Catalog # T5402

COVID-19 Surveillance Test Kit

Validation Report

For Research Use Only. Not for use in diagnostic procedures.

September 17, 2020

01 PERFORMANCE EVALUATION

ANALYTICAL SENSITIVITY

1.1 LIMIT OF DETECTION (LOD)

The LoD studies establish the lowest detectable concentration of SARS-CoV-2 (genome copies) at which 95% of all replicates test positive. The LoD of the COVID-19 Surveillance Test Kit was determined by twenty one replicates of nine different dilutions of SARS-Related Coronavirus 2 (SARS-CoV-2), Isolate USA-WA1/2020, Heat Inactivated (BEI Resources, NR-52286) with known titer (1.16 x 10^6 genome copies/µL). Respiratory matrix was prepared from negative saliva specimens with SARS-CoV-2 being directly spiked onto the saliva specimens. The saliva plus the spiked SARS-CoV-2 specimens were lysed by adding Chai Enzymatic DNA/RNA extraction Buffer 10X (Cat # R05220) at a 1:10 ratio to each specimen. Real-Time RT-qPCR assays were performed using Sahara One-Step RT-qPCR with UNG Master Mix (Cat # R02210). The test uses proprietary primers and probes that are designed to detect nucleocapsid (N) gene sequences in SARS-CoV-2 and Ribosomal Protein Lateral Stalk Subunit P0 *(RPLP0)* gene in saliva which acts as both an internal control and an extraction control to confirm the performance of the extraction. LoD screening was conducted by testing dilutions of inactive SARS-CoV-2 virus (BEI # NR-52286) prepared in negative saliva with 20 replicates for each concentration. The LoD was determined as the lowest concentration (genome copies/µL) where ≥ 95% of the replicates were positive on Bio-Rad CFX96 Real-Time PCR Detection System. The study results that are summarized in Table 1 below show that the LoD for the COVID-19 Surveillance Test Kit is 5.8 genome copies of SARS-CoV-2 per µL of saliva (5.8 copies/µL). The LoD for the COVID-19 Surveillance Test Kit was also confirmed on Chai Open qPCR machine (Table 2).

Table 1. Confirmation of the COVID-19 Surveillance Test Kit LoD on Bio-Rad CFX96 Real-Time PCR Detection System

Concentration (copies/µL)	N Gene		RPLP0	
	Positive Replicates Detected	Mean Cq Value (SD)	Positive Replicates Detected	Mean Cq Value (SD)
58.00	20/20	31.89 (0.18)	20/20	26.45 (0.05)
29.00	20/20	32.94 (0.24)	20/20	26.64 (0.06)
14.50	20/20	34.90 (0.42)	20/20	26.58 (0.08)
7.25	20/20	35.46 (0.63)	20/20	26.47 (0.12)
5.80	20/20	35.94 (0.70)	20/20	26.78 (0.09)
2.90	16/20	38.33 (1.88)	20/20	26.63 (0.11)
1.45	5/20	40.65 (2.42)	20/20	26.44 (0.07)

Table 2. Confirmation of the COVID-19 Surveillance Test Kit LoD on Chai Open qPCR

Concentration (copies/µL)	N Gene		RPLP0	
	Positive Replicates Detected	Mean Cq Value (SD)	Positive Replicates Detected	Mean Cq Value (SD)
58.00	20/20	32.61 (0.40)	20/20	25.00 (0.13)
29.00	20/20	34.66 (1.12)	20/20	25.04 (0.15)
14.50	20/20	35.95 (0.83)	20/20	25.26 (0.11)
7.25	20/20	34.57 (1.31)	20/20	25.15 (0.13)
5.80	20/20	34.36 (1.35)	20/20	25.08 (0.16)
2.90	18/20	35.28 (1.04)	20/20	25.12 (0.15)
1.45	14/20	35. 67 (1.47)	20/20	25.16 (0.09)

1.2 INCLUSIVITY/ ANALYTICAL REACTIVITY

To evaluate the analytical reactivity (inclusivity) of COVID-19 Surveillance Test for SARS-CoV-2, *in silico* analysis was performed with the sequences of primers and probe for N gene on all the publicly available sequences for severe acute respiratory syndrome coronavirus 2 in the National Center for Biotechnology Information (NCBI) Genbank as of September 15, 2020 (n= 23,735) to demonstrate the predicted inclusivity of the detection assay. The database search included GenBank+RefSeq sequences. BLASTn analysis shows 100% homology of the N primer and probe sequences to the available sequences.

02 ANALYTICAL SPECIFICITY

2.1 EXCLUSIVITY/ CROSS-REACTIVITY

Cross-reactivity of the Chai COVID-19 Surveillance Kit was evaluated using both *in silico* analysis and wet testing. BLASTn analysis queries of the primer and probe sequences for N gene SARS-CoV-2 RT-qPCR assays were performed against public domain nucleotide sequences. The database search parameters were set as follows: 1) The nucleotide collection consists of GenBank+EMBL+DDBJ+PDB+RefSeq sequences, but excludes EST, STS, GSS, WGS, TSA, patent sequences as well as phase 0, 1, and 2 HTGS sequences and sequences longer than 100Mb; 2) The database is non-redundant. Identical sequences have been merged into one entry, while preserving the accession, GI, title and taxonomy information for each entry; 3) Database was updated on October 3, 2019; 4) The search parameters automatically adjust for short input sequences and the expect threshold is 1000; 5) The match and mismatch scores are 1 and -3, respectively; 6) The penalty to create and extend a gap in an alignment is 5 and 2

was used to determine the cross-reactivity of the COVID-19 Surveillance Test Kit. The primer and probe individual sequences for N gene showed no sequence homology with human genome, other coronaviruses, and human microflora in upper and lower respiratory systems that would lead to potential false positives for RT-qPCR results. The N gene primer and probe sequences only showed >80% homology with SARS-coronavirus; however, no cross-reactivity was detected with wet testing. List of microorganisms evaluated for cross-reactivity against the primer and probe for SARS-CoV-2 N gene from the COVID-19 Surveillance Test Kit by *in silico* analysis and wet testing are shown in Table 3 and 4. No cross-reactivity was predicted or observed.

Table 3. *In silico* cross-reactivity study result

Microorganism	Tax ID	N Gene % Homology		
		Forward	Reverse	Probe
Human coronavirus 229E	taxid:11137	65	45	42
Human coronavirus OC43	taxid:31631	50	40	38
Human coronavirus HKU1	taxid:290028	45	40	46
Human coronavirus NL63	taxid:277944	45	40	53
MERS-coronavirus	taxid:1335626	55	45	53
SARS-coronavirus	taxid:694009	95	81	92
Human adenovirus B1	taxid:565302	50	45	38
Human Metapneumovirus (hMPV)	taxid:162145	70	45	38
Human parainfluenza virus 4a	taxid:11224	45	41	34
Human parainfluenza virus 4b	taxid:11226	50	36	34
Human parainfluenza virus 1	taxid:188538	45	36	34
Human parainfluenza virus 2 (strain Toshiba)	taxid:11214	0	0	0
Human parainfluenza virus 2 (strain Greer)	taxid:11213	0	0	0
Influenza A virus	taxid:11320	60	59	50
Influenza B virus	taxid:11520	55	59	42
Enterovirus	taxid:12059	70	54	50

Respiratory syncytial virus	taxid:12814	60	45	34
Rhinovirus	taxid:12059	70	54	50
Herpes simplex virus 1	taxid:10298	50	45	42
Herpes simplex virus 2	taxid:10310	50	50	38
Chlamydia pneumoniae	taxid:83558	60	73	42
Haemophilus influenzae	taxid:727	70	54	50
Legionella pneumophila	taxid:446	70	59	50
Streptococcus pneumoniae	taxid:1313	70	68	53
Pneumocystis jirovecii	taxid:42068	55	54	53
Pseudomonas aeruginosa	taxid:287	75	63	50
Actinomyces viscosus	taxid:1656	70	50	46
Candida albicans	taxid:5476	75	59	54
Staphylococcus aureus	taxid:1280	55	59	54
Staphylococcus epidermidis	taxid:1282	65	59	50
Staphylococcus pyogenes aureus	taxid:1280	55	59	54
Streptococcus pyogenes	taxid:1314	70	59	50
Streptococcus salivarius	taxid:1304	65	54	58
Streptococcus mutans	taxid:1309	65	59	50
Lactobacillus johnsonii	taxid:525330	0	0	0
Porphyromonas gingivalis	taxid:837	60	59	46
Mycobacterium tuberculosis	taxid:1773	65	59	58
Moraxella catarrhalis	taxid:480	65	54	50
Corynebacterium diphtheriae	taxid:1717	65	63	53
Nocardia sp.	taxid:1817	60	77	65

Bacteroides oralis	taxid:28134	45	36	42
Chlamydophila pneumoniae	taxid:83558	60	72	42
Mycoplasma pneumoniae	taxid:2104	50	50	46
Bordetella pertussis	taxid:520	0	54	58

Table 4. Wet testing cross-reactivity study result

Pathogen	Concentration	N Gene (Positive Amplification)	RPLP0 Cq
Human coronavirus, 229E	⩾10^5 pfu/mL	0/3	24.62
SARS-CoV	⩾10^5 pfu/mL	0/3	24.78
Human Parainfluenza Virus 2	⩾10^5 pfu/mL	0/3	24.56
Human Parainfluenza Virus 4A	⩾10^5 pfu/mL	0/3	24.44
Human Parainfluenza Virus 4B	⩾10^5 pfu/mL	0/3	24.82
Human parainfluenza virus 1	⩾10^5 pfu/mL	0/3	24.75
Rhinovirus	⩾10^5 pfu/mL	0/3	24.44
Enterovirus	⩾10^5 pfu/mL	0/3	24.82
MERS-CoV	⩾10^5 pfu/mL	0/3	25.10
Human metapneumovirus	⩾10^5 pfu/mL	0/3	24.77
Human Coronavirus NL63	⩾10^5 pfu/mL	0/3	24.64
Avian Infectious Bronchitis Virus (IBV)	⩾10^5 pfu/mL	0/3	24.54
Influenza A	⩾10^5 pfu/mL	0/3	24.90
Influenza B	⩾10^5 pfu/mL	0/3	24.81
Respiratory syncytial virus	⩾10^5 pfu/mL	0/3	24.63
Mycobacterium tuberculosis	⩾10^6 CFU/mL	0/3	24.71
Candida albicans	⩾10^6 CFU/mL	0/3	24.75
Streptococcus salivarius	⩾10^6 CFU/mL	0/3	24.81

Streptococcus pneumoniae	⩾10^6 CFU/mL	0/3	24.58
Streptococcus pyogenes	⩾10^6 CFU/mL	0/3	24.66
Bordetella pertussis	⩾10^6 CFU/mL	0/3	24.76
Pseudomonas aeruginosa	⩾10^6 CFU/mL	0/3	24.82

2.2 ENDOGENOUS AND EXOGENOUS INTERFERENCE SUBSTANCES STUDIES

An endogenous and exogenous interfering substances study was performed to determine if common interferents that could be present in respiratory samples including saliva could impact Chai COVID-19 Surveillance Test Kit performance. Each endogenous/exogenous interfering substance was evaluated at the highest medically relevant concentration (worst case) with samples spiked at 3X LoD (positive contrived sample consisting of spiked inactivated virus in negative saliva matrix). Prepared samples with each interfering substance were extracted with the Enzymatic DNA/RNA Extraction Buffer 10X and three replicates were tested on the Bio-Rad CFX96 Real-Time PCR Detection System. The list of tested interfering substances are shown in Table 5. No interference from the endogenous substances was observed.

Table 5. Endogenous/Exogenous Interfering Substances Evaluated in Interference Testing

Interfering Substance	Tested Concentration	Mean Cq	
		N Gene	RPLP0
Nasal Mist (Oxymetazoline Hydrochloride)	15% (v/v)	36.76	25.78
Nasal allergy Spray (Triamcinolone Acetonide)	5% (v/v)	35.56	25.55
Zicam (Luffa Operculata)	5% (v/v)	36.20	26.11
Flonase (Fluticasone Propionate)	5% (v/v)	35.66	25.78
Mucin	20 µg/mL	35.18	25.41
Zanamivir	75 µg/mL	35.66	25.55
Tobramycin	4 µg/mL	35.65	25.70
Menthol	500 µg/mL	35.09	25.55
Mouthwash	5% (v/v)	34.63	28.40
Toothpaste	0.50% (v/v)	36.50	25.32

Nicotine	30 µg/mL	36.74	25.35
Blood	2% (v/v)	36.42	25.32

03 POOLING AND SENSITIVITY FACTOR VALIDATION

We evaluated the sensitivity of sample pooling and the effect of sensitivity factor on Chai COVID-19 Surveillance Test Kit performance. Two saliva sample pools were generated by combining one negative saliva sample with spiked heat-inactivated SARS-CoV-2 (BEI Resources, NR-52286) at the LoD level and three or seven negative saliva samples (n = 3 and n = 8). The first pool (n = 4) was tested using the sensitivity factor of 2 (see COVID-19 Surveillance Test Kit Product Manual for more details), and the second pool (n = 8) was tested using the sensitivity factor of 3. The replicates from each pool were run on both Bio-Rad CFX96 Real-Time PCR Detection System and Chai Open qPCR. The individual positive was used as the control to compare the sensitivity of COVID-19 Surveillance Test Kit in individual and pooled samples. The result from this study is summarized in Table 6.

Table 6. Testing of pooled saliva samples with the Chai COVID-19 Surveillance Test Kit

Sample	Sensitivity Factor	Number of Replicates	N Gene Cq (Positive Amplification)		RPLP0 Cq	
			Bio-Rad CFX96	Chai Open qPCR	Bio-Rad CFX96	Chai Open qPCR
Pool #1 (n = 4)	2	2	35.08 (2/2)	35.28 (2/2)	26.36	25.29
Pool #2 (n = 8)	3	3	37.38 (2/3)	36.28 (1/3)	25.96	25.31
Individual Positive	N/A	3	32.50 (3/3)	34.07 (3/3)	26.53	25.69

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