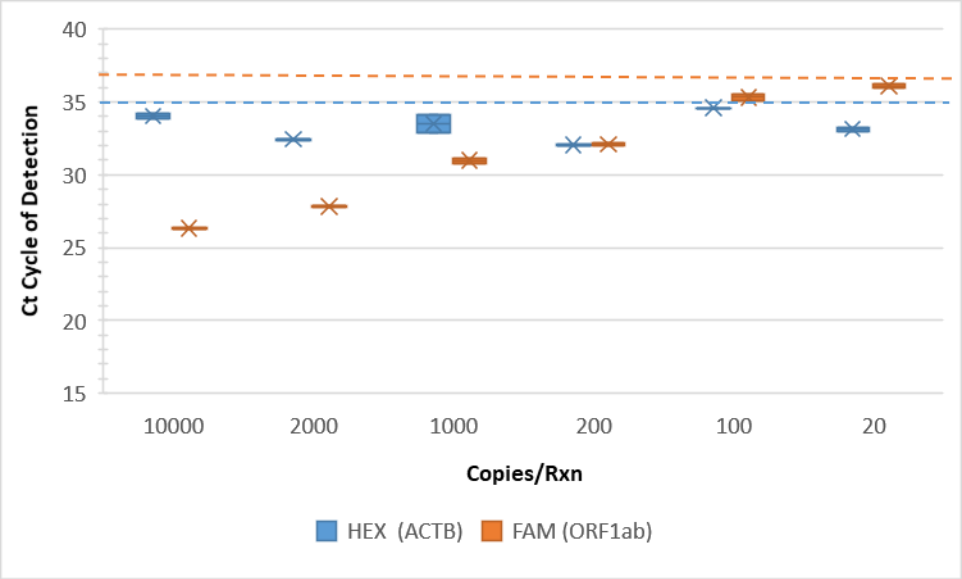

Figure 1: Average Ct values of two replicates of SARS-CoV-2 gene Orf-1ab (FAM channel) and of and human β-Actin (ACTB) (HEX channel/green). The dashed lines indicate the threshold for the valid detection of either of the two genes (FAM: Ct<37, HEX: Ct<35).

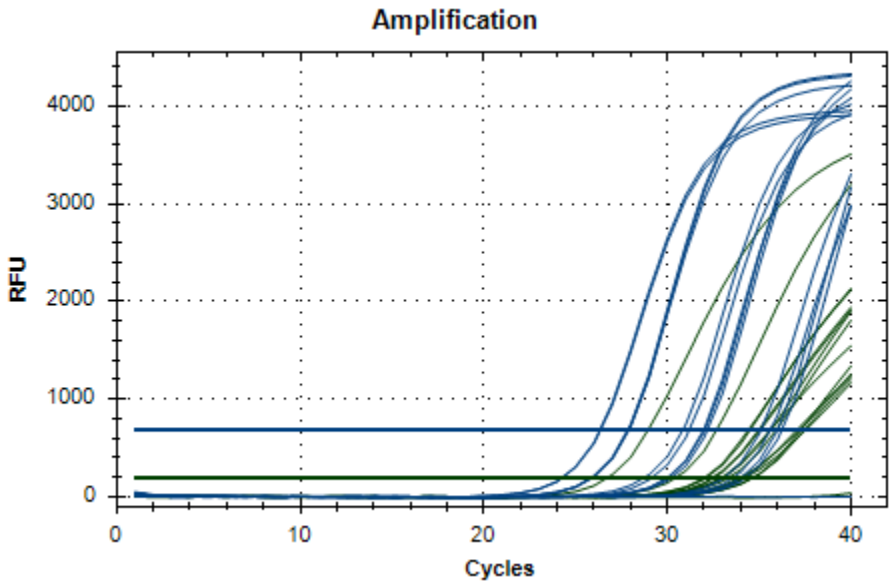

Figure 2: Amplification plots of targets SARS-CoV-2 Orf-1ab (FAM channel/blue) and human β-Actin (ACTB) (HEX channel/green).

Experiment II

Table 4. A summarizing table of the collected data of spiked samples used undiluted (A-C) or diluted 1:5 (A5-C5) and 1:1 (B2-C2) as template in the final RT-qPCR reaction. Numbers correspond to the Ct of three replicates per sample for the amplification of the Orf1ab SARS-CoV-2 and the human β-Actin gene targets.

Samples	Volume Spiked	Copies/Rxn	HEX	FAM
B	10	1000	32.85	31.14
			32.84	31.25
			32.59	31.38
C	10	100	33.15	35.08
			33.29	35.65
			33.41	35.53
B5	2	200	31.95	31.3
			32.22	31.41
			32.22	31.48
C5	2	20	32.55	36.7
			32.57	36.09
			32.75	36.13
B2	5	500	32.34	32.39
			32.3	32.3
			32.31	32.21
C2	5	50	32.24	35.6
			32.58	35.14
			32.42	36.64

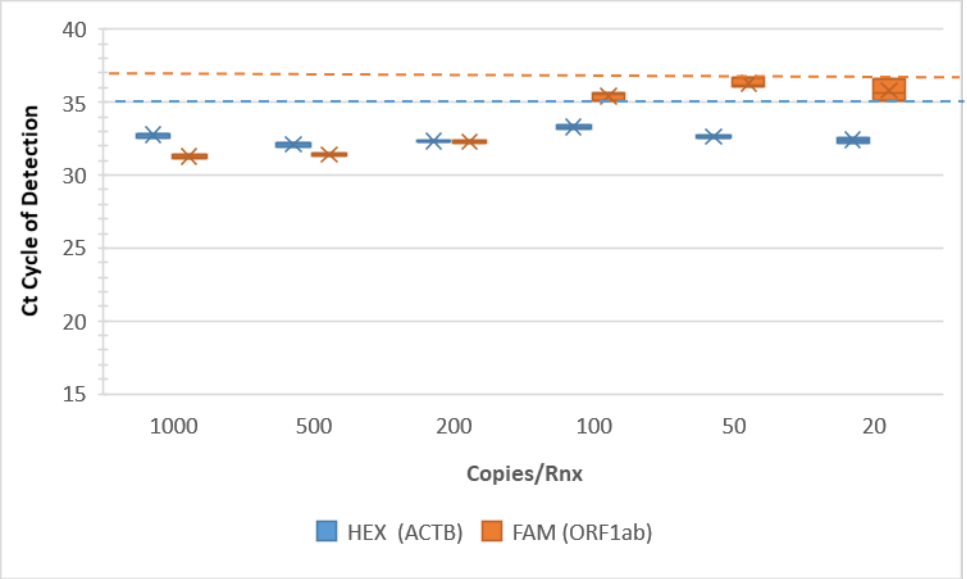

Figure 3: Average Ct values of three replicates of SARS-CoV-2 gene Orf-1ab (FAM channel) and of and human β-Actin (ACTB) (HEX channel/green). The dashed lines indicate the threshold for the valid detection of either of the two genes (FAM: Ct<37, HEX: Ct<35).

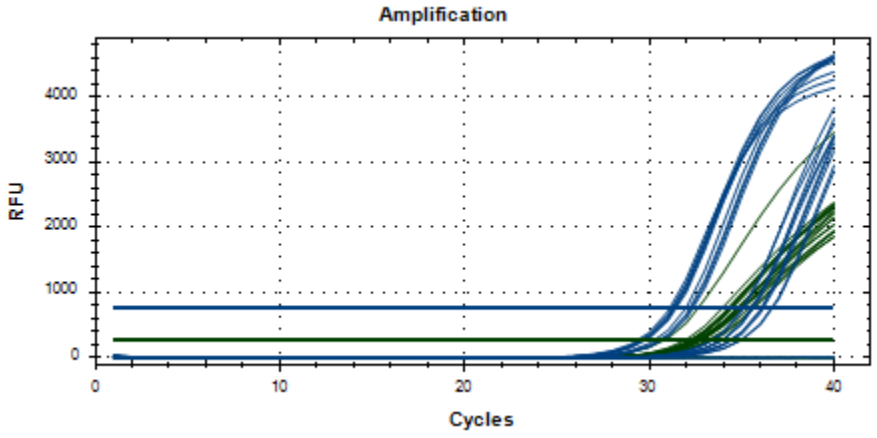

Figure 4: Amplification plots of targets SARS-CoV-2 Orf-1ab (FAM channel/blue) and human β-Actin (ACTB) (HEX channel/green).

4. Conclusions

- iSWAB-MB-EL is compatible with the BGI *"Real-Time Fluorescent RT-PCR Kit for Detecting SARS-CoV-2"* assay. Spiked heat inactivated virus can be successfully detected with a LoD of 20 cp/rxn (300 cp/µl) directly from the collection buffer without introducing any pretreatment steps, such as heating or/and Proteinase K treatment.
- Different dilutions of the template were tested to determine the one that detects both assay targets. Our lowest virus concentration, 300 cp/µl, was consistently detected (Ct<37) along with the human background (Ct<35) regardless of using undiluted or diluted sample.
- Undiluted template (10 µl) straight from the collection tube worked at 100 cp/rxn, that corresponds to 300 cp/µl of sample that was tested. Given the Ct values of ORF1ab (35.42), concentrations below 150 cp/µl could go undetected.
- Diluting the sample 1:2 or 1:5 did not result in better (earlier) detection but interestingly, 1:5 was slightly better than 1:2 when detecting the ORF1ab gene.

R&D Report	**Name**	**Date**
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