

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 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 set of primers comprises a first primer comprising a first oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6 and 27-31, 40, or a complement thereof, and a second primer comprising a second oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 7-20, and 41, or a complement thereof; and wherein the detectable probe of the one or more detectable probes comprises a third oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 21-26, 32, and 42-43 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 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 any combinations of the above.

- 5 6. The method of any one of claims 1 to 5, wherein the first primer comprises an oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 1-3 and 27-31, or a complement thereof; the second primer comprises an oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 7-14, or a complement thereof; and the detectable probe comprises an oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 21-23 and 42, or a complement thereof.
- 10 7. The method of claim 6 further comprising a first primer comprising an oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 4-6, and 40, or a complement thereof; a second primer comprising an oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 15-20, and 41, or a complement thereof; and a detectable probe comprising an oligonucleotide sequence selected from the group consisting of SEQ ID NOs:
15 24-26, 32 and 43, or a complement thereof.
8. The method of any one of claims 1 to 5, wherein the first primer comprises an oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 4-6, or a complement thereof; the second primer comprises an oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 15-20, or a complement thereof; and the detectable probe comprises an
20 oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 24-26 and 32, or a complement thereof.
9. The method of any one of claims 1 to 5, 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,
25 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
30 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 and an oligonucleotide probe

- comprising the oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 25 and 32.
10. The method of any one of claims 1 to 5, wherein the first primer comprises an oligonucleotide sequence of SEQ ID NO: 40, or a complement thereof; the second primer comprises an oligonucleotide sequence of SEQ ID NO: 41, or a complement thereof; and the detectable probe comprises an oligonucleotide sequence of SEQ ID NO: 43, 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: 1-6 and 27-31, and 40 or a complement thereof;
 - a second primer comprising a second oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 7-20, and 41, or a complement thereof; and
 - a third fluorescently detectably labeled oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 21-26, 32, and 42-43, 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 and 12, wherein the sample is a biological sample selected from a nasopharyngeal sample or an oropharyngeal sample.
14. The kit of any one of claims 11 to 13 further comprising nucleoside triphosphates, nucleic acid polymerase, and buffers necessary for the function of the nucleic acid polymerase.
15. The kit of any one of claims 11 to 14, wherein at least one of the first, second, and third oligonucleotide sequences comprises at least one modified nucleotide.
16. The kit of any one of claims 11 to 15, wherein the first primer comprises an oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 1-3 and 27-31, or a complement thereof; the second primer comprises an oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 7-14, or a complement thereof; and the detectable probe comprises an oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 21-23, and 42, or a complement thereof.
17. The kit of claim 16 further comprising a first primer comprising an oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 4-6, and 40, or a complement thereof; a second primer comprising an oligonucleotide sequence selected from the group consisting of

SEQ ID NOs: 15-20, and 41, or a complement thereof; and a detectable probe comprising an oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 24-26, 32, and 43, or a complement thereof.

18. The kit of any one of claims 11 to 15, wherein the first primer comprises an oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 4-6, or a complement thereof; the second primer comprises an oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 15-20, or a complement thereof; and the detectable probe comprises an oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 24-26 and 32, or a complement thereof.
19. The kit of any one of claims 11 to 15, wherein the first primer comprises an oligonucleotide sequence of SEQ ID NO: 40, or a complement thereof; the second primer comprises an oligonucleotide sequence of SEQ ID NO: 41, or a complement thereof; and the detectable probe comprises an oligonucleotide sequence of SEQ ID NO: 43, or a complement thereof.
20. A method for simultaneously detecting Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), influenza A, and influenza B in a sample comprising:
- performing an amplifying step, wherein the sample is contacted with a first set of primers, a second set of primers, and a third set of primers to produce one or more amplification products if SARS-CoV-2, and/or influenza A, and/or influenza B is present in the sample; wherein the first set of primers produces an amplification product if SARS-CoV-2 is present in the sample, the second set of primers produces an amplification product if influenza A is present in the sample, the third set of primers produces an amplification product if influenza B is present in the sample;
 - performing a hybridizing step wherein the amplification product(s) are contacted with three or more detectable probes, wherein the three or more detectable probes includes at least one probe specific for the amplification products of each of the first, the second and the third sets of primers; and detecting the presence or absence of the amplified products, wherein the presence of the amplified product is indicative of the presence of SARS-CoV-2, influenza A, and/or influenza B in the sample and wherein the absence of the amplified product is indicative of the absence of SARS-CoV-2, influenza A, and/or influenza B in the sample.
21. The method of claim 20, wherein the first set of primers comprises a first primer comprising a first oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6 and 27-31, 40, or a complement thereof, and a second primer comprising a second oligonucleotide

sequence selected from the group consisting of SEQ ID NOs: 7-20, and 41, or a complement thereof; and wherein the first detectable probe of the one or more detectable probes comprises a third oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 21-26, 32, and 42-43 or a complement thereof.

- 5 22. The method of claim 21, wherein the first set of primers comprises a forward primer comprising an oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 1-3, 27-31, and a reverse primer comprising an oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 7-14; and wherein the first detectable probe comprises an oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 21-23, and 42.
- 10 23. The method of claim 22, wherein the first set of primers comprises a forward primer comprising an oligonucleotide sequence of SEQ ID NO: 1 or 27 and a reverse primer comprising an oligonucleotide sequence of SEQ ID NO: 7, and the first detectable probe for detecting the amplification product comprises an oligonucleotide sequence of SEQ ID NO: 21 or 42.
- 15 24. The method of any one of claims 20 to 23, wherein the second set of 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 the third set of 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 wherein the
20 the second detectable probe comprises an oligonucleotide sequence of SEQ ID NO: 35 or 44, and the third detectable probe comprises an oligonucleotide sequence of SEQ ID NO: 38 or 45.
- 25 25. The method of any one of claims 20 and 22 to 24 further comprising a fourth set of primers that produces an 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 a fourth detectable probe that specifically hybridizes to the fourth amplification product.
- 30 26. The method of claim 25, wherein the fourth set of primers comprises a forward primer comprising an oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 4-6, and a reverse primer comprising an oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 15-20; the fourth detectable probe comprises an oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 24-26 and 32.
27. The method of claims 26, wherein the fourth set of primers comprises a forward primer comprising an oligonucleotide sequence of SEQ ID NO: 5 and a reverse primer comprising

- an oligonucleotide sequence of SEQ ID NO: 15 and the fourth detectable probe for detecting the amplification product comprises an oligonucleotide sequence of SEQ ID NO: 25 or 32.
28. The method of any one of claims 20 and 22 to 24 further comprising a fourth set of primers that produces an amplification product if SARS-CoV-2 target nucleic acids are present in the sample, wherein the fourth set of primers comprises a forward primer comprising an oligonucleotide sequence of SEQ ID NO: 40 and a reverse primer comprising an oligonucleotide sequence of SEQ ID NO: 41 and the fourth detectable probe for detecting the amplification product comprises an oligonucleotide sequence of SEQ ID NO: 43.
29. A kit for simultaneously detecting one or more nucleic acids of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), one or more nucleic acids of influenza A and one or more nucleic acids of influenza B in a sample comprising:
- a) a first set of primers that produces a first amplification product if SARS-CoV-2 is present in the sample, a second set of primers that produces a second amplification product if influenza A is present in the sample, and a third set of primers that produces a third amplification product if influenza B is present in the sample;
- b) three or more detectable probes comprising a first detectable probe that specifically hybridizes to the first amplification product, a second detectable probe that specifically hybridizes to the second amplification product, and a third detectable probe that specifically hybridizes to the third amplification product.
30. The kit of claim 29, wherein the first set of primers comprises a first primer comprising a first oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6 and 27-31, 40, or a complement thereof, and a second primer comprising a second oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 7-20, and 41, or a complement thereof; and wherein the first detectable probe of the one or more detectable probes comprises a third oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 21-26, 32, and 42-43 or a complement thereof.
31. The kit of claims 30, wherein the first set of primers comprises a forward primer comprising an oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 1-3, 27-31, and a reverse primer comprising an oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 7-14; and wherein the first detectable probe comprises an oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 21-23, and 42.
32. The kit of claim 31, wherein the first set of primers comprises a forward primer comprising an oligonucleotide sequence of SEQ ID NO: 1 or 27 and a reverse primer comprising an

oligonucleotide sequence of SEQ ID NO: 7, and the first detectable probe for detecting the amplification product comprises an oligonucleotide sequence of SEQ ID NO: 21 or 42.

33. The kit of any one of claims 29 to 32, wherein the second set of 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 the third set of 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 wherein the second detectable probe comprises an oligonucleotide sequence of SEQ ID NO: 35 or 44, and the third detectable probe comprises an oligonucleotide sequence of SEQ ID NO: 38 or 45.
34. The kit of any one of claims 29 and 31 to 33 further comprising a fourth set of primers that produces a fourth 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 a fourth detectable probe that specifically hybridizes to the fourth amplification product.
35. The kit claim 34, wherein the fourth set of primers comprises a forward primer comprising an oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 4-6, and a reverse primer comprising an oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 15-20, and the fourth detectable probe comprises an oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 24-26, and 32.
36. The kit of claim 35, wherein the fourth set of primers comprises a forward primer comprising an oligonucleotide sequence of SEQ ID NO: 5 and a reverse primer comprising an oligonucleotide sequence of SEQ ID NO: 15 and the fourth detectable probe for detecting the amplification product comprises an oligonucleotide sequence of SEQ ID NO: 25 or 32.
37. The kit of any one of claims 29 and 31 to 33 further comprising a fourth set of primers that produces a fourth amplification product if SARS-CoV-2 target nucleic acids are present in the sample, wherein the fourth set of primers comprises a forward primer comprising an oligonucleotide sequence of SEQ ID NO: 40 and a reverse primer comprising an oligonucleotide sequence of SEQ ID NO: 41 and the fourth detectable probe for detecting the amplification product comprises of an oligonucleotide sequence of SEQ ID NO: 43.