REAL-TIME ANALYZERS

Providing Chemical & Biological Information When & Where You Need It

COVID-19 Saliva Antigen Test Preliminary Data

FDA Guidelines for PERFORMANCE EVALUATION

1) **Limit of Detection (LoD) (Analytical Sensitivity):** The study used SARS-Related Coronavirus 2, Isolate USA-WA1/2020, heat Inactivated (BEI Resources, NR-52286, Lot: 70037779). The viral isolate was spiked into saliva specimen (Lee Biosolutions, Maryland Heights, MO). The saliva specimens were confirmed by PCR to be Covid-19 negative. Dilutions were carried out from $1.6x10^4$ to $2x10^2$ $TCID_{50}$ per mL.

The *COVID-19 Saliva Antigen Test* LoD is $1.6x10^3$ $TCID_{50}$ per mL

(SARS-CoV-2), isolate USA-WA1/2020, 1.6×10^5 $TCID_{50}$ per mL					
Dilution	1/10	1/100	1/200	1/400	1/800
Conc.	1.6×10^4	**1.6×10^3**	8×10^2	4×10^2	2×10^2
5 replicates	100% (5/5)	**100% (5/5)**	60% (3/5)	0% (0/5)	NA
20 replicates	100% (20/20)	**100% (20/20)**	50% (10/20)	0% (0/20)	NA

2) **Cross-reactivity (Analytical Specificity):** Cross-reactivity studies were performed to demonstrate that the test does not react with related pathogens, high prevalence disease agents and normal or pathogenic flora that are reasonably likely to be encountered in the clinical specimen. The organisms in the table below were prepared at and 10^6 plaque forming units/mL for viruses and 10^8 colony forming units/mL for bacteria in pooled saliva. ALL of the listed organisms tested NEGATIVE using the ***COVID-19 Saliva Antigen Test kit***.

Recommended List of Organisms

High priority organisms likely in the circulating area
Adenovirus
Respiratory syncytial virus
Haemophilus influenzae
Streptococcus pneumoniae
Candida albicans

3) **Studies to support Point-of-Care claim:** Twenty kits were provided to 20 non-laboratory personnel, and asked to test their own saliva following the instruction sheet that is provided with the kit (see attached). Once completed they were asked if their test indicated that they were Positive or Negative for Infection, or the test was Invalid. 19 of 20 participants tested themselves Negative. One person was inebriated and could not provide the required 0.5 mL of saliva. The robustness of the test is underway, and will be evaluated by testing a saliva sample containing **$1.6x10^4$ $TCID_{50}$ per mL** saliva inactivated SARS-CoV-2 (10 times the LOD) in which 0.3, 0.5 or 1 mL saliva will be

added to the Sample Vial, the buffer will or will not be added to the sample, and in each case, 2, 3, or 4 drops of treated sample will be added to the LFA, and results will be evaluated after 10, 12, and 15 minutes. This requires 54 Kits.

4) **<u>Clinical Evaluation</u>: If you intend to seek a claim for saliva or oral fluid, you should test at least 30 positive specimens with paired PCR results from an NP swab. Tests should demonstrate a minimum sensitivity of ≥ 80% for all sample types submitted.**

A limited study of saliva samples were performed consisting of samples collected 1) from 14 volunteers (co-workers and family members who also had PCR measurements), 2) 15 purchased saliva samples (Boca Biolistics) with threshold cycle (Ct) values ranging from 13 to 32, and 3) 10 purchased nasal samples (Lee Biosolutions) with Ct values ranging from 12 to 26. For 22 of the 25 (88%) PCR POSITIVE samples the ***COVID-19 Saliva Antigen Test*** Kit correctly produced two lines indicating POSITIVE INFECTION. In 22 of 22 PCR NEGATIVE samples the ***COVID-19 Saliva Antigen Test*** Kit correctly produced one line indicating NEGATIVE INFECTION. For one of the negative samples, the saliva was well below the required amount, and was considered an INVALID result.