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(71) Applicant *(for all designated States except US)*: **QUEST DIAGNOSTICS INVESTMENTS INCORPORATED** [US/US]; 300 Delaware Avenue, Wilmington, DE 19899 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **CHEN, Jules** [US/US]; 20340 Bickford Drive, Walnut, California 91789 (US). **KONG, Lilly I.** [US/US]; 2524 Palomino Drive, Covina, California 91724 (US). **LEE, Ming-Chou** [US/US]; 26251 Verona Place, Mission Viejo, California 92692 (US). **CHEN, Fan** [US/US]; 1214 Hearthside Court, Fullerton, California 92831 (US). **TABB, Michelle M.** [US/US]; 2317 N. Linwood Street, Santa Ana, California 92705 (US). **AYE, Michael** [US/US]; 9031 Crocus Avenue, Fountain Valley, California 92708 (US).

(74) Agent: **WILSON, Barry**; Foley & Lardner LLP, 11250 El Camino Real, Suite 200, San Diego, CA 92130 (US).

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(54) **Title**: MULTIPLEX DETECTION ASSAY FOR INFLUENZA AND RSV VIRUSES

FIGURE 1. *Influenza A* Virus, Matrix Protein 1 and Matrix Protein 2 Genes (M gene), Segment 7; GenBank Accession No. CY002353 (SEQ ID NO: 1)

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   1 agcaaaagca ggtagatatt gaaagatgag ccttctaacc gaggtcgaaa cgtatgttct
  61 ctctatcgtt ccatcaggcc ccctcaaagc cgaaatcgcg cagagacttg aagatgtctt
 121 tgctgggaaa aacacagatc ttgaggctct catggaatgg ctaaagacaa gaccaattct
 181 gtcacctctg actaagggga ttttggggtt tgtgttcacg ctcaccgtgc ccagtgagcg
 241 aggactgcag cgtagacgct ttgtccaaaa tgcccttaat gggaatgggg atccaaataa
 301 catggacaaa gcagtcaaac tgtatagaaa acttaagagg gagataacat tccatggggc
 361 caaagaaata gcactcagtt attctgctgg tgcacttgct agttgcatgg gcctcatata
 421 caataggatg ggggctgtaa ccaccgaagt ggcatttggc ctggtatgtg caacatgtga
 481 acagattgct gactcccagc acaggtctca taggcaaatg gtggcaacaa ccaatccatt
 541 aataaaacat gagaacagaa tggttttggc cagcactaca gctaaagcta tggagcaaat
 601 ggctggatca agtgagcagg cagcggaggc catggagatt gctagtcagg ccaggcaaat
 661 ggtgcaggca atgagaaccg ttgggactca tcctagttcc agtactggtc taagagatga
 721 tcttcttgaa aatttgcaga cctatcagaa acgaatggga gtacagatgc agcgattcaa
 781 gtgacccgct tgttgttgct gcgagtatca ttgggatctt gcacttgata ttgtggattc
 841 ttgatcgtct ttttttcaaa tgcatctatc gactcttcaa acacggcctg aaaagagggc
 901 cttctacgga aggagtacct gagtctatga gggaagaata tcggaaggaa cagcagaatg
 961 ctgtggatgc tgacgacagt cattttgtca gcatagagct ggagtaaaaa actaccttgt
1021 ttctact
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(57) **Abstract**: The present invention generally relates to a molecular test of *Influenza A, Influenza B*, Respiratory Syncytial Virus A, and Respiratory Syncytial Virus B in order to identify patients with a viral infection. Accordingly methods and compositions are disclosed to determine the presence or absence of a viral pathogen in a sample containing one or more target nucleic acids from the M gene of *Influenza A, Influenza B*, Respiratory Syncytial Virus A, and/or Respiratory Syncytial Virus B.

AMENDED CLAIMS
received by the International Bureau on 20 july 2009

New Claims

1. A method for identifying the presence or absence of a pathogen in a sample wherein said pathogen is selected from Respiratory Syncytial Virus A (RSV-A) or Respiratory Syncytial Virus B (RSV-B), comprising amplifying the RSV-A M gene, the RSV-B M gene, or both in a multiplex primer extension reaction, and detecting the presence or absence of Respiratory Syncytial Virus A or Respiratory Syncytial Virus B M gene or fragment thereof in said sample, wherein the presence of the RSV A M gene or fragment thereof in the sample indicates that the sample contains the pathogen RSV A, and the presence of the RSV B M gene or fragment thereof in the sample indicates that the sample contains the pathogen RSV B.

2. The method of claim 1, wherein the step of detecting comprises:

(a) contacting said sample with one or more primers suitable for amplifying said pathogen gene or fragment thereof;

(b) performing a primer extension reaction comprising the primers of step (a) under conditions suitable to produce a reaction product from said pathogen gene or fragment thereof if said pathogen gene or fragment thereof is present in said sample; and

(c) detecting the presence or absence of said reaction product, thereby determining the presence or absence of a viral pathogen in said sample.

3. The method of any of claims 1-2, wherein said method comprises real-time PCR.

4. The method of any of claims 1-3, wherein said primer suitable for amplifying said pathogen gene or fragment thereof is a Scorpion primer.

5. The method of any of claims 1-4, wherein said Respiratory Syncytial Virus A M gene or fragment thereof comprises at least 15 contiguous nucleotides that are at least 90% identical to the sequence of SEQ ID NO: 34, or a complement thereof.

6. The method of any of claims 1-4, wherein said Respiratory Syncytial Virus B M gene or fragment thereof comprises at least 15 contiguous nucleotides that are at least 90% identical to the sequence of SEQ ID NO: 35, or a complement thereof.

7. The method of any of claims 1-4, wherein at least one primer suitable for amplifying a Respiratory Syncytial Virus A M gene or fragment thereof comprises a sequence selected from the group consisting of: SEQ ID NOs: 21, 22, 23 and complements thereof.

8. The method of any of claims 1-4, wherein said Respiratory Syncytial Virus A M gene or fragment thereof is detected using a probe comprising the sequence of SEQ ID NO: 24, or a complement thereof.

9. The method of any of claims 1-4, wherein at least one primer suitable for amplifying a Respiratory Syncytial Virus B M gene or fragment thereof comprises a sequence selected from the group consisting of: SEQ ID NOs: 25, 26, 27 and complements thereof.

10. The method of any of claims 1-4, wherein said Respiratory Syncytial Virus B M gene or fragment thereof is detected using a probe comprising the sequence of SEQ ID NO: 28, or a complement thereof.

11. A method of diagnosing a subject for infection with a Respiratory Syncytial viral pathogen wherein said pathogen is selected from Respiratory Syncytial Virus A (RSV-A) or Respiratory Syncytial Virus B (RSV-B), comprising amplifying the RSV-A M gene, the RSV-B M gene, or both in a multiplex primer extension reaction, and detecting the presence or absence of Respiratory Syncytial Virus A or Respiratory Syncytial Virus B M gene or fragment thereof in said sample,

wherein the presence of the RSV A M gene or fragment thereof in the sample indicates that the sample contains the pathogen RSV A, and the presence of the RSV B M gene or fragment thereof in the sample indicates that the sample contains the pathogen RSV B.

12. The method of claim 11, wherein the step of evaluating comprises:

(a) contacting said biological sample with one or more primers suitable for amplifying said pathogen gene or fragment thereof;

(b) performing a primer extension reaction comprising the primers of step (a) under conditions suitable to produce a reaction product from said pathogen gene or fragment thereof if said pathogen gene or fragment thereof is present in said sample; and

(c) detecting the presence or absence of said reaction product, thereby determining the presence or absence of a viral pathogen in said sample.

13. The method of any one of claims 11-12, wherein said method comprises real-time PCR.

14. The method of any one of claims 11-13, wherein said primer suitable for amplifying said pathogen gene or fragment thereof is a Scorpion primer.

15. The method of any one of claims 11-14, wherein said Respiratory Syncytial Virus A M gene or fragment thereof comprises at least 15 contiguous nucleotides that are at least 90% identical to the sequence of SEQ ID NO: 34, or a complement thereof.

16. The method of any one of claims 11-14, wherein said Respiratory Syncytial Virus B M gene or fragment thereof comprises at least 15 contiguous nucleotides that are at least 90% identical to the sequence of SEQ ID NO: 35, or a complement thereof.

17. The method of any one of claims 12-14, wherein at least one primer suitable for amplifying a Respiratory Syncytial Virus A M gene or fragment thereof comprises a sequence selected from the group consisting of: SEQ ID NOs: 21, 22, 23 and complements thereof.

18. The method of any one of claims 11-14, wherein said Respiratory Syncytial Virus A M gene or fragment thereof is detected using a probe comprising the sequence of SEQ ID NO: 24, or a complement thereof.

19. The method of any one of claims 12-14, wherein at least one primer suitable for amplifying a Respiratory Syncytial Virus B M gene or fragment thereof comprises a sequence selected from the group consisting of: SEQ ID NOs: 25, 26, 27 and complements thereof.

20. The method of any one of claims 11-14, wherein said Respiratory Syncytial Virus B M gene or fragment thereof is detected using a probe comprising the sequence of SEQ ID NO: 28, or a complement thereof.

21. A method for identifying the presence or absence of a pathogen in a sample, comprising contacting the sample with amplification primers suitable for amplifying any two of:

(a) the *Influenza A* M gene or fragment thereof;

(b) the *Influenza B* M gene or fragment thereof;

(c) the Respiratory Syncytial Virus A M gene or fragment thereof; and

(d) the Respiratory Syncytial Virus B M gene or fragment thereof;

but not (a) and (b) only,

performing a multiplex primer extension reaction; and

detecting the presence or absence of the primer extension reaction products, wherein the presence of one or more of the genes or fragments specified in (a), (b), (c), or (d) indicates that the sample contains the associated pathogen.

22. The method of claim 21, wherein said method comprises detecting the presence or absence of any three of said pathogen genes or fragments thereof.

23. The method of any one of claims 21-22, wherein said method comprises detecting the presence or absence of each of said pathogen genes or fragments thereof.

24. The method of any one of claims 21-23, wherein said *Influenza A* M gene or fragment thereof comprises at least 15 contiguous nucleotides that are at least 90% identical to the sequence of SEQ ID NO: 32, or a complement thereof.

25. The method of any one of claims 21-24, wherein said *Influenza B* M gene or fragment thereof comprises at least 15 contiguous nucleotides that are at least 90% identical to the sequence of SEQ ID NO: 33, or a complement thereof.

26. The method of any one of claims 21-25, wherein said Respiratory Syncytial Virus A M gene or fragment thereof comprises at least 15 contiguous nucleotides that are at least 90% identical to the sequence of SEQ ID NO: 34, or a complement thereof.

27. The method of any one of claims 21-6, wherein said Respiratory Syncytial Virus B M gene or fragment thereof comprises at least 15 contiguous nucleotides that are at least 90% identical to the sequence of SEQ ID NO: 35, or a complement thereof.

28. The method of claim 28, wherein said method comprises real-time PCR.

29. The method of any one of claims 28-29, wherein at least one of said primers suitable for amplifying said pathogen genes or fragments thereof is a Scorpion primer.

30. The method of any one of claims 28-30, wherein at least one primer suitable for amplifying an *Influenza A* M gene or fragment thereof comprises a sequence selected from the group consisting of: SEQ ID NOs: 13, 14, 15 and complements thereof.

31. The method of any one of claims 28-31, wherein said *Influenza A* M gene or fragment thereof is detected using a probe comprising the sequence of SEQ ID NO: 16, 40 or complements thereof.

32. The method of any one of claims 28-32, wherein at least one primer suitable for amplifying an *Influenza B* M gene or fragment thereof comprises a sequence selected from the group consisting of: SEQ ID NOs: 17, 18, 19 and complements thereof.

33. The method of any one of claims 28-33, wherein said *Influenza B* M gene or fragment thereof is detected using a probe comprising the sequence of SEQ ID NO: 20, or a complement thereof.

34. The method of any one of claims 28-34, wherein at least one primer suitable for amplifying a Respiratory Syncytial Virus A M gene or fragment thereof comprises a sequence selected from the group consisting of: SEQ ID NOs: 21, 22, 23 and complements thereof.

35. The method of any one of claims 28-35, wherein said Respiratory Syncytial Virus A M gene or fragment thereof is detected using a probe comprising the sequence of SEQ ID NO: 24, or a complement thereof.

36. The method of any one of claims 28-36, wherein at least one primer suitable for amplifying a Respiratory Syncytial Virus B M gene or fragment thereof comprises a sequence selected from the group consisting of: SEQ ID NOs: 25, 26, 27 and complements thereof.

37. The method of any one of claims 28-37, wherein said Respiratory Syncytial Virus B M gene or fragment thereof is detected using a probe comprising the sequence of SEQ ID NO: 28, or a complement thereof.

38. A method of diagnosing a subject for infection with an *Influenza* or Respiratory Syncytial viral pathogen, comprising contacting a biological sample from the subject with

amplification primers suitable for amplifying for any two of:

(a) the *Influenza A* M gene or fragment thereof;

(b) the *Influenza B* M gene or fragment thereof;

(c) the Respiratory Syncytial Virus A M gene or fragment thereof; and

(d) the Respiratory Syncytial Virus B M gene or fragment thereof;

but not (a) and (b) only,

performing a multiplex primer extension reaction; and

detecting the presence or absence of the primer extension reaction products, wherein the presence of one or more of said genes or fragments thereof specified in (a), (b), (c), or (d) indicates that the individual is infected with the associated organism.

39. The method of claim 39, wherein said method comprises evaluating a biological sample from the subject for the presence or absence of any three of said pathogen genes or fragments thereof.

40. The method of any one of claims 39-40, wherein said method comprises evaluating a biological sample from the subject for the presence or absence of each of said pathogen genes or fragments thereof.

41. The method of any one of claims 39-41, wherein said *Influenza A* M gene or fragment thereof comprises at least 15 contiguous nucleotides that are at least 90% identical to the sequence of SEQ ID NO: 32, or a complement thereof.

42. The method of any one of claims 39-42, wherein said *Influenza B* M gene or fragment thereof comprises at least 15 contiguous nucleotides that are at least 90% identical to the sequence of SEQ ID NO: 33, or a complement thereof.

43. The method of any one of claims 39-43, wherein said Respiratory Syncytial Virus A M gene or fragment thereof comprises at least 15 contiguous nucleotides that are at least 90% identical to the sequence of SEQ ID NO: 34, or a complement thereof.

44. The method of any one of claims 39-44, wherein said Respiratory Syncytial Virus B M gene or fragment thereof comprises at least 15 contiguous nucleotides that are at least 90% identical to the sequence of SEQ ID NO: 35, or a complement thereof.

45. The method of claim 46, wherein said method comprises real-time PCR.

46. The method of any one of claims 46-47, wherein at least one of said primers suitable for amplifying said pathogen genes or fragments thereof is a Scorpion primer.

47. The method of any one of claims 46-48, wherein at least one primer suitable for amplifying an *Influenza A* M gene or fragment thereof comprises a sequence selected from the group consisting of: SEQ ID NOs: 13, 14, 15 and complements thereof.

48. The method of any one of claims 46-49, wherein said *Influenza A* M gene or fragment thereof is detected using a probe comprising the sequence of SEQ ID NO: 16, 40 or complements thereof.

49. The method of any one of claims 46-50, wherein at least one primer suitable for amplifying an *Influenza B* M gene or fragment thereof comprises a sequence selected from the group consisting of: SEQ ID NOs: 17, 18, 19 and complements thereof.

50. The method of any one of claims 46-51, wherein said *Influenza B* M gene or fragment thereof is detected using a probe comprising the sequence of SEQ ID NO: 20, or a complement thereof.

51. The method of any one of claims 46-52, wherein at least one primer suitable for amplifying a Respiratory Syncytial Virus A M gene or fragment thereof comprises a sequence selected from the group consisting of: SEQ ID NOs: 21, 22, 23 and complements thereof.

52. The method of any one of claims 46-53, wherein said Respiratory Syncytial Virus A M gene or fragment thereof is detected using a probe comprising the sequence of SEQ ID NO: 24, or a complement thereof.

53. The method of any one of claims 46-54, wherein at least one primer suitable for amplifying a Respiratory Syncytial Virus B M gene or fragment thereof comprises a sequence selected from the group consisting of: SEQ ID NOs: 25, 26, 27 and complements thereof.

54. The method of any one of claims 46-55, wherein said Respiratory Syncytial Virus B M gene or fragment thereof is detected using a probe comprising the sequence of SEQ ID NO: 28, or a complement thereof.

55. An isolated nucleic acid comprising a nucleotide sequence that is at least 90% identical to at least 20 contiguous nucleotides, further comprising a sequence selected from the group consisting of: SEQ ID NOs: 32, 33, 34, 35 and complements thereof, wherein said nucleic acid is less than 300 nucleotides in length.

56. The nucleic acid of claim 57, wherein said nucleic acid of the sequence of SEQ ID NO: 32 is less than 200 nucleotides in length.

57. The nucleic acid of claim 57, wherein said nucleic acid of the sequence of SEQ ID NO: 33 is less than 200 nucleotides in length.

58. The nucleic acid of claim 57, wherein said nucleic acid of the sequence of SEQ ID NO: 35 is less than 200 nucleotides in length.

59. The nucleic acid of claim 57, wherein said nucleic acid comprises the nucleotide sequence of SEQ ID NO: 32, or a complement thereof.

60. The nucleic acid of claim 57, wherein said nucleic acid comprises the nucleotide sequence of SEQ ID NO: 33, or a complement thereof.

61. The nucleic acid of claim 57, wherein said nucleic acid comprises the nucleotide sequence of SEQ ID NO: 34, or a complement thereof.

62. The nucleic acid of claim 57, wherein said nucleic acid comprises the nucleotide sequence of SEQ ID NO: 35, or a complement thereof.

63. A kit comprising at least 2 of (a), (b), (c) or (d) but not (a) and (b) only, wherein:

(a) is one or more primers suitable for detecting or amplifying an *Influenza A* M gene or fragment thereof and a first probe capable of specifically hybridizing to the *Influenza A* M gene or fragment thereof;

(b) is one or more primers suitable for detecting or amplifying an *Influenza B* M gene or fragment thereof and a second probe capable of specifically hybridizing to the *Influenza B* M gene or fragment thereof;

(c) is one or more primers suitable for detecting or amplifying a Respiratory Syncytial Virus A M gene or fragment thereof and a third probe capable of specifically hybridizing to the Respiratory Syncytial Virus A M gene or fragment thereof; and

(d) is one or more primers suitable for detecting or amplifying a Respiratory Syncytial Virus B M gene or fragment thereof and a fourth probe capable of specifically hybridizing to the Respiratory Syncytial Virus B M gene or fragment thereof;

and wherein said first probe, second probe, third probe, and fourth probe may each comprise one or more detectable labels.

64. The kit of claim 65, comprising at least three of (a), (b), (c), and (d).

65. The kit of any one of claims 65-66, comprising (a), (b), (c), and (d).

66. The kit of any one of claims 65-67, wherein at least one of said primers suitable for amplifying said pathogen genes or fragments thereof is a Scorpion primer.

67. The kit of any one of claims 65-68, wherein one or more primers suitable for amplifying an *Influenza A* M gene or fragment thereof comprises a sequence selected from the group consisting of: SEQ ID NOs: 13, 14, 15 and complements thereof.

68. The kit of any one of claims 65-69, wherein one or more primers suitable for amplifying an *Influenza B* M gene or fragment thereof comprises a sequence selected from the group consisting of: SEQ ID NOs: 17, 18, 19 and complements thereof.

69. The kit of any one of claims 65-70, wherein one or more primers suitable for amplifying a Respiratory Syncytial Virus A M gene or fragment thereof comprises a sequence selected from the group consisting of: SEQ ID NOs: 21, 22, 23 and complements thereof.

70. The kit of any one of claims 65-71, wherein one or more primers suitable for amplifying a Respiratory Syncytial Virus B M gene or fragment thereof comprises a sequence selected from the group consisting of: SEQ ID NOs: 25, 26, 27 and complements thereof.

71. The kit of any one of claims 65-72, wherein said first probe has the sequence of SEQ ID NO: 16, 40 or complements thereof.

72. The kit of any one of claims 65-73, wherein said second probe has the sequence of SEQ ID NO: 20 or a complement thereof.

73. The kit of any one of claims 65-74, wherein said third probe has the sequence of SEQ ID NO: 24 or a complement thereof.

74. The kit of any one of claims 65-75, wherein said fourth probe has the sequence of SEQ ID NO: 28 or a complement thereof.

75. The kit of any one of claims 65-76, wherein said kit comprises at least one primer suitable for amplifying each of said pathogen genes or fragment thereof.