

We claim:

1. A method for detecting an influenza H5 nucleic acid in a sample, comprising: amplifying an influenza H5 nucleic acid using primers, wherein the primers comprise a nucleic acid sequence having at least 95% sequence identity to the nucleotide sequence set forth as SEQ ID NO: 17 and a nucleic acid sequence having at least 95% sequence identity to the nucleotide sequence set forth as SEQ ID NO: 18 or wherein the primers comprise a nucleic acid sequence having at least 95% sequence identity to the nucleotide sequence set forth as SEQ ID NO: 22 and a nucleic acid sequence having at least 95% sequence identity to the nucleotide sequence set forth as SEQ ID NO: 23, wherein the primer is capable of hybridizing under very high stringency conditions to an influenza H5 nucleic acid sequence set forth as SEQ ID NO:46 or 47 and capable of amplifying the influenza H5 nucleic acid;

contacting the sample with at least one probe comprising at least 95% sequence identity to the nucleotide sequence set forth as SEQ ID NO: 19 or comprising at least 95% sequence identity to the nucleotide sequence set forth as SEQ ID NO: 24, wherein the probe is capable of hybridizing under very high stringency conditions to the influenza A nucleic acid sequence set forth as SEQ ID NO:46 or 47; and detecting hybridization between the influenza H5 virus nucleic acid and the probe, wherein the detection of hybridization indicates the presence of the influenza H5 nucleic acid in the sample.

2. The method according to claim 1, wherein the probe consists essentially of the nucleic acid sequence set forth as SEQ ID NO:19 or 24.

3. The method according to claim 1, wherein the probe is labeled.

4. The method of claim 3, wherein the probe is radiolabeled, fluorescently-labeled, biotin-labeled, enzymatically-labeled, or chemically-labeled.

5. The method according to claim 3, wherein detecting hybridization comprises detecting a change in signal from the labeled probe during or after hybridization relative to signal from the label before hybridization.
6. The method according to claim 3, wherein the probe is labeled with a fluorophore.
7. The method according to claim 3, wherein the probe is labeled with a fluorescence quencher.
8. The method according to claim 1, wherein the influenza H5 nucleic acid is amplified by polymerase chain reaction (PCR), real-time PCR, reverse transcriptase-polymerase chain reaction (RT-PCR), real-time reverse transcriptase-polymerase chain reaction (rt RT-PCR), ligase chain reaction, or transcription-mediated amplification (TMA).
9. The method according to claim 8, wherein the influenza virus nucleic acid is amplified by rt RT-PCR.
10. The method according to claim 1, wherein the primer consists essentially of the nucleic acid sequence set forth as SEQ ID NO: 17, 19, 22 or 23.
11. The method according to claim 1, wherein amplifying the influenza virus nucleic acid comprises contacting the sample with at least one forward primer comprising a nucleic acid sequence at least 95% identical to the nucleotide sequence set forth as SEQ ID NO: 17 or 22, and at least one reverse primer comprising a nucleic acid sequence at least 95% identical to the nucleotide sequence set forth as SEQ ID NO: 18 or 23, wherein the forward and reverse primers are capable of hybridizing to and amplifying the influenza H5 nucleic acid sequence.

12. The method according to claim 11, wherein the forward and reverse primer comprise multiple forward and reverse primers that are specific for influenza H5 and comprise SEQ ID NO: 17 and SEQ ID NO:18 or SEQ ID NO: 22 and SEQ ID NO: 23.

13. The method according to claim 1, further comprising:
amplifying an influenza A nucleic acid using primers, wherein the primers comprise a nucleic acid sequence having at least 95% sequence identity to the nucleotide sequence set forth as SEQ ID NO: 3 or having at least 95% sequence identity to the nucleotide sequence set forth as SEQ ID NO: 4, wherein the primers are capable of hybridizing under very high stringency conditions to an influenza A nucleic acid sequence set forth as SEQ ID NO:42 and are capable of amplifying the influenza A nucleic acid.

14. The method according to claim 1, wherein the sample is a biological sample obtained from a subject.

15. The method of claim 40, wherein the presence of an influenza H5 nucleic acid in the biological sample indicates the presence of an influenza H5 infection in the biological sample obtained from a subject.

16. The method according to claim 14, wherein the biological sample is obtained from bronchoalveolar lavage, tracheal aspirates, sputum, nasopharyngeal aspirates, oropharyngeal aspirates, or saliva.

17. A kit for detecting an influenza nucleic acid in a sample, comprising:
a probe comprising a nucleic acid sequence at least 95% identical to SEQ ID NO: 19 or 24, wherein the probe is specific for influenza subtype H5;
a set of primers, wherein the set comprises one or more forward primers 15 to 40 nucleotides in length comprising a nucleic acid sequence comprising a nucleic acid sequence at least 95% identical to a nucleic acid sequence set forth as SEQ ID NO: 17 and one or more reverse primers 15 to 40 nucleotides in length comprising a nucleic acid sequence at least 95% identical to a nucleic acid sequence set forth as SEQ ID NO:18, or

wherein the set comprises one or more forward primers 15 to 40 nucleotides in length comprising a nucleic acid sequence comprising a nucleic acid sequence at least 95% identical to a nucleic acid sequence set forth as SEQ ID NO: 22 and one or more reverse primers 15 to 40 nucleotides in length comprising a nucleic acid sequence at least 95% identical to a nucleic acid sequence set forth as SEQ ID NO:23, wherein the set of primers is capable of hybridizing to and directing the amplification of the influenza A nucleic acid; and
instructions for hybridizing the probe to the influenza nucleic acid within the sample.

18. The kit according to claim 17, wherein the set of primers comprises a nucleic acid sequence at least 95% identical to a nucleic acid sequence set forth as SEQ ID NO:17 and a nucleic acid sequence at least 95% identical to a nucleic acid sequence set forth as SEQ ID NO:18.

19. The kit according to claim 17, wherein the set of primers comprises a nucleic acid sequence at least 95% identical to a nucleic acid sequence set forth as SEQ ID NO:22 and a nucleic acid sequence at least 95% identical to a nucleic acid sequence set forth as SEQ ID NO:23.

20. The kit according to claim 17, wherein the probe is labeled.

21. The kit according to claim 20, wherein the probe is radiolabeled, fluorescently-labeled, biotin-labeled, enzymatically-labeled, or chemically-labeled.

22. The kit according to claim 20, wherein the probe is labeled with a fluorophore.

23. The kit according to claim 20, wherein the probe is labeled with a fluorescence quencher.

24. A method for diagnosing an influenza H5 infection in a subject suspected of having an influenza H5 infection comprising:

providing a sample obtained from a subject, wherein the sample comprises nucleic acids from the subject;

amplifying an influenza A nucleic acid from the sample using primers, wherein the primers comprise a nucleic acid sequence having at least 95% sequence identity to the nucleotide sequence set forth as SEQ ID NO:17 and at least 95% sequence identity to the nucleotide sequence set forth as SEQ ID NO: 18, or wherein the primers comprise a nucleic acid sequence having at least 95% sequence identity to the nucleotide sequence set forth as SEQ ID NO:22 and at least 95% sequence identity to the nucleotide sequence set forth as SEQ ID NO: 23, wherein the primer is capable of hybridizing under very high stringency conditions to an influenza H5 nucleic acid sequence set forth as SEQ ID NO:46 or 47 and capable of amplifying the influenza H5 nucleic acid;

contacting the sample with one or more the nucleic acid probes comprising at least 95% sequence identity to the nucleotide sequence set forth as SEQ ID NO:19 or 24, wherein the probe is capable of hybridizing under very high stringency conditions to the influenza A nucleic acid sequence set forth as SEQ ID NO:46 or 47; and

detecting hybridization between an influenza H5 virus nucleic acid sequence present in the sample and the probe, wherein the detection of hybridization indicates that the subject is infected with influenza H5 virus.

CLAIMS

1. A method for detecting an influenza H5 nucleic acid in a sample, comprising:
amplifying an influenza H5 nucleic acid using primers, wherein the primers comprise a nucleic acid sequence having at least 95% sequence identity to the nucleotide sequence set forth as SEQ ID NO: 17 and a nucleic acid sequence having at least 95% sequence identity to the nucleotide sequence set forth as SEQ ID NO: 18 or wherein the primers comprise a nucleic acid sequence having at least 95% sequence identity to the nucleotide sequence set forth as SEQ ID NO: 22 and a nucleic acid sequence having at least 95% sequence identity to the nucleotide sequence set forth as SEQ ID NO: 23, wherein the primer is capable of hybridizing under very high stringency conditions to an influenza H5 nucleic acid sequence set forth as SEQ ID NO:46 or 47 and capable of amplifying the influenza H5 nucleic acid;
contacting the sample with at least one probe comprising at least 95% sequence identity to the nucleotide sequence set forth as SEQ ID NO: 19 or comprising at least 95% sequence identity to the nucleotide sequence set forth as SEQ ID NO: 24, wherein the probe is capable of hybridizing under very high stringency conditions to the influenza A nucleic acid sequence set forth as SEQ ID NO:46 or 47; and
detecting hybridization between the influenza H5 virus nucleic acid and the probe, wherein the detection of hybridization indicates the presence of the influenza H5 nucleic acid in the sample.
2. The method according to claim 1, wherein the probe consists essentially of the nucleic acid sequence set forth as SEQ ID NO:19 or 24.
3. The method according to claim 1, wherein the probe is labeled.
4. The method of claim 3, wherein the probe is radiolabeled, fluorescently-labeled, biotin-labeled, enzymatically-labeled, or chemically-labeled.
5. The method according to claim 3, wherein detecting hybridization comprises detecting a change in signal from the labeled probe during or after hybridization relative to signal from the label before hybridization.

6. The method according to claim 3, wherein the probe is labeled with a fluorophore.
7. The method according to claim 3, wherein the probe is labeled with a fluorescence quencher.
8. The method according to claim 1, wherein the influenza H5 nucleic acid is amplified by polymerase chain reaction (PCR), real-time PCR, reverse transcriptase- polymerase chain reaction (RT-PCR), real-time reverse transcriptase-polymerase chain reaction (rt RT-PCR), ligase chain reaction, or transcription-mediated amplification (TMA).
9. The method according to claim 8, wherein the influenza virus nucleic acid is amplified by rt RT-PCR.
10. The method according to claim 1, wherein the primer consists essentially of the nucleic acid sequence set forth as SEQ ID NO: 17, 18, 22 or 23.
11. The method according to claim 1, wherein amplifying the influenza virus nucleic acid comprises contacting the sample with at least one forward primer comprising a nucleic acid sequence at least 95% identical to the nucleotide sequence set forth as SEQ ID NO: 17 or 22, and at least one reverse primer comprising a nucleic acid sequence at least 95% identical to the nucleotide sequence set forth as SEQ ID NO: 18 or 23, wherein the forward and reverse primers are capable of hybridizing to and amplifying the influenza H5 nucleic acid sequence.
12. The method according to claim 11, wherein the forward and reverse primer comprise multiple forward and reverse primers that are specific for influenza H5 and comprise SEQ ID NO: 17 and SEQ ID NO:18 or SEQ ID NO: 22 and SEQ ID NO: 23.
13. The method according to claim 1, further comprising:
amplifying an influenza A nucleic acid using primers, wherein the primers comprise a nucleic acid sequence having at least 95% sequence identity to the nucleotide sequence set forth as SEQ ID NO: 3 or having at least 95% sequence identity to the nucleotide sequence set forth as SEQ ID NO: 4, wherein the primers are capable of hybridizing under very high stringency conditions to an influenza A nucleic acid sequence set forth as SEQ ID NO:42 and are capable of amplifying the influenza A nucleic acid.

14. The method according to claim 1, wherein the sample is a biological sample obtained from a subject.
15. The method of claim 1, wherein the presence of an influenza H5 nucleic acid in the biological sample indicates the presence of an influenza H5 infection in the biological sample obtained from a subject.
16. The method according to claim 14, wherein the biological sample is obtained from bronchoalveolar lavage, tracheal aspirates, sputum, nasopharyngeal aspirates, oropharyngeal aspirates, or saliva.
17. A kit for detecting an influenza nucleic acid in a sample, comprising:
a probe comprising a nucleic acid sequence at least 95% identical to SEQ ID NO: 19 or 24, wherein the probe is specific for influenza subtype H5;
a set of primers, wherein the set comprises one or more forward primers 15 to 40 nucleotides in length comprising a nucleic acid sequence comprising a nucleic acid sequence at least 95% identical to a nucleic acid sequence set forth as SEQ ID NO: 17 and one or more reverse primers 15 to 40 nucleotides in length comprising a nucleic acid sequence at least 95% identical to a nucleic acid sequence set forth as SEQ ID NO:18, or wherein the set comprises one or more forward primers 15 to 40 nucleotides in length comprising a nucleic acid sequence comprising a nucleic acid sequence at least 95% identical to a nucleic acid sequence set forth as SEQ ID NO: 22 and one or more reverse primers 15 to 40 nucleotides in length comprising a nucleic acid sequence at least 95% identical to a nucleic acid sequence set forth as SEQ ID NO:23, wherein the set of primers is capable of hybridizing to and directing the amplification of the influenza A nucleic acid; and
instructions for hybridizing the probe to the influenza nucleic acid within the sample.
18. The kit according to claim 17, wherein the set of primers comprises a nucleic acid sequence at least 95% identical to a nucleic acid sequence set forth as SEQ ID NO:17 and a nucleic acid sequence at least 95% identical to a nucleic acid sequence set forth as SEQ ID NO:18.

19. The kit according to claim 17, wherein the set of primers comprises a nucleic acid sequence at least 95% identical to a nucleic acid sequence set forth as SEQ ID NO:22 and a nucleic acid sequence at least 95% identical to a nucleic acid sequence set forth as SEQ ID NO:23.
20. The kit according to claim 17, wherein the probe is labeled.
21. The kit according to claim 20, wherein the probe is radiolabeled, fluorescently-labeled, biotin-labeled, enzymatically-labeled, or chemically-labeled.
22. The kit according to claim 20, wherein the probe is labeled with a fluorophore.
23. The kit according to claim 20, wherein the probe is labeled with a fluorescence quencher.
24. A method for diagnosing an influenza H5 infection in a subject suspected of having an influenza H5 infection comprising:
 - providing a sample obtained from a subject, wherein the sample comprises nucleic acids from the subject;
 - amplifying an influenza A nucleic acid from the sample using primers, wherein the primers comprise a nucleic acid sequence having at least 95% sequence identity to the nucleotide sequence set forth as SEQ ID NO:17 and at least 95% sequence identity to the nucleotide sequence set forth as SEQ ID NO: 18, or wherein the primers comprise a nucleic acid sequence having at least 95% sequence identity to the nucleotide sequence set forth as SEQ ID NO:22 and at least 95% sequence identity to the nucleotide sequence set forth as SEQ ID NO: 23, wherein the primer is capable of hybridizing under very high stringency conditions to an influenza H5 nucleic acid sequence set forth as SEQ ID NO:46 or 47 and capable of amplifying the influenza H5 nucleic acid;
 - contacting the sample with one or more the nucleic acid probes comprising at least 95% sequence identity to the nucleotide sequence set forth as SEQ ID NO:19 or 24, wherein the probe is capable of hybridizing under very high stringency conditions to the influenza A nucleic acid sequence set forth as SEQ ID NO:46 or 47; and
 - detecting hybridization between an influenza H5 virus nucleic acid sequence present in the sample and the probe, wherein the detection of hybridization indicates that the subject is infected with influenza H5 virus.