Additional information for "Scalable and Resilient SARS-CoV-2 testing in an Academic Centre- Figures"

CCC test validity

The CCC test was bench-marked against the reference laboratory N gene assay using 56 current clinical samples processed in parallel using the Hologic Panther platform. 27 specimens were processed in duplicate through the CCC pipeline from 150µL inactivated virus, through RNA extraction and RT-PCR (**Figure 2A**) and a second batch of 29 clinical specimens was processed independently (**Figure 2B**), all with a parallel test of the same sample processed through the N gene assay on the Hologic Panther platform. As the reference laboratory assay does not contain a human internal control, there was no mechanism for testing 'swab failures' with the N gene assay. As part of the validation, 3 untested 'blank' swabs were submitted, yielding a negative result by the reference laboratory assay, but due to the absence of signal in the human internal control were classified as swab fails by the CCC test (**Figure 2C**). 5 of the clinical samples submitted were classified as 'low level positives' by the N gene assay with Ct values of >38.0. These 5 samples were all repeated using the N gene assay and again returned high Ct values and are therefore classified as borderline positives (**Supplementary Figure 1**). Overall, the diagnostic sensitivity of the CCC test is 92.86% with a specificity of 100% and a high degree of accuracy in the detection of SARS-CoV-2.

CCC test specificity

Cross-reactivity of the SARS-CoV-2 RT-PCR probes in the BGI kit was tested by performing the CCC RT-PCR test using specimens from 10 patients known to be infected with other viruses such as flu, RSV, rhinovirus, metapneumovirus and parainfluenza virus (**Figure 2A**). Specimens from patients with other viruses resulted in COVID-19 Ct values 'undetermined' or above 37.0 in the assay (negative result) demonstrating a high degree of assay specificity against SARS-CoV-2.

The specificity of the SARS-CoV-2 RT-PCR assay was further tested against RNA extracted from human cell lines (1:1 mixture of A549 and HT1080 cell lines) compared to a single positive control sample. Only the positive control well displayed SARS-CoV-2 amplification in the RT-PCR. All human cell lines were negative at 1:125 dilution (**Supplementary Figure 2A**). We next monitored our test specificity during live pipeline runs by assessing the Ct values from the SARS-CoV-2 amplification for wells containing elution buffer and RT-PCR master mix only (non-sample wells). Twenty-four out of twenty-five non-sample wells processed in our initial 3 live runs had an undetermined Ct value reflecting the absence of an amplified product in these wells (**Supplementary Figure 2B**). 48 samples of water were tested alongside a positive control to assess the occurrence of inherent late-cycle amplification or non-specific signal from using this RT-PCR kit. Late Ct signals for the internal control (>35) and COVID-19 (>37) targets were observed in 2 of 48 and 1 of 48 wells respectively (**Supplementary Figures 3A and B**). When the experiment was repeated using guanidinium inactivation buffer and RNA elution buffer, the signal was present in 8 out of 48 and 2 out of 48 wells respectively (**Supplementary Figures 3C and D**). No overlap was seen for the 2 targets, confirming that late signals did not represent contamination from positive control. The Ct values reported in the guanidinium and RNA elution buffer controls support the thresholds set for calling positive, negative and failed samples as documented in the manufacturer's instructions. The reproducibility of the kits used for the CCC test was determined by comparing two different batches of RT-PCR kit reagents (BGI). Four serial dilutions of the positive control from the kit were assessed in columns of a 96 well plate alongside 2 columns of negative controls. Both batches gave linear results following serial dilution. For the negative control wells, both batches gave similar degrees of late stage amplification; AUTO analysis yielded Ct's above 38 for COVID-19 and above 37 for the internal control (**Supplementary Figure 4**).

CCC test sensitivity

In an effort to ascertain the CCC test sensitivity, particularly relevant to specimens with low viral concentration, a linearity analysis was performed by testing 10-fold serial dilutions of RNA

extracted from a COVID-19 positive patient followed by RT-PCR. Starting with a Ct value of 21.3 in the original sample, a linear response was observed to 10^6 dilution from the original RNA eluate (**Figure 3A**).

CCC test reproducibility

The reproducibility of the CCC test was measured using a sample from a patient with confirmed COVID-19 diagnosis. The sample was diluted 100 fold in viral inactivation buffer and individual aliquots were subject to 5 independent repeats of the CCC pipeline through RNA extraction and RT-PCR to determine Ct values (**Figure 3B**). The coefficient of variation for CCC test precision was calculated as 0.0166.