KIT AND METHOD FOR DETECTING SARS-COV-2

FIELD OF THE INVENTION

[0001] The present invention relates to kit and method for detecting Coronavirus, and more particularly to kit and method for detecting SARS-CoV-2.

BACKGROUND OF THE INVENTION

[0002] Respiratory illness coronavirus disease 2019 (COVID-19) broke out at the end of 2019. In recent months, medical communities around the world have been rallying to develop reliable and rapid diagnosis tests for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which is the causing virus of the disease.

[0003] Main methods to detect COVID-19 include molecular diagnostics, serology testing and medical imaging (CT scan). The first validated molecular diagnostic test for COVID-19 was designed from Charité Institute of Virology in Berlin, Germany in January of 2020. Since then, hundreds of molecular tests have been designed to detect SARS-CoV-2; most of them employ RT-PCR technology for amplification and detection of genetic material from SARS-CoV-2. Other methods include isothermal amplification, full genome sequencing, microarray or lab-on-chip technologies and CRISPR.

[0004] The most prominent issue with RT-PCR method is its long reaction time. Most of the available kits for COVID-19 detection based on RT-PCR or Real-Time RT-PCR (rRT-PCR) technology have a turnaround time of more than 30 minutes. The rRT-PCR assay developed by School of Public Health, The University of Hong Kong (HKU) is one of the fastest assays, and still requires 28

minutes. Plus, to achieve a short turnaround time for RT-PCR based test, a high-performance thermocycler is mandatory.

[0005] Some isothermal technique-based coronavirus tests are available for COVID-19 detection, such as COVID-19 LAMP Kit from Eiken (not real-time; turnaround time around 30 minutes) and 2019-nCoV Kit based on CPA technique from Bioustar. However, most of them still have a turnaround time around 30 minutes or longer for nucleic acid amplification and detection.

[0006] Therefore, a fast and accurate test of SARS-CoV-2 that has the potential in point-of-care testing (POCT) use is still in need in COVID-19 outbreak control and management.

SUMMARY OF THE INVENTION

[0007] An object of the embodiments of the present invention is to provide kit and method for detecting SARS-CoV-2.

[0008] According to an aspect of an embodiment of the present invention, there is provided a kit for detecting SARS-CoV-2, and the kit includes at least one of a first primer set and a second primer set. The first primer set is selected from the group consisting of: (a) primers including nucleotide sequences of SEQ ID NOs: 1 to 4, or nucleotide sequences having at least 90% identity to SEQ ID NOs: 1 to 4; (b) primers including nucleotide sequences of SEQ ID NOs: 7 to 10, or nucleotide sequences having at least 90% identity to SEQ ID NOs: 7 to 10; (c) primers including nucleotide sequences of SEQ ID NOs: 13 to 16, or nucleotide sequences having at least 90% identity to SEQ ID NOs: 13 to 16; (d) primers including nucleotide sequences of SEQ ID NOs: 19 to 22, or nucleotide sequences having at least 90% identity to SEQ ID NOs: 19 to 22; and (e) primers including nucleotide sequences of SEQ ID NOs: 25 to 28, or nucleotide sequences having at

least 90% identity to SEQ ID NOs: 25 to 28. The second primer set is selected from the group consisting of: (f) primers including nucleotide sequences of SEQ ID NOs: 31 to 34, or nucleotide sequences having at least 90% identity to SEQ ID NOs: 31 to 34; (g) primers including nucleotide sequences of SEQ ID NOs: 37 to 40, or nucleotide sequences having at least 90% identity to SEQ ID NOs: 37 to 40; and (h) primers including nucleotide sequences of SEQ ID NOs: 43 to 46, or nucleotide sequences having at least 90% identity to SEQ ID NOs: 43 to 46.

[0009] In some embodiments, the primer set (a) further includes primers including nucleotide sequences of SEQ ID NOs: 5 and 6, or nucleotide sequences having at least 90% identity to SEQ ID NOs: 5 and 6. In some embodiments, the primer set (b) further includes primers including nucleotide sequences of SEQ ID NOs: 11 and 12, or nucleotide sequences having at least 90% identity to SEQ ID NOs: 11 and 12. In some embodiments, the primer set (c) further includes primers including nucleotide sequences of SEQ ID NOs: 17 and 18, or nucleotide sequences having at least 90% identity to SEQ ID NOs: 17 and 18. In some embodiments, the primer set (d) further includes primers including nucleotide sequences of SEQ ID NOs: 23 and 24, or nucleotide sequences having at least 90% identity to SEQ ID NOs: 23 and 24. In some embodiments, the primer set (e) further includes primers including nucleotide sequences of SEQ ID NOs: 29 and 30, or nucleotide sequences having at least 90% identity to SEQ ID NOs: 29 and 30. In some embodiments, the primer set (f) further includes primers including nucleotide sequences of SEQ ID NOs: 35 and 36, or nucleotide sequences having at least 90% identity to SEQ ID NOs: 35 and 36. In some embodiments, the primer set (g) further includes primers including nucleotide sequences of SEQ ID NOs: 41 and 42, or nucleotide sequences having at least 90% identity to SEQ ID NOs: 41 and 42. In some embodiments, the primer set (h) further includes primers

including nucleotide sequences of SEQ ID NOs: 47 and 48, or nucleotide sequences having at least 90% identity to SEQ ID NOs: 47 and 48.

[0010] According to another aspect of the embodiment of the present invention, there is provided a method for detecting SARS-CoV-2. The method includes the steps of: (a) contacting a biological sample with the above-mentioned kit; (b) amplifying nucleic acids of SARS-CoV-2, if present, from the biological sample; and (c) detecting a presence of amplified nucleic acids of SARS-CoV-2.

[0011] In an embodiment, the method further includes a step of extracting nucleic acids from the biological sample before the step (a).

[0012] In an embodiment, in the step (b), the nucleic acids are amplified by reverse transcription loop-mediated isothermal amplification.

[0013] The use of the word "a" or "an" when used in conjunction with the term "comprising/including" in the claims and/or the specification may mean "one," but it is also consistent with the meaning of "one or more," "at least one," and "one or more than one."

[0014] The use of the term "or" in the claims is used to mean "and/or" unless explicitly indicated to refer to alternatives only or the alternative are mutually exclusive, although the disclosure supports a definition that refers to only alternatives and "and/or."

[0015] Other objects, features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating specific embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

[0016] FIGS. 1 to 5 show the amplification plots for SARS-CoV-2 detection with the primer sets N_1, N_2, N_3, N_4, and N_5;

[0017] FIG. 6 shows the amplification plots with the primer set N_5 and 16 copies of the plasmid DNA template;

[0018] FIG. 7 shows the specificity analysis of the primer set N_5;

[0019] FIGS. 8 to 10 show the amplification plots for SARS-CoV-2 detection with the primer sets Orf1ab_2, Orf1ab_3, and Orf1ab_7;

[0020] FIG. 11 shows the amplification plots with the primer set Orf1ab_7 and 19 copies of the plasmid DNA template; and

[0021] FIG. 12 shows the specificity analysis of the primer set Orf1ab_7.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

[0022] The present invention will now be described more specifically with reference to the following embodiments. It is to be noted that the following descriptions of the embodiments of this invention are presented herein for purpose of illustration and description only; it is not intended to be exhaustive or to be limited to the precise form disclosed.

[0023] The embodiment of the present invention provides a kit that qualitatively detects SARS-CoV-2 nucleic acid extracted from human upper and lower respiratory specimens (such as nasopharyngeal and oropharyngeal swabs, sputum, lower respiratory tract aspirates, bronchoalveolar lavage) in real-time. This kit works with thermocycler or isothermal instrument for the detection of SARS-CoV-2 infections. It is intended to be used as an aid in the diagnosis of SARS-CoV-2 infection and for professional use.

[0024] The embodiment of the present invention utilizes reverse transcription loop-mediated isothermal amplification (RT-LAMP) technology to detect the presence of SARS-CoV-2 in the human upper and lower respiratory specimens.

[0025] In an embodiment, the kit contains ready-to-use control materials and reagents for sample detection. When the SARS-CoV-2 is present in the indicated specimen, target regions of the virus genome are amplified, and the real-time fluorescence signal would be detected with the increased amount of amplicons generated. A fixed cut-off value for the test is used to report test results.

[0026] In an embodiment, the kit includes positive control and negative control reagents as part of routine quality control testing. The reagents are provided to aid the user in detection of reagent deterioration, adverse environmental or test conditions, or variation in operator performance that may lead to test errors.

[0027] SARS-CoV-2 belongs to RNA viruses. The embodiment of the present invention is capable of qualitative detection of SARS-CoV-2 based on reverse transcription loop-mediated isothermal amplification (RT-LAMP) technology. RT-LAMP utilizes reverse transcriptase to synthesize cDNA from virus RNA in the sample followed by isothermal amplification of a DNA polymerase. Two target regions of the SARS-CoV-2 RNA including gene N and gene Orf1ab are to be amplified by two sets of specifically designed primers. Intercalating dyes binds to the double stranded amplicons, generating real-time signal that is detectable by the optical system in either a real-time PCR or an isothermal instrument. Reactions with signals above a predefined baseline before the cut-off time indicate SARS-CoV-2 positivity for the tested sample. Lack of such signal before the cut-off time is indicative of negativity of SARS-CoV-2 in

the tested sample. In the embodiment, a case is confirmed positive only when both targets are detected.

[0028] The following describes an example of the kit and method for detecting SARS-CoV-2 according to the embodiment of the present invention. Particularly, the kit contains ready-to-use control materials and reagents for sample detection. The kit includes two monoplex assays (N assay and Orf1ab assay), both of which are designed for specific detection of SARS-CoV-2. The kit includes positive control (PC) and negative control (NC) reagents as part of routine quality control testing, wherein the positive control is RNA template containing target gene sequences, and the negative control is water. The reagents are provided to aid the user in detection of reagent deterioration, adverse environmental or test conditions, or variation in operator performance that may lead to test errors.

[0029] The target genes and the primer sequences are listed in Table 1. In N assay, there are five primer sets, namely N_1, N_2, N_3, N_4, and N_5, each of which is capable of detecting gene N of SARS-CoV-2. In Orf1ab assay, there are three primer sets, namely Orf1ab_2, Orf1ab_3, and Orf1ab_7, each of which is capable of detecting gene Orf1ab of SARS-CoV-2. Each primer set includes six specifically designed primers named FIP, BIP, F3, B3, LF, and LB, wherein FIP and BIP are inner primers, F3 and B3 are outer primers, and LF and LB are loop primers. As shown in Table 1, SEQ ID NOs: 1 to 30 are designed to detect the gene N of SARS-CoV-2, and SEQ ID NOs: 31 to 48 are designed to detect the gene Orf1ab of SARS-CoV-2.

Table 1

Target	Set Name	Primer Name	Primer Sequence	SEQ ID NO.
N	N_1	N_FIP_1	TGTTTTGATCGCGCCCCACTGATTACGTTTGGTGGACCCTC	1

		N_BIP_1	ATACTGCGTCTTGGTTCACCGCATTGGAACGCCTTGTCCTC	2
		N_F3_1	TGGACCCCAAAATCAGCG	3
		N_B3_1	AGCCAATTTGGTCATCTGGA	4
		N_LF_1	CTGGTTACTGCCAGTTGAATCT	5
		N_LB_1	CAACATGGCAAGGAAGACCTTAA	6
	N_2	N_FIP_2	CCACTGCGTTCTCCATTCTGGTAAATGCACCCCGCATTACG	7
		N_BIP_2	GGCGCGATCAAAACAACGTCGTGCCATGTTGAGTGAGAGC	8
		N_F3_2	TGGACCCCAAAATCAGCG	9
		N_B3_2	GCCTTGTCCTCGAGGGAAT	10
		N_LF_2	TGAATCTGAGGGTCCACCAAA	11
		N_LB_2	GCCCCAAGGTTTACCCAATAA	12
	N_3	N_FIP_3	GCGTCAATATGCTTATTCAGCAAAAGACCTACACAGGTGCCAT	13
		N_BIP_3	ATACAAAACATTCCCACCAACAGATTCTGTCTCTGCGGTAAG	14
		N_F3_3	ATGGAAGTCACACCTTCG	15
		N_B3_3	GGAAGAAGAGTCACAGTTTG	16
		N_LF_3	GAAATTTGGATCTTTGTCATCCAATTTG	17
		N_LB_3	AGAAGGCTGATGAAACTCAAGC	18
	N_4	N_FIP_4	CAGCCTTCTTCTTTTTGTCCTTTTTCTGAATAAGCATATTGACGCA	19
		N_BIP_4	CTTACCGCAGAGACAGAAGAAACATGTTGCAATTGTTTGGAGAA	20
		N_F3_4	CCAAATTTCAAAGATCAAGTCAT	21
		N_B3_4	TCAGCACTGCTCATGGAT	22
		N_LF_4	CTCTGTTGGTGGGAATGTTTTGTA	23
		N_LB_4	GCAAACTGTGACTCTTCTTCCT	24
	N_5	N_FIP_5	CTTGAGTTTCATCAGCCTTCTTCTTCGCATACAAAACATTCCCA	25
		N_BIP_4	CTTACCGCAGAGACAGAAGAAACATGTTGCAATTGTTTGGAGAA	26
		N_F3_5	GTCATTTTGCTGAATAAGCATAT	27
		N_B3_4	TCAGCACTGCTCATGGAT	28

		N_LF_5	TGTCCTTTTTAGGCTCTGTTGG	29
		N_LB_4	GCAAACTGTGACTCTTCTTCCT	30
Orf1ab	Orf1ab_2	nCoV_Orf1ab_FIP_2	TTTGTGGTAATAAACACCCAAAAATGGAGGTACTACTTTAG ATTCGAAGACC	31
		nCoV_Orf1ab_BIP_2	AAGTTGGATGGAAAGTGAGTTCAGAGTCCATAAGAAAAGGC TGAGAG	32
		nCoV_Orf1ab_F3_2	TGAGAAGTCTAACATAATAAGAGGC	33
		nCoV_Orf1ab_B3_2	GAAATTACCCTGTTTTCCTTCAAG	34
		nCoV_Orf1ab_LF_2	AGTAGCGTTATTAACAATAAGTAGG	35
		nCoV_Orf1ab_LB_2	TTTATTCTAGTGCGAATAATTGCA	36
	Orf1ab_3	nCoV_Orf1ab_FIP_3	CCTGAGGGAGATCACGCACGGAAAACAGGGTAATTTCAAAA ATC	37
		nCoV_Orf1ab_BIP_3	TTCGGCTTTAGAACCATTGGTAGATCAAATAACTTCTATGTA AAGCAAGTAAA	38
		nCoV_Orf1ab_F3_3	GCCTTTTCTTATGGACCTTGA	39
		nCoV_Orf1ab_B3_3	CCAACCTGAAGAAGAATCACC	40
		nCoV_Orf1ab_LF_3	AATAGGCGTGTGCTTAGAATATATT	41
		nCoV_Orf1ab_LB_3	ATAGGTATTAACATCACTAGGTTTCA	42
	Orf1ab_7	nCoV_Orf1ab_FIP_7	TGAGTTGTTGACATGTTCAGCCCTTACTCCTACTTGGCGTG	43
		nCoV_Orf1ab_BIP_7	ACCCATTGGTGCAGGTATATGCGTGTAGGCAATGATGGATT GA	44
		nCoV_Orf1ab_F3_7	CAGAAGTCCCTGTTGCTATTC	45
		nCoV_Orf1ab_B3_7	AGAGTAAGCAACTGAATTTTCTG	46
		nCoV_Orf1ab_LF_7	CGTGTTTGAAAAACATTAGAACCT	47
		nCoV_Orf1ab_LB_7	TTATCAGACTCAGACTAATTCTCCT	48

[0030] The primer sets listed in Table 1 are tested to verify their detection capability for SARS-CoV-2. The reaction setup is as follows. The kit includes primer mix, reaction buffer, enzyme mix, and dye. The primer mix includes at least one of the primer sets listed in Table 1, and each primer set includes inner primers (FIP and BIP), outer primers (F3 and B3), and loop primers (LF and LB).

The reaction buffer contains 10X isothermal buffer, 1-2 mM dNTP, 8-12 mM $MgSO_4$ and 0.5-1.5 M Betaine. The enzyme mix includes 8-16 units of DNA polymerase and 1-10 units of reverse transcriptase. The dye is DNA intercalating dye. The RT-LAMP thermal protocol may be 65°C for 15 minutes in any real time PCR or isothermal instrument. For example, the primer test experiments in the present embodiments are performed on Bio-Rad CFX96 Real-Time PCR machine at 65°C. As to the fluorescence reading frequency, it may be adjusted in different platforms.

[0031] For N assay, FIGS. 1 to 5 show the amplification plots for SARS-CoV-2 detection with the primer sets N_1, N_2, N_3, N_4, and N_5. In the tests, the fluorescence reading was taken every 60 seconds. The "cycle" number on X-axis indicates the reading times. As the Bio-Rad CFX96 machine takes about 10 seconds in each reading, one "cycle" is approximately 70 seconds. In the embodiments, from FIGS. 1 to 5, it is clear that the primer sets N_1, N_2, N_3, N_4, and N_5 can all amplify the plasmid DNA containing the target N gene successfully without NTC (No Template Control) amplification. All reactions were done in duplicate.

[0032] In the embodiments, the primer set N_5 has a relatively better amplification curve than the other primer sets in N assay, and the limit of detection (LoD) and the specificity of the primer set N_5 are further tested. In these tests, the fluorescence reading was taken every 20 seconds. As the Bio-Rad CFX96 machine takes about 10 seconds in each reading, one "cycle" is approximately 30 seconds. The reaction was cutoff at 31 cycles, which was close to 15 minutes.

[0033] FIG. 6 shows the amplification plots with the primer set N_5 and 16 copies of the plasmid DNA template. It can be seen from FIG. 6 that the primer set N_5 has a limit of detection (LoD) of 16 copies with the plasmid DNA containing

the target gene for N_5, and 22 of 23 repeats were amplified within 15 minutes with no NTC amplification.

[0034] FIG. 7 shows the specificity analysis of the primer set N_5. In the analysis, the plasmid DNA containing the target gene for N_5 was used as the positive control, and other non-target templates (DNA or RNA, see Table 2) isolated from representative strains were used for cross-reactivity testing. Table 2 lists the respiratory related pathogens where the non-target templates isolated from in the specificity analysis. In the embodiment, it can be seen from FIG. 7 that the amplification only occurred when correct template was used for this primer set, and no amplification occurred for all other non-target templates. That is to say, the primer set N_5 is specific to SARS-CoV-2 detection.

Table 2

No.	Strain	Copies/μl	Nucleic Acid
1	*Streptococcus pneumoniae*	1.56 × 10^7	DNA
2	Influenza A H1N1	2 × 10^9	RNA
3	Influenza A Wuhan	7.3 × 10^8	RNA
4	Influenza A H1N1 PR8	6 × 10^8	RNA
5	*Haemophilus influenzae*	7.59 × 10^5	DNA
6	Rhinovirus 1B	6-6.88 × 10^8	RNA
7	Parainfluenza type 2	1.6 × 10^9	RNA
8	Parainfluenza type 3	1.4 × 10^9	RNA
9	Adenovirus 1	5.1-10.2 × 10^7	DNA
10	Adenovirus 3	2.35-4.71 × 10^7	DNA
11	Adenovirus 4	7.7-15.3 × 10^7	DNA
12	Adenovirus 29	1-2 × 10^8	DNA
13	Adenovirus 31	3.08-6.16 × 10^7	DNA
14	Adenovirus 40	1.44-2.88 × 10^8	DNA

15	Cytomegalovirus	7.93 × 10^6	DNA
16	Herpes simplex virus type 1	2.12 × 10^7	DNA
17	Herpes simplex virus type 2	9.28 × 10^6	DNA
18	Measles	2 × 10^9	RNA
19	Respiratory Syncytial Virus	6.4 × 10^8	RNA
20	Influenza B	6.1 × 10^8	RNA
21	*E. coli O157:H7*	5.52 × 10^4	DNA
22	*Staphylococcus aureus*	2.46 × 10^4	DNA
23	*Streptococcus pyrogenes*	4.85 × 10^4	DNA
24	*Enterococcus faecalis (type strain) 20478*	1.6 × 10^5	DNA
25	*Streptococcus agalactiae*	1.03 × 10^5	DNA
26	229E	2 × 10^8	RNA
27	NL63	2 × 10^8	RNA
28	OC43	2 × 10^8	RNA

[0035] For Orf1ab assay, FIGS. 8 to 10 show the amplification plots for SARS-CoV-2 detection with the primer sets Orf1ab_2, Orf1ab_3, and Orf1ab_7. In the tests, the fluorescence reading was taken every 60 seconds. The "cycle" number on X-axis indicates the reading times. As the Bio-Rad CFX96 machine takes about 10 seconds in each reading, one "cycle" is approximately 70 seconds. In the embodiments, from FIGS. 8 to 10, it is clear that the primer sets Orf1ab_2, Orf1ab_3, and Orf1ab_7 can all amplify the plasmid DNA containing the target Orf1ab gene successfully without NTC amplification.

[0036] In the embodiments, the primer set Orf1ab_7 has a relatively better amplification curve than the other primer sets in Orf1ab assay, and the limit of detection (LoD) of the primer set Orf1ab_7 is further tested. In the tests, the

fluorescence reading was also taken every 60 seconds, and one "cycle" is approximately 70 seconds. FIG. 11 shows the amplification plots with the primer set Orf1ab_7 and 19 copies of the plasmid DNA template. It can be seen from FIG. 11 that the primer set Orf1ab_7 has a limit of detection (LoD) of 19 copies with the plasmid DNA containing the target gene for Orf1ab_7, and 20 of 20 repeats were amplified within 15 minutes with no NTC amplification.

[0037] Further, the specificity of the primer set Orf1ab_7 is further tested. In the tests, the fluorescence reading was taken every 20 seconds, and one "cycle" is approximately 30 seconds. The reaction was cutoff at 31 cycles, which was close to 15 minutes. FIG. 12 shows the specificity analysis of the primer set Orf1ab_7. In the analysis, the plasmid DNA containing the target gene for Orf1ab_7 was used as the positive control, and other non-target templates (DNA or RNA, see Table 2) isolated from representative strains were used for cross-reactivity testing. In the embodiment, it can be seen from FIG. 12 that the amplification only occurred when correct template was used for this primer set, and no amplification occurred for all other non-target templates. That is to say, the primer set Orf1ab_7 is specific to SARS-CoV-2 detection.

[0038] From the above embodiments, it is clear that each primer set in N assay and Orf1ab assay may all amplify the plasmid DNA containing the target gene successfully without NTC amplification. In other words, each primer set in N assay and Orf1ab assay is capable of independently detecting SARS-CoV-2. In addition, the primer set of an embodiment is designed for LAMP reaction, and includes six primers named FIP, BIP, F3, B3, LF, and LB. In the embodiment, FIP, BIP, F3, and B3 are essential primers for LAMP reaction, and LF and LB are optional primers to accelerate the reaction. Therefore, in the embodiment, each

primer set is also workable when includes only four primers of FIP, BIP, F3, and B3.

[0039] To further guarantee detection accuracy, the kit for detecting SARS-CoV-2 according to the embodiment of the present invention uses two monoplex assays, i.e. N assay and Orf1ab assay. In N assay, the primer set may be one of the primer sets of N_1, N_2, N_3, N_4, and N_5, and in Orf1ab assay, the primer set may be one of the primer sets of Orf1ab_2, Orf1ab_3, and Orf1ab_7. In the assays, the cut-off time may be set to be 15 minutes. Reactions with signals above a predefined baseline before the cut-off time indicate SARS-CoV-2 virus positivity for the tested sample. Lack of such signal before the cut-off time is indicative of negativity of SARS-CoV-2 virus in the tested sample. In the embodiment, a case is confirmed positive only when both target genes are detected. Table 3 summarizes the result analysis and interpretation.

Table 3

N	Orf1ab	PC	NC	Result Interpretation
+	+	+	–	SARS-CoV-2 detected
–	+	+	–	SARS-CoV-2 suspected
+	–	+	–	SARS-CoV-2 suspected
–	–	+	–	SARS-CoV-2 not detected
Any result	Any result	–	Any result	Invalid Result
Any result	Any result	Any result	+	Invalid Result

[0040] Moreover, the primers of the present disclosure are not limited to the ones having sequences totally the same as SEQ ID NO: 1 to 48. It is noted that the hybridizing sequences need not have perfect complementarity to provide stable hybrids. In many situations, stable hybrids will form where fewer than about 10% of the bases are mismatches. Further, there may be genetic variations in some

SARS-CoV-2 strains. Therefore, the primers including sequences with at least 90% identity to SEQ ID NO: 1 to 48 should possess similar specificity to the original ones and thus can also be used for detection of SARS-CoV-2.

[0041] Therefore, the embodiment of the present invention provides a kit for detecting SARS-CoV-2, and the kit includes a first primer set and a second primer set. The first primer set is selected from the group consisting of: (a) primers including nucleotide sequences of SEQ ID NOs: 1 to 4, or nucleotide sequences having at least 90% identity to SEQ ID NOs: 1 to 4; (b) primers including nucleotide sequences of SEQ ID NOs: 7 to 10, or nucleotide sequences having at least 90% identity to SEQ ID NOs: 7 to 10; (c) primers including nucleotide sequences of SEQ ID NOs: 13 to 16, or nucleotide sequences having at least 90% identity to SEQ ID NOs: 13 to 16; (d) primers including nucleotide sequences of SEQ ID NOs: 19 to 22, or nucleotide sequences having at least 90% identity to SEQ ID NOs: 19 to 22; and (e) primers including nucleotide sequences of SEQ ID NOs: 25 to 28, or nucleotide sequences having at least 90% identity to SEQ ID NOs: 25 to 28. The second primer set is selected from the group consisting of: (f) primers including nucleotide sequences of SEQ ID NOs: 31 to 34, or nucleotide sequences having at least 90% identity to SEQ ID NOs: 31 to 34; (g) primers including nucleotide sequences of SEQ ID NOs: 37 to 40, or nucleotide sequences having at least 90% identity to SEQ ID NOs: 37 to 40; and (h) primers including nucleotide sequences of SEQ ID NOs: 43 to 46, or nucleotide sequences having at least 90% identity to SEQ ID NOs: 43 to 46.

[0042] In some embodiments, each primer set may further include the loop primers LF and LB to accelerate the reaction. Therefore, in some embodiments, the primer set (a) further includes primers including nucleotide sequences of SEQ ID NOs: 5 and 6, or nucleotide sequences having at least 90% identity to SEQ ID

NOs: 5 and 6; the primer set (b) further includes primers including nucleotide sequences of SEQ ID NOs: 11 and 12, or nucleotide sequences having at least 90% identity to SEQ ID NOs: 11 and 12; the primer set (c) further includes primers including nucleotide sequences of SEQ ID NOs: 17 and 18, or nucleotide sequences having at least 90% identity to SEQ ID NOs: 17 and 18; the primer set (d) further includes primers including nucleotide sequences of SEQ ID NOs: 23 and 24, or nucleotide sequences having at least 90% identity to SEQ ID NOs: 23 and 24; the primer set (e) further includes primers including nucleotide sequences of SEQ ID NOs: 29 and 30, or nucleotide sequences having at least 90% identity to SEQ ID NOs: 29 and 30; the primer set (f) further includes primers including nucleotide sequences of SEQ ID NOs: 35 and 36, or nucleotide sequences having at least 90% identity to SEQ ID NOs: 35 and 36; the primer set (g) further includes primers including nucleotide sequences of SEQ ID NOs: 41 and 42, or nucleotide sequences having at least 90% identity to SEQ ID NOs: 41 and 42; and the primer set (h) further includes primers including nucleotide sequences of SEQ ID NOs: 47 and 48, or nucleotide sequences having at least 90% identity to SEQ ID NOs: 47 and 48.

[0043] The primer sets are designed to hybridize and amplify the target genes. Therefore, the embodiment of the present invention also provides a method for detecting SARS-CoV-2. The method includes the steps of: (a) contacting a biological sample with the above-mentioned kit; (b) amplifying nucleic acids of SARS-CoV-2, if present, from the biological sample; and (c) detecting a presence of amplified nucleic acids of SARS-CoV-2.

[0044] In an embodiment, the method further includes a step of extracting nucleic acids from the biological sample before the step (a).

[0045] In an embodiment, in the step (b), the nucleic acids are amplified by reverse transcription loop-mediated isothermal amplification.

[0046] In an embodiment, the primer set N_5 has a relatively better amplification curve than the other primer sets in N assay, the primer set Orf1ab_7 has a relatively better amplification curve than the other primer sets in Orf1ab assay, and the primer set N_5 and the primer set Orf1ab_7 are selected to detect SARS-CoV-2. Therefore, the embodiment of the present invention further provides a kit for detecting SARS-CoV-2, and the kit includes a first primer set and a second primer set. The first primer set includes primers including nucleotide sequences of SEQ ID NOs: 25 to 28, or nucleotide sequences having at least 90% identity to SEQ ID NOs: 25 to 28, and the second primer set includes primers including nucleotide sequences of SEQ ID NOs: 43 to 46, or nucleotide sequences having at least 90% identity to SEQ ID NOs: 43 to 46. Also, the first primer set may further include primers including nucleotide sequences of SEQ ID NOs: 29 and 30, or nucleotide sequences having at least 90% identity to SEQ ID NOs: 29 and 30, and the second primer set may further include primers including nucleotide sequences of SEQ ID NOs: 47 and 48, or nucleotide sequences having at least 90% identity to SEQ ID NOs: 47 and 48. Besides, the embodiment of the present invention also provides a method for detecting SARS-CoV-2 using the above-mentioned kit.

[0047] In addition, each primer set in N assay and Orf1ab assay is capable of independently detecting SARS-CoV-2. Therefore, the embodiment of the present invention further provides a kit for detecting SARS-CoV-2, and the kit includes at least one of a first primer set and a second primer set. The first primer set is selected from the group consisting of: (a) primers including nucleotide sequences of SEQ ID NOs: 1 to 4, or nucleotide sequences having at least 90% identity to

SEQ ID NOs: 1 to 4; (b) primers including nucleotide sequences of SEQ ID NOs: 7 to 10, or nucleotide sequences having at least 90% identity to SEQ ID NOs: 7 to 10; (c) primers including nucleotide sequences of SEQ ID NOs: 13 to 16, or nucleotide sequences having at least 90% identity to SEQ ID NOs: 13 to 16; (d) primers including nucleotide sequences of SEQ ID NOs: 19 to 22, or nucleotide sequences having at least 90% identity to SEQ ID NOs: 19 to 22; and (e) primers including nucleotide sequences of SEQ ID NOs: 25 to 28, or nucleotide sequences having at least 90% identity to SEQ ID NOs: 25 to 28. The second primer set is selected from the group consisting of: (f) primers including nucleotide sequences of SEQ ID NOs: 31 to 34, or nucleotide sequences having at least 90% identity to SEQ ID NOs: 31 to 34; (g) primers including nucleotide sequences of SEQ ID NOs: 37 to 40, or nucleotide sequences having at least 90% identity to SEQ ID NOs: 37 to 40; and (h) primers including nucleotide sequences of SEQ ID NOs: 43 to 46, or nucleotide sequences having at least 90% identity to SEQ ID NOs: 43 to 46. As well, the embodiment of the present invention also provides a method for detecting SARS-CoV-2 using the above-mentioned kit.

[0048] The present disclosure has the following advantages. First, the present disclosure is based on one-step real-time RT-LAMP reaction, so the kit may be used in thermocycler or isothermal platforms with real-time fluorescence detection, and thus it has universal applicability. Moreover, due to the specific primer design of LAMP reaction and the nature of the enzymes used in the present disclosure, tests may be done within 15 minutes, so the present disclosure has the advantage of reduced reaction time. Further, the two monoplex assays provides specific and sensitive detection. Therefore, the present disclosure offers a possible solution as a Point-of-Care molecular diagnostic test for SARS-CoV-2.

[0049] Moreover, multiple SARS-CoV-2 variants are circulating globally. Several new variants emerged in late 2020, most notably UK B.1.1.7 lineage and South Africa B.1.351 lineage, which do not impact the kit of the present disclosure since the kit does not target the spike region. What is more, LAMP is quite tolerant to mismatches in target genes and the kit of the present disclosure uses two monoplex assays (N assay and Orf1ab assay) to further guarantee accuracy.

[0050] While the invention has been described in terms of what is presently considered to be the most practical and preferred embodiments, it is to be understood that the invention does not need to be limited to the disclosed embodiment. On the contrary, it is intended to cover various modifications and similar arrangements included within the spirit and scope of the appended claims which are to be accorded with the broadest interpretation so as to encompass all such modifications and similar structures.

WHAT IS CLAIMED IS:

1. A kit for detecting SARS-CoV-2, comprising a first primer set and a second primer set,

wherein the first primer set is selected from the group consisting of:

(a) primers comprising nucleotide sequences of SEQ ID NOs: 1 to 4;

(b) primers comprising nucleotide sequences of SEQ ID NOs: 7 to 10;

(c) primers comprising nucleotide sequences of SEQ ID NOs: 13 to 16;

(d) primers comprising nucleotide sequences of SEQ ID NOs: 19 to 22; and

(e) primers comprising nucleotide sequences of SEQ ID NOs: 25 to 28;

wherein the second primer set is selected from the group consisting of:

(f) primers comprising nucleotide sequences of SEQ ID NOs: 31 to 34;

(g) primers comprising nucleotide sequences of SEQ ID NOs: 37 to 40; and

(h) primers comprising nucleotide sequences of SEQ ID NOs: 43 to 46.

2. The kit according to claim 1, wherein the primer set (a) further includes primers comprising nucleotide sequences of SEQ ID NOs: 5 and 6.

3. The kit according to claim 1, wherein the primer set (b) further includes primers comprising nucleotide sequences of SEQ ID NOs: 11 and 12.

4. The kit according to claim 1, wherein the primer set (c) further includes primers comprising nucleotide sequences of SEQ ID NOs: 17 and 18.

5. The kit according to claim 1, wherein the primer set (d) further includes primers comprising nucleotide sequences of SEQ ID NOs: 23 and 24.

6. The kit according to claim 1, wherein the primer set (e) further includes primers comprising nucleotide sequences of SEQ ID NOs: 29 and 30.

7. The kit according to claim 1, wherein the primer set (f) further includes primers comprising nucleotide sequences of SEQ ID NOs: 35 and 36.

8. The kit according to claim 1, wherein the primer set (g) further includes

primers comprising nucleotide sequences of SEQ ID NOs: 41 and 42.

9. The kit according to claim 1, wherein the primer set (h) further includes primers comprising nucleotide sequences of SEQ ID NOs: 47 and 48.

10. A method for detecting SARS-CoV-2, comprising the steps of:

(a) contacting a biological sample with the kit of claim 1;

(b) amplifying nucleic acids of SARS-CoV-2, if present, from the biological sample by reverse transcription loop-mediated isothermal amplification; and

(c) detecting a presence of amplified nucleic acids of SARS-CoV-2.

11. The method according to claim 10 further comprising a step of extracting nucleic acids from the biological sample before the step (a).

12. A kit for detecting SARS-CoV-2, comprising a first primer set and a second primer set, wherein the first primer set includes primers comprising nucleotide sequences of SEQ ID NOs: 25 to 28, and the second primer set includes primers comprising nucleotide sequences of SEQ ID NOs: 43 to 46.

13. The kit according to claim 12, wherein the first primer set further includes primers comprising nucleotide sequences of SEQ ID NOs: 29 and 30, and the second primer set further includes primers comprising nucleotide sequences of SEQ ID NOs: 47 and 48.

14. A method for detecting SARS-CoV-2, comprising the steps of:

(a) contacting a biological sample with the kit of claim 12;

(b) amplifying nucleic acids of SARS-CoV-2, if present, from the biological sample by reverse transcription loop-mediated isothermal amplification; and

(c) detecting a presence of amplified nucleic acids of SARS-CoV-2.

15. The method according to claim 14 further comprising a step of extracting nucleic acids from the biological sample before the step (a).

16. A kit for detecting SARS-CoV-2, comprising a first primer set or a second primer set,

wherein the first primer set is selected from the group consisting of:

(a) primers comprising nucleotide sequences of SEQ ID NOs: 1 to 4;

(b) primers comprising nucleotide sequences of SEQ ID NOs: 7 to 10;

(c) primers comprising nucleotide sequences of SEQ ID NOs: 13 to 16;

(d) primers comprising nucleotide sequences of SEQ ID NOs: 19 to 22; and

(e) primers comprising nucleotide sequences of SEQ ID NOs: 25 to 28;

wherein the second primer set is selected from the group consisting of:

(f) primers comprising nucleotide sequences of SEQ ID NOs: 31 to 34;

(g) primers comprising nucleotide sequences of SEQ ID NOs: 37 to 40; and

(h) primers comprising nucleotide sequences of SEQ ID NOs: 43 to 46.

17. A method for detecting SARS-CoV-2, comprising the steps of:

(a) contacting a biological sample with the kit of claim 16;

(b) amplifying nucleic acids of SARS-CoV-2, if present, from the biological sample by reverse transcription loop-mediated isothermal amplification; and

(c) detecting a presence of amplified nucleic acids of SARS-CoV-2.