Implementation of Reverse Transcriptase PCR Testing for Severe Acute Respiratory Syndrome Coronavirus 2 under the US Food and Drug Administration Emergency Use Authorization

To the Editor:

On January 31, 2020, the US Department of Health and Human Services declared a public health emergency related to coronavirus disease 2019, and on February 9, 2020, the US Food and Drug Administration (FDA) issued a guidance document to fast-track the availability of tests for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) that were developed to control the emergence of this rapidly spreading virus (1). On March 13, 2020, at >1 month after the public health declaration, the president of the United States declared the pandemic a national emergency. The FDA guidance document established emergency use authorization (EUA) guidelines for those wishing to develop assays. At the time, however, little guidance was provided for those laboratories wishing to verify these EUA assays once they became commercially available.

The first published genetic sequences of SARS-CoV-2 were by investigators in China, France, and Germany in early 2020, allowing for the development of molecular-based assays to detect the virus (2). The CDC published primer and probe sequences as "Research Use Only 2019–Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Primer and Probe" and not for use for diagnostic testing under the FDA's authorization of the CDC 2019-nCoV Real-Time RT-PCR Diagnostic Panel (CDC-EUA). These primer and probe sequences were directed against 3 regions of the SARS-CoV-2 *N* gene (*N1*, *N2*, and *N3*) and a human control gene, RNAseP (*RPP30*) (3). The CDC later made this assay available and, on February 4, 2020, received EUA status for use by laboratories certified in the United States under the CLIA to perform high-complexity testing. These initial kits were distributed to public health laboratories, but a manufacturing issue resulted in an amendment to the procedure, delaying widespread testing among public health laboratories by approximately 2 weeks.

The FDA guidance document provided recommendations for assay developers that included determination of the limit of detection by spiking genomic RNA or virus into artificial or residual clinical matrices and a dilution series of 3 replicates per concentration with 20 replicates of the final limit of detection concentration. In the absence of known positive samples, laboratories needed to test a minimum of 30 contrived positive and 30 negative samples. In silico analysis of primer or probe sequences against published viral sequences could be performed to document inclusivity and cross-reactivity. Once completed, developers could commence clinical testing immediately but were required to notify and submit an EUA request to the FDA within 15 days. This guidance document provided specific instructions for those developing EUA tests but did not provide guidance for those laboratories wanting to use purchased EUA assays.

Our initial concerns were about the availability of these test kits, given increasing demand from public health laboratories, the multiwell reaction setup per sample, and EUA approval for using a specific real-time PCR instrument. Furthermore, the initial CDC-EUA instructions for use allowed the use of only 2 different Qiagen DSP kits for viral RNA isolation, but these kits were sequestered for distribution to CDC and public health laboratories. Laboratories wanting to perform clinical testing with the CDC-EUA assay were left to use non-DSP versions of these extraction kits. Initial feedback from the FDA suggested that this "off-label" use would require a new validation and EUA submission. Later, the FDA suggested that use of alternative extraction kits was acceptable and may or may not require an unspecified "bridging study." Because of this uncertainty, we decided to move forward with a verification study based on the FDA EUA guidance for laboratory-developed tests that also met the criteria for verification of in vitro diagnostic devices under the normal regulatory framework of a CLIA-certified laboratory.

We performed an evaluation study to ensure the virus-specific primers, probes, and amplification conditions were working properly using positive, negative, and no template control, 3 residual nasopharyngeal swab specimens from individuals not suspected of SARS-CoV-2 infection, and a positive RNA sample provided by the New Hampshire Public Health Laboratories. We began a series of experiments to verify the assay performance that included 52 previously tested samples and contrived samples using isolate USA-WA1/2020 (lot 70033700; BEI Resources). These samples were tested in 6 runs with the CDC-EUA assay to assess accuracy, specificity, precision, and limit of detection.

Verifying and performing molecular testing under an FDA EUA designation during a pandemic posed several challenges. Stringent initial guidelines set forth by the FDA and CDC, the many EUA assays with different claims requiring different verification strategies, supply chain issues, the need for high- and low-throughput molecular testing with unrealistic demands

on turnaround times, the need to test symptomatic and asymptomatic individuals, and the endless demands on staff affected our testing capacity.

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