Instruction for Use

CodeCheck™ SARS-CoV-2 RT-PCR Kit

Catalog # KD0119-96

Specification: 96 Reactions/kit

For Prescription Use Only

Validation of this test has not been reviewed by FDA. Review under the EUA program is pending

For *In Vitro* Diagnostic (IVD) Use Only

V.A/1

Contents

1 Name

CodeCheck™ SARS-CoV-2 RT-PCR Kit

2 Intended Use

The CodeCheck™ SARS-CoV-2 RT-PCR Kit is a real-time RT-PCR test intended for the qualitative detection of the SARS-CoV-2 RNA in nasopharyngeal and oropharyngeal swab specimens from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform high complexity tests, or by similarly qualified non-U.S. laboratories.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in the upper respiratory during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. 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 positive results to the appropriate public health authorities.

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

The CodeCheck™ SARS-CoV-2 RT-PCR Kit is intended for use by qualified and trained clinical laboratory personnel specifically instructed and trained in the techniques of real- time PCR and *in vitro* diagnostic procedures. Validation of this test has not been reviewed by FDA. Review under the EUA program is pending.

3 Principles of the Procedure

ORF1ab and N genes of SARS-CoV-2 RNA are targets of CodeCheck™ SARS-CoV-2 RT-PCR Kit. The kit includes primer/probe sets, the fluorophore FAM is for ORF1ab gene probe and Texas Red is for N gene probe. The kit also includes a primer/probe set to detect human β-actin as an internal control (IC) intended to examine the source of detected specimens. The IC probe is labeled with fluorophore JOE which uses an independent fluorescence detection channel from SARS-CoV-2 targets (Table 3.1.;3.2.).
In addition, the kit utilizes external Positive (PC) and Negative (NC) controls. The PC contains synthetic RNA templates of SARS-CoV-2 ORF1ab and N targets, the NC is molecular grade nuclease-free water.

Table 3.1. Detection Target Information

Target	Target genes
SARS-CoV-2	ORF1ab
	N gene
Internal Control: Human Housekeeping Gene	β-actin

Table 3.2. Fluorescence Information

Target genes	5' Fluorophore	3' Quencher
ORF1ab	FAM	BHQ1
N gene	Texas Red	BHQ2
β-actin	JOE	BHQ1

The kit does NOT include a "Reference dye"
(E.g. Set up the reference dye to "None" in the ABI 7500 / 7500 Fast program)

4 Materials Required and Provided

Label Name	Quantity (vial)	Volume (µL)	Description
PCR Reaction Mix A	1	680	Primer/probe sets mix for ORF1ab and N gene of SARS-CoV-2, and human β-actin genes
PCR Reaction Mix B	1	1400	*Taq* polymerase, reverse transcriptase, and PCR buffer
Negative Control	1	800	Nuclease-free water
Positive Control	1	800	Recombinant RNA containing sequences of ORF1ab and N genes of SARS-CoV-2

5 Materials Required but Not Provided

5.1 Compatible PCR Instruments

- Applied Biosystems™ 7500 Real-Time PCR Instrument System with software V1.4.1 or above
- Applied Biosystems™ 7500 Fast Real-Time PCR Instrument System with software V1.4.1 or above
- Roche® Cobas® z 480system with software V1.5.0 or above

5.2 Extraction Kits

- QIAamp® Viral RNA Mini Kit (QIAGEN, catalog # 52904 or 52906)

5.3 Other Instruments and Consumables

- Vortex mixer and microcentrifuge
- Pipette (10 µL, 200 µL, 1000 µL)
- Pipette tips with filter
- 96-well PCR plate or optical 8-tube strip
- Sealing Film or PCR optical cap
- 1.5 mL DNase/RNase free microcentrifuge tubes and racks
- Disposable powder-free gloves and laboratory gowns
- Cold blocks or ice

6 Warnings and Precautions

- For Prescription Use only.
- For in vitro diagnostic use only.
- This test has not been FDA cleared or approved.
- Validation of this test has not been reviewed by FDA. Review under the EUA program is pending.
- A statement such as "The test has been validated but FDA's independent review of this validation is pending" should be included in test reports to healthcare providers.
- Positive results are indicative of the presence of SARS-CoV-2 RNA.
- Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.
- Healthcare providers should include the following statement in each clinical report: "This test has been validated but FDA's independent review of this validation is pending".
- Follow standard precautions. All patient specimens and positive controls shall be considered potentially infectious and handled accordingly.
- Do not eat, drink, smoke, use cosmetics or wear contact lenses in areas where there are reagents and human specimens.
- Handle all specimens as infectious and follow safe laboratory procedures. Refer to Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with 2019-nCoV https://www.cdc.gov/coronavirus/2019-nCoV/lab-biosafety-guidelines.html.
- Only use the protocol described in this package insert. Deviations from the protocol may give erroneous results.
- Use personal protective equipment consistent with current guidelines for the handling of potentially infectious samples.
- Amplification technologies such as PCR are sensitive to accidental introduction of PCR products from previous amplification reactions. Incorrect results could occur if either the clinical specimen or the real-time reagents used in the amplification step are contaminated by accidental introduction of amplification product (amplicon). Workflow of PCR laboratory must be in a unidirectional manner.
- Change aerosol barrier pipette tips when transferring liquid manually.
- During sample preparation, it is essential to compliance with good laboratory practice that can minimize the risk of cross-contamination between samples due to the inadvertent introduction of nucleases into samples during and after the extraction procedure. Proper aseptic technique should always be applied when working with nucleic acids.
- Maintain separate, dedicated equipment (e.g., pipettes, microcentrifuges) and supplies (e.g., microcentrifuge tubes, pipette tips) for assay setup and treatment of extracted nucleic acids.
- Dispose of unused kit reagents and human specimens according to local, state, and federal regulations. Work surfaces, pipettes, and centrifuges shall be cleaned and decontaminated with cleaning products such as 10% bleach, to minimize risk of nucleic acid contamination. Residual bleach shall be removed using 70% ethanol.

7 Reagent Storage, Handling and Stability

- All the components of CodeCheck™ SARS-CoV-2 RT-PCR kit should be stored at -20±5℃ except during preparation and use.
- CodeCheck™ SARS-CoV-2 RT-PCR kit should be packaged and shipped using dry ice.
- Place on ice or cold-block and keep cold during preparation and use.
- The maximum number of repeated freezing and thawing is 5 times. Use the reagents within 3 days once un-caped.
- Keep away from light.
- Always check the expiration date before use and do not use expired reagent. The shelf storage life of CodeCheck™ SARS-CoV-2 RT-PCR kit is 8 months at -20±5℃.

8 Specimen Collection, Transportation and Storage

8.1 Specimen Collection

Please refer to https://www.cdc.gov/coronavirus/2019-nCoV/lab/guidelines-clinical-specimens.html for information on collection of appropriate specimens for SARS-CoV-2 testing and follow specimen collection devices manufacturer instructions for proper collection methods.

8.2 Specimen Transportation

All the specimens must be packaged and shipped in accordance with the current edition of the International Air Transport Association (IATA) Dangerous Goods Regulations and Guidance of the Center for Disease Control and Prevention (CDC).

8.3 Specimen Storage

Specimens can be stored at 2-8°C for up to 72 hours after being collected before the preparation.

9 Assay Procedure

9.1 RNA Extraction

- Use QIAamp® Viral RNA Mini Kit (QIAGEN, catalog # 52904 or 52906) to extract the RNA from specimens.
- Follow the manual of QIAamp® Viral RNA Mini instructions for the viral RNA purification.

9.2 RT- PCR Protocol

9.2.1 PCR Amplification Mix

a. In the reagent preparation zone, take out the CodeCheck™ SARS-CoV-2 RT-PCR Kit from the freezer and open the package. Thaw PCR Reaction Mix A, PCR Reaction Mix B, Negative Control, and Positive Control at room temperature prior to use. Place on ice or cold block; Keep cold during preparation and use.

b. Gently invert PCR Reaction Mix A and PCR Reaction Mix B 8 to 10 times to mix, then quick spin to collect reagents at the bottom of the tube. Place the tube on the ice or cold block.

c. Determine the number of reactions (N) to set up per assay. It is necessary to make excess reaction mix for the Positive Control (PC), Negative Control (NC) and for pipetting error. Use the following guide to determine N (n refers to the number of patients' samples):

$$N = n + PC + NC + 1$$

d. Calculate the amount of PCR amplification mix (Table 9.2.1.).

Table 9.2.1 RT-PCR Reaction mixture

Reagent	Amount (μL)	
	Single Reaction	N Reactions
PCR Reaction Mix A	6.5	N× 6.5
PCR Reaction Mix B	13.5	N×13.5
Total	20	N×20

e. Dispense each reagent into each 1.5 mL labeled microcentrifuge tube. After adding the reagents, mix reaction mixtures by pipetting up and down. DO NOT vortex.

f. Perform a quick spin to collect reagents at the bottom.

9.2.2 Nucleic Acid Template and Controls Addition

a. After centrifugation, place the tubes containing extracted nucleic acid samples on the cold rack.

b. Mix reaction mixtures by pipetting up and down, then spin quickly to collect contents at the bottom of the tube. Aliquot 20 µL of amplification mixture into each PCR reaction tube.

c. Carefully pipette 5.0 µL of the negative control, the template of specimens and positive control into appropriate PCR reaction tubes. Keep other sample wells covered during addition. Change tips after each addition.

d. Cap the entire reaction tubes and move them to the PCR amplification zone.

9.2.3 Software Setting

For "Applied Biosystems™ 7500 Real-Time PCR System" and "Applied Biosystems™ 7500 Fast Real-Time PCR System":
Please refer to the manufacture's manual for general instruction

a. New Experiment → enter the name of this experiment
Select sequentially **7500 (96 Wells)** → **Quantitation-Standard Curve** → **TaqMan Reagents** → **Standard***
(*Must select ***standard*** mode on Applied Biosystems™ 7500 Fast Real-Time PCR System)

b. Plate Setup → Define Targets and Samples→ Define targets → Add New Target → Target information set up (Table 9.2.3.-1)
Assign Targets and Sample →Click a well from View Plate Layout →activate all targets and tasks →select **None** for passive reference

c. Channel Setting (Table 9.2.3.-1)

Table 9.2.3.-1 Channel Setting information

Target Name	Reporter	Quencher	Color*
Target 1	FAM	None	Color 1
Target 2	TEXAS RED	None	Color 2
Target 3	JOE	None	Color 3

*Choose three different colors for each of the three targets.

d. RT-PCR Conditions Setting (Table 9.2.3.-2)

Table 9.2.3.-2 RT-PCR Conditions

Stage	Reps	Temp	Time	Data Collection
1	1	42℃	10 min	
2	1	95℃	10 sec	
3	45	95℃	5 sec	
		54℃	15 sec	
		70℃	30 sec	√

e. Analysis

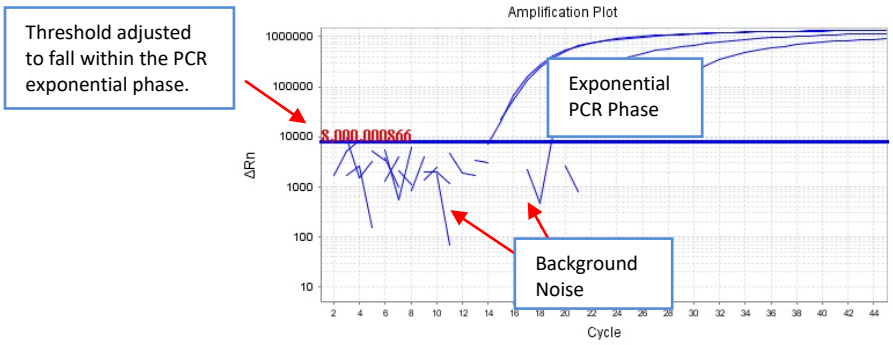
1) Click Analysis. →Amplification Plot, under Plot Settings tab →select sequentially ΔRn vs Cycle (default) →Linear →Target. Set the baseline start at cycle 3 and end at cycle 15.

2) Under options tab, select target (Reporter) to be adjusted > Adjust the Threshold value manually.

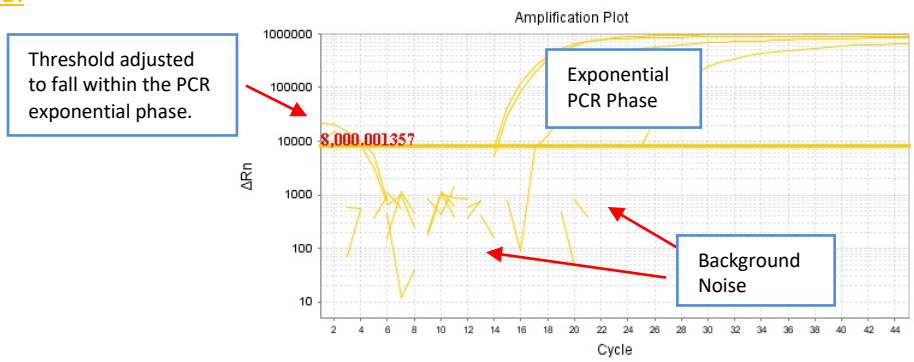
3) Ct values will be calculated after adjusting threshold. To review a Ct value of a sample, click the well as shown in the figure below. In the Target drop down, select the target for review.

Examples of a positive sample amplification curve (FAM in blue, TEXAS RED in yellow, and JOE in pink):

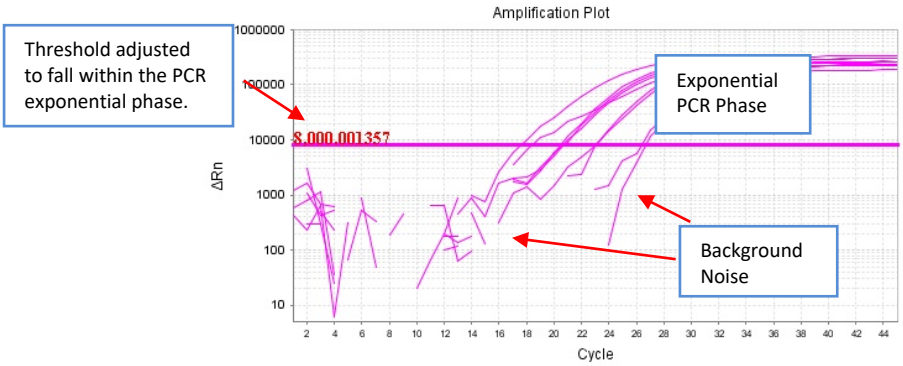
FAM:



TEXAS RED:



JOE:



For "Roche® Cobas® z 480" (software v1.5.0):

Please refer to the manufacture's manual for general instruction

a. Click **New Experiment** to set up experiment.

b. In the drop-down menu next to **Detection Format**, select **Multi Color Hydrolysis Probe** for target information set up (Table9.2.3.-3).

Table 9.2.3.-3 Target information set up

Integration Time Mode	Filter Combination	Filter Combination Selection*	
		Excitation Filter	Emission Filter
Dynamic	FAM	465	510
	TEXAS RED	540	610
	JOE	540	580

*** Filter Combination Selection** can be set by clicking **Detection Formats** on the **Tools** menu.

c. Designate individual program under **Program Name**, and set temperature and time parameters for each program in the **Program Temperature Targets** panel below, referring to the steps, number of cycles, temperature, and duration (Table 9.2.3.-4). Use (+) and (–) buttons to add or delete steps in the interface.

Table 9.2.3.-4 RT-PCR Conditions

Program name	Cycles	Target(℃)	Running Time	Analysis Mode	Acquisition Mode
1	1	42	00：10：00	None	None
2	1	95	00：00：10	None	None
3	45	95	00：00：05	Quantification	None
		54	00：00：15		None
		70	00：00：30		Single

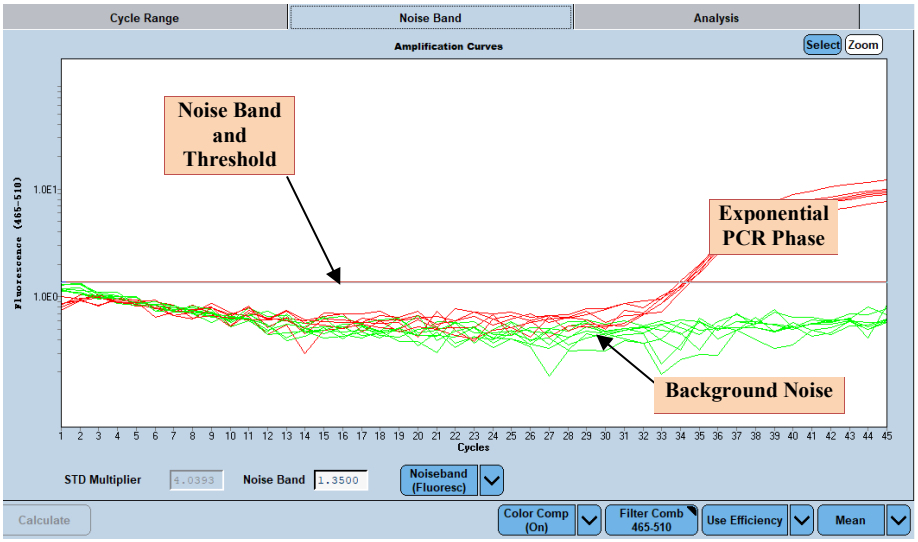
- Click **Save As Template to save the program**. The template can be used for future experiments by clicking **Apply Template**.
- After editing subset and defining all sample names for this experiment, select **Start Run** to run the test.
- After running, click **Analysis** on the left panel to open the analysis interface.
- To adjust **Noise Band** and **Threshold** of different channels, select **Filter Comb**:
  - To adjust the **Noise Band** parameter, click **Noise Band** tab. The optimal position of the Noise Band should be as low as possible, without any background noise, and as high as necessary, where it clearly crosses all sample

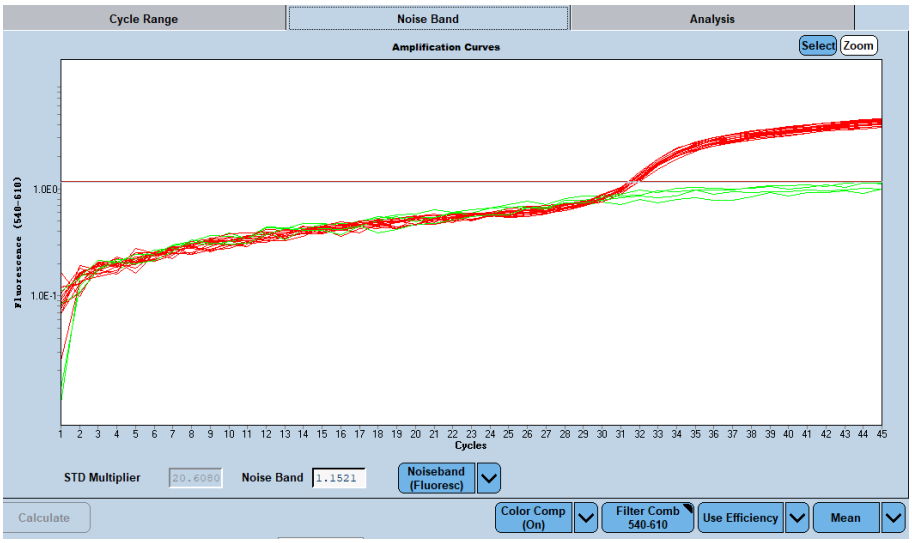
- curves in the lower part of the log-linear phase.
- To adjust the **Threshold** parameter, set Threshold to **Threshold (Auto)**.
- Click **Calculate** in the bottom left of the screen to apply the change. The results will be analyzed.
- On the upper left corner of the interface, select a well, and corresponding Ct value will be shown by dragging the bar to the right.

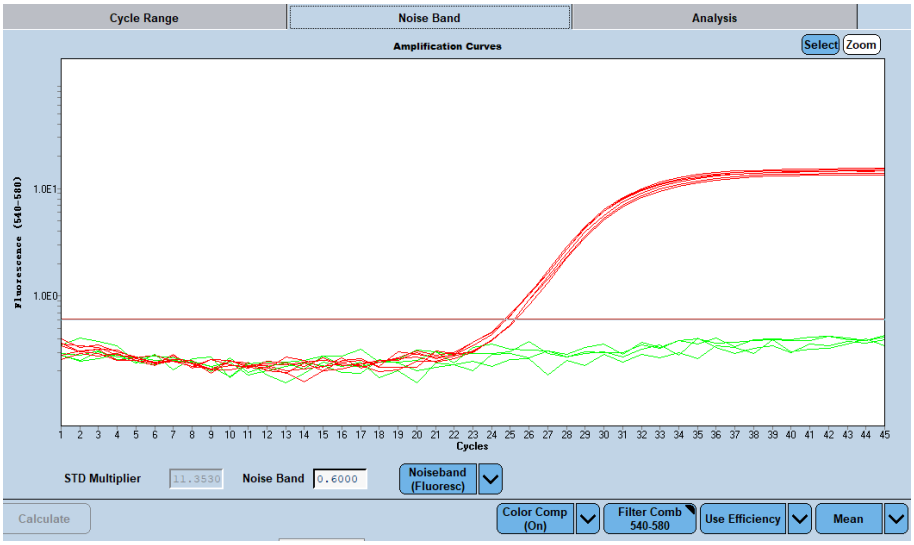
- To export results, right-click and select **Export Table**.

- Observe the Ct values of target FAM (top screenshot below), target Texas Red (middle screenshot below), and JOE (internal reference, bottom screenshot below) of samples and determine the result of samples referring to instructions for use of the CodeCheck™ SARS-CoV-2 RT-PCR Kit for detecting SARS- CoV-2.

Examples of a positive sample amplification curve (Running with Roche® Cobas® z480), FAM, TEXAS RED and JOE are arranged from top to bottom:







10 Interpretation of Results

All test controls must be examined prior to interpretation of patient results. If PC and NC controls are not valid, the patient results cannot be interpreted (Table 10.1.).

Human β-actin is used as an internal control (IC) for evaluation of sampling. The probe of β-actin is labeled with JOE which uses an independent fluorescence detection channel from SARS-CoV-2 targets. All clinical samples exhibit β-actin amplification curves that cross the threshold line with the Ct value < 36.

If the β-actin assay does not produce a positive result for human clinical specimens, interpret as follows:

- If one of the ORF1ab and N is positive, even in the absence of a positive β-actin, the result must be considered valid. It is possible that some samples may have β-actin amplification curves with Ct value >36 or fail to exhibit β-actin amplification curves due to low cell numbers in the original clinical specimen. A negative β-actin signal does not preclude the presence of SARS-CoV-2 RNA in a clinical specimen.

- If the ORF1ab, N and β-actin are all negative for the specimen, the result should be considered invalid. If residual specimen is available, repeat the extraction procedure and repeat the test. If all markers remain negative after re-test, report the result as invalid and a new specimen should be collected if possible.

Table 10.1. Interpretation of Positive and Negative Controls

Control Type	External Control Name	Expected Ct Value	ORF1ab (FAM) Result	N (Texas Red) Result	Interpretation
Positive Control	**PC**	<36	+	+	Control has Passed
			+	-	<u>Control Failure</u> Failure in lysis and extraction procedure, Substantial reagent failure including primer and probe integrity
			-	+	
			-	-	
Negative Control	**NC**	None Detected	-	-	Control has Passed
			+	+	<u>Control failure</u> Reagent and /or environmental contamination
			+	-	
			-	+	

If the Ct values of controls are expected, refer to result interpretation for patient samples Table 10.2. below to determine the infection status:

Table 10.2. Interpretation of Results for Patient Samples

ORF1ab Ct<40	N Ct<40	β-actin Ct<36	Interpretation	Report	Action
+	+	+	SARS-CoV-2 Detected	Positive	Report to sender and appropriate public health authorities
+	+	-	SARS-CoV-2 Detected	Positive	Report to sender and appropriate public health authorities
-	+	-	SARS-CoV-2 Detected	Positive	Report to sender and appropriate public health authorities
+	-	-	SARS-CoV-2 Detected	Positive	Report to sender and appropriate public health authorities
+	-	+	SARS-CoV-2 Detected	Positive	Report to sender and appropriate public health authorities
-	+	+	SARS-CoV-2 Detected	Positive	Report to sender and appropriate public health authorities
-	-	+	SARS-CoV-2 None Detected	Negative	Report to sender and appropriate public health authorities
-	-	-	Invalid Result	Invalid	Repeat extraction and RT-PCR procedure
+	-	-	SARS-CoV-2 Detected	Positive	Report to sender and appropriate public health authorities
-	+	-	SARS-CoV-2 Detected	Positive	Report to sender and appropriate public health authorities

11 Performance Characteristics

11.1 Limit of Detection

The Limit of Detection (LoD) studies determined the lowest detectable SARS-CoV-2 viral RNA concentration that yield greater than or equal to 95% of all (true positive) replicates test positive with the CodeCheck™SARS-CoV-2 RT-PCR Kit. All sample replicates were prepared by spiking the standard SARS-CoV-2 viral genomic RNA obtained from the National Institute of Metrology of China (NCRM; # GBW(E)091099, lot #:2020-02) into negative clinical nasopharyngeal (NP) swab specimen matrix.

a. The Following studies were processed by using QIAamp® Viral RNA Mini Kit (QIAGEN, catalog #52906) and run on the Roche® Cobas® z480 PCR detection system (software v1.5.0).

In the first part of this study, the tentative LoD was conducted at 3 different concentration levels with 5 replicate measurements at each concentration of 10000, 1000 and 100 copies/mL, respectively.

The final LoD was confirmed by conducting at 4 different concentration levels with 20 replicate measurements at each concentration of 800, 400, 200, and 100 copies/mL, respectively.

Table 11.1.1. Tentative LoD Study Result

Concentrations (copies/mL)	ORF1ab		N		β-actin		Detection Rate
	Average Ct	SD	Average Ct	SD	Average Ct	SD	
10000	31.34	0.520	30.78	0.109	18.30	0.117	5/5
1000	34.91	0.273	34.76	0.211	18.24	0.160	5/5
100	NA	NA	NA	NA	18.35	0.155	0/5

Table 11.1.2. LoD Confirmation Study Result

Concentrations (copies/mL)	ORF1ab		N		β-actin		Detection Rate
	Average Ct	SD	Average Ct	SD	Average Ct	SD	
800	35.82	0.520	35.34	0.109	18.08	0.117	20/20
400	37.66	0.507	38.37	0.902	18.38	0.106	20/20
200	NA	NA	NA	NA	18.28	0.145	8/20
100	NA	NA	NA	NA	18.44	0.268	1/20

b. ABI 7500 detection system (software V2.0.6)

The QIAamp® Viral RNA Mini Kit (QIAGEN, catalog # 52906) has been validated with the CodeCheck™SARS-CoV-2 RT-PCR Kit. The extraction kit requires 140 µL sample input and yields 60 µL of purified nucleic acid eluent.
In the first part of this study, the tentative LoD was conducted at 3 different concentration levels with 5 replicate measurements at each concentration of 10000, 1000 and 100 copies/mL, respectively.

The final LoD was confirmed by conducting at 4 different concentration levels with 20 replicate measurements at each concentration of 800, 400, 200, and 100 copies/mL, respectively.

Table 11.1.3. Tentative LoD Study Result

Concentrations (copies/mL)	ORF1ab		N		β-actin		Detection Rate
	Average Ct	SD	Average Ct	SD	Average Ct	SD	
10000	30.97	0.196	30.67	0.305	28.42	0.082	5/5
1000	34.55	1.23	35.2	0.305	28.56	0.162	5/5
100	NA	NA	NA	NA	28.84	0.110	2/5

Table 11.1.4. LoD Confirmation Study Result

Concentrations (copies/mL)	ORF1ab		N		β-actin		Detection Rate
	Average Ct	SD	Average Ct	SD	Average Ct	SD	
800	34.56	0.923	34.47	0.932	27.25	0.48	20/20
400	37	1.193	37.72	0.748	27.12	0.45	20/20
200	39.45	0.793	NA	NA	27.31	0.386	15/20
100	NA	NA	NA	NA	27.32	0.369	2/20

Conclusion:

The LoD of CodeCheck™ SARS-CoV-2 kit is 400 copies/mL.

11.2 Inclusivity

The CodeCheck™ SARS-CoV-2 RT-PCR Kit primers and probes were designed based on SARS-CoV-2 sequences published on GenBank (https://www.ncbi.nlm.nih.gov/genbank/sars-cov-2-seqs/) and 2019nCoVR (https://bigd.big.ac.cn/ncov/lang=en). The design of primers and probes targeting on ORF1ab and N gene was finished on March 21, 2020. It was shown these primers and probes are specific to NCBI Reference Sequence NC_045512.2 by Nucleotide BLAST®. The BLAST+ software (Version 2.10.1, downloaded from ftp://ftp.ncbi.nlm.nih.gov/blast/executables/blast+/LATEST/ , search in SARS-CoV-2 (taxid:2697049) database including 35,635 sequences on Oct 9, 2020) showed that 39 variants lie in 5'-end of forward primers, 39 variants lie in 5'-end of reverse primers, and 46 variants lie in the middle of probes. All these variants are single nucleotide polymorphisms. After excluding ambiguous nucleotides (N), a total number of 47 SNPs were identified as shown in the table below. (Table 11.2.1)

Table 11.2.1

	Accordance	Discordance	Discordance Type	Significant discordance	Location
ORF1ab_forward primer	100% alignments (26777 sequences)	95% alignments (14 sequences)	SNP	2 sequences with single nucleotide mismatched	Pos. 8 of 5'-end Pos. 6 of 3'-end
ORF1ab_reverse primer	100% alignments (26691 sequences)	94% alignments (20 sequences)	SNP	3 sequences with the same single nucleotide mismatched	Pos. 5 of 5'-end
ORF1ab_probe	100% alignments (26689 sequences)	95~96% alignments (17 sequences)	SNP	4 sequences with same single nucleotide mismatched	Pos. 7 of 5'-end
N_forward primer	100% alignments (26789 sequences)	90~95% alignments (25 sequences)	SNP	2 sequences with the same single nucleotide mismatched 5 sequences with the same single nucleotide mismatched	Pos. 10 of 5'-end Pos. 8 of 5'-end
N_ reverse primer	100% alignments (26743 sequences)	95% alignments (19 sequences)	SNP	10 sequences with the same single nucleotide mismatched 2 sequences with another single nucleotide mismatched	Pos. 5 of 5'-end Pos. 8 of 5'-end Pos. 11 of 5'-end
N_ probe primer	100% alignments (26775 sequences)	95% alignments (29 sequences)	SNP	6 sequences with a same single nucleotide mismatched 6 sequences with another same single nucleotide mismatched 4 sequences with another same single nucleotide mismatched 3 sequences with another single nucleotide mismatched	Pos. 10 of 5'-end Pos. 11 of 5'-end Pos. 5 of 5'-end Pos. 9 of 5'-end Pos. 11 of 3'-end Pos. 6 of 5'-end

Conclusion:

None of the mismatches are located within 4 sites of the 3'-end, and all the mismatches are presented only once in one sequence. Thus, the PCR amplification component of the assay is tolerant to all identifiable variants in publicly available sequence data. of these variants are predicted to impact on the assay performance.

11.3 Cross-Reactivity

Evaluation of analytical specificity of the kit was conducted using both *in-silico* analysis and wet-testing against pathogenic organisms mainly found in the human respiratory tract (Table 11.3.1. and 11.3.2 respectively).

11.3.1 *In-silico* Analysis

BLASTn analysis queries of the CodeCheck™ SARS-CoV-2 RT-PCR Kit primers and probes (1 ORF1ab primer/probe set and 1 N primer/probe set) were performed against public domain nucleotide sequences with the following database search parameters:

Mask low complexity regions = Yes
Expectation value = 10
Match/Mismatch = Match 2 Mismatch -3
Gap Costs = Existence 5 Extension 2
Max number of hit sequence = 250
Mask lower case = No
Mask low complexity regions = Yes
Number of threads = 16
Filter out redundant results = No

Table 11.3.1. *In-silico Cross-Reactivity* Analysis

	Microorganism	Ref.Sequence	ORF1ab (2primer, 1 probe)	N (2 primer, 1 probe)
1	Human coronavirus 229E	NC_002645.1	No alignment found	No alignment found
2	Human coronavirus OC43	NC_006213.1	No alignment found	No alignment found
3	Human coronavirus HKU1	NC_006577.2	No alignment found	No alignment found
4	Human coronavirus NL63	NC_005831.2	No alignment found	No alignment found
5	SARS-coronavirus	NC_004718.3	<80% alignment	<80% alignment
6	MERS-coronavirus	NC_019843.3	No alignment found	No alignment found
7	Human adenovirus 1	AC_000017.1	No alignment found	No alignment found
8	Human adenovirus 2	AC_000007	No alignment found	No alignment found
9	Human adenovirus 3	DQ086466.1	No alignment found	No alignment found
10	Human Metapneumovirus	NC_039199.1	No alignment found	No alignment found
11	Human parainfluenza virus 1	NC_003461.1	No alignment found	No alignment found
12	Human parainfluenza virus 2	AB176531.1	No alignment found	No alignment found
14	Human parainfluenza virus 4a	NC_021928.1	No alignment found	No alignment found

	Microorganism	Ref.Sequence	ORF1ab (2primer, 1 probe)	N (2 primer, 1 probe)
15	Human parainfluenza virus 4b	AB543337.1	No alignment found	No alignment found
16	Influenza A (H1N1)	GCF_000865725.1	No alignment found	No alignment found
17	Influenza A (H5N1)	GCF_000864105.1	No alignment found	No alignment found
18	Influenza B virus	NC_002204.1	No alignment found	No alignment found
19	Human enterovirus B	NC_001472.1	No alignment found	No alignment found
20	Respiratory syncytial virus	NC_001803.1	No alignment found	No alignment found
21	Human rhinovirus C	NC_009996.1	No alignment found	No alignment found
22	Chlamydia pneumoniae TW-183	NC_005043.1	No alignment found	No alignment found
23	Haemophilus influenzae type B	DM109591.1	No alignment found	No alignment found
24	Legionella pneumophila NCTC12273	NZ_LR134380.1	No alignment found	No alignment found
25	Mycobacterium tuberculosis H37Rv	NC_000962.3	No alignment found	No alignment found
26	Streptococcus pneumoniae NCTC7465	NZ_LN831051.1	No alignment found	No alignment found
27	Streptococcus pyogenes NCTC8198	NZ_LN831034.1	No alignment found	No alignment found
28	Bordetella pertussis 18323	NC_018518.1	No alignment found	No alignment found
29	Mycoplasma pneumoniae FH	NZ_CP010546.1	No alignment found	No alignment found
30	Pneumocystis jirovecii RU7	GCF_001477535.1	No alignment found	No alignment found
31	*Candida albicans*	Database (BLAST www.candidagenome.org)	No alignment found	No alignment found
32	Pseudomonas aeruginosa PAO1	NC_002516.2	No alignment found	No alignment found
33	Staphylococcus epidermidis ATCC 14990	NZ_CP035288.1	No alignment found	No alignment found
34	*Streptococcus salivarius NCTC8618*	NZ_LR134274.1	No alignment found	No alignment found
35	Staphylococcus aureus NCTC 8325	NC_007795.1	No alignment found	No alignment found

Conclusion:
The *in-silico* cross-reactivity analysis showed that no alignment was found between primer/probe and microorganism listed in the table. That proves the homology between one of the primers/probes and any sequence present in the targeted microorganism is less than 80%.

11.3.2 Wet-testing Analysis

The CodeCheck™ SARS-CoV-2 RT-PCR Kit was used to test contrived nasopharyngeal (NP) swab preparations of microorganisms which are similar to SARS-CoV-2 species which can cause similar symptoms with SARS-CoV-2, including Epstein-Barr (EB) virus, human cytomegalovirus, Haemophilus influenzae, Staphylococcus aureus, Streptococcus pneumoniae, Streptococcus pyogenes, Klebsiella pneumoniae, Aspergillus fumigatus, Candida albicans, Candida glabrata and Cryptococcus neoformans. RNA extraction was performed with the QIAamp Viral RNA Mini Kit (QIAGEN, catalog # 52906) and testing on the ABI 7500 Real Time PCR System (software V2.0.6). The testing was performed in triplicate.

Table 11.3.2 Wet Testing Cross-Reactivity Results

Sample name	Concentration	Replicate 1				Replicate 2				Replicate 3				Detection rate
		Ct value			Result	Ct value			Result	Ct value			Result	
		ORF1ab	N	β-actin		ORF1ab	N	β-actin		ORF1ab	N	β-actin		
Epstein-Barr (EB) virus	1×10^5 pfu/mL	NA	NA	18.27	Negative	NA	NA	18.07	Negative	NA	NA	18.19	Negative	0/3
Human cytomegalovirus	1×10^5 pfu/mL	NA	NA	19.87	Negative	NA	NA	19.54	Negative	NA	NA	19.48	Negative	0/3
Haemophilus influenzae	1×10^7 CFU/mL	NA	NA	21.65	Negative	NA	NA	21.24	Negative	NA	NA	21.44	Negative	0/3
Staphylococcus aureus	2×10^6 CFU/mL	NA	NA	24.91	Negative	NA	NA	24.32	Negative	NA	NA	24.84	Negative	0/3
Streptococcus pneumoniae	3×10^6 CFU/mL	NA	NA	21.36	Negative	NA	NA	21.32	Negative	NA	NA	21.54	Negative	0/3
Streptococcus pyogenes	7×10^6 CFU/mL	NA	NA	23.35	Negative	NA	NA	23.22	Negative	NA	NA	23.19	Negative	0/3
Klebsiella pneumoniae	2×10^6 CFU/mL	NA	NA	24.24	Negative	NA	NA	24.12	Negative	NA	NA	24.37	Negative	0/3
Aspergillus fumigatus	1×10^6 CFU/mL	NA	NA	21.53	Negative	NA	NA	21.45	Negative	NA	NA	21.54	Negative	0/3
Candida albicans	3×10^7 CFU/mL	NA	NA	18.15	Negative	NA	NA	18.31	Negative	NA	NA	18.11	Negative	0/3
Candida glabrata	1×10^7 CFU/mL	NA	NA	19.19	Negative	NA	NA	19.27	Negative	NA	NA	19.15	Negative	0/3
Cryptococcus neoformans	1×10^7 CFU/mL	NA	NA	22.52	Negative	NA	NA	22.43	Negative	NA	NA	22.85	Negative	0/3

Conclusion:

There were no cross reactions between the pathogens tested.

11.4 Clinical Performance

The clinical performance of the CodeCheckSARS-CoV-2 RT-PCR Kit was established by testing 90 previously collected residual nasopharyngeal samples identified as SARS-CoV-2 positive or negative with the cobas SARS-CoV-2 on the Roche 6800 system. Samples were tested with the CodeCheckSARS-CoV-2 RT-PCR Kit according to the instructions for use on the Applied BioSytems 7500 PCR system. The results are presented the table below.

CodeCheck™ SARS-CoV-2 RT-PCR Kit Clinical Agreement

		cobas SARS-CoV-2	
		Positive	**Negative**
CodeCheck™ SARS-CoV-2 RT-PCR Kit	Positive	44	2
	Negative	1	43

Positive percent agreement: 97.8%, 95% CI (88.4-99.6)
Negative percent agreement: 95.6%, 95% CI (85.2-98.8)

12 Technical Support

Manufacturer:

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14 Symbols

REF	Reference Number
Rx ONLY	Prescription Use Only
IVD	In Vitro Diagnostic Medical Device
LOT	Lot Number
	Temperature limit
	Use By
	Manufacturer
	Caution
	Keep away from sunlight
	Sufficient for
	Consult Instruction for Use