Doherty Institute

THE UNIVERSITY OF MELBOURNE

The Royal Melbourne Hospital

A joint venture between The University of Melbourne and The Royal Melbourne Hospital

Final Report

Post-market validation of the Beijing Genomics Institute (BGI) SARS-CoV-2 Real Time PCR platform

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Executive Summary

Currently, reverse transcription polymerase chain reaction (RT-PCR) performed on upper respiratory tract swab samples is the main approach used in Australian laboratories to detect of SARS-CoV-2, the virus that causes COVID-19 disease[1]. To meet the urgent need for diagnostic testing in Australia the Therapeutic Goods Administration (TGA) has expedited approvals (with conditions) for SARS-CoV-2 diagnostic tests based on the studies that manufacturers were able to provide at the time of application. To supplement this data, robust post market validation of SARS-CoV-2 diagnostic kits that are listed on the Australian Register of Therapeutic Goods (ARTG) is desirable.

This study presents a post-market validation study of the Beijing Genomics institute (BGI) MGISP-960 robotic nucleic extraction instrument, paired with either the SLAN 96S real-time PCR analyser, or Bioer Line Gene 9600 PCR analyser, and using the MGIEasy Magnetic Beads Virus DNA/RNA Extraction Kit (Ref 1000021043), and BGI Real-time fluorescent RT-PCR kit for detecting 2019-nCoV (Ref MFG030010) collectively referred to here as 'the BGI-Platform'. This equipment and reagents have been made available to selected Australian laboratories for diagnostic use.

The study employed a 271 sample validation panel comprising 156 positive and 115 negative samples, randomly assembled. These were of necessity simulated samples, using inactivated cell-culture-grown SARS-CoV-2, and other respiratory viruses spiked into a representative variety of fluids, since patient samples were not available in sufficient volumes to parallel test in three laboratories.

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The BGI equipment and test kits were tested at two collaborating laboratories: 'Laboratory 2', and 'Laboratory3'. Both laboratories tested the whole 271 sample panel in parallel with the Victorian Infectious Diseases Reference Laboratory, Doherty Institute.

A set of exercises only done in Melbourne separately compared the BGI MGISP-960 robotic nucleic extraction instrument, and the SLAN 96S real-time PCR analyser with Doherty Institute test methods. In addition different lot numbers of MGIEasy Magnetic Beads Virus DNA/RNA Extraction Kit (Ref 1000021043), and BGI Real-time fluorescent RT-PCR kit for detecting 2019-nCoV (Ref MFG030010) were compared with each other and Doherty Institute reagents.

The most dilute sample reliably detected (Limit of detection, LOD) was calculated for each test, along with the accuracy in detecting true positive samples and true negative samples (Operational Sensitivity and specificity), the latter including samples containing no target, and samples containing respiratory viruses other than SARS-CoV-2. Precision in testing replicates of the same sample was examined compared to an objective standard test (droplet digital PCR), together with comparison of the graphical distribution of raw RT-PCR test output values (Cycle threshold values, Ct values) for each laboratory.

In summary, the BGI platform was shown to have a Limit of Detection, and Operational Sensitivity and Specificity that were comparable to the reference test methods; Sensitivity being slightly higher and specificity slightly lower. This was fairly consistently achieved in two BGI-user Laboratories. The manufacturer's Instructions for Use (IFU) dictate retesting of samples with low reactivity (Ct values above

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38), and a number of such values were recorded on testing negative panel samples, especially by one laboratory. Best practice would be to use a second assay using a different gene target for this retesting, consistent with Australian guidelines. This extra testing may be onerous for test users if low reactivity is commonplace in clinical use of the test, as this study suggests might be the case in some scenarios.

One lot number of BGI Real-time fluorescent RT-PCR reagents gave noticeably lower specificity than another, albeit still relatively high, and varied considerably from the overall specificity achieved testing the panel that is described above. Otherwise different lot numbers of MGIEasy Magnetic Beads Virus DNA/RNA Extraction Kit and BGI Real-time fluorescent RT-PCR reagents gave comparable performance. Some differences in precision between replicates of the same sample were observed, but these were most evident in one sample type: (swabs diluted in UTM) and with one batch of MGIEasy Magnetic Beads Virus DNA/RNA Extraction Kit reagents. Overall the precision was acceptable for a qualitative test.

Hence, no single issue has been identified to suggest that the BGI platform is not fit for purpose in Australian laboratories. Best practice in executing confirmatory testing of samples producing Ct values > 38 will be important in use. Similarly, close monitoring of consistency in performance of Lot numbers, especially of RT-PCR reagents, will be important to ensure optimal performance in clinical use

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1. Introduction

Timely, scalable and accurate diagnostic testing for SARS-CoV-2 underpins the public health response to COVID-19 disease, and clinical care of the infected individual. RT-PCR performed on respiratory samples is the main diagnostic modality for detection of acute COVID-19 disease.[1] This is being done in Australia under an emergency exemption from the Therapeutic Goods Administration (TGA), initially for Public Health Laboratory Network of Australasia (PHLN)-member public health laboratories, and more recently extended to hospital and community laboratories.

To meet the urgent need for diagnostic testing in Australia the TGA has expedited approvals (with conditions) for SARS-CoV-2 diagnostic tests based on the studies that manufacturers were able to provide at the time of application. To supplement this data, robust post market validation of SARS-CoV-2 diagnostic kits that are listed on the Australian Register of Therapeutic Goods (ARTG) is desirable.

Here, we present a post-market validation study of the Beijing Genomics institute (BGI) MGISP-960 robotic nucleic extraction instrument, together with either the SLAN 96S real-time PCR analyser, or Bioer LineGene 9600 PCR analyser, and using the MGIEasy Magnetic Beads Virus DNA/RNA Extraction Kit (Ref 1000021043), and BGI Real-time fluorescent RT-PCR kit for detecting 2019-nCoV (Ref MFG030010). This equipment and reagents have been made available to selected Australian laboratories for diagnostic use.

Post-market validation of the Beijing Genomics Institute (BGI) SARS-CoV-2 Real Time PCR platform continued

2. Methods

2.1 Participating Laboratories

The three participating laboratories in this study were Laboratory 1: Victorian Infectious Diseases Reference Laboratory (VIDRL), Doherty Institute, Melbourne, 'Laboratory 2', and 'Laboratory3'.

2.2 Validation Panel

A sample panel was developed to test performance of the BGI CoVID-19 RT-PCR Platforms. The panel comprises 271 mock respiratory tract samples. There were 156 positive and 115 negative samples. Samples were randomised within the validation panel as presented to testing laboratories.

Positive samples were all dilutions of gamma-irradiated (inactivated) SARS-CoV-2 (strain VIC/01/202). Samples were prepared by spiking a dilution series of gamma-irradiated SARS-CoV-2 into each sample matrix. Actual residual clinical samples were not available in sufficient volumes to permit parallel testing in three laboratories. In addition replicating real clinical samples obviates potential uncertainty about samples discrepant between reference and test systems, since known quantities of virus are present in each positive sample. The preparation of virus and spiked samples is described in greater detail below.

The positive samples comprise two groups representing samples for which the MGISP-960/MGIEasy Magnetic Beads Virus DNA/RNA Extraction Kit is validated according to the manufacturer's Instructions for Use (IFU)[3]. The first is a four-fold dilution series of gamma-irradiated SARS-CoV-2 virus isolate in virus

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transport medium (VTM) to simulate eluate from respiratory swabs. These were diluted 1/4000 to 1/262,144,000 with quadruplicate replicates of each (a total of 36 samples). The second comprise VTM (n= 72), 10% saliva diluted in VTM (n=24), and de-identified previously negative human nose and throat swabs in commercial transport medium (Copan UTM, CA USA)) (n=24), spiked with differing final dilutions of gamma-irradiated SARS-CoV-2 virus stock.

The remaining samples were negative controls (n=115) which were a mixture of previously screened negative human swabs (n=33), 10% saliva diluted in VTM (n=49), VTM (n=9), and VTM or 10% saliva diluted in VTM spiked with other seasonal respiratory viruses including influenza A, influenza B, Parainfluenza 1,2 and 3, rhinovirus, enterovirus (Coxsackie A16), respiratory syncytial virus (RSV), human metapneumovirus, adenovirus, measles virus, parvovirus and seasonal human coronaviruses 229E and OC43 (n=24). These were a dilution of cell culture grown isolates from the Doherty Institute's reference collection with Ct values ranging from 25 to 30 (data not shown).

This complete panel of 271 samples was used to compare the performance of the complete nucleic acid extraction and RT-PCR workflows of the three participating laboratories. Sensitivity and specificity were compared, and LOD was examined using the four fold dilution series present in four fold replicates that is described above.

A full listing of panel samples is provided in Appendix A.

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A subset of samples n=90, comprising two duplicate sets of 45 samples, each comprising 15 positive samples, and 30 negative samples, was used to perform a sub-study which individually compared the MGISP-960 high-throughput automated extraction and liquid handling workstation (MGI Tech Co., Ltd., China), and the BGI Real-time fluorescent RT-PCR kit for detecting SARS-CoV-2 on the BGI SLAN-96S real-time PCR system (BGI, China) with the respective reference methods. In addition this sub-panel was used to compare performance of different lot numbers of the MGIEasy Magnetic Beads Virus DNA/RNA Extraction Kit (MGI Tech Co., Ltd., China), and the BGI Real-time fluorescent RT-PCR kit for detecting SARS-CoV-2, with each other.

Virus

Virus stock for artificial spiking of samples was SARS-CoV-2/Vic/01/2020 isolate grown in vero cells that had been subjected to 50Kgray of gamma-irradiation and verified as inactive by subsequent virus isolation attempts and sub-culture passaging. Primary stocks of 1/100 and 1/1000 dilution of virus were prepared in VTM for subsequent dilution into all matrices used for preparation of mock-positive samples.

Mock negative and positive samples

Samples were prepared using 3 alternative dilution matrices. These include VTM prepared in-house (Essential Minimum Eagles medium (EMEM) (Sigma, UK) with 2% Foetal Bovine serum (FBS)(Bovogen Keilor East, Australia); VTM with 10% volume of cell-clarified human saliva) and SARS-CoV-2 negative screened human nose and throat swabs in Copan swabs in Universal Transport Medium (UTM) (Copan 306C, CA USA). Mock positive samples had varying dilutions of stock gamma-irradiated virus added to each sample pool prior

to aliquoting into individual replicates. Mock negative samples had no addition of gamma-irradiated virus added.

After preparation and assembly of the test panel samples, aliquoting of 3 identical copies in 1.5ml tubes (Cat # 2231-50, SSI bio), numbered 1-18 to 101-353 was done at the Doherty Institute. All three panels were then frozen at -80°C.

One test panel was provided to Laboratory 3 and then thawed for testing. A second panel was thawed for testing at Laboratory 1. The third panel was shipped frozen to Laboratory 2. The panel was verified received in good condition, and was subsequently thawed for testing.

2.3 Test Methods

2.3.1 Doherty Institute

2.3.1.1 Nucleic Acid Extraction and Reverse transcription

The validation panel samples for SARS-CoV-2 RNA detection were extracted using a QIAamp 96 DNA QIAcube HT kit (Ref 51331, Lot No. 166014792 Qiagen, Germany) on the QIAcube HT System (Qiagen, Germany) according to manufacturer's instructions. In brief, a 200uL volume of each sample was used for extraction and eluted in a 60uL volume of elution buffer into a 96-well elution tray. Reverse transcription of viral RNA to complimentary DNA (cDNA) was performed using Bioline SensiFAST cDNA synthesis kit (Cat. No. CSA-01148, Lot B081600 Bioline Reagents Ltd. UK) according to manufacturers instruction. In brief, a 10uL

volume of eluted nucleic acid was added to 10uL of SensiFAST cDNA reaction mix, heat linearized at 25˚C for 10 minutes then incubated at 42˚C for 15 minutes before denaturation at 85 ˚C for 5 minutes on an Applied Biosystems SimpliAmp thermal cycler (Thermo Fisher Scientific, Singapore).

2.3.1.2 SARS-CoV-2 real-time PCR

An in-house RT-PCR TaqMan assay targeting the SARS-CoV-2 RdRP gene was used. Primers and probe sequences targeting the RdRP gene have been previously described by Caly et al.[2] Briefly, 3ul of cDNA was added to a commercial real-time PCR master mix (Ref. z-PFAST-LR-10ml, Lot JN-00037-0007 PrecisionFast qPCR Master Mix with LowRox, Primer Design Ltd.,UK) in a 20ul reaction mix containing primers and probe with a final concentration of 0.9µM and 0.2µM for primer and the probe, respectively. An in-house positive extraction control, a negative control and a positive control were included with each PCR run. Thermal cycling and real-time PCR analysis for all assays were performed on an ABI 7500 FAST real-time PCR system (Applied Biosystems, Foster City, CA) with the thermal cycling profile: 95°C for 2 min, followed by 45 PCR cycles of 95°C for 5 sec and 60°C for 25 sec. Results were interpreted individually per run of 96 samples. A positive result was interpreted from uniform S-shaped amplification profiles where the threshold line was set to intersect with the mid-point of the linear amplification phase of the amplification-curve. Any result up to Ct of 40 is considered positive. Cycle thresholds of 40-45 are examined with further testing.

2.3.2 Laboratory 2

2.3.2.1 Nucleic Acid Extraction

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The validation panel samples for SARS-CoV-2 were extracted using a MGIEasy Magnetic Beads Virus DNA/RNA Extraction Kit (MGI Tech Co., Ltd., China) (Ref 1000021043, Lot No. H0056) on the MGISP-960 high-throughput automated extraction and liquid handling workstation (MGI Tech Co., Ltd., China) according to manufacturer's Instructions For Use (IFU) [3]. In brief, 180uL of each sample was used for extraction and eluted in 30uL of elution buffer into a 96-well elution tray.

2.3.2.2 SARS-CoV-2 real-time PCR

A BGI Real-time fluorescent RT-PCR kit for detecting SARS-CoV-2 was used (Lot 6220200425) according to the manufacturer's IFU[4]. This assay uses primers and probe sequences targeting the ORF1ab gene. An internal control (Human β-actin), a negative control and a positive control were included with each PCR run. 10ul of nucleic acid extract was added to the PCR mix. Thermal cycling and real-time PCR analysis were performed on the Bioer LineGene 9600 real-time PCR detection system (Bioer Technology, China) with the thermal cycling profile: 50°C for 20 min, 95°C for 10 min, followed by 40 PCR cycles of 95°C for 15 sec and 60°C for 30 sec. Results were interpreted individually per run of 96 samples. A positive result was interpreted from uniform S-shape amplification profiles with the Ct value <38. A negative result is required to have a satisfactory Human β-actin Ct of ≤32. Results with unsatisfactory Human β-actin amplification are to be re-tested unless a uniform S-shape amplification profile of the SARS-CoV-2 target with the Ct value ≤38 is present.

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2.3.3 Laboratory 3

2.3.3.1 Nucleic Acid Extraction

The Validation panel samples for SARS-CoV-2 were extracted using a MGIEasy Magnetic Beads Virus DNA/RNA Extraction Kit (MGI Tech Co., Ltd., China). Two extraction kit lot numbers (Ref 1000021043, Lot No.s H0058 and H0059) were used for 90 samples from the panel that had duplicate samples, so that 45 samples were extracted using both lot numbers. The remaining validation panel was extracted using kit lot No H0059. Extraction was performed on the MGISP-960 high-throughput automated extraction and liquid handling workstation (MGI Tech Co., Ltd., China) according to manufacturer's IFU[3].

2.3.3.2 SARS-CoV-2 real-time PCR

The above mentioned BGI Real-time fluorescent RT-PCR kit for detecting SARS-CoV-2 Lot # 6220200318 and #62220200319 was used according to the manufacturer's IFU[4]. An additional negative (PBS) and positive (γ -irradiated SARS-CoV-2 VIDRL isolate diluted 1/1,000) extraction controls were also included with each PCR run. A 10ul volume of nucleic acid extract was added to the PCR mix. Thermal cycling and real-time PCR analysis were performed on the BGI SLAN-96S real-time PCR system (BGI, China) with the above mentioned thermal cycling profile. Results were interpreted individually per run of 96 samples. Results were interpreted individually per run of 96 samples. A positive result was interpreted from uniform S-shape amplification profiles with the Ct value ≥38. A negative result is required to have a satisfactory Human β-actin Ct of ≤ 32. Results with unsatisfactory Human β-actin amplification are to be re-tested unless a uniform S-shape amplification profile of the SARS-CoV-2 target with the Ct value ≤ 38 is present.

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2.3.4 Digital Droplet PCR for Absolute Quantification of SARS-CoV-2, Doherty Institute

A 4-fold dilution series (1/4000 to 1/262,144,000) of spiked gamma irradiated SARS-CoV-2 was prepared in 3 different sample matrices as described above (i.e. VTM, 10% saliva diluted in VTM and negative human nose and throat swabs in commercial transport media). For each dilution point two separate extractions was performed using the QIAamp 96 DNA QIAcube HT kit on the QIAcube HT system. The purified nucleic acid was then converted to cDNA as described above. Prior to performing digital droplet PCR (ddPCR) the cDNA samples were first tested in an in-house qualitative assay, using targets RdRP gene[2] and N gene[1], to determine the sample's relative viral load through the cycle threshold (Ct) measure for each gene target. PCR amplification was carried out with the same thermal cycling profile as the in-house RdRP RT-PCR assay.

For ddPCR, primers and probe from the qualitative N gene RT-PCR was used to prepare a ddPCR Supermix (ddPCR Supermix for Probes, Cat. #1863024, Batch 64330928, Bio-Rad, Bio-Rad Laboratories. Inc, USA). The Supermix was prepared with the same concentration of primers and probe as the qualitative assay. 3µl of cDNA template was added to the Supermix for a final reaction volume of 25µl. Droplets were generated from this mixture on the QX200 Droplet Generator (Bio-Rad, USA) as per manufacturer's instructions and run on a thermal cycler (Thermo Scientific Arktik Thermal Cycler, Thermo Fisher Scientific) with thermal cycling conditions: 95˚C for 10 min, followed by 40 cycles of 95˚C for 30 sec and 60˚C for 1 min, 98˚C for 10 min and hold 4˚C. A QX200 Droplet Reader (Bio-Rad, USA) was used to take fluorescence readings. Each dilution point was tested in the ddPCR in quadruplicate (2 replicates for each of the 2 extracts per dilution point) and the mean quantity was calculated.

2.3.5 Statistical analysis

Data analysis was managed using GraphPad Prism (v7.05). Nonlinear regression, Gaussian distribution and One-Way ANOVA with Dunn's multiple comparisons, were performed as deployed in GraphPad Prism.

2.4 Validation Method

2.4.1 On-Site Validation Laboratory 3

Performance of the MGIEasy Magnetic Beads Virus DNA/RNA Extraction Kit on the MGISP-960 high-throughput automated extraction workstation, and BGI Real-time fluorescent RT-PCR SLAN 96-S RT-PCR machine (collectively referred to below as the 'BGI Platform') were compared head to head with the Doherty Institute QIAGEN QIAcube extraction robot and Applied Biosystems 7500 RT-PCR machine using Doherty Institute in-house RdRP gene RT-PCR.

The BGI platform and test were examined in situ at Laboratory 3. Protocols used for the BGI platform were the Laboratory 3 standard operating procedures (SOP) for this equipment, and assays were performed according to the manufacturer's IFU[3, 4].

Personnel were an experienced scientist from Doherty Institute's Virus Identification Laboratory, working collaboratively with an experienced Laboratory 3 Molecular scientist.

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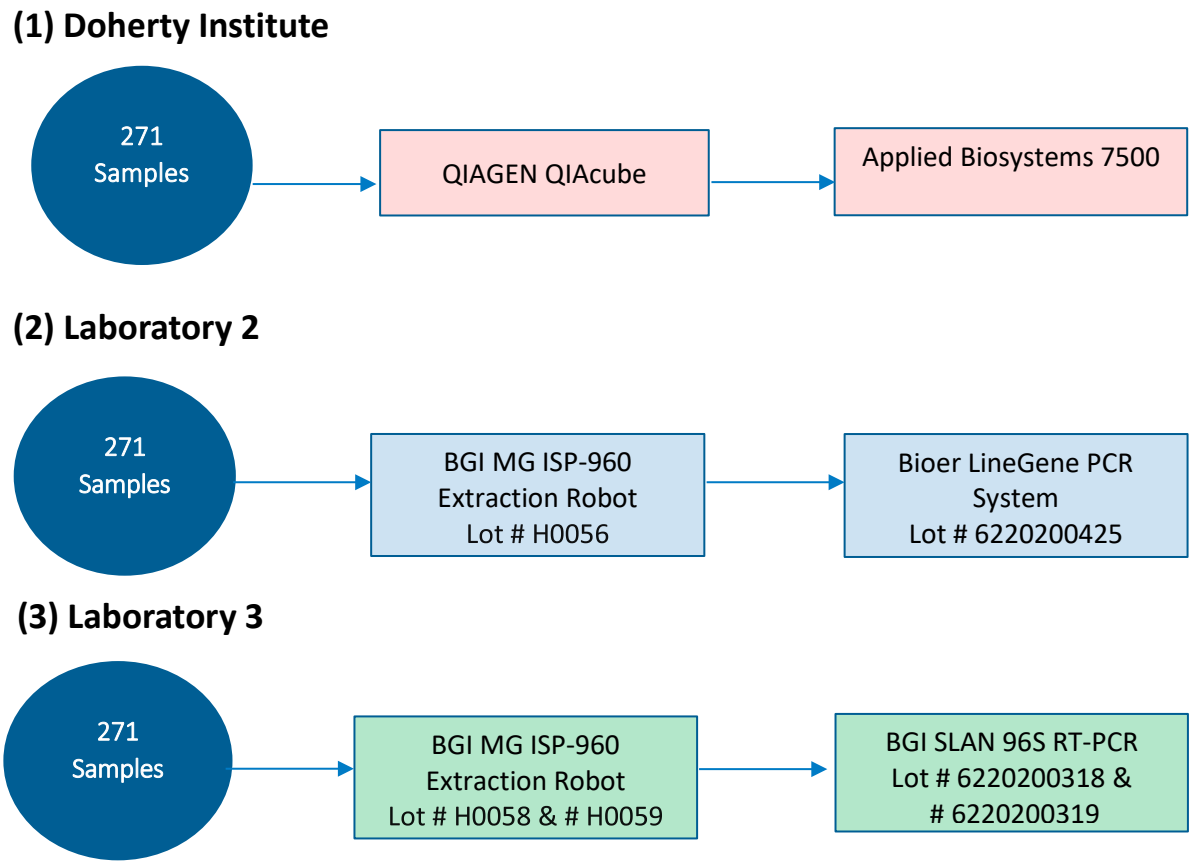
In the first exercise (Fig 1. Exercise 1) the whole BGI platform comprising MGISP-960 Extraction Robot, and SLAN 96-S RT-PCR machine with the Doherty Institute reference platform, in testing the entire 271 sample panel.

Figure 1. Exercise #1



2.4.2 Validation Panel Analysis Laboratory 2

The Validation panel described above was also shipped to Laboratory 2 as described above. Testing of the 271 sample panel (Fig 1. Exercise 1) at Laboratory 2 compared the performance of the BGI MGISP-960 extraction robot, and Bioer LineGene PCR System RT-PCR machine, with MGIEasy Magnetic Beads Virus DNA/RNA Extraction Kit and BGI Real-time fluorescent RT-PCR compared head to head with the Doherty Institute Reference platform and reagents.

Protocols were the Laboratory 2 Standard Operating Procedures (SOP), and assays were performed according to the manufacturer's IFU[3,4]. Personnel were Laboratory 2 Senior Molecular Scientific Staff.

2.4.3 Sub-study Laboratory 3

An additional sub-study was done as part of the Laboratory 3 on-site evaluation using 90 selected samples from within the 271 sample panel. The 90 samples comprised two duplicate sets of 45 samples, each made up of 15 positive and 30 negative samples, as described above in Section 2.1. These samples were used to make the following comparisons:

- Firstly (Fig 2. Laboratory 3 Sub-study) RT-PCR was compared between the BGI Real-time fluorescent RT-PCR kit using the SLAN 96-S RT-PCR analyser, and the Doherty Institute ABI 7500 RdRP in-house RT-PCR assay.

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- Secondly (Fig 2. Laboratory 3 Sub-study) nucleic acid extraction using the BGI MGISP-960 high-throughput automated extraction workstation was compared to nucleic acid extraction using the QIAGEN QIAcube.

- Thirdly (Fig 2. Laboratory 3 Sub-study) performance was compared for two different lot numbers of the MGIEasy Magnetic Beads Virus DNA/RNA Extraction Kit (#H0058, and #H0059), and two different lot numbers of the BGI Real-time fluorescent RT-PCR kit for detecting SARS-CoV-2 (#6220200318, and #6220200319).

To execute these studies separate copies of the 90 sample panel underwent nucleic acid extraction at the Doherty Institute using the QIAGEN QIAcube, and at Laboratory 3 using the BGI MGISP-960 high-throughput automated extraction workstation. The resulting eluates were split into two aliquots and frozen at -80C. Each aliquot was subsequently tested, one at Laboratory 3 using the BGI Real-time fluorescent RT-PCR kit and SLAN 96-S RT-PCR machine protocol, and the other underwent RT-PCR analysis using the Doherty Institute ABI 7500 RdRP in-house RT-PCR assay.

At Laboratory 3 45 samples were extracted using MGIEasy Magnetic Beads Virus DNA/RNA Extraction Kit Lot #H0058, and the other 45 duplicate samples using Lot #H0059. In turn 45 extraction eluates underwent RT-PCR at Laboratory 3 using the BGI Real-time fluorescent RT-PCR kit for detecting SARS-CoV-2 Lot #6220200318, and 45 duplicate extraction eluates underwent RT-PCR using Lot #6220200319. Equipment, SOP, assay performance, and personnel were as described above.

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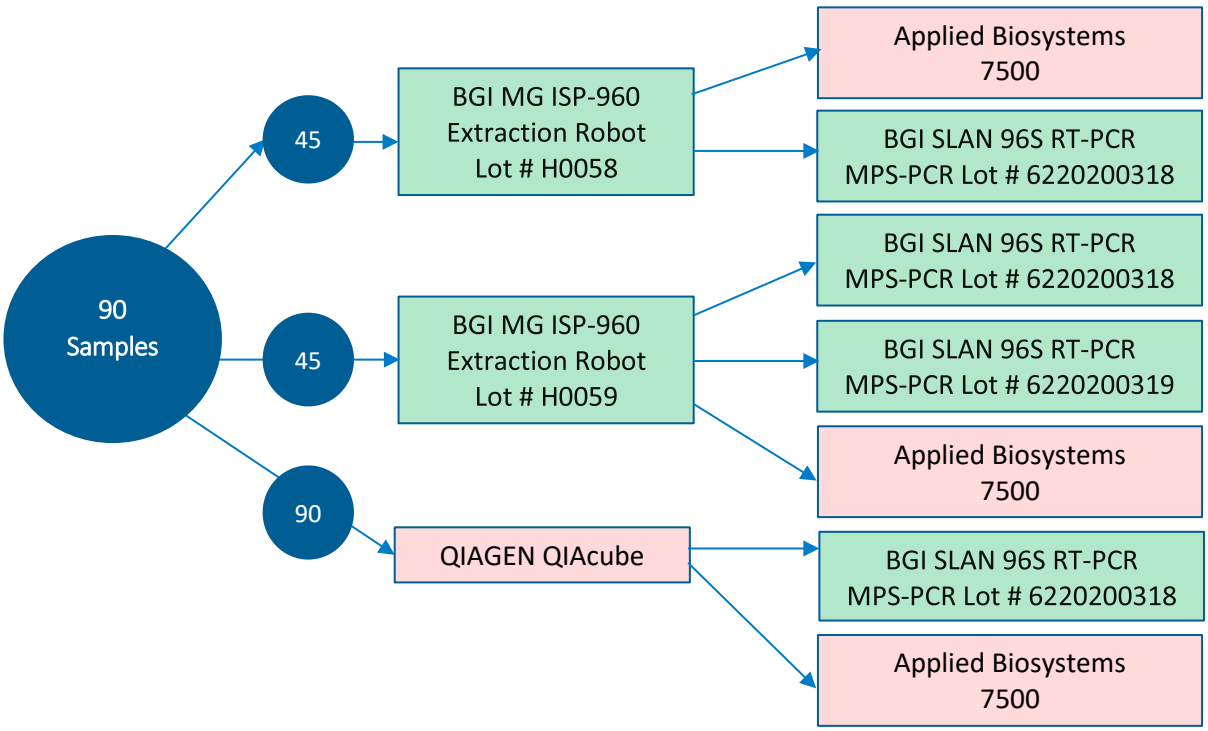
Figure 2. Exercise #2

Laboratory 3: Sub-study



The sub-analysis described above in which the performance of BGI extraction robot, and BGI RT-PCR analyser were separately examined at Laboratory 3 was not attempted with Laboratory 2. It was considered not feasible to ship interstate the extracted eluates from the nucleic acid extraction robots of the Doherty Institute and Laboratory 2 due to the fragility of extracted viral RNA. The robustness of results of any RT-PCR analysis of RNA extracts after such shipping could not be relied on.

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3. Results

3.1 Limits-of-Detection

A 4-fold dilution series of virus in VTM starting at 1/4000 dilution of stock virus, as described above was used to determine LoD for each of the three laboratories.

The LoD for each laboratory's testing platform was defined as the highest dilution at which all four replicate samples were detected. Laboratory 1, Laboratory 2 and Laboratory 3 had LoD of 1/16,384,000, 1/65,536,000 and 1/65,536,000, respectively (Table 1). It is worth noting that only 3 out of 4 replicates were detected at 1/16,384,000 for Laboratory 2 but since all replicates were detected at the next dilution the latter was scored as the LoD.

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Table 1. Limit-of-detection of SARS-CoV-2 for each laboratory testing platform was determined by testing serial dilutions of a stock virus.

	Laboratory 1[a]		**Laboratory 2[b]**		**Laboratory 3[c]**	
Sample dilution[1]	Detections/ replicates	Ct value Mean[2] (SD[3])	Detections/ replicates	Ct value Mean[2] (SD[3])	Detections/ replicates	Ct value (Mean[2]/SD[3])
1/4,000	4/4	25.0 (0.9)	4/4	21.8 (0.1)	4/4	21.4 (0.1)
1/16,000	4/4	28.4 (0.9)	4/4	24.5 (0.1)	4/4	23.8 (0.3)
1/64,000	4/4	29.7 (0.8)	4/4	26.5 (0.2)	4/4	25.8 (0.4)
1/256,000	4/4	32.2 (0.4)	4/4	28.5 (0.1)	4/4	27.9 (0.3)
1/1,024,000	4/4	33.8 (0.3)	4/4	30.7 (0.4)	4/4	29.9 (0.4)
1/4,096,000	4/4	36.4 (0.4)	4/4	32.7 (0.1)	4/4	32.2 (0.5)
1/16,384,000	**4/4**	**37.8 (1.1)**	3/4	34.3[#] (0.7[δ])	4/4	34.3 (0.4)
1/65,536,000	0/4	N/A	**4/4**	**36.5 (0.2)**	**4/4**	**35.6 (0.7)**
1/262,144,000	1/4	40.2[*]	3/4	38.0[#] (0.7 [δ])	3/4	36.7[#] (0.7 [δ])

[a] Doherty Institute. [b] Laboratory 2. [c] Laboratory 3. [1] Diluted samples were from the VTM matrix. [2] Mean Ct is based on Ct values of 4 replicate samples. SD[3] Standard deviation. [*] Ct value based on a single detection. [#] Ct value based on the mean of 3 detected replicates. [δ] Standard deviation from Ct values of 3 detections. N/A Not applicable. Grey shaded boxes indicate the LOD dilution point for each laboratory.

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3.2 Operational Sensitivity

Sensitivity of the testing platform used in each laboratory was assessed on 271 samples. These were made up of 156 spiked positive samples and 115 true negative samples. The test sensitivity of Laboratory 1, 2 and 3 was demonstrated to be 94.2%, 98.1% and 99.4%, respectively (Table 2).

Table 2. Detection of positive SARS-CoV-2 spiked material (n=156)

	Laboratory 1[a]	**Laboratory 2[b]**	**Laboratory 3[b]**
False negative	9/156	3/156	1/156
Positivity rate	94.2%	98.1%	99.4%

[a] Doherty Institute. [b] Laboratory 2. [c] Laboratory 3.
Sensitivity was calculated from the number of false negative results within the positive spiked material of sample size 156.

3.3 Specificity

The specificity of each laboratory's testing platform was established using all 115 negative SARS-CoV-2 samples (Appendix 1). Specificity was determined to be 100%, 99.1% and 97.5% for Laboratory 1, laboratory 2 and Laboratory 3, respectively (Table 4). Specificity was further evaluated on a subset of 24 samples (9 VTM samples and 15 samples of 10% saliva in VTM) for test cross-reactivity to other common respiratory virus agents (Table 5). These included samples spiked with parvovirus B19, parainfluenza virus 1-3,

Post-market validation of the Beijing Genomics Institute (BGI) SARS-CoV-2 Real Time PCR platform continued

coronavirus OC43, coronavirus 229E, rhinovirus, respiratory syncytial virus, adenovirus, influenza virus A and B, human metapneumovirus, Coxsackie virus and measles virus. All samples were negative for SARS-CoV-2 by Laboratory 1. For Laboratory 2, sample 253 (influenza virus A in saliva matrix) and sample 260 (coronavirus-229E in saliva matrix), were excluded from analysis after returning invalid results (internal reference target Ct>32 or not detected). The remaining 22 samples were negative for SARS-CoV-2 by Laboratory 2. For Laboratory 3, sample 259 (coronavirus-OC43 in saliva matrix) and sample 260 (coronavirus-229E in saliva matrix) were excluded from analysis due to their internal reference target having a Ct>32. The remaining 22 samples were negative for SARS-CoV-2 by Laboratory 3. Of note were 4 samples from laboratory 3: sample 236 (parainfluenza type-1 in saliva matrix), sample 246 (adenovirus type-5 in saliva matrix, sample 181 (rhinovirus in VTM matrix) and sample 191 (influenza virus A in VTM matrix) that generated a Ct value above 38 for SARS-CoV-2. These were interpreted as not SARS-CoV-2 positive as per manufacturer's IFU.

Post-market validation of the Beijing Genomics Institute (BGI) SARS-CoV-2 Real Time PCR platform continued

Table 4. Summary of specificity of each laboratory testing platform for the detection of SARS-CoV-2 (n=115).

	Laboratory 1[a]	Laboratory 2[b]	Laboratory 3[c]
False positives	0/115	1/115	3/115
Specificity	100%	99.1%	97.5%

[a] Doherty Institute. [b] Laboratory 2. [c] Laboratory 3.
Specificity rate was calculated from the number of negative detections divided by the 115 total true negative sample size.

Table 5. Results of samples matrices containing non-SARS-CoV-2 respiratory virus agents.

Sample #	Matrix	Spiked respiratory virus	Laboratory 1	Laboratory 2	Laboratory 3
169	10% saliva sample	Parvovirus	ND	ND	ND
236	10% saliva sample	Parainfluenza-1	ND	ND	ND[#]
239	10% saliva sample	Parainfluenza-2	ND	ND	ND
240	10% saliva sample	Parainfluenza-3	ND	ND	ND
242	10% saliva sample	CoV-OC43	ND	ND	ND
243	10% saliva sample	Rhinovirus	ND	ND	ND
244	10% saliva sample	RSV	ND	ND	ND
246	10% saliva sample	Adenovirus type-5	ND	ND	ND[#]
253	10% saliva sample	Influenza virus A	ND	ND*	ND
254	10% saliva sample	Influenza virus B	ND	ND	ND

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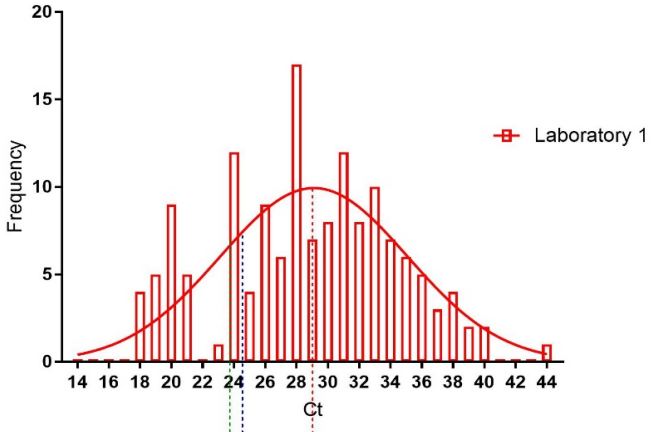
255	10% saliva sample	Human metapneumovirus	ND	ND	ND
256	10% saliva sample	CA16	ND	ND	ND
257	10% saliva sample	Measles virus	ND	ND	ND
259	10% saliva sample	CoV-OC43	ND	ND	ND**
260	10% saliva sample	CoV-229E	ND	ND**	ND**
131	VTM	CoV-229E	ND	ND	ND
161	VTM	Rhinovirus	ND	ND	ND
175	VTM	CoV-229E	ND	ND	ND
181	VTM	Rhinovirus	ND	ND	ND#
191	VTM	Influenza virus A	ND	ND	ND#
225	VTM	Influenza virus A	ND	ND	ND
5	VTM	Influenza virus A	ND	ND	ND
9	VTM	CoV-229E	ND	ND	ND
15	VTM	Rhinovirus	ND	ND	ND

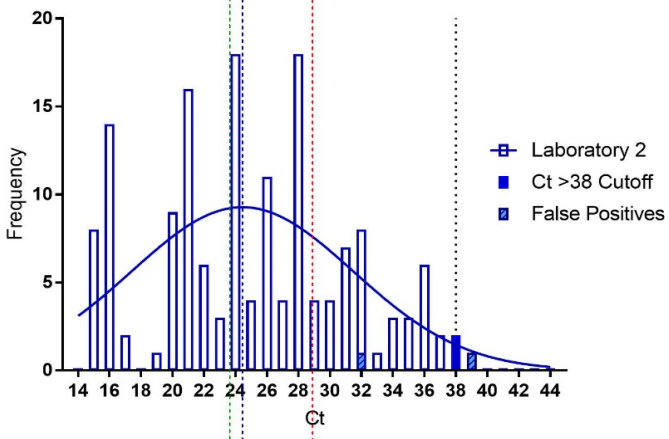
CoV, coronavirus; RSV, respiratory syncytial virus; CA16, Coxsackievirus A16; ND not detected; # SARS-CoV-2 Ct>38 is considered not detected, * Internal reference target not detected, ** Internal reference target Ct>32

Post-market validation of the Beijing Genomics Institute (BGI) SARS-CoV-2 Real Time PCR platform continued

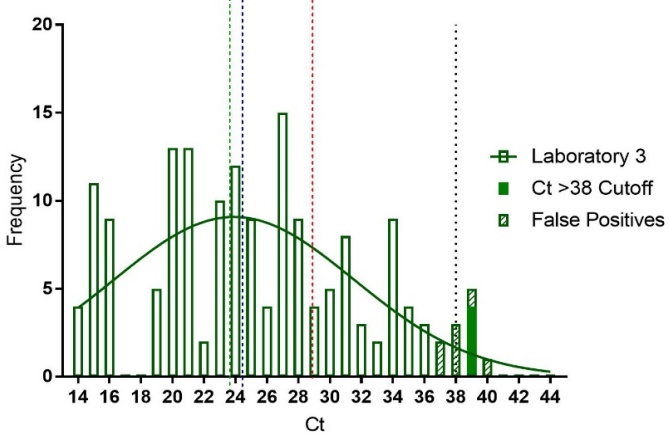
(a)



(b)



(c)



Post-market validation of the Beijing Genomics Institute (BGI) SARS-CoV-2 Real Time PCR platform continued

Figure 3: Gaussian distribution of Ct frequency for (a) Laboratory 1 (29.10 ±5.98), (b) Laboratory 2 (24.44 ±7.05) and (c) Laboratory 3 (23.90 ±7.63). Values represented are mean ±SD. Outliers are represented as solid fill histograms for Ct values greater than 38 for BGI workflows and false positives represented as hatched histograms. These outliers were excluded in the distribution analysis.

3.4 Cycle threshold (Ct) value frequency distribution

During specificity data analysis it was noted that the BGI platform gave Ct values for a number of negative samples, both samples containing no target and samples containing other respiratory viruses. In line with the manufacturers IFU these did not meet the criteria for positivity, sometimes due to invalid values for the internal reference control (≥32), and more frequently because the IFU states that Ct ≥38 require retest. This was more commonly seen in data from Laboratory 3. Hence the overall specificity values achieved by the BGI-users, laboratory 2 & 3 were high after removal of invalid samples from analysis.

The distribution of Ct values produced by each of the three participating laboratories was calculated and the relative frequency displayed as a Gaussian distribution for each data sets (Fig 3 a-c). Outliers are represented as solid fill histograms for Ct values greater than 38, which are not considered positive until retesting is completed consistent with the IFU. True false positives are represented as hatched histograms at their respective Ct values. These latter outliers were excluded in the Gaussian distribution analysis. Differences were noted. Frequency data for Laboratory 1 had a mean Ct of 29.10 and a tighter standard deviation of ±5.98, when compared to both BGI workflows at Laboratory 2 and Laboratory 3. The mean values for

Laboratory 2 and Laboratory 3 were a mean and standard deviation of 24.44 ±7.05 and 23.90 ±7.63, respectively.

As well as the greater spread of Ct values from BGI-users Laboratory 2 and 3, there was an increased frequency of Ct values at 38 and above; most commonly in data from Laboratory 3 (Fig 3 c) where there was a small but distinct peak.

3.5 Comparison of Lot Numbers

3.5.1 Comparing Sensitivity of Nucleic Acid Extraction kits and RT-PCR kits.

Nucleic acid was extracted from 45 samples (30 positive spiked material samples and 15 true negative samples using the MGIEasy Magnetic Beads Virus DNA/RNA Extraction Kit. Two different lot numbers (#H0058 and #H0059) at Laboratory 3 were used in this exercise to assess the performance of the BGI's extraction efficiency. The sensitivity was 96.7% and 100% for Lot number #H0058 and #H0059, respectively, when the same BGI SLAN 96S RT-PCR kit (lot #6220200318) was used (Table 6). When the same MGIEasy Magnetic Beads Virus DNA/RNA Extraction Kit was used the sensitivity was 100% for both BGI SLAN 96S RT-PCR kit numbers. The same 45 samples and an additional 45 samples (90 total) were extracted at Laboratory 1 using the QIAamp 96 DNA QIAcube HT Kit on the QIAcube HT robotic and tested in Laboratory 3 with the same BGI RT-PCR kit above. This combination yielded a sensitivity of 95% (Table 7). The same analysis was performed at Laboratory 1 across the 3 separate extractions as described above. The in-house RT-PCR detection method was consistently used at Laboratory 1. The sensitivity was determined to be 93.3% for BGI

extraction (lot #H0058), 90.0% for BGI extraction (lot #H0059) and 88.3% for the Qiagen extraction (Table 6-7).

3.5.2 Comparing Specificity of Nucleic Acid Extraction kits and RT-PCR kits.

Forty-five samples were extracted for nucleic acid using the MGIEasy Magnetic Beads Virus DNA/RNA Extraction Kit. The specificity was 93.8% for Lot number #H0058 and 100% for Lot number #H0059 when the same BGI SLAN 96S RT-PCR kit (lot #6220200318) was used (Table 8). When the same MGIEasy Magnetic Beads Virus DNA/RNA Extraction Kit was used the specificity was 93.8% and 100% for BGI SLAN 96S RT-PCR kit Lot number #6220200319 and #6220200318, respectively.

The same 45 samples and an additional 45 samples (90 total) were extracted at Laboratory 1 using the QIAamp 96 DNA QIAcube HT Kit on the QIAcube HT robotic and tested in Laboratory 3 with the same BGI RT-PCR kit above. This combination yielded a specificity of 93.8% (Table 9). The same analysis was performed at Laboratory 1 across the 3 separate extractions as described above. The in-house RT-PCR detection method was consistently used at Laboratory 1. The specificity was determined to be 100% for all extraction methods assessed (Table 8-9).

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3.5.3 Comparative Sensitivity and Specificity of Nucleic Acid Extraction Methods on the ABI 7500 real-time PCR System.

The sensitivity for Laboratory 1 using the MGIEasy Magnetic Beads Virus DNA/RNA Extraction Kit on the MGISP-960 extraction robot was 93.3%/90.0% compared to 88.3% for the Qiagen QIAamp 96 DNA QIAcube HT Kit with the Qiagen QIAcube HT System. The specificity was 100% irrespective of the nucleic extraction kit/platform used.

3.5.4 Comparative Sensitivity and Specificity of Nucleic Acid Extraction Methods on the BGI SLAN-96S real-time PCR system.

The sensitivity for Laboratory 3 using the MGIEasy Magnetic Beads Virus DNA/RNA Extraction Kit on the MGISP-960 extraction robot was 96.7%/100%/100% compared to 95.0% for the Qiagen QIAamp 96 DNA QIAcube HT Kit with the Qiagen QIAcube HT System. The specificity using the MGIEasy Magnetic Beads Virus DNA/RNA Extraction Kit on the MGISP-960 extraction robot was 93.8%/93.8%/100% compared to 93.8% for the Qiagen QIAamp 96 DNA QIAcube HT Kit with the Qiagen QIAcube HT System

Table 6. Detection of positive spiked material with MGIEasy Magnetic Beads Virus DNA/RNA Extraction Kit (n=30)

	Laboratory 1 #1	Laboratory 1 #2	Laboratory 3 #1	Laboratory 3 #2	Laboratory 3 #3
False Negatives	2	3	1	0	0
Sensitivity (%)	93.3	90.0	96.7	100	100

True positive rate defined by number of false negatives within the 30 sample size. Laboratory 1 #1 used BGI Extraction Lot# H0058 and Laboratory 1 #2 used BGI Extraction Lot# H0059. Laboratory 3 #1 RT-PCR Lot# 6220200318, Laboratory 3 #2 RT-PCR Lot # 6220200319 and Laboratory 3 #3 RT-PCR Lot# 6220200318.

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Table 7. Detection of positive spiked material with Qiagen QIAamp 96 DNA QIAcube HT Kit (n=60)

	Laboratory 1	Laboratory 3
False Negatives	7	3
Sensitivity (%)	88.3	95.0

True positive rate defined by number of false negatives within the 60 sample size. Laboratory 3 RT-PCR Lot# 6220200318.

Table 8. Specificity of detection across laboratories using MGIEasy Magnetic Beads Virus DNA/RNA Extraction Kit (n=15)

	Laboratory 1 #1	Laboratory 1 #2	Laboratory 3 #1	Laboratory 3 #2	Laboratory 3 #3
False Positives	0	0	1	1	0
Specificity (%)	100	100	93.8	93.8	100

False positive rate defined by number of false positives within the 15 sample size. Laboratory 1 #1 used BGI Extraction Lot# H0058 and Laboratory 1 #2 used BGI Extraction Lot# H0059. Laboratory 3 #1 RT-PCR Lot# 6220200318, Laboratory 3 #2 RT-PCR Lot # 6220200319 and Laboratory 3 #3 RT-PCR Lot# 6220200318.

Table 9. Specificity of detection across laboratories using Qiagen QIAamp 96 DNA QIAcube HT Kit (n=30)

	Laboratory 1	Laboratory 3
False Positives	0	2
Specificity (%)	100.0	93.8

False positive rate defined by number of false positives within the 30 sample size. Laboratory 3 RT-PCR Lot# 6220200318.

3.6 One-way ANOVA (Dunn's multiple comparisons)

A One-way ANOVA with Dunn's multiple comparisons was analysed for all variables, including extraction methods and RT-PCR reagents for both Laboratory 1 and Laboratory 3. A standard curve for each specimen type of VTM and swabs in UTM was produced via Droplet Digital PCR (ddPCR) using in-house N-gene probe

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and primers to generate absolute Ct values, as described in the Methods section. The standard curves were as follows for VTM and swabs, respectively, where x is copies/mL and y is Ct values:

$$y = -3.367 \times 10^{-6}x + 32.08$$

$$y = -2.040 \times 10^{-6}x + 31.54$$

When each variable was compared to the above standard curves and absolute Ct values, there was a significant difference in Ct values of BGI extracted material for both Laboratory 3 BGI SLAN 96S RT-PCR kit Lot numbers, when swabs in UTM was analysed. For Lot number #6220200319 the calculated p-value was 0.0022 and Lot number #6220200318 the calculated p-value of 0.0271 (Table 10). There was no significant variation when VTM specimens were used across both extraction methods and RT-PCR instruments/reagents for either Laboratory (data not shown).

Both specimen types showed significant Ct variation within replicates when BGI Virus DNA/RNA Extraction Kit was used for nucleic acid extraction at Laboratory 3, and then analysed in Laboratory 1 using the in-house RdRP RT-PCR. This significant difference was further pronounced when MGIEasy Magnetic Beads Virus DNA/RNA Extraction Kit Lot number #H0059 was used for nucleic acid extraction, and again when swabs in UTM was analysed. The VTM specimens also showed Ct variation within replicates for the same workflow as described above, however the variation was less pronounced in comparison to the swab specimens in UTM. Significant p-values are shown in grey area within Table 10 and Table 11.

Table 10. One-way ANOVA (Dunn's multiple comparisons) for swabs in UTM specimens

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	Statistical Significance	Adjusted p-Value
Standard Curve vs. BGI Extraction + Laboratory 3 #2	**	0.0022
Standard Curve vs. BGI Extraction + Laboratory 3 #3	*	0.0271
BGI Extraction: Laboratory 1 #1 vs. Laboratory 3 #2	**	0.0058
BGI Extraction: Laboratory 1 #2 vs. Laboratory 3 #2	**	0.0028
BGI Extraction: Laboratory 1 #2 vs. Laboratory 3 #3	*	0.0334

Laboratory 1 #1 used BGI Extraction Lot# H0058 and Laboratory 1 #2 used BGI Extraction Lot# H0059. Laboratory 3 #1 RT-PCR Lot# 6220200318, Laboratory 3 #2 RT-PCR Lot # 6220200319 and Laboratory 3 #3 RT-PCR Lot# 6220200318.

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Table 11. One-way ANOVA (Dunn's multiple comparisons) for VTM specimens

	Statistical Significance	Adjusted p-Value
BGI Extraction: Laboratory 1 #2 vs. Laboratory 3 #2	*	0.0419
BGI Extraction: Laboratory 1 #2 vs. Laboratory 3 #3	*	0.0299

Laboratory 1 #1 used BGI Extraction Lot# H0058 and Laboratory 1 #2 used BGI Extraction Lot# H0059. Laboratory 3 #1 RT-PCR Lot# 6220200318, Laboratory 3 #2 RT-PCR Lot # 6220200319 and Laboratory 3 #3 RT-PCR Lot# 6220200318.

4. Discussion

A 271 sample validation panel comprising 156 positive and 115 negative samples was used to assess the Beijing Genomics institute (BGI) MGISP-960 robotic nucleic extraction instrument, paired with either the SLAN 96S real-time PCR analyser (Laboratory 3), or Bioer LineGene 9600 PCR analyser (Laboratory 2), and using the MGIEasy Magnetic Beads Virus DNA/RNA Extraction Kit (Ref 1000021043), and BGI Real-time fluorescent RT-PCR kit for detecting 2019-nCoV (Ref MFG030010): 'the BGI platform'.

The Limit of Detection (LoD) for the BGI platform was found to be a four-fold dilution more sensitive at both participating BGI-user laboratories than the Doherty Institute in-house RT-PCR, although one BGI-user laboratory did fail to detect one sample replicate at a lower dilution. Had the definition of LoD been rigidly applied this would have been scored as a less sensitive LoD. More input RNA is loaded into the BGI RT-PCR

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assay than the reference method (10µl compared to 1.5µl), and this may contribute significantly to the observed relative LoD.

Operational Sensitivity of the BGI platform was also found to be high. Sensitivity was 98.1% (Laboratory 2) and 99.4% (Laboratory 3) for the BGI platform compared to 94.2% for the reference method (Laboratory 1), consistent with the observed difference in LoD described above. Overall Operational Specificity was determined to be 99.1% (Laboratory 2) and 97.5% (Laboratory 3) compared to 100% for the reference method (Laboratory 1). Hence the most sensitive test was the least specific, and vice-versa, although differences were small. Specificities of 100% were also demonstrated for both users of the BGI platform in testing the sub-group of 24 samples containing other respiratory viruses, but only after spurious reactivity in several samples containing other respiratory viruses were excluded from analysis, by either invalid results in the test internal control, or recorded Ct values of >38, which according to the IFU may not be reported as positive without retesting. Results of retesting were not studied in this exercise. In addition it should be noted that such high Operational Specificity is less likely to have been achieved had this part of the study been done predominantly using one (#6220200319) of the two lot numbers of RT-PCR reagents which were subsequently compared in the sub-study done at Laboratory 3 (see below).

Examination of the frequency distribution of Ct values recorded by each laboratory revealed differences in the spread of values. The BGI platform data shows a greater spread of Ct values, with higher standard deviations about the mean (+/-7.05, Laboratory 2 and +/-7.63, Laboratory 3) than the reference method (+/-5.98, Laboratory 1). Potentially of greater interest was a small but distinct secondary peak of Ct values

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beginning at Ct 38, present in data from Laboratory 3, and to a lesser extent from Laboratory 2. Retesting of such samples showing Ct >38 is required by the IFU[4]. Although consistent with the IFU, simply retesting such samples using the BGI platform may run the risk that Ct >38 are obtained on re-test, and this might contribute to false positive results if reported. However retesting such samples with a different test, which employs a different gene target would be recommended practice, both consistent with PHLN guidelines for SARS-COV-2 laboratory diagnosis[5], and still consistent with the wording of the IFU. Reflexing to a second test potentially mitigates possible contribution of high Ct results to falsely positive test results, but it still represents potentially onerous levels of retesting if numbers of such high Ct results recorded by Laboratory 3 are representative.

It is not possible to draw conclusions regarding the difference in proportion of samples with Ct>38 observed between the two BGI-user laboratories Laboratory 2 and Laboratory 3, since extraction kit and RT-PCR kit lot numbers, RT-PCR analyser, and personnel among other variables all differ between the two laboratories.

Relative sensitivity of lot numbers of the MGIEasy Magnetic Beads Virus DNA/RNA Extraction Kit used at Laboratory 3 did vary a little between 96.7% (#H0058) and 100% (#H0059) when compared using the same Lot number (#6220200318) of the BGI Real-time fluorescent RT-PCR kit for detecting 2019-nCoV. However the opposite trend in sensitivity was observed when these same two MGIEasy Magnetic Beads Virus DNA/RNA Extraction Kit Lot numbers were used with the reference RT-PCR method in Laboratory 1 (#H0058, 93.3%, and #H0059, 90%). Hence this variation is of doubtful significance. Use of the reference QIAamp

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QIAcube HT kit with BGI Real-time fluorescent RT-PCR kit (Lot #6220200318) for detecting 2019-nCoV yielded a sensitivity of 95% by way of comparison.

Specificity of lot numbers #H0058 and #H0059 of the MGIEasy Magnnetic Beads Virus DNA/RNA Extraction Kit used at Laboratory 3 did not vary; both being 93.8% when the same Lot number (#6220200318) of the BGI Real-time fluorescent RT-PCR kit for detecting 2019-nCoV was used. Use of the reference QIAamp QIAcube HT kit with the same Lot number of the BGI Real-time fluorescent RT-PCR kit (Lot #6220200318) for detecting 2019-nCoV yielded the same sensitivity of 93.8%. However specificity was found to be 100% using either of the two MGIEasy Magnetic Beads Virus DNA/RNA Extraction Kit Lot numbers #H0058 and #H0059 together with the reference RT-PCR method in Laboratory 1. One hundred percent versus 93.8% was a bigger difference in specificity within this sub-study than was observed when the entire BGI-platform was compared to the reference method (see above) and high values of 99.1% and 97.5% were obtained for Operational Specificity by the BGI-users.

Sensitivity of the BGI Real-time fluorescent RT-PCR kit for detecting 2019-nCoV did not vary between lot numbers #6220200318 and #6220200319; both 100%, when the same lot number of the MGIEasy Magnetic Beads Virus DNA/RNA Extraction Kit was used (Lot #H0059). However specificity of different Lot numbers of the BGI Real-time fluorescent RT-PCR kit for detecting 2019-nCoV did vary considerably between 93.8% (#6220200319), and 100% (#6220200318) when the same lot number of the MGIEasy Magnetic Beads Virus DNA/RNA Extraction Kit was used (Lot #H0059). This may have contributed to the observed difference is specificity between the substudy described here, and the high specificities achieved with the whole 271

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sample panel, mostly done using lot numbers other than #6220200319 (i.e. predominantly with #6220200318).

The use of droplet digital PCR (ddPCR) provided objective quantification of selected samples from within the panel: 4-fold dilution series of cell culture-grown SARS-Cov-2 spiked into VTM, swabs, and 10% saliva diluted in VTM. This allowed statistical comparison of the degree of precision achieved in testing sample replicates using one-way Analysis of Variance (ANOVA) statistics. Overall the observed precision between methods was similar. However, statistically significant differences in precision were seen between reference ddPCR Ct values and BGI platform Ct values for swabs diluted in UTM, with both lot numbers of the BGI Real-time fluorescent RT-PCR kit for detecting 2019-nCoV Lot numbers (#6220200319, p=0.0022 and #6220200318, p=0.0027), using the same lot number of the MGIEasy Magnetic Beads Virus DNA/RNA Extraction Kit (Lot #H0059). This observation did not extend to other sample types. Both swabs in UTM, and VTM samples demonstrated significant variation within replicates when the MGIEasy Magnetic Beads Virus DNA/RNA Extraction Kit was used at Laboratory 3, and RT-PCR was subsequently done by the reference method at Laboratory 1. This was more pronounced with Lot #H0059.

In summary the BGI platform was shown to have a LoD, and Operational Sensitivity and Specificity that was comparable to the reference method using the 271 sample panel of simulated clinical samples of cell-culture grown SARS-CoV-2, and other respiratory viruses spiked into sample matrices. This was fairly consistently achieved in two BGI-user Laboratories. A potential tendency of the BGI RT-PCR assay to generate high Ct values of 38 and above for negative samples is mitigated with a threshold for retesting at Ct >38 specified in

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the IFU. Use of reflex testing to a second assay using a different gene target for this retesting would be consistent with Australian PHLN guidelines, and could mitigate any inherent risk of false positive reactivity.

One lot number of BGI Real-time fluorescent RT-PCR reagents gave noticeably lower specificity than another, and varied considerably from the overall Operational Specificity achieved when testing the entire 271 sample panel. Otherwise different lot numbers of MGIEasy Magnetic Beads Virus DNA/RNA Extraction Kit and BGI Real-time fluorescent RT-PCR reagents gave similar performance. Some statistically significant differences in precision between sample replicates were observed, but these were more evident with swabs diluted in UTM than with other samples, and with one batch of MGIEasy Magnetic Beads Virus DNA/RNA Extraction Kit reagents. Over all specimen types the degree of inter-sample precision achieved was acceptable in a qualitative assay.

Hence no single issue has been identified to suggest that the BGI platform is not fit for purpose in Australian laboratories. Best practice in executing confirmatory testing of samples producing Ct values ≥ 38 will be important in use. Similarly, close monitoring of consistency in performance of Lot numbers, especially of RT-PCR reagents, will be important to ensure optimal performance in clinical use

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6. References

1. Corman VM, Landt O, Kaiser M, MolenKamp R, Meijer A, Chu Dk, et al. 2020. Detection of 2019 Novel Coronavirus (2019- nCoV) by real-time RT-PCR. Eurosurveillance.; 25 (3).
2. Caly L, Druce J. Roberts J, Bond K, Tran T, Kostecki R et al. 2020. Isolation and rapid sharing of the 2019 novel coronavirus (SARS-CoV-2) from the first patient diagnosed with COVID-19 in Australia. Med J Aust. Doi:10.5694/mja2.50569.
3. Manufacturer's Instructions For Use (IFU), Virus DNA/RNA Extraction Kit (MGI Tech Co., Ltd., MGIEasy Magnetic Beads Virus DNA/RNA Extraction Kit MGI Tech Co., Ltd., 2/F, Building 11. Beishan Industrial Zone, Yantian District, Shenzen, China, 518083.
4. Manufacturer's Instructions For Use (IFU), Real-time fluorescent RT-PCR kit for detecting 2019-nCoV, MFG030010. BGI Biotechnology (Wuhan) Co., Ltd, Building B2, Zone B/C/D, Wuhan National Bioindustry Base, NO.666 Ga0xin Avenue, East Lake High-tech Development Zone, Wuhan.
5. Public Health Laboratory Network (PHLN) guidance on laboratory testing for SARS-CoV-2 (the virus that causes COVID-19). Https://www.health.gov.au/resources/publications/phln- guidance-on-laboratory-testing-for-sars-cov-2-the-virus-that-causes-covid-19. Cited 5 June 2020

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Appendix A. Line listing of Panel Samples and SARS-CoV-2 RT-PCR Results

Sample number	Specimen matrix	Positive spiked material	SARS-CoV-2 RT-PCR result[#]		
			Laboratory 1	Laboratory 2	Laboratory 3
1	VTM	SARS-CoV-2	24.1	19.9	19.3
2	VTM	SARS-CoV-2	26.1	22.4	21.5
3	VTM	SARS-CoV-2	28.6	24.3	23.4
4	VTM	None	ND	ND	ND
5	VTM	Influenza virus A	ND	ND	ND
6	VTM	SARS-CoV-2	30.6	26.5	25.8
7	VTM	None	ND	ND	ND
8	VTM	SARS-CoV-2	32.5	28.7	27.3
9	VTM	CoV-229E	ND	ND	ND
10	VTM	SARS-CoV-2	31.0	26.6	25.5
11	VTM	None	ND	ND	39.0
12	VTM	SARS-CoV-2	24.0	20.4	19.7
13	VTM	SARS-CoV-2	26.1	22.3	21.5
14	VTM	SARS-CoV-2	28.8	24.4	23.6
15	VTM	Rhinovirus	ND	ND	ND
16	VTM	SARS-CoV-2	33.0	28.6	27.7
17	VTM	SARS-CoV-2	33.0	31.6	30.0
18	VTM	1/1,024,000	33.7	31.3	30.2
101	Saliva	None	ND	ND	ND
102	Saliva	None	ND	ND	37.1
103	Saliva	None	ND	ND	ND
104	Saliva	SARS-CoV-2	18.5	15.6	14.9
105	Saliva	SARS-CoV-2	18.9	15.7	15.2
106	Saliva	None	ND	ND	ND
107	Saliva	None	ND	ND	ND
108	Saliva	SARS-CoV-2	19.8	16.4	15.9

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109	Saliva	None	ND	ND	ND
110	Saliva	None	ND	ND	ND
111	Saliva	None	ND	ND	ND
112	Saliva	None	ND	ND	39.1
113	Saliva	SARS-CoV-2	18.1	15.5	15.7
114	Saliva	None	ND	ND	ND
115	Saliva	None	ND	ND	ND
116	Saliva	None	ND	ND	ND
117	Saliva	SARS-CoV-2	20.5	16.3	15.9
118	Saliva	None	ND	ND	ND
119	Saliva	None	ND	ND	39.9
120	Saliva	None	ND	ND	ND
121	VTM	SARS-CoV-2	23.9	21.5	21.2
122	VTM	SARS-CoV-2	31.0	28.3	27.9
123	VTM	SARS-CoV-2	31.5	28.5	28.1
124	VTM	SARS-CoV-2	34.1	31.9	30.8
125	VTM	SARS-CoV-2	40.2	36.8	34.9
126	VTM	SARS-CoV-2	24.1	21.4	21.1
127	VTM	None	ND	ND	38.8
128	Saliva	None	ND	ND	ND
129	VTM	SARS-CoV-2	27.2	24.9	24.6
130	VTM	SARS-CoV-2	27.2	24.8	24.6
131	VTM	CoV-229E	ND	ND	ND
132	VTM	SARS-CoV-2	28.6	25.7	25.0
133	VTM	None	ND	ND	ND
134	Saliva	None	ND	ND	ND
135	VTM	SARS-CoV-2	24.4	21.0	21.2
136	VTM	None	ND	ND	ND
137	VTM	SARS-CoV-2	34.8	32.1	31.6
138	VTM	SARS-CoV-2	26.1	26.2	23.1

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139	VTM	SARS-CoV-2	33.0	29.4	29.3
140	VTM	SARS-CoV-2	28.8	25.6	25.1
141	Saliva	None	ND	ND	ND
142	Saliva	None	ND	ND	ND
143	Saliva	None	ND	ND	ND
144	Saliva	None	ND	39.2	ND
145	Saliva	None	ND	ND	ND
146	Saliva	None	ND	ND	ND
147	Saliva	None	ND	ND	ND
148	Saliva	None	ND	ND	39.2
149	Saliva	SARS-CoV-2	19.2	15.4	15.1
150	Saliva	None	ND	ND	ND
151	Saliva	None	ND	ND	ND
152	Saliva	None	ND	ND	39.7
153	Saliva	None	ND	ND	ND
154	Saliva	None	ND	ND	ND
155	Saliva	SARS-CoV-2	20.2	16.6	16.2
156	Saliva	None	ND	ND	ND
157	Saliva	None	ND	ND	ND
158	Saliva	None	ND	ND	ND
159	Saliva	None	ND	ND	ND
160	Saliva	None	ND	ND	38.6
161	VTM	Rhinovirus	ND	ND	ND
162	VTM	SARS-CoV-2	37.0	28.6	28.0
163	Saliva	SARS-CoV-2	20.6	16.5	15.8
164	Saliva	None	ND	ND	ND
165	Saliva	SARS-CoV-2	19.7	15.6	15.2
166	VTM	SARS-CoV-2	24.7	21.7	20.8
167	VTM	SARS-CoV-2	37.6	38.4	35.3
168	VTM	SARS-CoV-2	27.6	24.6	24.2

Post-market validation of the Beijing Genomics Institute (BGI) SARS-CoV-2 Real Time PCR platform continued

169	Saliva	Parvovirus	ND	ND	ND
170	Saliva	None	ND	ND	ND
171	Saliva	SARS-CoV-2	19.7	15.6	14.8
172	VTM	SARS-CoV-2	31.2	27.8	27.1
173	VTM	SARS-CoV-2	24.3	21.6	20.9
174	VTM	SARS-CoV-2	26.4	23.6	23.1
175	VTM	CoV-229E	ND	ND	ND
176	Saliva	None	ND	ND	ND
177	Saliva	None	ND	ND	40.0
178	VTM	SARS-CoV-2	31.3	28.5	28.0
179	VTM	SARS-CoV-2	35.5	32.0	31.1
180	VTM	SARS-CoV-2	ND	35.1	34.9
181	VTM	Rhinovirus	ND	ND	39.3
182	VTM	SARS-CoV-2	33.0	29.7	29.4
183	VTM	SARS-CoV-2	28.3	24.9	24.3
184	VTM	SARS-CoV-2	28.0	24.8	24.3
185	VTM	SARS-CoV-2	30.9	27.7	27.3
186	VTM	SARS-CoV-2	26.5	23.5	23.1
187	VTM	SARS-CoV-2	31.0	27.9	27.3
188	VTM	SARS-CoV-2	30.6	27.9	27.1
189	VTM	SARS-CoV-2	29.0	25.5	25.0
190	Saliva	SARS-CoV-2	19.8	16.7	15.7
191	VTM	Influenza virus A	ND	ND	39.7
192	Saliva	None	ND	ND	ND
193	VTM	SARS-CoV-2	31.2	28.6	27.5
194	VTM	SARS-CoV-2	32.7	29.8	28.9
195	VTM	None	ND	ND	ND
196	VTM	SARS-CoV-2	24.4	21.4	20.5
197	VTM	SARS-CoV-2	35.1	31.8	31.2
198	VTM	SARS-CoV-2	35.2	31.9	31.2

Post-market validation of the Beijing Genomics Institute (BGI) SARS-CoV-2 Real Time PCR platform continued

199	Saliva	SARS-CoV-2	20.4	16.8	16.2
200	VTM	None	ND	ND	ND
201	VTM	SARS-CoV-2	39.6	ND	34.8
202	Saliva	SARS-CoV-2	21.8	17.2	16.3
203	Saliva	None	ND	32.8	ND
204	Saliva	None	ND	ND	ND
205	Saliva	SARS-CoV-2	18.8	15.9	14.8
206	Saliva	None	ND	ND	ND
207	Saliva	SARS-CoV-2	25.1	16.8	16.2
208	Saliva	None	ND	ND	ND
209	Saliva	SARS-CoV-2	20.9	16.4	15.8
210	Saliva	SARS-CoV-2	20.4	16.4	15.7
211	Saliva	None	ND	ND	ND
212	VTM	SARS-CoV-2	24.3	21.3	20.6
213	VTM	SARS-CoV-2	ND	35.4	33.8
214	VTM	SARS-CoV-2	32.5	29.4	28.9
215	Saliva	SARS-CoV-2	20.3	16.8	16.3
216	Saliva	None	ND	ND	ND
217	VTM	SARS-CoV-2	34.7	31.7	31.2
218	Saliva	SARS-CoV-2	21.0	16.6	16.2
219	VTM	SARS-CoV-2	31.8	28.5	27.3
220	Saliva	None	ND	ND	ND
221	VTM	SARS-CoV-2	30.8	28.0	27.4
222	Saliva	SARS-CoV-2	20.1	17.1	16.3
223	VTM	SARS-CoV-2	27.4	24.5	24.1
224	VTM	SARS-CoV-2	26.1	23.4	22.8
225	VTM	Influenza virus A	ND	ND	ND
226	VTM	None	ND	ND	ND
227	VTM	SARS-CoV-2	29.4	25.5	24.9
228	Saliva	SARS-CoV-2	21.1	15.5	14.7

Post-market validation of the Beijing Genomics Institute (BGI) SARS-CoV-2 Real Time PCR platform continued

229	Saliva	None	ND	ND	ND
230	VTM	SARS-CoV-2	35.1	32.6	31.2
231	VTM	SARS-CoV-2	39.7	35.8	35.2
232	VTM	SARS-CoV-2	38.4	36.0	34.2
233	VTM	SARS-CoV-2	25.3	20.9	20.4
234	Saliva	SARS-CoV-2	21.2	16.9	16.2
235	VTM	SARS-CoV-2	31.7	28.4	27.4
236	Saliva	Parainfluenza-1	ND	ND	39.2
237	VTM	SARS-CoV-2	24.4	20.9	20.5
238	VTM	SARS-CoV-2	35.3	31.7	31.4
239	Saliva	Parainfluenza-2	ND	ND	ND
240	Saliva	Parainfluenza-3	ND	ND	ND
241	Saliva	SARS-CoV-2	21.8	16.8	16.4
242	Saliva	CoV-OC43	ND	ND	ND
243	Saliva	Rhinovirus	ND	ND	ND
244	Saliva	RSV	ND	ND	ND
245	VTM	SARS-CoV-2	28.4	24.6	24.1
246	Saliva	Adenovirus type-5	ND	ND	39.4
247	Saliva	SARS-CoV-2	20.0	16.5	15.8
248	Saliva	None	ND	ND	37.7
249	VTM	SARS-CoV-2	26.0	21.5	20.6
250	VTM	SARS-CoV-2	28.0	24.7	24.0
251	VTM	SARS-CoV-2	44.9	34.3	34.5
252	VTM	SARS-CoV-2	26.4	21.0	20.4
253	Saliva	Influenza virus A	ND	ND	ND
254	Saliva	Influenza virus B	ND	ND	ND
255	Saliva	hMPV	ND	ND	ND
256	Saliva	CA16	ND	ND	ND
257	Saliva	Measles virus	ND	ND	ND
258	VTM	SARS-CoV-2	24.0	21.3	20.7

Post-market validation of the Beijing Genomics Institute (BGI) SARS-CoV-2 Real Time PCR platform continued

259	Saliva	CoV-OC43	ND	ND	ND
260	Saliva	CoV-229E	ND	ND	ND
261	VTM	1/64,000	28.6	26.5	26.1
262	VTM	1/4,000	24.4	21.8	21.5
263	VTM	1/65,536,000	ND	36.3	35.7
264	Swab	SARS-CoV-2	28.1	20.3	19.7
265	Swab	SARS-CoV-2	33.5	22.4	26.0
266	Swab	None	ND	ND	ND
267	Swab	None	ND	ND	38.0
268	VTM	1/16,000	27.6	24.6	24.0
269	VTM	SARS-CoV-2	33.0	30.6	30.1
270	Swab	SARS-CoV-2	28.2	24.2	20.7
271	Swab	None	ND	ND	ND
272	VTM	1/16,384,000	38.3	34.5	34.9
273	VTM	1/262,144,000	ND	ND	37.4
274	Swab	SARS-CoV-2	31.0	28.8	24.1
275	Swab	SARS-CoV-2	34.2	26.8	28.0
276	VTM	1/256,000	32.2	28.7	28.2
277	Swab	SARS-CoV-2	30.5	28.7	23.8
278	Swab	None	ND	ND	ND
279	Swab	None	ND	ND	ND
280	VTM	1/4,096,000	36.1	32.7	32.6
281	VTM	1/262,144,000	ND	38.7	ND
282	Swab	SARS-CoV-2	30.7	28.2	23.5
283	Swab	None	ND	ND	ND
284	Swab	None	ND	ND	ND
285	Swab	None	ND	ND	ND
286	VTM	1/4,000	24.2	21.7	21.4
287	Swab	SARS-CoV-2	35.9	26.6	28.2
288	VTM	1/4,000	25.1	21.9	21.3

Post-market validation of the Beijing Genomics Institute (BGI) SARS-CoV-2 Real Time PCR platform continued

289	Swab	None	ND	ND	ND
290	Swab	None	ND	ND	ND
291	Swab	None	ND	ND	ND
292	Swab	None	ND	ND	ND
293	VTM	1/256,000	32.4	28.5	27.6
294	Swab	SARS-CoV-2	33.0	22.4	25.8
295	Swab	None	ND	ND	ND
296	Swab	None	ND	ND	ND
297	Swab	None	ND	ND	ND
298	Swab	None	ND	ND	ND
299	VTM	1/1,024,000	33.4	30.5	29.8
300	VTM	1/4,096,000	36.9	32.6	32.6
301	VTM	1/16,384,000	38.9	ND	34.2
302	Swab	SARS-CoV-2	25.5	20.4	20.1
303	Swab	SARS-CoV-2	32.0	28.5	23.4
304	Swab	SARS-CoV-2	34.8	22.0	26.2
305	Swab	SARS-CoV-2	33.5	26.7	27.8
306	Swab	None	ND	ND	ND
307	VTM	1/4,000	26.1	21.7	21.2
308	Swab	None	ND	ND	ND
309	Swab	None	ND	ND	ND
310	VTM	1/16,000	29.6	24.4	24.0
311	VTM	1/1,024,000	34.1	30.5	30.2
312	Swab	SARS-CoV-2	28.3	20.3	19.9
313	Swab	SARS-CoV-2	31.1	20.8	21.8
314	Swab	SARS-CoV-2	29.0	21.6	21.7
315	Swab	None	ND	ND	ND
316	Swab	None	ND	ND	ND
317	VTM	1/64,000	29.9	26.4	26.1
318	Swab	SARS-CoV-2	32.1	22.5	25.6

Post-market validation of the Beijing Genomics Institute (BGI) SARS-CoV-2 Real Time PCR platform continued

319	VTM	1/65,536,000	ND	36.4	36.5
320	Swab	SARS-CoV-2	29.3	24	21.2
321	VTM	1/4,096,000	36.4	32.7	32.0
322	Swab	None	ND	ND	ND
323	Swab	SARS-CoV-2	28.1	24.4	20.8
324	Swab	None	ND	ND	ND
325	Swab	None	ND	ND	ND
326	Swab	None	ND	ND	ND
327	Swab	None	ND	ND	ND
328	VTM	1/64,000	30.4	26.7	25.7
329	VTM	1/256,000	32.5	28.4	28.1
330	VTM	1/16,384,000	36.3	33.6	34.1
331	Swab	SARS-CoV-2	27.5	20.9	21.5
332	Swab	None	ND	ND	ND
333	Swab	None	ND	ND	ND
334	Swab	None	ND	ND	ND
335	VTM	1/262,144,000	40.2	37.9	36.7
336	Swab	None	ND	ND	ND
337	Swab	None	ND	ND	ND
338	Swab	None	ND	ND	ND
339	VTM	1/1,024,000	34.0	30.6	29.3
340	Swab	None	ND	ND	ND
341	VTM	1/65,536,000	ND	36.8	34.8
342	VTM	1/262,144,000	ND	37.4	36.1
343	Swab	SARS-CoV-2	28.2	24.5	20.2
344	VTM	1/16,000	28.2	24.5	23.4
345	VTM	1/16,000	28.1	24.3	23.6
346	VTM	1/4,096,000	36.1	32.7	31.6
347	Swab	SARS-CoV-2	29.6	21.2	22.0
348	Swab	SARS-CoV-2	38.5	26.4	27.2

Post-market validation of the Beijing Genomics Institute (BGI) SARS-CoV-2 Real Time PCR platform continued

349	VTM	1/256,000	31.7	28.4	27.7
350	VTM	1/65,536,000	ND	36.4	35.3
351	Swab	SARS-CoV-2	28.4	20.2	19.3
352	VTM	1/64,000	30.0	26.2	25.2
353	VTM	1/16,384,000	37.8	34.9	33.9