Technical Bulletin

Performance Characteristics of SARS-CoV-2 (COVID-19) PCR

TO: Medical Staff and Clients

FROM: Dr. Wesley Kim, MD
Medical Director, DLS and QMC West

Dr. Amy Woron, PhD
Manager, DLS Molecular

Dr. A. Christian Whelen, PhD
VP & Technical Director
Microbiology & Molecular

Dr. Ana Ortega-Lopez, MD
Medical Director QMC Punchbowl,
North Hawaii and Molokai

DATE: April 6, 2020

SUBJECT: Performance characteristics of SARS-CoV-2 (COVID-19) molecular tests are excellent

Many laboratories and companies are validating tests under FDA Emergency Use Authorization (EUA), which may give the false impression that they are substandard, especially with molecular tests. Validation studies under EUA are with smaller numbers than usual, but the product designs are excellent.

Each assay has a minimum of 2 target sequences, with an internal fluorescent probe for real time detection. The primers and probes in 3 systems that DLS has examined (CDC, DLS-developed, and commercial) are designed to be specific. In silico analysis shows that sequences of all probes and primers have no cross reactivity to other respiratory flora or pathogen sequences, including SARS and MERS, and 100% match to SARS-COV-2 (COVID-19) sequences.

The analytical sensitivity is demonstrated by replicates at the limit of detection (LOD), which in all assays was less than 100 targets. The package insert for our commercial assay hit 100% of 50 samples at 1.5 and 4 times the LOD. Our LOD study demonstrated we could detect 20-60 copies in triplicate (matches company LOD calculation of 25-50 copies), and 2 of 3 replicates at 2-6 copies. Our DLS-developed assay LOD was about 2x higher, but still <100. The CDC assay has an LOD of 5 copies.

Our commercial assay was correct with 100% of 100 negative samples and our DLS-developed assay was correct with 100% of 32 negative samples and 3 pools of 5 samples each, further verifying no cross-reactivity indicated in the in silico analysis.

Clearly no test is perfect, or should I say no testing process is perfect. Risk of false positives will primarily be high titer specimens that leaked and contaminate other specimens, or cross-over contamination during testing (which we closely monitor in all of our molecular assays). Risk of false negatives would occur at or below the LOD and/or related to sampling – but these risks are very low because of low prevalence and very high virus titers in true infections (unless the timing of the sampling is way off). Many checks are in place to catch labeling errors, but this can be a rare occurrence.

DLS encourages providers to follow Department of Health (DOH) guidelines, (https://health.hawaii.gov/docd/for-healthcare-providers/news-updates/), as well as those of **their respective institutions**.

Please refer any questions to Dr, Amy Woron, Manager - DLS Microbiology Lab at 441-5436, or DLS Client Services at 589-5101.