Technical Alert – Update #1

Possible False Positive SARS-CoV-2 (COVID-19) NAAT

TO: Medical Staff, Clients, and Medical Laboratories

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SUBJECT: Rapid NAAT Instruments Occasionally Report False Positives

Many companies are producing high quality SARS-COV-2 molecular tests under FDA Emergency Use Authorization (EUA), however, even high quality assays are not infallible. These COVID-19 testing methods were validated, implemented, and emergency approved in the US with less data and a shorter timeline than usual. As such, it is still not completely clear how many of these assays will truly perform in the field. Based on early experience with the Cepheid GeneXpert and Abbott ID Now, there are concerns that these instruments are occasionally overcalling background signal as a positive result. Since implementation, we have identified suspect results at 4 of our 6 testing locations.

Each assay has a minimum of 2 target sequences, with internal fluorescent probes for real time detection. The primers and probes are designed to be specific with little to no cross reactivity to other respiratory flora or pathogen sequences, including SARS and MERS, and match SARS-COV-2 (COVID-19) sequences.

The Cepheid instrument is presently set by the manufacturer to interpret a single target positive with very poor amplification efficiency (high Cycle Threshold [Ct] and/or atypical curve) as "DETECTED". These often cannot be confirmed positive when tested on other systems using similar targets. So raw data are audited before results are released. If only one of two targets OR if any Ct value is ≥40, it's reported as presumptive positive pending confirmation by an alternate method. Confirmation testing is expedited either in-house or sent to the DOH State Laboratories – usually within 24 hours.

The Abbott instrument does not use PCR and does not generate Cts. It uses rapid isothermal amplification with true positives detected in as few as 3 minutes. "Positives" that signal later in the 13 minute incubation are suspicious, so we established a 5-minute breakpoint. If the instrument calls a specimen positive after 5 minutes, it's reported presumptive positive pending confirmation by an alternate method. Confirmation testing is the same as above.

Following confirmation, the "presumptive" result will be amended to a final "DETECTED" or "NOT DETECTED" result. In the case where the initial presumptive positive result is determined to be a false positive, and a final negative result is released, the ordering physician, institution, and infection control will be notified.

As more data regarding the performance of all the various methods become available, we will continue to update our community and make adjustments to our protocols and processes to ensure we provide the most accurate results possible to guide appropriate patient care.

If you have any questions, please call Dr. Amy Woron at 441-5436 or DLS client services at 589-5101.

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