

The methods for the simultaneous detection of SARS-CoV-2, influenza A and influenza B by RT-PCR in a single test tube or single well may also include simultaneously detecting by RT-PCR in a single test tube or single well additional viruses that may cause respiratory disease in humans, especially in the upper respiratory tract. Examples of these viruses include but are not limited to:

- 5 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).

A method for detecting SARS-CoV-2 in a sample is provided, comprising performing an amplifying step including contacting the sample with a set of primers to produce an amplification product if SARS-CoV-2 is present in the sample; performing a hybridizing step including
 10 contacting the amplification product with one or more detectable probes; and detecting the presence or absence of the amplified product, wherein the presence of the amplified product is indicative of the presence of SARS-CoV-2 in the sample and wherein the absence of the amplified product is indicative of the absence of SARS-CoV-2 in the sample; wherein the set of primer comprises or consists of an oligonucleotide sequence selected from the group consisting of SEQ
 15 ID NOs: 1-20, 27-31, and 40-41 or a complement thereof; and wherein the one or more detectable probes comprises or consists of an oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 21-26, 32, and 42-43 or a complement thereof.

In one aspect, the present disclosure provides a method of detecting Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) in a sample, the method comprising:

- 20 - 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
 25 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

- 5 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.

In one example, a multiplex method for detecting SARS-CoV-2 in a sample is provided, comprising performing an amplifying step including contacting the sample with a first set of
10 primers to produce an amplification product if SARS-CoV-2 is present in the sample and a second set of primers to produce an amplification product if SARS-CoV-2 is present in the sample; performing a hybridizing step including contacting the amplification products with at least one detectable probe hybridizing to an amplification product produced by the first primer pair and with
15 at least one second detectable probe hybridizing to an amplification product produced by the second primer pair; and detecting the presence or absence of the amplified product(s), wherein the presence of the amplified product(s) is indicative of the presence of SARS-CoV-2 in the sample and wherein the absence of the amplified product is indicative of the absence of SARS-CoV-2 in the sample; wherein the first set of primers comprises or consists of a forward primer oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 1-3, and 27-31, and
20 a reverse primer oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 7-14; and the second set of primers comprises or consists of a forward primer oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 4-6, and 40 and a reverse primer oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 15-20, and 41; and wherein the at least one detectable probe hybridizing to an amplification product produced by the

In another aspect, the present disclosure provides 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;
 - 5 - 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
 - 10 - 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.
- 15 In one example, the kit can include probes already labeled with donor and corresponding acceptor moieties, e.g., another fluorescent moiety or a dark quencher, or can include fluorophoric moieties for labeling the probes. The kit can also include nucleoside triphosphates, nucleic acid polymerase, and buffers necessary for the function of the nucleic acid polymerase. The kit can also include a package insert and instructions for using the primers, probes, and fluorophoric moieties to detect