Summary of performance evaluation for the SaliVIR Rapid COVID19 vRNA Test

Following FDA EUA guidelines

Limit of Detection (LoD) - Analytical Sensitivity

To determine the LoD of the device utilising the entire test system from sample preparation to detection.

The LoD was determined by spiking SARS-CoV-2 RNA (ORF, E, N; ATCC® VR-3276SD™) into negative saliva matrices collected using the SaliVIR Collect kit. A 2-3X dilution series at viral load concentrations equivalent to 100,000 copies/ml to 200 copies/ml was performed (with four replicates per concentration, Table 1). The samples containing the RNA spike in were subsequently extracted using the SaliVIR Bead Xtract Viral RNA/DNA kit and eluted in 50µl of elution buffer. 5µl of elution were processed according to SaliVIR COVID19 Rapid 1 STEP QRT-PCR kit protocol in a BioRad CFX96 qPCR instrument.

Table 1. Summary of LoD dilution series results for the SaliVIR Rapid COVID19 vRNA Test.

Concentration of viral RNA (copies/ml)	Mean N1 Cq	Positive replicates	Mean N2 Cq	Positive replicates
100000	28.29	100% (4/4)	28.90	100% (4/4)
50000	29.47	100% (4/4)	30.07	100% (4/4)
20000	30.62	100% (4/4)	31.47	100% (4/4)
10000	31.81	100% (4/4)	32.78	100% (4/4)
5000	33.72	100% (4/4)	34.13	100% (4/4)
2500	35.26	100% (4/4)	35.20	100% (4/4)
1000	35.80	100% (4/4)	36.09	100% (4/4)
500	37.04	100% (4/4)	38.92	100% (4/4)
200	38.57	100% (4/4)	N/A	0% (0/4)

In order to confirm the final LoD concentration, 20 replicates were tested, following the same protocol, at 750 copies/ml and 500 copies/ml respectively (Table 2). This study confirmed

that the LoD of the SaliVIR Rapid COVID19 vRNA Test is 750 copies/ml (20/20 replicates positive for SARS-CoV-2).

Table 2. Summary of results to confirm the LoD for the SaliVIR Rapid COVID19 vRNA Test.

Concentration of viral RNA (copies/ml)	Mean N1 Cq	Positive replicates	Mean N2 Cq	Positive replicates
750	35.57	100% (20/20)	35.98	100% (20/20)
500	35.29	90% (18/20)	36.39	90% (18/20)

Inclusivity

To document the results of an inclusivity study that demonstrates that all the strains of SARS-CoV-2 that can be detected by the proposed molecular assay.

The SaliVIR Rapid COVID19 vRNA Test comprises only primers and probes designed by CDC from the CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel without any changes. Inclusivity of the CDC Diagnostic panel has been previously established (inclusivity = 100%).

Cross-reactivity (Analytical Specificity):

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 SaliVIR Rapid COVID19 vRNA Test comprises only primers and probes designed by CDC from the CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel without any changes. Cross-reactivity of the CDC Diagnostic panel has been previously established (cross-reactivity=0%, analytical specificity=100%).

Clinical study

A contrived clinical study was designed according to FDA recommendations:

In the absence of known positive samples available for testing, you should confirm the performance of your assay with a series of contrived clinical specimens by testing a minimum of 30 contrived reactive specimens and 30 non-reactive specimens in a randomized blinded fashion. Contrived reactive specimens can be created by spiking RNA or inactivated virus into leftover individual clinical specimens representing unique patients. If claiming multiple specimen types, you should perform the contrived clinical testing described above for one representative upper respiratory tract matrix and one representative lower respiratory tract matrix. Please note the representative matrix should be the most challenging. Twenty of the contrived clinical specimens should be spiked at a concentration

of 1x-2x LoD, with the remainder of specimens spanning the assay testing range. FDA defines the acceptance criteria for the performance as 95% agreement at 1x-2x LoD, and 100% agreement at all other concentrations and for negative specimens.

A contrived clinical study was performed to evaluate the performance of the SaliVIR Rapid COVID19 vRNA Test. A total of 78 clinical respiratory samples were considered: 48 positive contrived saliva samples and 30 negative contrived saliva samples. All positive contrived clinical samples were generated by spiking SARS-CoV-2 RNA (ORF, E, N; ATCC® VR-3276SD™) at different viral load concentrations into negative samples.

As shown in Table 3, the positive agreement was 100% (28/28) for concentrations in the 1.3-133.3X LoD range and 100% (20/20) for the samples at 1X LoD (overall positive agreement 100%, 48/48). The negative agreement was 100% (30/30). Therefore, the results of the clinical evaluation were considered acceptable for this specimen type.

Table 3. Clinical performance of the SaliVIR Rapid COVID19 vRNA Test with contrived saliva samples.

Contrived sample type	Concentration of viral RNA	Number of samples	Mean N1 Cq	N1 positive samples	Mean N2 Cq	N2 positive samples
Positive	133.3X LoD	4	28.29	100% (4/4)	28.90	100% (4/4)
Positive	66.7X LoD	4	29.47	100% (4/4)	30.07	100% (4/4)
Positive	26.7X LoD	4	30.62	100% (4/4)	31.47	100% (4/4)
Positive	13.3X LoD	4	31.81	100% (4/4)	32.78	100% (4/4)
Positive	6.7X LoD	4	33.72	100% (4/4)	34.13	100% (4/4)
Positive	3.3X LoD	4	35.26	100% (4/4)	35.20	100% (4/4)
Positive	1.3X LoD	4	35.80	100% (4/4)	36.09	100% (4/4)
Positive	1X LoD	20	35.57	100% (20/20)	35.98	100% (20/20)
Negative	0X LoD	30	N/A	100% (30/30)	N/A	100% (30/30)