

CLAIMS:

1. A method of detecting Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) in a sample, the method comprising:

- performing an amplifying step comprising contacting the sample with a set of primers to produce an amplification product if a SARS-CoV-2 nucleic acid is present in the sample;
- performing a hybridizing step comprising contacting the amplification product with one or more detectable probes; and
- detecting the presence or absence of the amplification product, wherein the presence of the amplification product is indicative of the presence of SARS-CoV-2 in the sample and wherein the absence of the amplification product is indicative of the absence of SARS-CoV-2 in the sample;

wherein the first primer comprises an oligonucleotide sequence of SEQ ID NO: 1 or 27-29; the second primer comprises an oligonucleotide sequence of SEQ ID NO: 7; and the detectable probe comprises an oligonucleotide sequence of SEQ ID NO: 21 or 42, or a complement thereof; or

wherein the first primer comprises an oligonucleotide sequence of SEQ ID NO: 40; the second primer comprises an oligonucleotide sequence of SEQ ID NO: 41; and the detectable probe comprises an oligonucleotide sequence of SEQ ID NO: 43, or a complement thereof.

2. The method of claim 1, wherein:

the hybridizing step comprises contacting the amplification product with the detectable probe that is labeled with a donor fluorescent moiety and a corresponding acceptor moiety; and the detecting step comprises detecting the presence or absence of fluorescence resonance energy transfer (FRET) between the donor fluorescent moiety and the acceptor moiety of the probe, wherein the presence or absence of fluorescence is indicative of the presence or absence of SARS-CoV-2 in the sample.

3. The method of claim 2, wherein said amplification step employs a polymerase enzyme having 5' to 3' nuclease activity.

4. The method of any one of claims 1 to 3, wherein the sample is a biological sample selected from a nasopharyngeal sample or an oropharyngeal sample.

5. The method of any one of claims 1 to 4 further comprising performing the amplifying step in the presence of a second set of primers comprising a first primer comprising an oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 4-6 and a second

primer comprising an oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 15-20 to produce a second amplification product if SARS-CoV-2 or SARS-CoV-2 and other coronavirus target nucleic acids from the subgenus Sarbecovirus is present in the sample; and performing the hybridizing step in the presence of a detectable probe comprising an oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 24-26 and 32, or a complement thereof, wherein detecting the presence of the second amplification product is indicative of the presence of SARS-CoV-2 or SARS-CoV-2 and other coronavirus target nucleic acids from the subgenus Sarbecovirus in the sample and wherein the absence of the amplification product is indicative of the absence of SARS-CoV-2 or SARS-CoV-2 and other coronavirus target nucleic acids from the subgenus Sarbecovirus in the sample.

6. The method of any one of claims 1 to 4, wherein the set of primers for amplification of the SARS-CoV-2 includes a plurality of first primers, a plurality of second primers, and a plurality of detectable probes,

wherein the plurality of first primers is the combination of a first primer comprising the oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 1 and 27, and a first primer comprising the oligonucleotide sequence of SEQ ID NO: 5;

wherein the plurality of second primers is the combination of a second primer comprising the oligonucleotide sequence of SEQ ID NO: 7 and a second primer comprising the oligonucleotide sequence of SEQ ID NO: 15; and

wherein the plurality of detectable probes is the combination of an oligonucleotide probe comprising the oligonucleotide sequence of SEQ ID NO: 21 or 42, or a complement thereof and an oligonucleotide probe comprising the oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 25 and 32, or a complement thereof.

7. The method of any one of claims 1 to 6, further comprising a method of detecting a nucleic acid from one or more other viruses, in parallel, wherein the one or more other viruses is selected from the group consisting of influenza virus, bat-coronavirus, severe acute respiratory syndrome (SARS) coronavirus (SARS-CoV) and Middle East respiratory syndrome (MERS) coronavirus (MERS-CoV), Coronavirus (229E, NL63, OC43, HKU1), Respiratory Syncytial Virus, Human Metapneumovirus, Adenovirus (B,E,U,C), Enterovirus, Rhinovirus, and Human Parainfluenza Virus (1, 2, 3, 4), and combinations thereof.

8. The method of claim 7, wherein the method is for simultaneously detecting Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), influenza A, and influenza B in a sample further comprising performing the amplifying step in the presence of a set of influenza

A primers to produce an amplification product if influenza A is present in the sample; and a set of influenza B primers to produce an amplification product if influenza B is present in the sample; and performing the hybridizing step in the presence of one or more detectable influenza A probes and one or more influenza B probes, wherein detecting the presence of the influenza A amplification product is indicative of the presence of influenza A in the sample and wherein the absence of the influenza A amplification product is indicative of the absence of influenza A in the sample; and wherein detecting the presence of the influenza B amplification product is indicative of the presence of influenza B in the sample and wherein the absence of the influenza B amplification product is indicative of the absence of influenza B in the sample.

9. The method of claim 8, wherein the set of influenza A primers comprises a forward primer comprising an oligonucleotide sequence of SEQ ID NO: 33, and a reverse primer comprising an oligonucleotide sequence of SEQ ID NO: 34; and one of the one or more detectable influenza A probes comprises an oligonucleotide sequence of SEQ ID NO: 35 or 44, or a complement thereof.

10. The method of any one of claims 8 to 9, wherein the set of influenza B primers comprises a forward primer comprising an oligonucleotide sequence of SEQ ID NO: 36, and a reverse primer comprising an oligonucleotide sequence of SEQ ID NO: 37; and one of the one or more detectable influenza B probes comprises an oligonucleotide sequence of SEQ ID NO: 38 or 45, or a complement thereof.

11. A kit for detecting a nucleic acid of SARS-CoV-2 comprising:

- a first primer comprising a first oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 1 and 27-29;
- a second primer comprising a second oligonucleotide sequence of SEQ ID NOs: 7; and
- a third fluorescently detectably labeled oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 21 and 42, or a complement, the third detectably labeled oligonucleotide sequence configured to hybridize to an amplicon generated by the first primer and the second primer; or
- a first primer comprising a first oligonucleotide sequence of SEQ ID NO: 40;
- a second primer comprising a second oligonucleotide sequence of SEQ ID NOs: 41; and
- a third fluorescently detectably labeled oligonucleotide sequence of SEQ ID NO: 43, or a complement, the third detectably labeled oligonucleotide sequence configured to hybridize to an amplicon generated by the first primer and the second primer.

12. The kit of claim 11, wherein the third detectably labeled oligonucleotide sequence comprises a donor fluorescent moiety and a corresponding acceptor moiety.
13. The kit of any one of claims 11 to 12 further comprising nucleoside triphosphates, nucleic acid polymerase, and buffers necessary for the function of the nucleic acid polymerase.
- 5 14. The kit of any one of claims 11 to 13, wherein at least one of the first, second, and third oligonucleotide sequences comprises at least one modified nucleotide.
15. The kit of any one of claims 11 to 14 further comprising a first primer comprising an oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 4-6; a second primer comprising an oligonucleotide sequence selected from the group consisting of SEQ ID
10 NOs: 15-20; and a detectable probe comprising an oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 24-26 and 32, or a complement thereof.
16. The kit of claim 15, wherein the first primer comprises an oligonucleotide sequence of SEQ ID NO: 5; the second primer comprises an oligonucleotide sequence of SEQ ID NO: 15; and the detectable probe comprises an oligonucleotide sequence of SEQ ID NO: 25 or 32, or a
15 complement thereof.
17. The kit of any one of claims 11 to 16, wherein the kit is for simultaneously detecting Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), influenza A, and influenza B and further comprises a set of influenza A primers and one or more detectable influenza A probes and a set of influenza B primers and one or more detectable influenza B probes.
- 20 18. The kit of claim 17, wherein the set of influenza A primers comprises a forward primer comprising an oligonucleotide sequence of SEQ ID NO: 33, and a reverse primer comprising an oligonucleotide sequence of SEQ ID NO: 34; and one of the one or more detectable influenza A probes comprises an oligonucleotide sequence of SEQ ID NO: 35 or 44; and/or the set of influenza B primers comprises a forward primer comprising an oligonucleotide
25 sequence of SEQ ID NO: 36, and a reverse primer comprising an oligonucleotide sequence of SEQ ID NO: 37; and one of the one or more detectable influenza B probes comprises an oligonucleotide sequence of SEQ ID NO: 38 or 45.