CHAI

COVID-19 Saliva Dx Test Kit

Instructions for Use

RT-qPCR assay for the detection of SARS-CoV-2

Catalog #T6010
100 tests/kit

This test kit has been validated by Chai pursuant to the FDA Policy for Coronavirus Disease-2019 Tests During the Public Health Emergency. FDA's independent review of this validation is pending.

Table of Contents

1. INTRODUCTION

1.1 INTENDED USE

The Chai COVID-19 Saliva Dx Test Kit is a RT-qPCR test intended for the qualitative detection of the SARS-CoV-2 virus in upper and lower respiratory specimens including nasopharyngeal swab in viral transport media (VTM), and saliva specimens collected without preservatives in a sterile container. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet requirements to perform high complexity tests.

Results are for the identification of SARS-CoV-2 RNA, which is generally detectable in respiratory specimens during the acute phase of infection. Positive results indicate the presence of SARS-CoV-2 RNA. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Laboratories within the United States and its territories are required to report all results to the appropriate public health authorities. Negative results indicate that the amount of SARS-CoV-2 present in the initial sample is lower than the limit of detection.

Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for public health decisions. Negative results should be combined with patient history, clinical observations and epidemiological information.

The COVID-19 Saliva Dx Test Kit is intended for use by qualified laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures. The Chai COVID-19 Saliva Dx Test Kit has been validated by Chai pursuant to the FDA Policy for Coronavirus Disease-2019 Tests During the Public Health Emergency. FDA's independent review of this validation is pending.

1.2 PRINCIPLES OF THE PROCEDURE

The COVID-19 Saliva Dx Test Kit is a qualitative, RT-qPCR (Reverse Transcription Quantitative Polymerase Chain Reaction) test in which specific primers and fluorescent probes are designed to detect SARS-CoV-2 RNA. The assay supports nasopharyngeal swabs stored in VTM and saliva specimens. RNA is initially extracted from the specimen with Chai Enzymatic DNA/RNA Extraction Buffer (10X) that uses chemical and enzymatic activity for lysing the sample at ambient temperature (20 – 30 ˚C). The specimen is first incubated at ambient temperature (20 – 30 ˚C) for 15 minutes to lyse. The enzyme is then inactivated during a subsequent 95 ˚C incubation step to impede RNA degradation. The extracted RNA is then reverse transcribed to complementary DNA (cDNA) and amplified using Real-Time PCR.

The assay utilizes hydrolysis probes that incorporate an oligonucleotide complementary to the target sequence with a fluorescent dye molecule (FAM or HEX) on its 5' end and quencher dye molecule on its 3' end. During PCR, the probes anneal to their specific target sequence located between the forward and reverse primers. Fluorescence is suppressed while the probe remains intact. During synthesis of the complementary DNA strand, the polymerase cleaves the probe, separating its quencher from the fluorescent dye molecule and generating a fluorescence signal. As additional fluorescent dye molecules are cleaved from their respective probes, the signal intensity increases. The fluorescence for each target is measured during each PCR cycle and a threshold value can be determined from the resulting amplification curve. This threshold value, or cycle of quantification (Cq), is proportional to the concentration of target in each reaction. No reference dye is required.

The kit has been designed and evaluated using the publicly available full and partial SARS-CoV-2 genome sequence. The assay detects a conserved region in the SARS-CoV-2 nucleocapsid (N) gene with FAM and a conserved region in human Ribosomal Protein Lateral Stalk Subunit P0 (*RPLP0*) gene with HEX. *RPLP0* is an endogenous control gene used as the internal/extraction control to confirm successful RNA extraction and RT-qPCR amplification. This human housekeeping gene displays no cross reactivity with SARS-CoV-2 and utilizes an intron-flanking primer and probe design to prevent amplification from genomic DNA. The assay also utilizes dUTP for DNA synthesis and includes a Uracil-DNA Glycosylase (UNG) carryover prevention system to prevent amplicon contamination from previous qPCR runs.

The test kit has been validated on Chai's dual channel Open qPCR instrument and on the Bio-Rad CFX96 Real-Time PCR Detection System.

1.3 WARNINGS AND PRECAUTIONS

1. The COVID-19 Saliva Dx Test Kit is intended for in vitro diagnostic use only.

2. For prescription use only.

3. The COVID-19 Saliva Dx Test Kit has not been FDA cleared or approved. Testing is limited to laboratories which are certified under the Clinical Laboratory Improvement Amendments (CLIA) of 1988, 42 U.S.C. §263a, and that meet requirements to perform high complexity tests.

4. Laboratories should include a statement such as 'the test has been validated but FDA's independent review of this validation is pending' in test reports to healthcare providers.

5. The COVID-19 Saliva Dx Test Kit is designed for the specific detection of RNA from SARS-CoV-2. The test is not intended for other viruses or pathogens.

6. Performance of the test has only been validated with nasopharyngeal swabs in VTM and saliva specimens.

7. Positive results do not rule out co-infection with other viruses or bacteria.

8. The test is subject to limitations and potential errors that may result from inadequate sample collection, handling, or transport, insufficient RNA extraction, and misuse of equipment. False positive or false negative results may occur.

9. Kit components must be stored at -20 ºC and must be fully thawed, homogenized, and centrifuged according to the instructions prior to use. Avoid repeated freeze-thaw cycles when possible.

10. Perform all RT-qPCR reaction setup in an environment that minimizes active air flow to prevent potential amplicon contamination.

11. The use of PPE (Personal Protective Equipment) is essential during sample collection and RT-qPCR reaction setup to minimize the risk of exposure to infectious viruses or unknown bacteria. Utilize disposable gloves and wear eye protection gear, a face mask, and lab coat when performing any part of the kit workflow. Change PPE frequently to avoid cross contamination between samples.

12. Sanitize the workspace and all equipment used for sample preparation and reaction setup in accordance with CDC Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with Coronavirus Disease 2019 (COVID-19).

13. Handle all samples, reagents, and waste materials as if they are capable of transmitting infection agents. Seal all reagents, controls, and sample tube caps when not in use. Keep materials away from skin, eyes, or mucous membranes and avoid ingesting or swallowing. Refer to CDC Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with SARS-CoV-2 (https://www.cdc.gov/coronavirus/2019-nCoV/lab-biosafety-guidelines.html).

14. Handle all specimens as if infectious using safe laboratory procedures. Dispose of used and unused reagents and waste in accordance with Country, Federal, State, and Local requirements.

15. Avoid handling the positive control when not required to prevent cross contamination and cause false positives. The template for the positive control should be added last.

16. PCR tube and cap strips or plates should not be opened after RT-qPCR to prevent amplicon contamination. Dispose of all hazardous materials and thoroughly decontaminate all equipment and preparation workspaces in accordance with local regulatory guidelines.

1.4. KIT COMPONENTS

The COVID-19 Saliva Dx Test Kit includes all components necessary for 100 tests (Table 1). Reagents should not be used past the expiration date.

Table 1. COVID-19 Saliva Dx Test Kit Components

Component	Quantity	Storage
Sahara One-Step RT-qPCR Master Mix with UNG	1.1 mL	-20 ˚C
COVID-19 Saliva Dx Oligo Mix (primers, probes)	415 µL	-20 ˚C
COVID-19 Saliva Dx Cofactor Buffer	415 µL	-20 ˚C
COVID-19 Saliva Dx Positive Control	110 µL	-20 ˚C
DNase/RNase-Free Distilled Water	500 µL	-20 ˚C to 25 ˚C

1.5 ADITIONAL REQUIRED MATERIALS

- Chai Enzymatic DNA/RNA Extraction Buffer, 10X (Cat # R05221)
- Real-Time PCR Thermocycler with FAM and HEX fluorophore detection
- Dry heating block capable of heating 1.5 mL tubes at 95 ˚C ±1 ˚C
- Benchtop centrifuge capable of spinning 1.5 mL tubes and PCR tubes or plates at 3,000 g (RCF)

- Micropipettes (range between 0.5 to 10 µL, 10 to 100 µL and 100 to 1000 µL)
- Non-aerosol filtered-barrier pipette tips
- 96-well PCR plate and optical plate films or PCR tubes and caps
- 1.5 mL microcentrifuge tubes

1.6 REAGENT STORAGE AND SHELF LIFE

- Store all components according to specification.
- All components should be stored and used together. Do not pool reagents across kits or lots and discard surplus components from the kit after use.
- Avoid excessive freeze-thaw cycles. Reagents can be aliquoted into smaller volumes and stored at -20 ˚C in order to preserve stability.
- Check the expiration date of all reagents prior to use. Do not use components beyond the listed expiration date.

1.7 SPECIMEN COLLECTION, HANDLING, AND STORAGE

The COVID-19 Saliva Dx Test Kit is designed for human nasopharyngeal swabs in VTM, and saliva specimens collected without preservatives in a sterile container.

For the packaging and transport of specimens, consult the current version of the International Air Transport Association (IATA) Dangerous Goods Regulation and follow shipping regulations for UN 3373 Biological Substance, Category B.

1.7.1 NASOPHARYNGEAL SWABS IN VTM

Swabs and VTM media for collecting specimens are not included in the kit. Adhere to CDC Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens for COVID-19 prior to specimen processing.

Human nasopharyngeal swabs in VTM can be stored at 2 – 8 ˚C for up to 72 hours after collection and at -70 ˚C or below for long term storage. For nasopharyngeal swabs in VTM, a collection volume of 1 – 5 mL is sufficient for testing.

1.7.2 SALIVA

Containers for collecting saliva specimens are not included in the kit. Collect saliva specimens using a sterile container. The container used should be empty, and not contain a preservative or any other material. The kit was tested with both sterile tubes and sterile cups. When available cups are preferred, as the wider mouth makes saliva collection easier.

Adhere to CDC Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens for COVID-19 prior to specimen processing.

Saliva specimens can be stored at ambient temperature for up to 16 hours, at 2 – 8 ˚C for up to 72 hours after collection, and at -70 ˚C or below for long term storage. For saliva samples, a minimum collection volume of 500 µL is recommended for ease of sample processing.

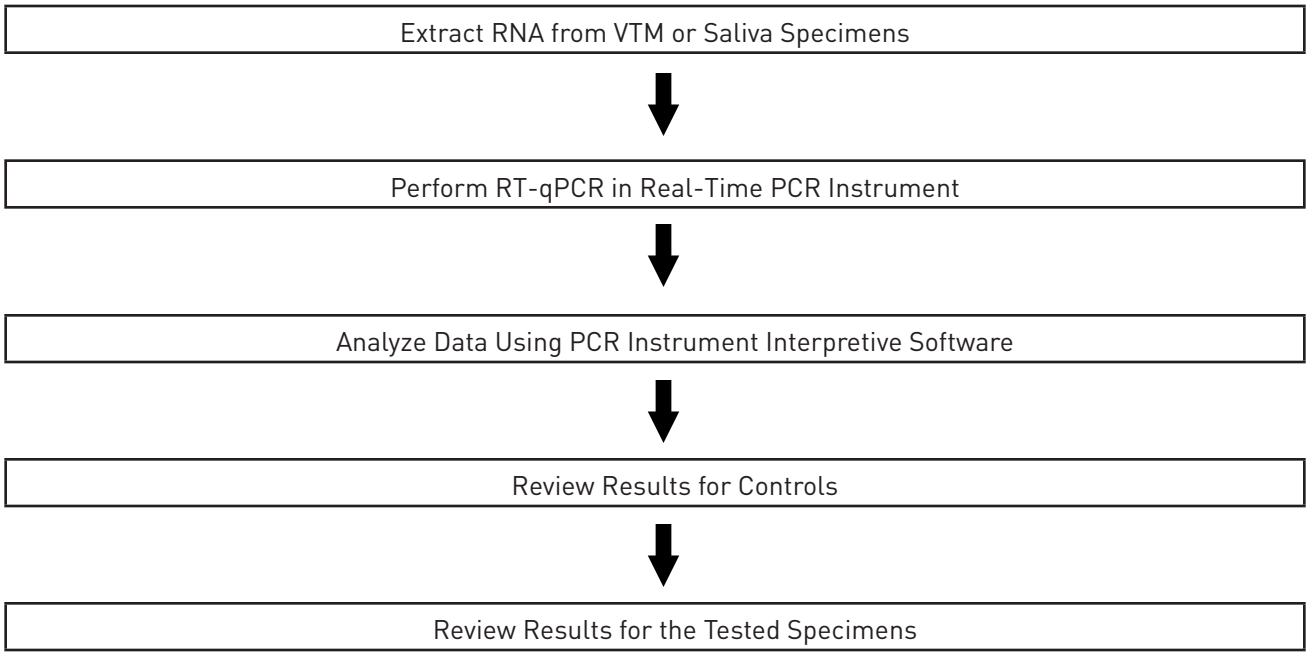
1.8 REAL-TIME PCR INSTRUMENTS VALIDATED FOR USE

The following real-time PCR instruments have been validated for use with the COVID-19 Saliva Dx Test Kit:

- Chai Open qPCR dual channel equipped with v1.1 Software (Catalog # E013201)
- Bio-Rad CFX96 Real-Time PCR Detection System equipped with CFX Maestro 2.0 Version 5

Please ensure that all instruments used have been installed, calibrated and maintained according to the manufacturer's instruction and recommendations.

2. WORKFLOW



2.1 SAMPLE LYSIS AND RNA EXTRACTION

Nasopharyngeal swabs in VTM and saliva specimens must be lysed to release viral genomic RNA from SARS-CoV-2 and endogenous control RNA from human cells.

Thaw the Enzymatic DNA/RNA Extraction Buffer and specimen (if frozen) completely at room temperature (25 ˚C) before proceeding with lysis and RNA extraction.

2.1.1 NASOPHARYNGEAL SWAB IN VTM

1. Homogenize the VTM specimen thoroughly by vortexing briefly.

2. Pipette 180 µL of the VTM specimen into a 1.5 mL tube.

3. Add 20 µL of Enzymatic DNA/RNA Extraction Buffer (10X) into the tube and mix contents completely by vortexing for 3 seconds.

4. Incubate the tube at ambient temperature (20 ˚C – 30 ˚C) for 15 minutes. Mix the lysate at least once by vortexing for 3 seconds halfway through the incubation time.

5. Incubate the tube at 95 ˚C for 10 minutes. The lysate is now ready for the RT-qPCR reaction.

6. Centrifuge the tube for 20 seconds at a minimum of 1,500 g (RCF). This may form a small white pellet at the bottom of the tube. Avoid disturbing the pellet; the supernatant will contain the extracted RNA.

2.1.2 SALIVA

1. Homogenize the saliva specimen thoroughly by vortexing briefly.

2. Pipette 180 µL of the saliva specimen into a 1.5 mL tube utilizing a 1000 µL pipette due to the viscosity of saliva. If viscosity interferes with proper pipetting of saliva, it may be necessary to cut the tip at a slight angle with a sterile scissors to enlarge the tip orifice.

3. Add 20 µL of Enzymatic DNA/RNA Extraction Buffer (10X) into the tube and mix contents completely by vortexing for 3 seconds.

4. Incubate the tube at ambient temperature (20 – 30 ˚C) for 15 minutes. Mix the lysate at least once by vortexing for 3 seconds halfway through the incubation time.

5. Incubate the tube at 95 ˚C for 10 minutes.

6. Centrifuge the tube for 20 seconds at a minimum of 3,000 g (RCF). This may form a small white pellet at the bottom of the tube. Avoid disturbing the pellet; the supernatant will contain the extracted RNA.

2.2 POST-LYSIS SAMPLE STORAGE

For long term storage, store the lysate at -70 ˚C or below. For saliva lysate, transfer the supernatant to a new 1.5 mL tube for storage to prevent the pellet from being resuspended in solution.

2.3 RT-qPCR REACTION SETUP

1. Completely thaw all reagents at room temperature (15 – 30 °C) prior to use. Thaw lysates if frozen at -20 °C or -80 °C. Homogenize the contents of all samples and reagents by vortexing or pipetting.

2. Briefly centrifuge all reagents at a minimum of 1,500 g (RCF). Set aside the COVID-19 Saliva Dx Positive Control and DNase/RNase-Free Water tubes to be used later for the Positive Control and No Template Control (NTC).

3. Prepare a Common Mix containing the Sahara One-Step RT-qPCR Master Mix with UNG, COVID-19 Saliva Dx Oligo Mix, and COVID-19 Saliva Dx Cofactor Buffer for the total number of PCR reactions required (Table 2). Count the Positive Control, No Template Control (NTC) and each sample lysate as an individual reaction. Include an excess 10% in the calculation to account for pipetting error. Pipette each Common Mix component into a 1.5 mL tube, then briefly vortex or mix by pipetting. Briefly centrifuge the tube at a minimum of 1,500 g (RCF).

4. Aliquot 17.5 µL of the Common Mix into each PCR tube or plate well.

5. Add the required template (Positive Control, NTC, or sample lysate) to a PCR tube or plate well.

    a. Prepare sample reactions by adding 2.5 µL of each lysate.
    b. Prepare the NTC reaction by adding 2.5 µL of DNAse/RNAse–Free Water.
    c. Prepare the Positive Control by adding 2.5 µL of COVID-19 Saliva Dx Positive Control. The Positive Control should be prepared last to minimize potential risk of contamination.

6. Seal the plate or tubes and centrifuge for 30 seconds at a minimum of 1,500 g (RCF).

7. Load the plate or tubes into the thermocycler and start the RT-qPCR run using the following cycling protocol (Table 3). The data collection point should be set at the final 62 ˚C annealing step. Configure the software to detect N gene SARS-CoV-2 target in FAM and the *RPLP0* gene as the internal/extraction control target in HEX.

Table 2. Common Mix for a Single Reaction

Component	Volume (µL) per Reaction	Volume (µL) per Reaction (including excess 10%)
Sahara One-Step RT-qPCR Master Mix with UNG	10.00	11.00
COVID-19 Saliva Dx Oligo Mix	3.75	4.13
COVID-19 Saliva Dx Cofactor Buffer	3.75	4.13
Total Common Mix	**17.50**	**19.26**

Table 3. RT-qPCR Protocol

Function	Temperature	Time	# Cycles
UNG activation	25 ºC	5 min	1
Reverse transcription	62 ºC	5 min	
Initial denaturation	95 ºC	30 sec	
Denaturation	95 ºC	10 sec	45
Annealing	62 ºC	40 sec	

3. POST RUN ANALYSIS

3.1 INTERPRETATION OF RESULTS

3.1.1 CONTROL RESULTS INTERPRETATION

Results are interpreted based on the presence or absence of targets in the FAM and HEX channels. A region of the SARS-CoV-2 nucleocapsid (N) gene is detected in FAM as the virus target and a region of the human Ribosomal Protein Lateral Stalk Subunit P0 (*RPLP0*) gene is detected in HEX as the internal/extraction control target. It is essential to assess the validity of all controls based on the presence or absence of the FAM and HEX targets (Table 4). The positive control should show amplification in both the FAM and HEX channels. The NTC should not show amplification in either channel. If the positive control and NTC are valid, then the specimen can be evaluated for the presence of SARS-CoV-2.

Table 4. Control results evaluation

Positive Control (N gene - FAM)	Positive Control (*RPLP0* - HEX)	NTC (N gene - FAM)	NTC (*RPLP0* - HEX)	Result
POS	POS	NEG	NEG	Valid
NEG	ANY	ANY	ANY	Invalid N gene positive control
ANY	NEG	ANY	ANY	Invalid *RPLP0* gene positive control
ANY	ANY	POS	ANY	Invalid N gene NTC control
ANY	ANY	ANY	POS	Invalid *RPLP0* gene NTC control

3.1.2 INTERPRETATION OF PATIENTS SPECIMENS

Assessment of clinical specimen test results should be performed after the positive control and NTC have been evaluated and determined to be valid. If the controls are not valid, the patient results cannot be interpreted and the test should be repeated. Each specimen should be evaluated for the presence or absence of the N gene SARS-CoV-2 target in FAM and the *RPLP0* internal/extraction control target in HEX. Amplification in FAM indicates that SARS-CoV-2 was detected in the patient's specimen. HEX amplification verifies successful RNA sample extraction from the patient's specimen, reverse transcription, and PCR amplification (Table 5). If the obtained results do not adhere to these guidelines, the existing specimens should be re-extracted and retested.

Table 5. Patient Specimens Evaluation

N gene (FAM)	***RPLP0* gene (HEX)**	**Sample Result**
POS	POS	SARS-CoV-2 Detected
	NEG	
NEG	POS	SARS-CoV-2 Not Detected
NEG	NEG	Invalid Sample: Repeat the test

3.2 TROUBLESHOOTING

PROBLEM	POSSIBLE CAUSE	SOLUTION
No FAM/HEX fluorescence signal detected in any reaction	Incorrect concentration or omitted Common Mix components	Verify that all components (Sahara One-Step RT-qPCR Master Mix with UNG, COVID-19 Saliva Dx Oligo Mix and Cofactor Buffer) are added to the Common Mix in the correct amount.
	Incorrect PCR protocol	Utilize the correct cycling protocol for the RT-qPCR run as specified above. Ensure that the correct data collection step is selected
No internal/extraction control amplification for the patient's specimen	Sample is not successfully lysed due to inadequate Enzymatic DNA/RNA Buffer	Store extraction buffer at the recommended temperature. Minimize time at room temperature and number of freeze-thaw cycles to ensure that samples are sufficiently lysed.
	Enzymes in Enzymatic DNA/ RNA Extraction Buffer not completely inactivated	Incubate the pooled sample at 95 °C for at least 10 minutes to fully inactivate all enzymes. Incomplete inactivation of the extraction buffer may result in degradation of reagents during the PCR run.
	PCR inhibition from improper specimen collection	Avoid using the specimen if there is a visible amount of blood in VTM or saliva, or sputum in saliva.
	PCR inhibition from cellular debris in the lysate	Spin down the lysate after heat incubation and before running the PCR to collect cell debris at the bottom of the tube. Only use the supernatant as the template for the PCR reaction.
	Insufficient number of cells in initial specimen	Cells tend to settle down very quickly. Ensure that each specimen is homogenized thoroughly by vortexing or pipetting up and down before aliquoting.

PROBLEM	POSSIBLE CAUSE	SOLUTION
Amplification in FAM and/or HEX channel of NTC reaction	Carry-over contamination	Take measures to avoid potential sources of contamination. Change pipettes tips between samples, clean pipettes, use filtered tips, etc. Change gloves regularly.
	Contamination of the extraction and/or RT-qPCR run preparation area	Clean surfaces and instruments with aqueous detergents, wash lab coats, and replace test tubes and tips in use. Ensure that all sample prep is performed in a still air environment. Preferably, separate the areas for RNA extraction, PCR set up, and PCR instruments.

3.3 LIMITATIONS

1. The Adhere to all instructions provided. Deviations from specified test procedures, storage conditions, materials, equipment, and/or recommended samples may lead to experiment failures or inconsistencies.

2. Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for public health decisions.

3. Performance of the test has only been validated with nasopharyngeal swabs in VTM and saliva specimens.

4. The detection of SARS-CoV-2 RNA may be affected by specimen collection, symptom presence, and/or stage of infection. Improper collection, storage, or transport of specimens may lead to false negative results.

5. The test does not rule out diseases caused by other bacterial or viral pathogens.

6. False negative results may occur if:

    - Specimens are improperly collected, handled, or stored
    - PCR inhibitors are present in the specimen
    - The amount of SARS-CoV-2 in the specimen is below the limit of detection of the test
    - Spontaneous mutations within the target sequence affect primer and/or probe binding

7. The impact of the administration of SARS-CoV-2 vaccines and/or therapeutics on the ability to detect SARS-CoV-2 RNA in patient specimens has not been evaluated.

4. PERFORMANCE EVALUATION

4.1 ANALYTICAL SENSITIVITY

4.1.1 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 Saliva Dx Test Kit was determined by twenty replicates of different dilutions of SARS-Related Coronavirus 2 (SARS-CoV-2), Isolate USA-WA1/2020, Heat Inactivated (BEI Resources, NR-52286) with known titers (3.75 x 10^5 genome copies/µL). Respiratory matrix was prepared from negative nasopharyngeal swab in VTM or saliva specimens with SARS-CoV-2 being directly spiked into the corresponding specimen.

The negative specimen plus the spiked SARS-CoV-2 were lysed by adding Enzymatic DNA/RNA extraction Buffer, 10X (Cat # R05221) 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 # R02311). The test uses proprietary primers and probes that are designed to detect nucleocapsid (N) gene sequences in SARS-CoV-2 and human Ribosomal Protein Lateral Stalk Subunit P0 (*RPLP0*) gene which acts as both an internal control and an extraction control to confirm the performance of the extraction. 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 6 and 7 below show that the LoD for the COVID-19 Saliva Dx Test Kit is 5.86 genome copies of SARS-CoV-2 per µL of nasopharyngeal swab in VTM (5.86 copies/µL) (Table 6) and 5.86 genome copies of SARS-CoV-2 per µL of saliva (5.86 copies/µL) (Table 7). The LoD of 5.86 copies/µL for the COVID-19 Saliva Dx Test Kit was also confirmed on Chai Open qPCR machine for both nasopharyngeal swabs in VTM (Table 8) and saliva (Table 9).

Table 6. Confirmation of the COVID-19 Saliva Dx Test Kit LoD of Nasopharyngeal Swab in VTM 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)
23.44	20/20	37.34 (0.50)	20/20	35.94 (0.31)
11.72	20/20	38.21 (0.86)	20/20	35.68 (0.39)
5.86	**19/20**	**38.93 (0.65)**	**20/20**	**35.92 (0.52)**
2.93	13/20	39.41 (0.95)	20/20	35.80 (0.43)
1.46	12/20	40.02 (1.08)	20/20	35.95 (0.39)

Table 7. Confirmation of the COVID-19 Saliva Dx Test Kit LoD of Saliva 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)
23.44	20/20	36.24 (0.32)	20/20	34.51 (0.18)
11.72	20/20	36.55 (0.56)	20/20	33.09 (0.25)
5.86	**19/20**	**36.83 (0.93)**	**20/20**	**31.80 (0.14)**
2.93	17/20	37.65 (0.74)	20/20	34.17 (0.21)
1.46	14/20	38.76 (1.27)	20/20	33.83 (0.20)

Table 8. Confirmation of the COVID-19 Saliva Dx Test Kit LoD of Nasopharyngeal Swab in VTM on Chai Open qPCR

Concentration (copies/µL)	N Gene		*RPLP0*	
	Positive Replicates Detected	Mean Cq Value (SD)	Positive Replicates Detected	Mean Cq Value (SD)
23.44	20/20	36.24 (0.41)	20/20	35.25 (0.90)
11.72	20/20	37.06 (0.56)	20/20	34.44 (0.36)
5.86	**20/20**	**37.75 (0.61)**	**20/20**	**35.07 (0.52)**
2.93	16/20	38.58 (0.73)	20/20	35.07 (0.25)
1.46	12/20	38.58 (0.57)	20/20	35.24 (0.32)

Table 9. Confirmation of the COVID-19 Saliva Dx Test Kit LoD of Saliva on Chai Open qPCR

Concentration (copies/µL)	N Gene		*RPLP0*	
	Positive Replicates Detected	Mean Cq Value (SD)	Positive Replicates Detected	Mean Cq Value (SD)
23.44	20/20	35.11 (0.24)	20/20	33.91 (0.33)
11.72	20/20	35.16 (0.37)	20/20	32.31 (0.23)
5.86	20/20	35.76 (0.40)	20/20	30.92 (0.17)
2.93	**20/20**	**36.90 (0.50)**	**20/20**	**33.66 (0.37)**
1.46	13/20	37.08 (0.44)	20/20	33.31 (0.24)

4.1.2 INCLUSIVITY/ANALYTICAL REACTIVITY

To evaluate the analytical reactivity (inclusivity) of COVID-19 Saliva Dx Test Kit 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.

4.2 ANALYTICAL SPECIFICITY

4.2.1 EXCLUSIVITY/CROSS-REACTIVITY

Cross-reactivity of the COVID-19 Saliva Dx Test 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 respectively. Greater than 80% homology to the primer and probe for the SARS-CoV-2 N gene was used to determine the cross-reactivity of the COVID-19 Saliva Dx 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. Additionally, SARS-CoV is not known to be currently

circulating in the human population, therefore it is highly unlikely to be present in patient specimens. List of microorganisms evaluated for cross-reactivity against the primer and probe for SARS-CoV-2 N gene from the COVID-19 Saliva Dx Test Kit by in silico analysis and wet testing are shown in Table 10 and 11. No cross-reactivity was predicted or observed.

Table 10. 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 11. 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

4.2.2 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 upper and lower respiratory samples including nasopharyngeal swabs and saliva could impact COVID-19 Saliva Dx 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 nasopharyngeal swabs and saliva matrix). Prepared samples with each interfering substance were extracted with the Chai 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 12 and Table 13 for nasopharyngeal swab in VTM and saliva, respectively. Three replicates were tested for each of the samples. No interference from the endogenous substances was observed in any of the replicates.

Table 12. Endogenous/Exogenous Interfering Substances Evaluated in Nasopharyngeal Interference Testing

Interfering Substance	Tested Concentration	Mean Cq	
		N Gene	*RPLP0*
Nasal Mist (Oxymetazoline Hydrochloride)	15% (v/v)	37.07	37.20
Nasal allergy spray (Triamcinolone Acetonide)	5% (v/v)	37.95	37.78
Zicam (Luffa Operculata)	5% (v/v)	37.76	38.72
Flonase (Fluticasone Propionate)	5% (v/v)	38.29	37.79
Mucin	20 µg/mL	37.19	36.43
Zanamivir	75 µg/mL	39.15	38.02
Tobramycin	4 µg/mL	38.01	37.93
Menthol	500 µg/mL	38.41	38.26
Blood	2% (v/v)	38.00	35.37

Table 13. Endogenous/Exogenous Interfering Substances Evaluated in Saliva Interference Testing

Interfering Substance	Tested Concentration	Mean Cq	
		N Gene	*RPLP0*
Nasal Mist (Oxymetazoline Hydrochloride)	15% (v/v)	40.63	30.78
Nasal allergy spray (Triamcinolone Acetonide)	5% (v/v)	37.38	29.37
Zicam (Luffa Operculata)	5% (v/v)	37.93	34.64
Flonase (Fluticasone Propionate)	5% (v/v)	37.42	30.32
Mucin	20 µg/mL	37.82	33.25
Zanamivir	75 µg/mL	39.42	35.19
Tobramycin	4 µg/mL	40.72	33.33
Menthol	500 µg/mL	37.35	33.31
Mouthwash (Listerine)	5% (v/v)	39.90	34.71
Toothpaste (Colgate Total Advanced Whitening)	0.25% (v/v)	36.72	27.75
Toothpaste (Crest Pro-Health Clear Mint)	0.25% (v/v)	37.52	30.04
Nicotine	30 µg/mL	39.38	33.53
Blood	2% (v/v)	36.99	30.12

4.3 CLINICAL EVALUATION

The performance of the COVID-19 Saliva Dx Test Kit was evaluated using 60 clinical paired nasopharyngeal and saliva specimens. The NP specimens were previously confirmed by an EUA authorized PCR test: 30 tested positive for SARS-CoV-2 RNA and 30 tested negative for SARS-CoV-2 RNA. Paired saliva specimens were collected in sterile tubes or cups without addition of any preservatives. Two types of sterile cups and one type of sterile tube were used in the collection to help ensure the type of container used does not affect results.

RNA from the paired nasopharyngeal and saliva specimens was subsequently extracted using the Chai Enzymatic DNA/RNA Extraction Buffer (10X), and tested using the COVID-19 Saliva Dx Test Kit on both Open qPCR and Bio-Rad CFX96 instruments.

Out of the 30 nasopharyngeal specimens confirmed positive by the EUA authorized PCR test, all 30 nasopharyngeal specimens tested positive with the COVID-19 Saliva Dx Test Kit. Out of the 30 paired saliva specimens, 29 tested positive with the COVID-19 Saliva Dx Test Kit while 1 tested negative.

Out of the 30 nasopharyngeal specimens confirmed negative by the EUA authorized PCR test, all 30 nasopharyngeal specimens tested negative with the COVID-19 Saliva Dx Test Kit. Out of the 30 paired saliva specimens, all 30 tested negative with the COVID-19 Saliva Dx Test Kit.

The results from these studies are described in Table 14 (nasopharyngeal) and Table 15 (saliva). All samples were blinded and randomized.

Table 14. Results of nasopharyngeal swabs in VTM tested by COVID-19 Saliva Dx Test Kit against an EUA authorized PCR test

		EUA Authorized PCR Test	
		Nasopharyngeal Swab	
COVID-19 Saliva Dx Test Kit		Positive	Negative
Nasopharyngeal Swab	Positive	30	0
	Negative	0	30
	Total	30	30
Positive agreement = 100% (30/30)			
Negative agreement = 100% (30/30)			

Table 15. Parallel testing of paired nasopharyngeal swabs and saliva specimens with the EUA authorized PCR test and COVID-19 Saliva Dx Test Kit

		EUA Authorized PCR Test	
		Nasopharyngeal Swab	
COVID-19 Saliva Dx Test Kit		Positive	Negative
Saliva	Positive	29	0
	Negative	1	30
	Total	30	30
Positive agreement = 96.67% (29/30)			
Negative agreement = 100% (30/30)			

5. ADDITIONAL SUPPORT

5.1 REVISION HISTORY

REVISION	DATE	DESCRIPTION
A	December 4, 2020	Initial Release

5.2 TRADEMARKS

Chai®, Open qPCR™ and Sahara™ are trademarks of Chai Inc. FAM™ and HEX™ are trademarks of Life Technologies, Inc. Bio-Rad® is a trademark of Bio-Rad Laboratories, Inc.

5.3 PRODUCT SUPPORT

For questions regarding this test, contact Chai Technical Support at support@chaibio.com or call toll-free at (800) 642-4002.

This page intentionally left blank

CHAI

990 Richard Ave, Suite 110
Santa Clara, CA 95050
www.chaibio.com

sales@chaibio.com
support@chaibio.com

Toll-free +1 (800) 642-4002
International +1 (650) 779-5577