Performance Characteristics

1. Limit of Detection (LoD) (Analytical Sensitivity)

The preliminary limit of detection (LoD) for covidSHIELD was determined to be 1,000 viral copies/mL using Gamma-irradiated SARS-CoV-2 spiked into fresh human saliva (SARS-CoV-2 negative) collected in 50 mL tubes (Corning item #352070) at 1.0x10^2, 5.0x10^2, 1.0x10^3, 2.5x10^3, 5.0x10^3, 1.0x10^4, 5.0x10^4, 1.0x10^5, and 5.0x10^5 viral copies/mL; four replicates per concentration. The preliminary LoD was determined to be 1,000 viral copies per milliliter. Final LoD for covidSHIELD was tested using the Thermo Fisher Sciences QuantStudio 3 with 30 individual extraction replicates at 1,000 copies/mL in different sources of saliva; all 30 samples tested positive.

2. Inclusivity (Analytical Sensitivity)

covidSHIELD is a modification of the previously Emergency Use Authorized Thermo Fisher Scientific Applied Biosystems TaqPath COVID-19 Combo Kit. The assay targets ORF1ab, nucleocapsid (N) gene, and spike (S) gene. Thermo Fisher Scientific’s in silico analysis was updated on October 6, 2020. Based upon BLAST analysis, the TaqPath™ COVID-19 Combo Kit maps with 100% homology to >99.99% of known SARS-CoV-2 isolates in GISAID and 100% of known isolates in GenBank databases.

3. Cross-Reactivity (Analytical Specificity)

The analytical specificity of covidSHIELD was demonstrated in silico under the EUA for the Thermo Fisher Scientific Applied Biosystems TaqPath COVID-19 Combo Kit. A full listing of cross-reactivity can be found in the Applied Biosystems IFU (https://www.fda.gov/media/136112/download). Based on this analysis, significant amplification of non-target sequences that could result in cross-reaction (false-positive results) or interference (false-negative results) is unlikely to occur. In addition, the University of Illinois conducted additional wet testing using a Thermo Fisher Scientific QuantStudio 3 to validate the specificity of our detection system to SARS-CoV-2. Saliva collected in a 50mL tube (Corning item #352070) was spiked with or without SARS-CoV-2 (gamma-irradiated virus, synthetic N- transcript), two other human coronaviruses (OC43, 229E), and SARS and MERS synthetic RNA; two replicates were tested for each. Among these samples, SARS-CoV-2 genes were only detected in the positive control, and SARS-CoV-2 samples, supporting specificity of the detection platform for SARS-CoV-2.

4. Clinical Evaluation

A prospective study was completed to assess the clinical performance of the covidSHIELD assay. 120 study participants who were symptomatic for COVID-19 infection provided saliva samples (self-collected with observation in either a 50mL tube (Corning item #352070) or via a straw (Smipam PLA straw, 7.75” x 0.25”) in a 4 mL tube (Duran Wheaton Kimble Life Sciences item #W985870) and either a nasopharyngeal (NP) or mid-turbinate (MT) sample collected by a healthcare professional. Samples were run on a comparator assay (Abbott RealTime SARS-CoV-2 assay performed on the Abbott m2000 System) and covidSHIELD (run on either a Thermo Fisher Scientific QuantStudio 7 Flex or 7 Pro). Of the 120, 31 were positive and 89 were negative according to comparator results. Of the 31 positives, the candidate assay identified 30 of the samples as positive, with one discordant positive result. Of the 89 negatives, the candidate assay
identified 88 of the samples as negative, with one discordant negative result. The positive percent agreement (PPA) and negative percent agreement (NPA) are therefore 96.8% and 98.9%, respectively. The lower bound of the two-sided 95% confidence interval for PPA and NPA are 82.2% and 93.2%, respectively.