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StrongStep®

Novel Coronavirus (SARS-CoV-2) Multiplex Real-Time PCR Kit (detection for three genes)

Instructions for Use (IFU)

StrongStep®
Novel Coronavirus (SARS-CoV-2)
Multiplex Real-Time PCR Kit -COVID-19 IFU DOC LM213955
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Σ 96 tests

StrongStep®
Novel Coronavirus (SARS-CoV-2)
Multiplex Real-Time PCR Kit (detection for three genes)

Sample Types	Extraction Platforms	PCR Platforms
Nasopharyngeal Swabs	FDA/CE IVD Extraction System, suitable for the directed sample types	Applied Biosystem® 7500 Real-Time PCR System; Bio-Rad CFX96
Oropharyngeal Swabs		
Sputum/Broncheoalveolar lavage fluid (BALF)		

Contents

1. Intended Use

This product is intended to be used to achieve qualitative detection of SARS-CoV-2 viral RNA extracted from nasopharyngeal swabs, oropharyngeal swabs, sputum and BALF from patients in association with an FDA IVD extraction system and the designated PCR platforms listed above. The kit is intended for use by laboratory trained personnel. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform moderate or high complexity tests.

2. Kit Components

Kit Components	Description	Amount & Package
SARS-CoV-2 rt-qPCR reagent	Lyophilized ready-to-use PCR beads in 8-Strip Tubes	12 X vacuum seal bags
	New 8-Strip Caps	
Positive control	Lyophilized Armored RNA containing target genes.	1 X 2.0 ml tube
Instructions for Use		1

3. Storage & Handling Conditions

Shipping at less than 37℃, and store at -20℃ for 12 months.
Attention should be paid to the "use by" date specified on the pack label and individual tube labels. On this date, the kit should be discarded following the disposal instructions in Section 8.

3.1. In Use Stability

The kit should be stored in the original packaging.
SARS-CoV-2 rt-qPCR reagents are vacuum-packed. They must be kept completely dry. Once opened, please use it immediately or store at -20℃ no more than 1 week within 1 freeze-thaw cycle.
After adding water, **positive control** is stable for 2 months, if stored at -20℃. Repeated thawing and freezing should be kept to a minimum and should not exceed 5 freeze-thaw cycles. It may be aliquoted into smaller volumes after resuspension, if necessary.

4. Identification of Material & Devices Required but Not Provided

4.1. Reagents

- Appropriate nucleic extraction system and/or kit (please refer to Section 9.1 'sample preparation procedure').
- Water for injection, available from hospital pharmacy

4.2. Equipment

- Benchtop centrifuge
- Vortex mixer
- Adjustable pipettes
- Pipette tips with filters
- Disposable gloves
- 1.5mL microcentrifuge tubes for extraction

4.3. Real-Time PCR Machine

- Appropriate Real-Time PCR instrument (please refer to **Section 6.3**).

4.4. Facilities/Training Requirements

- Samples should be handled in a Biosafety Level 2 facility, World Health Organization Interim guidance on laboratory biosafety from 12 Feb 2020 should be followed. Testing for the presence of COVID-19 should be performed in an appropriately equipped laboratory by staff trained to the relevant technical and safety procedures.

5. Background Information

The target is important to be screened for patient displaying relevant symptoms of Coronavirus.
Reliable and regular diagnosis of individuals for this target will help to ensure a reduction in the spread of infections but also help rapidly treat infected patients.
COVID-19 is caused by the SARS-CoV-2 virus. There is limited information available to characterize the spectrum of clinical illness associated with COVID-19 but it likely spreads to others when a person shows signs or symptoms of being sick (e.g., fever, coughing, difficulty breathing, etc.).

6. Product Description

StrongStep® Novel Coronavirus (SARS-CoV-2) Multiplex Real-Time PCR Kit (detection for three genes) is an in vitro diagnostic test based on Real-Time PCR technology, developed for specific detection of SARS-CoV-2 viral RNA. The probe system is based on the standard hydrolysis probe system known as TaqMan® Technology.
This product provides quadruple detections of three independent genes of SARS-CoV-2 in a single tube. Specific primers and probes were designed for the detection of conserved region of SARS-CoV-2's ORF1ab gene, S gene and N gene, respectively, avoiding non-specific interference of SARS2003 and Bat SARS-like virus strains. An internal Control (IC) amplifying human RNase P gene was used to identify possible PCR inhibition, to measure extraction purity and to confirm the integrity of the PCR run.
The kit is supplied as ready-to-use lyophilized PCR beads, shipping in ambient temperature.

6.1. Positive Control

The Positive Control Template (PCT) contains standardized concentrations of SARS-CoV-2 RNA (Coronavirus COVID-19) specific sequence in concentration 10 copies per μL.
To ensure PCR run validity, the PCT should produce Ct value ≤35 in FAM/HEX/ROX channel.

6.2. Extraction Kits / Instruments

The performance of the COVID-19 Coronavirus Real Time PCR Kit depends on the quantity of SARS-CoV-2 RNA present in the specimen and the efficiency and purity of nucleic acid extraction.
StrongStep® Novel Coronavirus (SARS-CoV-2) Multiplex Real-Time PCR Kit was developed to be used with an extraction system validated on the automated extraction system GenoXtract® using GXT DNA/RNA Extraction kit (Hain Lifescience GmbH (Brucker) Cat# 12.01.02) and QIAamp Viral RNA Mini Kit (QIAGEN, Cat#52906/ 52904).

6.3. Real-Time PCR instruments

StrongStep® Novel Coronavirus (SARS-CoV-2) Multiplex Real-Time PCR Kit was developed and validated to be used with the following Real-Time PCR instruments.

- Applied Biosystem® 7500 Real-Time PCR System (software version 2.3)
- Bio-Rad CFX Maestro (software version 1.1).
- The qPCR machine should meet the following requirements:
  1 fit 8 strip PCR tube volume 0.2 ml
  2 Have More than four detection channels:

Channel	Excitation (nm)	Emission (nm)	Pre-Calibrated Dyes
1.	470	525	FAM, SYBR Green I
2	523	564	VIC, HEX, TET, JOE
3	571	621	ROX, TEXAS-RED
4	630	670	CY5

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

7. Warning and Precautions

7.1. Safety Information

7.1.1. Samples

Testing for the presence of SARS-CoV-2 should be performed in appropriately equipped laboratories by

staff trained in the relevant technical and safety procedures. National guidelines on laboratory biosafety should be followed in all circumstances.

7.1.2.RNA Extraction Kit (FDA/CE IVD) Warnings

Please consult the relevant MSDS, available from the supplier, before using your chosen FDA/CE IVD extraction kit.

7.2. Handling and Procedural Requirements

7.2.1 General

- Always wear disposable gloves when handling kit components.
- Use separated working areas for specimen preparation, reaction set up and amplification.
- Supplies and equipment should be separated in each work area and not moved between them.
- When mixing reagents by pipetting up and down this should be done with a volume roughly equal to 50% of the total component volume

7.2.2. Preventing Template Contamination

- Positive control template contains a high copy number of templates. It should be opened and processed away from test samples and kit components to avoid cross-contamination.
- After each run has been set up and performed, clean work surfaces and equipment with a DNA/RNA remover.
- Never open post-amplification tubes. For further instruction for disposal see Section 8 Disposal Instructions.

7.2.3. Prevention of DNase Contamination

- Use DNase/RNase free disposable plastic ware and pipettes reserved for DNA/RNA work to prevent cross-contamination with DNase/RNase from shared equipment.
- Use DNase/RNase free filter tips throughout procedure to prevent aerosol and liquid contamination.

8. Limitations of Use

- The StrongStep® Novel Coronavirus (SARS-CoV-2) Multiplex Real-Time PCR Kit has been validated for use with oropharyngeal swab, nasopharyngeal swab, sputum, or BALF samples run on the Applied Biosystem® 7500 Real-Time and Bio-Rad CFX96 PCR System.
- The procedures in this handbook must be followed, as described. Any deviations may result in assay failure or cause erroneous results.
- Good laboratory practice is required to ensure the performance of the kit, with care required to prevent contamination of the kit components. Components should be monitored for contamination and any components thought to have become contaminated should be discarded as standard laboratory waste in a sealed pouch or zip-lock plastic bag.
- All samples should be handled as if they are infectious following proper biosafety precautions.
- Interpretation of results must account for the possibility of false negative and false positive results.
- False negative results may be caused by:
    - ✧ Unsuitable collection, handling and/or storage of samples.
    - ✧ Sample outside of viremic phase.
    - ✧ Failure to follow procedures in this handbook.
    - ✧ Use of unauthorized extraction kit or PCR platform.
- False positive results may be caused by:
    - ✧ Unsuitable handling of samples containing high concentration of SARS-CoV-2 viral RNA or positive control template.
    - ✧ Unsuitable handling of amplified product.
- All results should be interpreted by a health care professional in the context of patient medical history and clinical symptoms.
- This test cannot rule out diseases caused by other pathogens.
- A negative result for any PCR test does not conclusively rule out the possibility of infection.
- Reports to Healthcare providers should include the information that the StrongStep® Novel Coronavirus (SARS-CoV-2) Multiplex Real-Time PCR Kit has been validated but FDA's independent review under the EUA program of this validation is pending.

9. Procedure

9.1. Sample Preparation Procedure

Prepare at least 1 negative extraction control (NEC) each time an extraction is performed (i.e. DEPC-water). This NEC will serve as the negative control for the entire testing system.

	Nasopharyngeal swabs	Oropharyngeal swabs	Sputum** or BALF
Collection	Dacron or polyester flocked swabs in viral transport medium	Dacron or polyester flocked swabs in viral transport medium	Sterile container
Transport temperature*	4°C	4°C	4°C
Short-term storage (pre-extraction) *	4°C for ≤ 5 days	4°C for ≤ 5 days	4°C for ≤ 48 hours
Long-term storage (pre-extraction) *	-70°C for longer periods	-70°C for longer periods	-70°C for longer periods
Extraction System	FDA/CE IVD extraction system intended for use in the isolation of RNA	FDA/CE IVD extraction system intended for use in the isolation of RNA	FDA/CE IVD extraction system intended for use in the isolation of RNA
Extraction sample volume	700µL***	700µL***	700µL
Extraction elution volume	85µL	85µL	85µL

*These are World Health Organization (WHO) recommendations. Local regulations pertaining to sample handling must take priority.
**Sputum must be from the lower respiratory tract
***Sample refers to the viral transport medium provided in the sample container serving as the repository for the swab.

9.1. RNA Extraction

Please consult the IFU of the chosen FDA/CE IVD extraction system for full usage details.

9.2. Reaction Setup

- Open 8-Strip Tubes, add 13 µL H_2O (water for injection) to each SARS-CoV-2 RT-qPCR reagent tube
- Add 5µL of the following into the appropriate wells according to your setup:
  i. Sample(s)
  ii. PCT: resuspended in 50µL water for injection (vortex to mix)
  iii. NEC

iv. No template control (optional): add water for injection as template

Seal the tubes with new PCR CAPs, Vortex and centrifuge for 30s at 3000 rpm.

9.3. Programming the Real-Time PCR Instrument

Please refer to one of the following manuals for additional information on using the instrument:

- Applied Biosystem® 7500 Real-Time PCR system Relative Standard curve and comparative CT Experiments (Applied Biosystem).
- CFX96™ Touch Instruction Manual (© 2013, Bio-Rad Laboratories Inc.)

a) Enter the following amplification program:

Create a temperature profile on your instrument as follows:

Steps	Cycles	Temperature	Time	Detection Format
Reverse Transcription	1	50°C	15 minutes	N gene=FAM S gene=HEX ORF1ab gene=ROX IPC=Cy5
Pre-denature	1	95°C	3 minutes	
Thermal cycling	45	95°C	5 seconds	
		60°C*	60 seconds	

*Acquisition must be performed at the end of this stage

b) Ensure the well loaded with PCT are designated as "Sample Type - Standard" and assigned the appropriate concentration (see **Section 6.1**)

c) Ensure wells loaded with sample(s) are designated as "Sample Type – Unknown"; the software will automatically calculate quantities for these wells if amplification occurs.

9.4. Data Analysis

Before interpreting sample results, it is necessary to verify the success of the run. If the following criteria are not satisfied, then testing needs to be repeated:

a) NEC or No template control is free from amplification in the all channels

b) PCT produces a Ct <35 in FAM/HEX/ROX channels

For instrument specific guidance on correctly assigning Ct values please see below.

9.4.1. Bio-Rad® CFX96

Select the 'Ct Determination Mode' to Regression. This option can be accessed in the menu bar under 'View'.

9.4.2. Applied Biosystem® 7500 Real-Time PCR System

- Select "Graph Type: Linear"
- Select "Target: FAM"
- Untick box for "Threshold: Auto"
- Tick box for "Show: Threshold"
- Manually set the threshold line at the 1/10th of the End point fluorescence value for the PCT
- Select "Target: HEX"
- Untick box for "Threshold: Auto"
- Tick box for "Show: Threshold"
- Manually set the threshold line at the 1/10th of the End point fluorescence value for the PCT
- Select "Target: ROX"
- Untick box for "Threshold: Auto"
- Tick box for "Show: Threshold"
- Manually set the threshold line at the 1/10th of the End point fluorescence value for the PCT
- Select "Target: Cy5"
- Untick box for "Threshold: Auto"
- Tick box for "Show: Threshold"
- Manually set the threshold line at the 1/10th of the End point fluorescence value for Cy5 amplification curves.

9.5. Interpretation of Results

If all the data analysis criteria are fulfilled, then each sample can be assessed with the following metric:

Result	Result interpretation
FAM Ct≤38*	N gene positive (POS)
FAM Ct＞38	N gene negative (NEG)
HEX Ct≤38*	S gene positive (POS)
HEX Ct＞38	S gene negative (NEG)
ROX Ct≤38*	Orf1ab gene positive (POS)
ROX Ct＞38	Orf1abene negative (NEG)
Cy5 Ct≤38*	Rnase P gene positive (POS)
Cy5 Ct＞38	Rnase P gene negative (NEG)

*Please manually inspect amplification curves for all samples assigned a Ct value to verify the positive amplification.

According the result of N gene, S gene and Orf1ab gene, The results of for patient samples are interpreted as follows:

N gene	S gene	Orf1ab gene	Rnase P gene	Status	Result	Action
NEG	NEG	NEG	NEG	Invalid	NA	Repeat test. If the repeat result remains invalid, consider collecting a new specimen.
NEG	NEG	NEG	POS	Valid	SARS-CoV-2 Not Detected	Report results to healthcare provider. Consider testing for other viruses
Only one SARS-CoV-2 target POS			POS or NEG	Valid	SARS-CoV-2 Inconclusive*	Repeat test. If the repeat result remains inconclusive, additional confirmation testing should be conducted if clinically indicated.
Two or more SARS-CoV-2 targets POS			POS or NEG	Valid	Positive SARS-CoV-2	Report results to healthcare provider and appropriate public health authorities

*Samples with a result of SARS-CoV-2 Inconclusive shall be retested one time.

10. Performance Evaluation

The results for the StrongStep® Novel Coronavirus (SARS-CoV-2) Multiplex Real-Time PCR Kit performance evaluation have been generated on the Applied Biosystem® 7500 Real-Time PCR system.

10.1. Analytical Sensitivity

The analytical sensitivity was defined as the lowest concentration of analyte that could be reliably detected with 95% confidence. This was assessed via spiking 3 negative Sputum samples with known copy number template (Armored RNA containing target fragments of N gene, S gene and Orf1ab gene.). The eluates were then serially diluted to give the 11 contrivance levels that were tested over 3 days, producing at least 36 replicates for each concentration tested.

The spiking samples were purified by GenoXtract® using GXT DNA/RNA Extraction kit (Hain Lifescience GmbH (Brucker) Cat# 12.01.02). 700µL sample was used to extraction, elution volume was 85µL. concentrations in the table were quantified after extraction.

10.1.1. Analytical Sensitivity Results

This data demonstrates that the StrongStep® Novel Coronavirus (SARS-CoV-2) Multiplex Real-Time

PCR Kit detects 3.9 copies/uL of SARS-CoV-2 viral RNA with a confidence ≥95%. This concentration therefore serves as the limit of detection of the kit.

	ABI 7500 Data (FAM channel)			
Contrivance Level	Overall Mean Concentration (copies/μL)	% Replicate Detection	Mean Ct	Ct Standard Deviation
1	1000	100	26.13	1.286
2	500	100	27.03	1.304
3	250	100	28.15	1.353
4	125	100	29.17	1.372
5	62.5	100	29.99	1.351
6	31.5	100	31.15	1.343
7	15.8	100	32.20	1.125
8	7.9	100	33.17	1.427
9	3.9	97.2	34.21	2.464
10	1.9	72.2	35.17	2.077
11	1	19.4	35.20	0.790
	ABI 7500 Data (HEX channel)			
Contrivance Level	Overall Mean Concentration	% Replicate Detection	Mean Ct	Ct Standard Deviation
1	1000	100	26.10	1.386
2	500	100	26.90	1.404
3	250	100	28.02	1.373
4	125	100	29.04	1.373
5	62.5	100	29.86	1.353
6	31.5	100	31.02	1.447
7	15.8	100	32.27	1.125
8	7.9	100	33.14	1.427
9	3.9	100	34.09	2.564
10	1.9	97.2	35.14	2.077
11	1	76.9	35.26	0.790
	ABI 7500 Data (ROX channel)			
Contrivance Level	Overall Mean Concentration (copies/μL)	% Replicate Detection	Mean Ct	Ct Standard Deviation
1	1000	100	25.99	1.186
2	500	100	26.89	1.204
3	250	100	28.01	1.174
4	125	100	29.03	1.573
5	62.5	100	29.85	1.553
6	31.5	100	31.00	1.646
7	15.8	100	32.05	1.325
8	7.9	100	33.02	1.627
9	3.9	97.2	34.06	2.364
10	1.9	72.2	35.04	2.277
11	1	55.5	35.07	1.004

The CFX 96 (Bio-Rad®) qPCR machines were used to re-validate the analytical sensitivity to ensure that 3.9 copies/μl. 36 replicates of positive control template representing the analytical sensitivity concentration were tested.

Bio-Rad CFX96 (FAM Channel)

Contrivance Level	Overall Mean Concentration (copies/μL)	% Replicate Detection	Mean Ct	Ct Standard Deviation
1	15.8	100	31.79	0.266
2	7.9	100	32.87	0.341
3	3.9	100	33.92	0.564
Bio-Rad CFX96 (HEX Channel)				
Contrivance Level	Overall Mean Concentration (copies/μL)	% Replicate Detection	Mean Ct	Ct Standard Deviation
1	15.8	100	31.68	0.266
2	7.9	100	32.74	0.304
3	3.9	100	33.85	0.329
Bio-Rad CFX96 (ROX Channel)				
Contrivance Level	Overall Mean Concentration (copies/μL)	% Replicate Detection	Mean Ct	Ct Standard Deviation
1	15.8	100	31.67	0.071
2	7.9	100	32.75	0.078
3	3.9	100	33.87	0.105

10.2. Analytical Specificity

10.2.1 Inclusivity analytical sensitivity

In silico analysis was conducted to evaluate the extent of homology between the the StrongStep® Novel Coronavirus (SARS-CoV-2) Multiplex Real-Time PCR Kit and all SARS-CoV-2 sequences available in NCBI and in GISAID databases. A total of 11360 sequences (NCBI (n=2035); GISAID (n=9325)) collected from Africa, America, Europe, Oceania and Asia that were available through April 5 2020, were examined using BLAST to identify the extent of predicted assay inclusivity. Overall, >99% of available N gene, S gene and ORF1ab sequences exhibited 100% homology with The the StrongStep® Novel Coronavirus (SARS-CoV-2) Multiplex Real-Time PCR Kit primers and probes. For those sequences with <100% homology, there is a single mismatch located in the middle of one primer that is not expected to affect test performance. Based on in silico analysis, The the StrongStep® Novel Coronavirus (SARS-CoV-2) Multiplex Real-Time PCR Kti is predicted to detect all currently available SARS-CoV-2 sequences.

The result of the in inclusivity of the primers and probes

a. Sequences from GISAID (n=9325)

SARS-CoV-2 Target	Number of sequences	Numbers of Mismatches		
		Forward primers	probe	Reverse primer
N gene	9218(99.84%)	0	0	0
	12(0.12%)	1	0	0
	2(0.01%)	0	0	1
S gene	7163(100%)	0	0	0
Orf1ab gene	9324(99.99%)	0	0	0
	1(0.01%)	0	0	1

b. Sequences from NCBI (n=2035))

SARS-CoV-2 Target	Number of sequences	Numbers of Mismatches		
		Forward primers	probe	Reverse primer

N gene	2033(99.90%)	0	0	0
	1(0.05%)	1	0	0
	1(0.05%)	0	0	1
S gene	563(100%)	0	0	0
Orf1ab gene	1324(99.92%)	0	0	0
	1(0.08%)	0	0	1

10.2.2 Cross-reactivity

Samples of other pathogens with similar symptoms or belonging to the same class or kind were tested. Samples of other pathogens with similar symptoms or belonging to the same class or kind were tested, including Influenza A H1N1, Influenza A H3N2, Influenza B Victoria, Influenza B Yamagata, RSV A, RSV B, Coronavirus NL63, Coronavirus 229E, Coronavirus HKU, Streptococcus pneumoniae and Neisseria meningitidis. Viruses were tested at 10^3–10^5 TCID50/mL and Bacteria were tested at 10^5–10^6CFU/mL. StrongStep® Novel Coronavirus (SARS-CoV-2) Multiplex Real-Time PCR Kit showed no cross reactivity for the tested organisms.
The results of the in vitro specimen testing are presented below.

Organism Interpreted	Result*
Influenza A H1N1	SARS-CoV-2 not detected
Influenza A H3N2	SARS-CoV-2 not detected
Influenza B Victoria	SARS-CoV-2 not detected
Influenza B Yamagata	SARS-CoV-2 not detected
RSV A	SARS-CoV-2 not detected
RSV B	SARS-CoV-2 not detected
Coronavirus NL63	SARS-CoV-2 not detected
Coronavirus 229E	SARS-CoV-2 not detected
Coronavirus HKU	SARS-CoV-2 not detected
Coronavirus OC43	SARS-CoV-2 not detected
Streptococcus pneumoniae	SARS-CoV-2 not detected
Neisseria meningitidis	SARS-CoV-2 not detected

Results were interpreted according to **Section 9.5**.

10.3. Precision

Assay precision for the StrongStep® Novel Coronavirus (SARS-CoV-2) Multiplex Real-Time PCR Kit was determined by the repeated testing of clinical samples contrived to represent 3 viral load levels:
• 120 copies/reaction (24 copies/μL)
• 100 copies/reaction (20 copies/μL)
• 80 copies/reaction (16 copies/μL)
Precision was expressed in the form of the Ct standard deviation and coefficient of variation.

10.3.1. Repeatability

Repeatability was measured by analyzing 10 replicates of each sample on a single plate:

StrongStep® Novel Coronavirus N gene (FAM channel)			
Sample Concentration (copies/μL)	Mean Ct	% Replicate Detection	Coefficient of Variation (%)
24	31.65	100%	0.040
20	31.81	100%	0.052
16	32.15	100%	0.045
StrongStep® Novel Coronavirus S gene (HEX channel)			

Sample Concentration (copies/μL)	Mean Ct	% Replicate Detection	Coefficient of Variation (%)
24	31.64	100%	0.025
20	31.76	100%	0.028
16	32.12	100%	0.035
StrongStep® Novel Coronavirus Orf1ab gene (ROX channel)			
Sample Concentration (copies/μL)	Mean Ct	% Replicate Detection	Coefficient of Variation (%)
24	31.63	100%	0.030
20	31.79	100%	0.045
16	32.11	100%	0.035

10.3.2. Inter-instrument Reproducibility

Inter-instrument reproducibility was measured by running 10 replicates of each sample across 2 qPCR instruments.

StrongStep® Novel Coronavirus N gene (FAM channel)			
Sample Concentration (copies/μL)	Mean Ct	% Replicate Detection	Coefficient of Variation (%)
24	31.56	100%	2.16
20	31.84	100%	2.23
16	32.16	100%	2.58
StrongStep® Novel Coronavirus S gene (HEX channel)			
Sample Concentration (copies/μL)	Mean Ct	% Replicate Detection	Coefficient of Variation (%)
24	31.53	100%	2.16
20	31.89	100%	1.98
16	32.20	100%	2.11
StrongStep® Novel Coronavirus Orf1ab gene (ROX channel)			
Sample Concentration (copies/μL)	Mean Ct	% Replicate Detection	Coefficient of Variation (%)
24	31.48	100%	1.86
20	31.82	100%	1.91
16	32.15	100%	1.78

10.3.3. Operator Reproducibility

Three different operators tested 10 replicates of each sample with the StrongStep® Novel Coronavirus (SARS-CoV-2) Multiplex Real-Time PCR Kit to assess operator reproducibility. For each test, the same batch and the same instrument was used.

StrongStep® Novel Coronavirus N gene (FAM channel)			
Sample Concentration (copies/μL)	Mean Ct	% Replicate Detection	Coefficient of Variation (%)
24	31.48	100%	2.35
20	31.83	100%	2.38
16	32.15	100%	2.98
StrongStep® Novel Coronavirus S gene (HEX channel)			
Sample Concentration (copies/μL)	Mean Ct	% Replicate Detection	Coefficient of Variation (%)
24	31.59	100%	3.16

20	31.91	100%	2.98
16	32.23	100%	2.15
StrongStep® Novel Coronavirus Orf1ab gene (ROX channel)			
Sample Concentration (copies/μL)	Mean Ct	% Replicate Detection	Coefficient of Variation (%)
24	31.50	100%	2.86
20	31.83	100%	1.97
16	32.17	100%	1.88

10.3.4. Daily Reproducibility

Daily reproducibility was assessed by analyzing 40 replicates of each sample across 4 days, 10 replicates of each concentration per day. For each test, the same batch and the same instrument was used.

StrongStep® Novel Coronavirus N gene (FAM channel)			
Sample Concentration (copies/μL)	Mean Ct	% Replicate Detection	Coefficient of Variation (%)
24	31.53	100%	1.95
20	31.79	100%	2.38
16	32.15	100%	2.92
StrongStep® Novel Coronavirus S gene (HEX channel)			
Sample Concentration (copies/μL)	Mean Ct	% Replicate Detection	Coefficient of Variation (%)
24	31.48	100%	3.16
20	31.75	100%	2.97
16	32.12	100%	2.19
StrongStep® Novel Coronavirus Orf1ab gene (ROX channel)			
Sample Concentration (copies/μL)	Mean Ct	% Replicate Detection	Coefficient of Variation (%)
24	31.51	100%	1.85
20	31.78	100%	2.07
16	32.17	100%	2.15

10.4. Stability

A same batch kits were stored at four different temperatures: -20℃, 24 ℃, 37 ℃ and 56 ℃. The higher temperatures were used for the accelerated studies, which allowed for rapid stability predictions. The kits were removed weekly for nine weeks to perform functional testing. For each time point, the assay stored at -20 ℃ served as the control and was tested in parallel with the assay stored at higher temperatures. Analytical Sensitivity test was performed as 10.1. The LoD of the kit kept 3.9 copies/μL, and Coefficient of Variation is from 2.05 to 3.66.

As the LoD test result, the kit is stable for longer than 9 weeks when stored at -20℃, 24 ℃, 37 ℃ and 56 ℃. According to the following formula, derived from the Arrhenius Equation:

Predicted Stability =Accelerated Stability X $2^{\Delta T/10}$,

where ΔT is the difference between the normal storage temperature and the sample storage temperature. The greater this difference is, however, the less reliable the prediction. The kit's shelf life is tentatively set at 1 year when store at -20°C and transport under room temperature no more than 3 months. Since accelerated testing is a model and should be supported by real-time testing, we plan to continue to accumulate real-time data on a regular schedule.

10.5. Accuracy

Clinical evaluation of the StrongStep® Novel Coronavirus (SARS-CoV-2) Multiplex Real-Time PCR Kit was conducted with contrived nasopharyngeal swab and BALF specimens.

A total of 150 contrived positive specimens were tested:

• 50 contrived positive nasopharyngeal swab specimens

• 50 contrived positive BALF specimens

• 50 contrived positive Sputum specimens
Samples were contrived by spiking known concentrations of extracted SARS-CoV-2 viral genomic RNA, relative to the product LoD, into matrices which were determined to be negative by the StrongStep® Novel Coronavirus (SARS-CoV-2) Multiplex Real-Time PCR Kit prior to spiking in the RNA.
In addition to the contrived positive specimens, 100 negative specimens were tested:
• 50 negative nasopharyngeal swab specimens
• 50 negative samples BALF specimens
• 50 negative Sputum specimens
All negative samples yielded negative results.
Results for positive samples are shown in the tables below:

Nasopharyngeal swab Clinical Evaluation Study

Final RNA Concentration in Sample(copies/μL)	Number of Positives	Mean Ct N gene	Mean Ct S gene	Mean Ct ORF1ab
8	25/25	33.18	33.15	33.12
12	20/20	32.78	32.76	32.75
20	5/5	31.75	31.72	31.72

BALF Clinical Evaluation Study

Final RNA Concentration in Sample(copies/μL)	Number of Positives	Mean Ct N gene	Mean Ct S gene	Mean Ct ORF1ab
8	25/25	33.22	33.18	32.17
12	20/20	32.85	32.83	32.83
20	5/5	31.95	31.87	31.85

Sputum Clinical Evaluation Study

Final RNA Concentration in Sample(copies/μL)	Number of Positives	Mean Ct N gene	Mean Ct S gene	Mean Ct ORF1ab
8	25/25	33.54	33.49	33.50
12	20/20	33.07	32.97	33.04
20	5/5	32.19	32.09	32.20

Clinical evaluation of the StrongStep® Novel Coronavirus (SARS-CoV-2) Multiplex Real-Time PCR Kit was conducted with (Nasopharyngeal Swabs/ Oropharyngeal Swabs/Sputum/Broncheoalveolar lavage fluid (BALF)) in five Clinical trial center laboratories with 416 Nasopharyngeal Swabs, 101 Oropharyngeal Swabs,28 Sputum and 6 Broncheoalveolar lavage fluid (BALF)). The results were compared with that of Reference Reagent and the COVID-19 Diagnostic Criteria and the medical determination of disease process towards COVID-19.
Compared with the reference reagent, the positive agreement was 98.55% (95.82% ~ 99.70%), the negative agreement was 99.13% (97.47% ~ 99.82%) and total agreement was 98.91% (97.65% ~ 99.60%). The kappa value of the consistency analysis was 0.9768 (95%CI: 0.9583 ~ 0.9953).The results of the clinical evaluation show that the two reagents (methods) have a high degree of consistency and equivalent sensitivity and specificity in detecting COVID-19.

11. Quality Control

In accordance with Liming Bio-Products Co., Ltd. ISO 13485 certified Quality Management System, each batch of Kit is tested against predetermined specifications to ensure consistent product quality.

12. Technical Support

For Technical support, please contact our dedicated technical support team on:
Phone: +86(25)85288510 **E-mail**: pcr_tech@limingbio.com

13. Trademarks and Disclaimers

Trademarks: StrongStep® and

All other trademarks that appear in this IFU are the property of their respective owners.

14. Explanation of Symbols

Symbol	Explanation
IVD	In vitro diagnostics
	Manufacturer
REF	Catalogue number
	Suffices for
	Use by Date
	Temperature limit
LOT	Batch Code
EC REP	Authorized representative in the European Community

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