Catalog #: T1401

Coronavirus Environmental Test Kit

Validation Report

For research use only

May 1, 2020

PERFORMANCE EVALUATION

01 ANALYTICAL SENSITIVITY

1.1. LIMIT OF DETECTION (LoD)

The LoD studies establish the lowest detectable concentration of SARS-CoV-2 (genome copies) at which approximately 95% of all replicates test positive. The Coronavirus Environmental Kit was determined by twenty eight replicates of six different dilutions of SARS-Related Coronavirus 2, Isolate USA-WA1/2020, Heat Inactivated (BEI Resources, NR-52286) with known titer (1.16 x 106 genome copies/µL) that was directly spiked onto the environmental swabs from the kit. The virus was lysed by inserting the swab into One-Step DNA/RNA Extraction Buffer (Cat # R05210). Real-Time RT-qPCR assays were performed using Sahara One-Step RT-qPCR with UNG Master Mix (Cat # R02210). The N1 and N2 targets from the CDC 2019-nCoV Diagnostic Panel were used for detection. The LoD was determined as the lowest concentration (genome copies/swab) where ⩾ 95% of the replicates were positive.

The study results that are summarized in Table 1 below show that the LoD for the Coronavirus Environmental Kit is 290 copies/swab for environmental samples.

Table 1. Confirmation of the Coronavirus Environmental Kit Limit of Detection

Concentration (genome copies/swab)	Positive Replicates	Mean Cq Value	Standard Deviation
116000	28/28	28.54	0.16
11600	28/28	31.23	0.35
1160	28/28	35.31	0.43
580	28/28	36.38	0.71
290	28/28	37.35	0.81
145	26/28	38.5	1.17

1.2. SWABBING RECOVERY

The swabbing recovery of SARS-Related Coronavirus 2 particles from the surface was tested to further validate the LoD of the Coronavirus Environmental Kit. The swabbing recovery was determined by twenty eight replicates of five different dilutions of heat-inactivated SARS-Related Coronavirus 2, Isolate (BEI Resources, NR-52286) with known titer of 1.16 x 10_6 genome copies/µL. Five different dilutions of the virus were spotted onto a hard plastic surface. Next, the surface was swabbed and the virus lysed by inserting the swab into One-Step DNA/RNA Extraction Buffer (Cat # R05210). Real-Time RT-qPCR assays were performed using Sahara One-Step RT-qPCR with UNG Master Mix (Cat # R02210). The N1 and N2 targets from the CDC 2019-nCoV Diagnostic Panel were used for detection. The swabbing recovery was calculated by comparing the Cq of surface swabs with the swabs that were directly spiked by the virus at the same concentration. The genome copies of SARS-CoV-2 on the surface swabs were adjusted by incorporating the loss during recovery of the virus from the surface.

The study results that are summarized in Table 2 below demonstrate that the Coronavirus Environmental Kit provides an average of 63% swabbing recovery.

Table 2. Determination of the Coronavirus Environmental Kit Swabbing Recovery

Expected Concentration (genome copies/swab)	**Spiked Swab Mean Cq Value**	**Surface Swab Mean Cq Value**	**Cq Difference**	**Swabbing Recovery (%)**
116000	28.54	29.21	0.67	62.8%
11600	31.23	32.02	0.79	57.8%
1160	35.31	35.76	0.45	73.2%
580	36.38	37.11	0.73	60.3%
290	37.35	38.05	0.7	61.5%

02 ANALYTICAL SPECIFICITY: REACTIVITY/INCLUSIVITY

2.1. *IN SILICO* ANALYSIS OF PRIMER AND PROBE SEQUENCES

In Silico analysis was performed with the sequences of primer and probe for the CDC N1 and N2 RT-qPCR Diagnostics Panel with all the publicly available nucleic acid sequences for severe acute respiratory syndrome coronavirus 2 Genbank in NCBI as of May 1, 2020 (n= 2051) to demonstrate the predicted inclusivity of the detection assay. The database search included GenBank+RefSeq sequences. BLASTn analysis shows 100% homology of the N1 and N2 primer and probe sequences to the available sequences with the exception of one nucleotide mismatch with the N1 forward primer in one of the available sequences. The risk of a single mismatch causing loss of reactivity and a false negative result is very low since one to two mismatches are tolerated in a qPCR assay when the mismatch is not at the 3' end of the oligo.

2.2. SPECIFICITY/EXCLUSIVITY TESTING: *IN SILICO* ANALYSIS

BLASTn analysis queries of the primer and probe sequences for N1 and N2 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 respectively. *In Silico* analysis (greater than 80% homology to the primer and probe for the N1 and N2 targets) was used to determine the cross-reactivity of the Coronavirus Environmental Kit.

Combining the primer and probe for N1 and N2 individually showed no sequence homology with SARS coronavirus, Bat SARS-like, human genome, other coronaviruses, and human microflora that would lead to potential false positives for RT-qPCR results. Other organisms and viruses evaluated for cross-reactivity, by *in silico* analysis, against the primer and probe for N1 and N2 SARS-CoV-2 from the Coronavirus Environmental Kit are listed in Table 3.

As a result of analysis, there were no microorganisms with potential nonspecific or cross-reactive sequences.

Table 3. List of Evaluated Organisms for the Coronavirus Environmental Kit Cross-Reactivity

Viruses	**Bacteria**
Adenovirus	*Bordetella pertussis*
Enterovirus	*Candida albicans*
Human coronavirus 229E	*Chlamydia pneumoniae*
Human coronavirus HKU1	*Clostridium perfringens*
Human coronavirus NL63	*Escherichia coli*
Human coronavirus OC43	*Haemophilus influenzae*
Human metapneumovirus (hMPV)	*Legionella pneumophila*
Influenza A virus	*Listeria monocytogenes*
Influenza B virus	*Mycobacterium tuberculosis*
MERS-coronavirus	*Mycoplasma pneumoniae*
Norovirus	*Pneumocystis jirovecii (PJP)*
Parainfluenza virus 1-4	*Pseudomonas aeruginosa*
Respiratory syncytial virus	*Staphylococcus epidermidis*
Rhinovirus	*Streptococcus pneumoniae*
SARS-coronavirus	*Streptococcus pyogenes*
	Streptococcus salivarius

03 SURFACE MATERIAL INTERFERENCE STUDY

We tested potential interference/inhibition from the surface material. The performance of the Coronavirus Environmental Kit was validated on plastic, stainless steel, brass, rubber and ceramic surfaces. The gRNA was extracted from the swabs used to swab different surfaces using One-Step DNA/RNA Extraction Buffer. No interference/inhibition from the surface material was observed at tested concentration (10X LoD).

04 ENVIRONMENTAL PERFORMANCE EVALUATION

Environmental samples were obtained from shopping carts, ATMs, gas station pumps, and public restroom fixtures. The tested samples were collected during the COVID-19 pandemic between March 31, 2020 and April 14, 2020 from the San Francisco Bay Area.

The swabs used for environmental sampling which could be potentially contaminated by SARS-CoV-2 were inactivated immediately by inserting the swab in One-Step DNA/RNA Extraction Buffer and lysing the virus. The lysates were stored at -80°C until analysis. Each environmental sample was analysed in triplicate. In total, 36 different environmental samples from public spaces were tested, with five confirmed to be positive for SARS-CoV-2. Detection of positive environmental samples for SARS-CoV-2 during the shelter-in-place order in the San Francisco Bay Area further supports and validates the sensitivity of the Coronavirus Environmental Kit.