

COVID-19 Saliva Antigen At-Home Test Data

1) Limit of Detection (LoD) (Analytical Sensitivity): 20 of 20 samples of heat inactivated Isolate USA-WA1/2020 were successfully detected at **1.6x10³ TCID₅₀ per mL** (Table 1). 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 1.6x10⁴ to 2x10² TCID₅₀ per mL.

Table 1. *COVID-19 Saliva Antigen At-Home Test* Limit of Detection Data.

(SARS-CoV-2), isolate USA-WA1/2020, 1.6 × 10⁵ TCID₅₀ per mL					
Dilution	1/10	1/100	1/200	1/400	1/800
Conc.	1.6 x 10 ⁴	1.6 x 10³	8 x 10 ²	4 x 10 ²	2 x 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

2a) Cross-reactivity (Analytical Specificity): 19 of 19 related pathogens tested negative using the *COVID-19 Saliva Antigen At-Home Test* (Table 2). 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 measured **in pooled saliva**.

Table 2. *COVID-19 Saliva Antigen At-Home Test* Cross-Reactivity Data.

Virus/Bacteria	Source	Concentration	Result
Adenovirus	BEI, cell preparation	2.5 x 10 ⁷ TCID ₅₀ /ml	Negative
Respiratory syncytial virus	ATCC	4 x 10 ⁵ TCID ₅₀ /ml	Negative
Haemophilus influenzae	Hardy Diagnostics	3 x 10 ⁶ TCID ₅₀ /ml	Negative
Human Metapneumovirus	BEI, Inactive cell lysate	5 x 10 ⁵ TCID ₅₀ /ml	Negative
Enterovirus	BEI, cell preparation	2.4 x 10 ⁵ TCID ₅₀ /ml	Negative
Rhinovirus	BEI, Inactive cell lysate	2 x 10 ⁶ TCID ₅₀ /ml	Negative
Influenza A	BEI, Inactive cell lysate	6 x 10 ⁵ CEID ₅₀ /ml	Negative
Influenza B	BEI, Inactive cell lysate	5.3 x 10 ⁴ CEID ₅₀ /ml	Negative
Human coronavirus 229E	ZeptoMetrix	1 x 10 ⁵ TCID ₅₀ /ml	Negative
Human coronavirus OC43	ZeptoMetrix	1 x 10 ⁵ TCID ₅₀ /ml	Negative
Human coronavirus NL63	ZeptoMetrix	1 x 10 ⁵ TCID ₅₀ /ml	Negative
MERS	BEI, Inactive cell lysate	8.9 x 10 ⁵ TCID ₅₀ /ml	Negative
<i>Streptococcus pneumoniae</i>	ATCC	5 x 10 ⁶ cells/ml	Negative
<i>Streptococcus pyogenes</i>	ATCC	8 x 10 ⁵ cells/ml	Negative
<i>Mycoplasma pneumoniae</i>	ATCC	3.2 x 10 ⁶ cells/ml	Negative
<i>Chlamydia pneumoniae</i>	ATCC	7.5 x 10 ⁷ cells/ml	Negative
<i>Legionella pneumophila</i>	ATCC	5 x 10 ⁵ cells/ml	Negative
<i>Mycobacterium tuberculosis</i>	Univ. Rhode Island	6.3 x 10 ⁶ cells/ml	Negative
<i>Candida albicans</i>	ATCC	4 x 10 ⁶ cells/ml	Negative

2b) Microbial Interferent Studies: Contrived specimens of SARS-CoV-2 with the following interferents were prepared in **pooled saliva** (obtained from Lee BioSolutions) and tested for the interference with 3 replicates. In all SARS-CoV-2 was detected, i.e. tested positive, using the *COVID-19 Saliva Antigen At-Home Test*, indicate NO interference (Table 3).

Table 3. *COVID-19 Saliva Antigen At-Home Test* Interferent Data.

Interferent	Concentration	SARS-CoV-2 Concentration	Result n = 3
Influenza A		1.6x10³ TCID₅₀ per mL	Positive
<i>Streptococcus pneumoniae</i>	5 x 10 ⁶ cells/ml	1.6x10³ TCID₅₀ per mL	Positive
<i>Streptococcus mutans</i>	1 x 10 ⁷ cells/ml	1.6x10³ TCID₅₀ per mL	Positive
Human Blood, EDTA	5% v/v	1.6x10³ TCID₅₀ per mL	Positive
Acetaminophen	1 mg/ml	1.6x10³ TCID₅₀ per mL	Positive
Aspirin	1mg/ml	1.6x10³ TCID₅₀ per mL	Positive
Caffeine	5mg/ml	1.6x10³ TCID₅₀ per mL	Positive
Amoxicillin	5 mg/ml	1.6x10³ TCID₅₀ per mL	Positive

3) Clinical Evaluation: 50 of 50 (100%) volunteers that had PCR tests performed by state labs, self-administered the test the same day and correctly tested negative. 29 of 38 (76.32%) Positive saliva samples with Ct values, obtained from Boca Biolistics and Lee Biosolutions correctly tested positive. All 38 samples were tested 3 times by RTA lab personnel. 29 of 29 samples with Ct values from 13 to 29 correctly tested positive using the RTA kit. All 9 samples that incorrectly tested negative had Ct values from 30-33 (Tables 3 and 4).

Table 3. *COVID-19 Saliva Antigen At-Home Test* Sensitivity, Specificity, Predicted Positive, and Predicted Negative percents are provided for Ct 13-33 values and Ct 13-30 values.

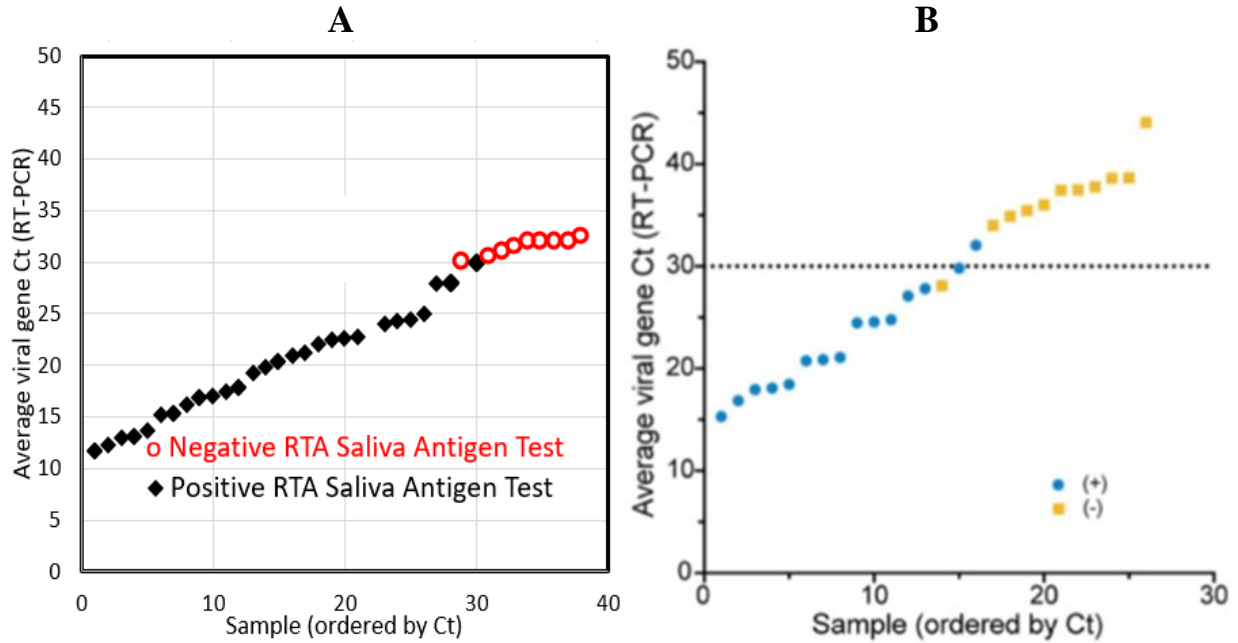
LFA Statistics	Sensitivity	Specificity	Predicted Positive	Predicted Negative
Total True Positive = a, Total False Positive = b, Total False Negative = c, Total True Negative = d				
	a/(a+c)	d/(d+b)	a/(a+b)	d/(d+c)
Ct =13-33	29/(29+9)	49/(49+0)	29/(29+0)	49/(49+9)
a=29, b=0, c=9, d=49	76.32%	100.00%	100.00%	84.48%
Ct =13-29	29/(29+0)	49/(49+0)	29/(29+0)	49/(49+0)
a=29, b=0, c=0, d=49	100.00%	100.00%	100.00%	100.00%

Table 4. *COVID-19 Saliva Antigen At-Home Test* compared to PCR for all positive samples.

Positive Samples X	PCR Ct Value	LFA Test	True Positives (Ct = 13 to 33)	True Positives (Ct 13-29)
1	21.2	Positive	1	1
2	22.5	Positive	1	1
3	19.8	Positive	1	1
4	23.0	Positive	1	1
5	16.2	Positive	1	1
6	22.6	Positive	1	1
7	12.2	Positive	1	1
8	17.1	Positive	1	1
9	24.5	Positive	1	1
10	13.7	Positive	1	1
11	15.3	Positive	1	1
12	32.0	Negative		
13	19.2	Positive	1	1
14	24.4	Positive	1	1
15	22.1	Positive	1	1
16	30.0	Negative		
17	17.5	Positive	1	1
18	17.7	Positive	1	1
19	11.6	Positive	1	1
20	13.1	Positive	1	1
21	15.3	Positive	1	1
22	16.9	Positive	1	1
23	20.4	Positive	1	1
24	22.8	Positive	1	1
25	24.1	Positive	1	1
26	31.5	Negative		
27	30.5	Negative		
28	32.5	Negative		
29	28	Positive	1	1
30	25	Positive	1	1
31	13	Positive	1	1
32	21	Positive	1	1
33	32	Negative		
34	30	Positive	1	1
35	28	Positive	1	1
36	31	Negative		
37	32	Negative		
38	32	Negative		
Total Pos. LFA			29	29
Total Pos. PCR			38	29

While RTA is still in the process of performing the Clinical Validation, the results as shown in Tables 3 and 4 for our saliva swab test are very favorable compared to a competitor's nasopharyngeal swab test (Graph 1). A number of studies suggest that the ideal antigen test can detect the virus 100% of the time at Ct values below 30. It is at these concentrations that people are most likely to infect others. This is an approximately 9 day window from days 3 to day 12 of the infection (Graph 2).

Graph 1. Comparison of A) RTA's *COVID-19 Saliva Antigen At-Home Test* to B) a competitor's nasopharyngeal swab test for the detection of infected patients. RTA's Table 4 data.



Graph 2. Graph of Log₁₀ RNA copies/mL as a function of days since infection. The peak infection at day five is 2.1x10⁸ (210 million) RNA copies/mL of nasal mucus.

