CHAI

COVID-19 Surveillance Test Kit

Product Manual

RT-qPCR surveillance assay for the detection of SARS-CoV-2 in populations

Catalog #T5402
100 tests/kit

Table of Contents

1. INTRODUCTION 1

2. PRINCIPLES OF THE PROCEDURE 1

3. WARNINGS AND PRECAUTIONS 2

4. KIT COMPONENTS AND STORAGE 3

5. REQUIRED MATERIALS NOT PROVIDED 3

6. REAGENT STORAGE, HANDLING, AND STABILITY 4

7. SPECIMEN COLLECTION, HANDLING, AND STORAGE 4

8. WASTE DISPOSAL 4

9. WORKFLOW 5

- 9.1 Saliva Speciment Collection
- 9.2 Preparing Saliva Specimens for Extraction
- 9.3 RNA Extraction
- 9.4 Post-Lysis Sample Storage
- 9.5 RT-qPCR Reaction Setup

10. INTERPRETATION OF RESULTS 12

11. LIMITATIONS 13

12. TROUBLESHOOTING 14

13. REFERENCES 15

14. REVISION HISTORY 16

15. TRADEMARKS 16

16. PRODUCT SUPPORT 16

1. INTRODUCTION

The COVID-19 Surveillance Test Kit is a RT-qPCR test intended for the qualitative detection of RNA from the SARS-CoV-2 virus in saliva. Saliva specimens are first self-collected under observation in individual collection containers. A partial quantity of each specimen is then transferred to a new container to create a pooled sample or run on a de-identified, individual basis. The intended use of this kit is general surveillance to inform health management decisions at the population level. The COVID-19 Surveillance Test Kit is not intended for diagnostic use.

The purpose of COVID-19 surveillance is to monitor the presence of the virus in a community or population. While diagnostic testing and screening provide results on an individual level, surveillance testing provides information on a population level to guide population level decision making. Testing may be performed on de-identified individual specimens or pooled specimens to avoid returning individual diagnostic results. Surveillance testing with individual saliva specimens may be performed by removing all identifiable information related to specimens prior to sample processing. Alternatively, individual saliva specimens may be pooled together and run in replicates. Pooling samples increases testing capacity with existing equipment and test kits while maintaining sufficient sensitivity for the detection of positive samples. Processing de-identified or pooled samples does not produce a patient-specific test result, so surveillance testing should not be reported to health agencies as diagnostic results. If routine surveillance testing reveals the presence of infection, decision makers can take additional action to ensure the safety in the population, such as recommending individual diagnostic testing or implementing temporary workplace closures.

The COVID-19 Surveillance Test Kit identifies SARS-CoV-2 RNA, which is generally detectable in respiratory and saliva specimens during the acute phase of COVID-19 infection. Positive results indicate the presence of SARS-CoV-2 RNA in the pooled sample. Negative results from pooled testing should not be considered definitive. Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for public health decisions. Positive individuals with low amounts of virus may evade detection in large pools as pooled testing can decrease the sensitivity of the assay. The limit of detection (LoD) is 5.8 viral copies per µl of saliva sample.

The COVID-19 Surveillance Test Kit is intended for use by qualified laboratory personnel specifically instructed and trained in the techniques of real-time PCR. Performance of the test has been validated with individual and pooled saliva specimens. The COVID-19 Surveillance Test Kit is only for population surveillance and is not intended for individual diagnostic use. The test kit has been validated on Chai's dual channel Open qPCR instrument and Bio-Rad CFX96 Touch™ Real-Time PCR Detection System, and is expected to be compatible with other Real-Time PCR instruments with FAM and HEX fluorophore detection.

2. PRINCIPLES OF THE PROCEDURE

The COVID-19 Surveillance Test Kit is a single-step RT-qPCR (Reverse Transcription Quantitative Polymerase Chain Reaction) test that utilizes probe-based multiplexing for the detection of SARS-CoV-2 RNA in saliva specimens. 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 humans. *RPLP0* acts as both an internal control and an extraction control to confirm the performance of the extraction. RNA is extracted from saliva specimens using Chai Enzymatic DNA/RNA Extraction Buffer 10X, which releases the nucleic acid from the cells and potential SARS-CoV-2 through chemical and enzymatic lysis reactions. After lysis, the enzymatic reaction is stopped by incubation at 95 °C. 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, 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. No cross reactivity with any other coronavirus family members that cause SARS-related diseases (i.e. MERS, SARS) has been found. The assay detects one region in the SARS-CoV-2 *N* gene with FAM and one region in the human endogenous control gene *RPLP0* gene with HEX. *RPLP0* amplification in human specimens confirms successful RNA extraction and RT-qPCR. This 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.

3. WARNINGS AND PRECAUTIONS

1. Read all instructions carefully prior to use. The COVID-19 Surveillance Test Kit is intended for general COVID-19 surveillance and has not been FDA cleared or approved.

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

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

4. 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.

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

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

7. 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.
8. 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).

9. Decontaminate work surfaces and equipment with appropriate disinfectants, such as 10% bleach, before and after using the area and the equipment.

10. 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.

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

4. KIT COMPONENTS AND STORAGE

The COVID-19 Surveillance Test Kit includes materials for 100 tests. Reagents should not be used past the expiration date.

Table 1. COVID-19 Surveillance Test Kit Components

Component	Quantity	Storage
Sahara One-Step RT-qPCR Master Mix with UNG	1.1 mL	-20 °C
COVID-19 Surveillance Oligo Mix	275 µL	-20 °C
COVID-19 Surveillance Cofactor Buffer	275 µL	-20 °C
COVID-19 Surveillance Positive Control	110 µL	-20 °C
DNase/RNase-Free Distilled Water	500 µL	-20 °C to 25 °C

5. REQUIRED MATERIALS NOT PROVIDED

- Chai Enzymatic DNA/RNA Extraction Buffer 10X
- 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 for 1.5 mL tubes and PCR tubes or plates
- Micropipettes for pipetting volumes between 1 to 10 µL, 10 to 100 µL, and 100 to 1000 µL
- Non-aerosol filtered-barrier pipette tips
- PCR tubes and caps or 96-well PCR plate and optical plate films
- 1.5 mL microcentrifuge tubes

6. REAGENT STORAGE, HANDLING, AND STABILITY

- 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. Common Mix should be prepared the same day as specimen processing and stored at 4 ˚C until ready for use.
- Check the expiration date of all reagents prior to use. Do not use components beyond the listed expiration date.

7. SPECIMEN COLLECTION, HANDLING, AND STORAGE

The COVID-19 Surveillance Test Kit is designed for human saliva specimens. Adhere to CDC Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens for COVID-19 prior to specimen processing. Containers for collecting saliva specimens are not included in the kit.

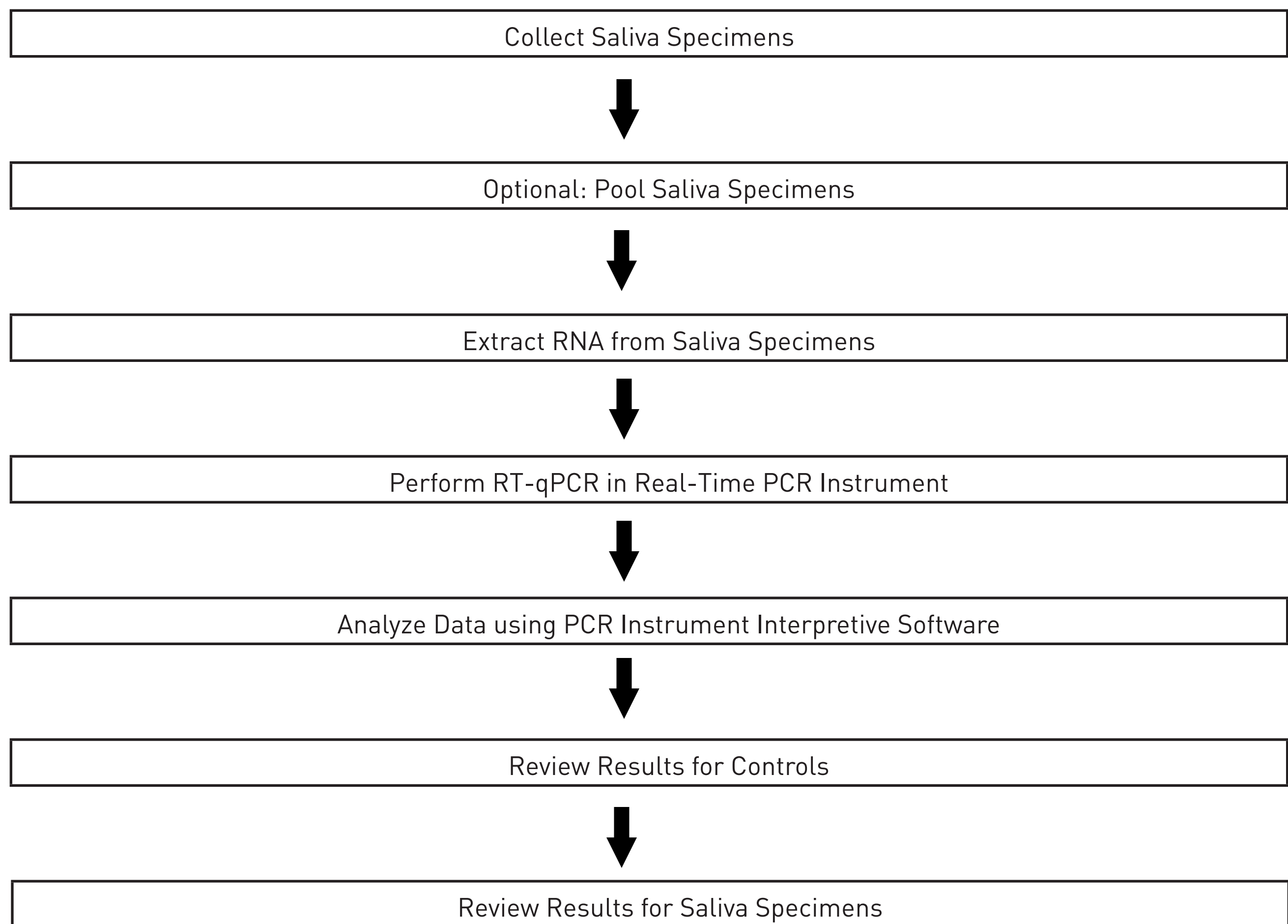
For the packaging and transport of specimens, consult the current version of the International Air Transport Association (IATA) Dangerous Goods Regulation and follow shipping regulations for UN 3373 Biological Substance, Category B.

Human saliva specimens can be stored at room temperature for up to 16 hours after collection, at 2 – 8 ˚C for up to 72 hours after collection and at -70 ˚C or below for long term storage. A minimum saliva volume of 500 μL is required from each subject prior to sample processing.

8. WASTE DISPOSAL

Handle all samples, controls, and kit components as potentially infectious waste. 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.

9. WORKFLOW



9.1 SALIVA SPECIMEN COLLECTION

Subjects should avoid eating, drinking, smoking, chewing gum, brushing teeth, or rinsing with mouthwash at least 30 minutes prior to sampling. Instruct the subject to wash their hands with soap for at least 20 seconds, and dry hands before collecting saliva. Refer to the Chai Knowledge Base for additional information regarding individual saliva specimen collection. Improper saliva specimen collection may lead to inconclusive results.

To collect a saliva specimen:

1. Uncap the sterile specimen collection container and hand it to the subject. The subject should spit/drool at least 0.5 – 1 mL of saliva into the specimen container and avoid touching the inside of the container or contaminating the outer surface of the container with saliva. Do not allow the subject to cough up sputum into the collection container as it may inhibit the specimen during RT-qPCR processing.

2. Immediately cap the container after specimen collection is complete and perform a visual analysis. The saliva specimen should be homogeneously cloudy. Discard the specimen if it contains large solid particles or blood.

9.2 PREPARING SALIVA SPECIMENS FOR EXTRACTION

COVID-19 human surveillance in populations may be performed using individual or pooled saliva specimens. For preparing individual saliva specimens, follow guidelines in 9.2.1. For preparing pooled saliva specimens, follow the instructions in 9.2.2.

9.2.1 INDIVIDUAL SALIVA SPECIMENS

Surveillance testing may be performed on de-identified individual saliva specimens. Ensure that all identifiable information (i.e., subject name) is removed from individual specimens prior to sample processing to avoid obtaining individual results.

1. Homogenize the saliva specimen by vortexing or pipetting up and down. Ensure that the specimen is completely homogenized and no white pellet remains at the bottom of the saliva container when aliquoting.

2. Transfer 100 µL of saliva into a sterile 1.5 mL screw-cap tube. Utilize a larger volume pipette (i.e., a 1000 µL pipette) due to the viscosity of saliva. Transferring saliva using smaller volumetric pipettes (i.e., 100 µL or 200 µL pipettes) may result in incorrect volume transfer. Each individual saliva sample should be transferred into its own 1.5 mL screw-cap tube.

3. Determine the number of sample reactions (S) for the run. The number of sample reactions will equal the number of individual saliva samples.

   S = Number of tubes with 100 µL of saliva

9.2.2 PREPARING POOLED SALIVA SPECIMENS

Alternatively, saliva specimens may be pooled to increase throughput and prevent obtaining individual results. The number of individuals to include in the pool, or Pool Size (n), can accommodate different infection scenarios and be optimised according to infrastructure constraints and the level at which action will be taken. To pool saliva specimens:

1. Homogenize each individual saliva specimen by vortexing or pipetting up and down. Ensure that the specimen is completely homogenized and no white pellet remains at the bottom of the saliva container when aliquoting.

2. Transfer 100 µL of saliva from each individual specimen into a single sterile 1.5 mL screw-cap tube. A maximum of 12 individual 100 µL saliva specimen aliquots can be transferred to the tube; if $n > 12$, use a sterile tube that can accommodate a larger volume. Utilize a larger volume pipette (i.e., a 1000 µL pipette) due to the viscosity of saliva. Pooling saliva specimens with smaller volumetric pipettes (i.e., 100 µL or 200 µL pipettes) may result in incorrect volume transfer.

3. Determine the number of Replicates required for each pool, then determine the total number of Sample reactions (S) for the run. The number of Sample reactions will equal the sum of all Replicates for all pools.

Saliva specimens are diluted when they are pooled, which reduces the analytical sensitivity of the test by increasing the limit of detection (LoD). The impact of decreased analytical sensitivity depends on the percent of subject specimens with viral genome equivalent close to the LoD (weak positives) in the tested population. The decrease in assay sensitivity due to pooling can be compensated for by incorporating a matrix pooling strategy and by running replicates from each pool. The number of Replicates (r) that should be run for each pooled sample will depend on the Pool Size (n) and the Sensitivity Factor (z) used.

The Sensitivity Factor (z) is a value used to determine the number of Replicates by dividing the Pool Size by a whole number between 2 and 4. A Sensitivity Factor of 2 is recommended for the highest degree of sensitivity, while a Sensitivity Factor of 4 will provide the lowest recommended level of sensitivity. A Sensitivity Factor of 5 or greater is not recommended for sensitive detection of SARS-CoV-2 in pooled samples. Once the Pool Size (n) and Sensitivity Factor (z) are determined, the number of Replicates for the pooled sample can be calculated using the following equation:

$r = n / z$

r: number of Replicates
n: Pool Size
z: Sensitivity Factor (between 2 - 4)

Example 1: A Pool Size of 6 individual saliva specimens (n = 6) with the best Sensitivity Factor of 2 (z = 2), will require 3 Replicates for the run.

$r = n / z$
$r = 6 / 2$
$r = 3$ Replicates

When the equation produces a value that is not a whole number, round up to the next whole number.

Example 2: A Pool Size of 13 individual saliva specimens (n = 13) with the least sensitive Sensitivity Factor of 4 (z = 4), will require 4 Replicates for the run.

$r = n / z$
$r = 13 / 4$
$r = 3.25$ → Round up to next whole number → 4 Replicates

The number of sample reactions will be the sum of the Replicates for all pooled samples. If multiple pools are created, add the number of Replicates from each pool to determine the number of sample reactions.

$S = r_{Pool\ 1} + rP_{ool\ 2} + r_{Pool\ 3} + \ldots..$

Example 3: Samples from three pools from a school are run with a Sensitivity Factor of 3 (z = 3). Pool A has 8 students, Pool B has 11 students, and Pool C has 6 students.

$r = n / z$
$r_{Pool\ 1} = 8 / 3 = 2.67 = 3$ Replicates

$r_{Pool\ 2} = 11 / 3 = 3.67 = 4$ *Replicates*
$r_{Pool\ 3} = 6 / 3 = 2$ *Replicates*

$S = r_{Pool\ 1} + r_{Pool\ 2} + r_{Pool\ 3}$
$S = 3 + 4 + 2$
$S = 9$ *Sample reactions*

9.3 RNA EXTRACTION

Individual or pooled saliva specimens must be lysed in order to release viral genomic RNA from SARS-CoV-2 and endogenous control RNA from human cells in saliva.

1. Homogenize the saliva specimen thoroughly by vortexing or pipetting.

   Add the required amount of Enzymatic DNA/RNA Extraction Buffer 10X to lyse the sample.

   For individual saliva specimens, add 11.1 µL of buffer into each sample tube. Mix the contents thoroughly by vortexing or pipetting.

   For pooled saliva specimens, determine the amount of buffer required by multiplying each Pool Size (n) by 11.1 and adding that volume (µL) to the pooled sample. homogenize the contents completely by vortexing or pipetting.

   Enzymatic DNA/RNA Extraction Buffer 10X (µL) = n x 11.1

   Example: A Pool Size of 8 individual specimens (n = 8) will require 88.8 µL of Enzymatic DNA/RNA Extraction Buffer 10X. Add 88.8 µL to the pooled saliva sample.

2. Incubate the tube at room temperature (20 °C – 30 °C) for 15 minutes. Mix the lysate at least once through the incubation by briefly vortexing or pipetting.

3. Incubate the tube at 95 °C for 10 minutes.

4. 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.

9.4 POST-LYSIS SAMPLE STORAGE

If the sample lysate will not be used for immediate PCR analysis, transfer the supernatant to a new 1.5 mL tube for storage to prevent the pellet from being resuspended in solution. Extracted RNA in the lysate is stable for up to 72 hours at -20 ˚C, 4˚C, or room temperature. For long term storage, store the lysate at -70 ˚C or below.

9.5 RT-qPCR REACTION SETUP

1. Ensure that all RT-qPCR reaction setup is in an area with still air (no active air flow) to prevent potential contamination. Completely thaw the Sahara One-Step RT-qPCR Master Mix with UNG,

COVID-19 Surveillance Oligo Mix, COVID-19 Surveillance Cofactor Buffer, COVID-19 Surveillance Positive Control, and DNase/RNase-Free Distilled water at ambient temperature. Thaw lysates if frozen at -20 °C or -80 °C. Homogenize the contents of all samples and reagent tubes by vortexing or pipetting.

2. Briefly centrifuge all reagents at a minimum of 3000 g (RCF). Set aside the COVID-19 Surveillance 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 Surveillance Oligo Mix, and COVID-19 Surveillance Cofactor Buffer for the total number of PCR reactions (R) required. For adequate quality control, one Positive Control and one No Template Control (NTC) should be processed with each run and will each count as an individual Reaction (2 Reactions total). Each Sample (S) calculated from Step 9.2 should be counted as an individual Reaction.

   R = 2 + S

   Example: Running two controls (Positive Control and NTC) and 3 Samples will require 5 Reactions.

   R = 2 + 3 = 5 Reactions

   Include an excess 10% in the calculation to account for pipetting error. The calculations for each component volume are as follows, where R is the number of total PCR reactions. **Table 2** shows the amount required for a single reaction. **Table 3** shows the Common Mix component required for 1 – 16 reactions.

   Volume of Sahara One-Step RT-qPCR Master Mix with UNG (μL) = 11.00 x R
   Volume of COVID-19 Surveillance Oligo Mix (μL) = 2.75 x R
   Volume of COVID-19 Surveillance Cofactor Buffer (μL) = 2.75 x R

Table 2. Common Mix for a single reaction setup

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 Surveillance Oligo Mix	2.50	2.75
COVID-19 Surveillance Cofactor Buffer	2.50	2.75
Total Common Mix	**15.00**	**16.50**

Table 3. Amount of each Common Mix component to pipette, 1 – 16 total reactions

Component	1	2	3	4	5	6	7	8
Sahara One-Step RT-qPCR Master Mix with UNG	11.00	22.00	33.00	44.00	55.00	66.00	77.00	88.00
COVID-19 Surveillance Oligo Mix	2.75	5.50	8.25	11.00	13.75	16.50	19.25	22.00
COVID-19 Surveillance Cofactor Buffer	2.75	5.50	8.25	11.00	13.75	16.50	19.25	22.00
Total	16.50	33.00	49.50	66.00	82.50	99.00	115.50	132.00

Component	9	10	11	12	13	14	15	16
Sahara One-Step RT-qPCR Master Mix with UNG	99.00	110.00	121.00	132.00	143.00	154.00	165.00	176.00
COVID-19 Surveillance Oligo Mix	24.75	27.75	30.25	33.00	35.75	38.50	41.25	44.00
COVID-19 Surveillance Cofactor Buffer	24.75	27.75	30.25	33.00	35.75	38.50	41.25	44.00
Total	148.50	165.00	181.50	198.00	214.50	231.00	247.50	264.00

4. Pipette each Common Mix component into a 1.5 mL tube, then briefly vortex or mix by pipetting. Briefly centrifuge the tube at 3,000 g (RCF).

5. Aliquot 15 µL of the Common Mix into each PCR tube or plate well.

6. Add the required template (Positive Control, NTC, or sample lysate) to a PCR tube or plate well as indicated in **Table 4**. Add the required number of samples first by pipetting 5 µL of the sample lysate into the same number of individual wells. For example, if 3 Samples will be run, pipette 5 µL of lysate into 3 separate wells. Prepare the NTC reaction by adding 5 µL of DNAse/RNAse–Free Water. Prepare the Positive Control by adding 5 µL of COVID-19 Surveillance Positive Control. The Positive Control should be prepared last to minimize potential contamination; immediately cap the tube after pipetting.

Table 4. Template to add for each Well Type

Well Type	Template to Add
Positive Control	5 µL of the COVID-19 Surveillance Positive Control
No Template Control (NTC)	5 µL of DNAse/RNAse - Free Water (Use the 0.5 mL tube provided)
Sample	5 µL of the individual or pooled sample lysate

7. Seal the plate or tubes and centrifuge for 30 seconds at a minimum of 3,000 g (RCF).

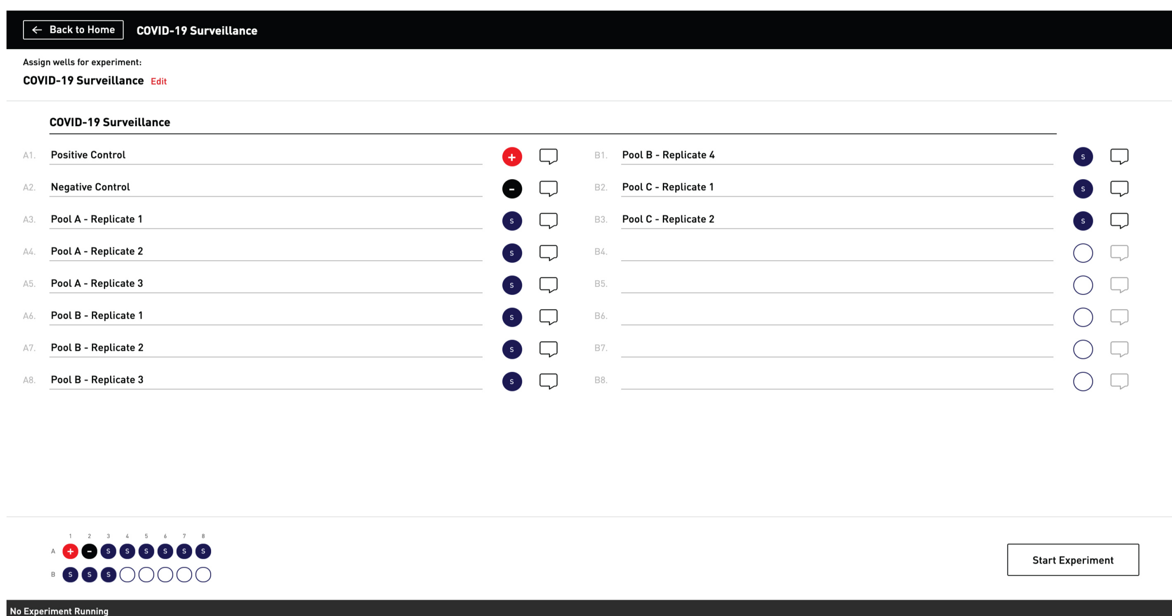
8. Load the plate or tubes into the thermocycler and start the RT-qPCR run using the following cycling protocol **(Table 5)**. The data collection point should be set at the final 62 ˚C annealing step. If required, configure the software to detect *N* gene SARS-CoV-2 target in FAM and the *RPLP0* gene endogenous control target in HEX.

   Alternatively, if using the Open qPCR Dual Channel thermocycler, select the "Run a Test Kit" option. Select Chai as the manufacturer and select "COVID-19 Surveillance" for the test kit option. Input your sample names and select "Run Experiment" to start the experiment.

Table 5. Template to add for each Well Type

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	40
Annealing	62 ºC	40 sec	

Figure 1. Well layout in Open qPCR



10. INTERPRETATION OF RESULTS

Results are interpreted based on the presence or absence of targets in the FAM and HEX fluorophore detection channels with the Real-Time PCR instrument. A region of the SARS-CoV-2 nucleocapsid *(N)* gene is detected in FAM for the COVID-19 target and a region of the human Ribosomal Protein Lateral Stalk Subunit P0 *(RPLP0)* gene is detected in HEX for the endogenous control target. It is essential to assess the validity of all controls and samples based on the presence or absence of the FAM and HEX targets. 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 samples can be evaluated for the presence of SARS-CoV-2.

Table 6. 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

Each individual sample or replicate from a pooled sample must be evaluated for the presence or absence of the *N* gene SARS-CoV-2 target in FAM and the *RPLP0* gene endogenous control target in HEX. Amplification in FAM indicates that SARS-CoV-2 was detected in the sample. HEX amplification verifies successful RNA sample extraction, reverse transcription, and PCR amplification. At least one Replicate from a pooled sample should show amplification in the FAM channel to call that pool as positive for SARS-CoV-2 infection.

Table 7. Sample results 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

Lack of amplification in both FAM and HEX channels indicates that PCR did not successfully take place for the SARS-CoV-2 and endogenous control targets. In this case, the result is invalid and the sample lysate should be rerun or a new saliva specimen(s) may need to be collected.

11. LIMITATIONS

1. All users, analysts, and any person involved with results interpretation and reporting should be trained to perform this test by a competent instructor. Each individual should demonstrate the ability to successfully perform the test and interpret results prior to performing the assay independently.

2. 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.

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

4. The COVID-19 Surveillance Test Kit is validated for use with saliva specimens. Other sample types should not be used.

5. The detection of SARS-CoV-2 RNA may be affected by specimen collection, symptom presence, stage of infection, pool size, number of replicates per pool, and/or the viral load of specimens.

6. Detection of targets does not indicate that the SARS-CoV-2 virus is infectious or the cause of clinical symptoms.

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

8. 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 under the limit of detection of the test
- Spontaneous mutations within the RT-qPCR target sequence affect primer and/or probe binding

9. Negative results indicate that the amount of SARS-CoV-2 present in the sample is lower than the limit of detection but do not guarantee the complete absence of the virus.

12. 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 Surveillance 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 endogenous control amplification for saliva specimen	Sample not successfully lysed due to inadequate	Store buffer at the recommended temperature and minimize time at room temperature to ensure that samples are sufficiently lysed.
	Enzymes in Enzymatic DNA/RNA Extraction Buffer not completely inactivated	Incubate the sample at 95 °C for at least 10 minutes to fully inactivate all enzymes. Incomplete inactivation of the buffer may result in degradation of reagents during the PCR run.
	PCR inhibition from improper saliva specimen collection	Avoid using the sample if there is a visible amount of blood or sputum in it. Ensure that the subject is only collecting saliva and is not coughing when spitting into the collection tube.
	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 saliva specimen	Saliva 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.
	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.

13. REFERENCES

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14. REVISION HISTORY

REVISION NUMBER	REVISION DATE	DESCRIPTION
A	September 14, 2020	Original Product Manual

15. TRADEMARKS

Chai®, Open qPCR™ and Sahara™ are trademarks of Chai Inc.
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16. PRODUCT SUPPORT

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

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