Influenza detection method and kit therefor

Field of the invention

The present invention relates to methods and reagents for detecting Influenza A and/or B virus.

Background of the art

Influenza A and B are an infectious diseases of animals caused by type A and type B strains respectively of the influenza virus. Molecular biology techniques like PCR and RT-PCR may be used for the detection of the virus. However, influenza viruses are prone to mutations.

Accordingly, there is a need for a specific and/or sensitive detection method for influenza A and B virus infection.

Summary of the invention

The present invention addresses the problems above, and in particular provides new and novel oligonucleotides for detection of Influenza A and/or B viruses. In particular, the present invention provides specific and sensitive primer and probe sequences for detection of influenza A/ B virus with swine-origin H1N1 subtyping confirmation. The sequences are provided in Table 1.

In one aspect the present invention provides at least one isolated oligonucleotide comprising, consisting essentially of or consisting of at least one nucleotide sequence selected from the group consisting of: SEQ ID NO:1 to SEQ ID NO:19, fragment(s), derivative(s), mutation(s), and complementary sequence(s) thereof. The oligonucleotide may be capable of binding to and/or being amplified from Influenza A and/or B virus.

In another aspect the present invention provides at least one pair of oligonucleotides comprising at least one forward primer and at least one reverse primer, wherein the forward primer comprises, consists essentially of or consists of at least one nucleotide sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:7, SEQ ID NO:10, SEQ ID NO:13 and SEQ ID NO:16, fragment(s), derivative(s), mutation(s), and complementary sequence(s) thereof and the reverse primer comprises, consists essentially of or consists of at least one nucleotide sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, SEQ ID NO:11, SEQ ID NO:14, SEQ ID NO:17 and SEQ ID NO:18, fragment(s), derivative(s), mutation(s), and complementary sequence(s) thereof.

In another aspect the present invention provides at least one pair of oligonucleotides comprising at least one forward primer and at least one reverse primer, wherein the forward primer comprises, consists essentially of or consists of SEQ ID NO:1, fragment(s), derivative(s), mutation(s), or complementary sequence(s) thereof and the reverse primer comprises, consists essentially of or consists of SEQ ID NO:2, fragment(s), derivative(s), mutation(s), or complementary sequence(s) thereof. The pair of oligonucleotides may be capable of binding to and/or being amplified from Influenza A virus.

In another aspect the present invention provides at least one set of oligonucleotides comprising a pair of oligonucleotides according to any aspect of the present invention and at least one probe comprising, consisting essentially of or consisting of the nucleotide sequence of SEQ ID NO:3, fragment(s), derivative(s), mutation(s), or complementary sequence(s) thereof.

In another aspect the present invention provides at least one pair of oligonucleotides comprising at least one forward primer and at least one reverse primer, wherein the forward primer comprises, consists essentially of or

consists of at least one nucleotide sequence of SEQ ID NO:4, SEQ ID NO:7, fragment(s), derivative(s), mutation(s), and complementary sequence(s) thereof and the reverse primer comprises, consists essentially of or consists of at least one nucleotide sequence of SEQ ID NO:5 or SEQ ID NO:8, fragment(s), derivative(s), mutation(s), or complementary sequence(s) thereof. The pair of oligonucleotides may be capable of binding to and/or being amplified from Influenza A virus. The influenza A virus may be H1N1 subtype of Influenza A virus. The influenza A virus may be swine origin H1N1 subtype of Influenza A virus.

In another aspect the present invention provides at least one set of oligonucleotides comprising a pair of oligonucleotides according to any aspect of the present invention and at least one probe comprising, consisting essentially of or consisting of the nucleotide sequence of SEQ ID NO:6 or SEQ ID NO:9, fragment(s), derivative(s), mutation(s), or complementary sequence(s) thereof.

In another aspect the present invention provides at least one pair of oligonucleotides comprising at least one forward primer and at least one reverse primer, wherein the forward primer comprises, consists essentially of or consists of SEQ ID NO:10, fragment(s), derivative(s), mutation(s), or complementary sequence(s) thereof and the reverse primer comprises, consists essentially of or consists of SEQ ID NO:11, fragment(s), derivative(s), mutation(s), or complementary sequence(s) thereof. The pair of oligonucleotides may be capable of binding to and/or being amplified from Influenza A virus. The influenza A virus may be H1N1 subtype of Influenza A virus.

In another aspect the present invention provides at least one set of oligonucleotides comprising a pair of oligonucleotides according to any aspect of the present invention and at least one probe comprising, consisting

essentially of or consisting of the nucleotide sequence of SEQ ID NO:12, fragment(s), derivative(s), mutation(s), or complementary sequence(s) thereof.

In another aspect the present invention provides at least one pair of oligonucleotides comprising at least one forward primer and at least one reverse primer, wherein the forward primer comprises, consists essentially of or consists of SEQ ID NO:13, fragment(s), derivative(s), mutation(s), or complementary sequence(s) thereof and the reverse primer comprises, consists essentially of or consists of SEQ ID NO:14, fragment(s), derivative(s), mutation(s), or complementary sequence(s) thereof. The pair of oligonucleotides according to any aspect of the present invention may be capable of binding to and/or being amplified from Influenza A virus. The influenza A virus may be H3N2 subtype of Influenza A virus.

In another aspect the present invention provides at least one set of oligonucleotides comprising a pair of oligonucleotides according to any aspect of the present invention and at least one probe comprising, consisting essentially of or consisting of the nucleotide sequence of SEQ ID NO:15, fragment(s), derivative(s), mutation(s), or complementary sequence(s) thereof.

In another aspect the present invention provides at least one pair of oligonucleotides comprising at least one forward primer and at least one reverse primer, wherein the forward primer comprises, consists essentially of or consists of SEQ ID NO:16, fragment(s), derivative(s), mutation(s), or complementary sequence(s) thereof and the reverse primer comprises, consists essentially of or consists of at least one of SEQ ID NO:17 or SEQ ID NO:18, fragment(s), derivative(s), mutation(s), or complementary sequence(s) thereof. The pair of oligonucleotides may be capable of binding to and/or being amplified from Influenza B virus.

In another aspect the present invention provides at least one set of oligonucleotides comprising a pair of oligonucleotides according to any aspect

of the present invention and at least one probe comprising, consisting essentially of or consisting of the nucleotide sequence of SEQ ID NO:19, fragment(s), derivative(s), mutation(s), or complementary sequence(s) thereof.

In another aspect the present invention provides at least one amplicon amplified from Influenza A and/or B virus using at least one forward primer comprising, consisting essentially of or consisting of at least one nucleotide sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:7, SEQ ID NO:10, SEQ ID NO:13 and SEQ ID NO:16, fragment(s), derivative(s), mutation(s), and complementary sequence(s) thereof and at least one reverse primer comprising, consisting essentially of or consisting of the nucleotide sequence from the group consisting of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, SEQ ID NO:11, SEQ ID NO:14, SEQ ID NO:17 and SEQ ID NO:18, fragment(s), derivative(s), mutation(s), and complementary sequence(s) thereof.

At least one probe comprising, consisting essentially of or consisting of the nucleotide sequence from the group consisting of SEQ ID NO:3, SEQ ID NO:6, SEQ ID NO:9, SEQ ID NO:12, SEQ ID NO:15 and SEQ ID NO:19, fragment(s), derivative(s), mutation(s), and complementary sequence(s) thereof may be capable of binding to the amplicon.

In another aspect the present invention provides at least one method of detecting the presence of Influenza A and/or B virus in a biological sample, the method comprising the steps of:

(a) providing at least one biological sample;

(b) contacting at least one oligonucleotide, a pair of oligonucleotides or a set of oligonucleotides according to any aspect of the present invention, with at least one nucleic acid in the biological sample,

and/or with at least one nucleic acid extracted, purified and/or amplified from the biological sample; and

(c) detecting any binding resulting from the contacting in step (b) whereby the virus is present when binding is detected.

In another aspect the present invention provides at least one kit for the detection of Influenza A and/or B virus, the kit comprising at least one oligonucleotide, a pair of oligonucleotides or a set of oligonucleotides according to any aspect of the present invention.

In another aspect the present invention provides at least one method of detecting the presence of Influenza A and/or B virus in a sample, wherein the method is substantially as described according to the whole content of the present invention.

In another aspect the present invention provides at least one oligonucleotide, fragment and/or derivative thereof, wherein the oligonucleotide is substantially as described according to the whole content of the present invention.

In another aspect the present invention provides at least one kit for the detection of the presence of Influenza A and/or B virus in a sample and/or in a subject, wherein the kit is substantially as described according to the whole content of the present invention. All primers and probes may be mixed to construct multiplex one-step fluorescence probe-based real-time PCR in one-tube with high specificity and high sensitivity.

Brief description of the figures

Figure 1 shows the results of a 4-plex RT-PCR, Detection of Flu A/swine H1N1/H3N2/ Flu B

Figure 2 shows the results of the PCR products which were analysed by Ethidium bromide - agarose gel electrophoresis.

Detailed description of the invention

Bibliographic references mentioned in the present specification are for convenience listed in the form of a list of references and added at the end of the examples. The whole content of such bibliographic references is herein incorporated by reference.

The term "comprising" is herein defined as "including principally, but not necessarily solely". Furthermore, the term "comprising" will be automatically read by the person skilled in the art as including "consisting of". The variations of the word "comprising", such as "comprise" and "comprises", have correspondingly varied meanings.

The term "mutation" is herein defined as a change in the nucleic acid sequence of a length of nucleotides. A person skilled in the art will appreciate that small mutations, particularly point mutations of substitution, deletion and/or insertion has little impact on the stretch of nucleotides, particularly when the nucleic acids are used as probes. Accordingly, the oligonucleotide(s) according to the present invention encompasses mutation(s) of substitution(s), deletion(s) and/or insertion(s) of at least one nucleotide. Further, the oligonucleotide(s) and derivative(s) thereof according to the present invention may also function as probe(s) and hence, any oligonucleotide(s) referred to herein also encompasses their mutations and derivatives.

The term "biological sample" is herein defined as a sample of any tissue and/or fluid from at least one animal and/or plant. In particular, a sample of any tissue and/or fluid from at least a human being.

The term "fragment" is herein defined as an incomplete or isolated portion of the full sequence of an oligonucleotide which comprises the active/binding site(s) that confers the sequence with the characteristics and function of the oligonucleotide. In particular, it may be shorter by at least one nucleotide or amino acid. More in particular, the fragment comprises the binding site(s) that enable the oligonucleotide to bind to HIV-1.

The term "derivative," is herein defined as the chemical modification of the oligonucleotides of the present invention, or of a polynucleotide sequence complementary to the oligonucleotides. Chemical modifications of a polynucleotide sequence can include, for example, replacement of hydrogen by an alkyl, acyl, or amino group.

Name	**Sequence**	**SEQ ID NO:**
Flu A, Forward Primer, 490:A145F	(5'-3')TTCTAACCGAGGTCGAAACGTA	1
Flu A, Reverse Primer, 488:A145R	(5'-3')ATTGGTCTTGTCTTTAGCCAYT	2
Flu A, Probe, 500:A145U (Fluorescent dye)	5'-CCCCTCAAAGCCGAGATCGC 3' (Quencher)	3
Swine-Origin H1N1 Set A, Forward Primer, 470:H1s138F	(5'-3')GGCTGCTTTGAATTTTACCAC	4
Swine-Origin H1N1 Set A, Reverse Primer, 471:H1s138R	(5'-3') TGTTGATTCCAGCTTTACCC	5
Swine-Origin H1N1 Set A, Probe, 475:138P(Fluorescent dye)	5'-GCGATAACACGTGCATGGAAAGT-3' (Quencher)	6
Swine-Origin H1N1 Set B, Forward Primer, 476:H1s142F : Primer Reverse,: Probe,:	(5'-3') AACTTTGGACTACCACGA	7
Swine-Origin H1N1 Set B, Reverse Primer, 477:H1s142R	(5'-3')TTTGACACTTTCCATGCAC	8

Swine-Origin H1N1 Set B, Probe, 478:142U (Fluorescent dye)	5'-ACGGCTGCTTTGAATTTTACCAC-3' (Quencher)	9
Seasonal H1N1, Forward Primer, 468:H1h218F	(5'-3')CCTGTTATGCACATTTACAGC	10
Seasonal H1N1, Reverse Primer, 469:H1h218R	(5'-3')CTAAGATCCACCCGGCAAC	11
Seasonal H1N1, Probe, 472:H1h218P (Fluorescent dye)	5'-CTAACAACTCGACCGACACTG-3' (Quencher)	12
Seasonal H3N2, Forward Primer, 482:H3,116F	(5'-3')AATGACAACAGCACGGCAAC	13
Seasonal H3N2, Reverse Primer, 483:H3,116R	(5'-3')TGAACCAGCTCAGTAGCATT	14
Seasonal H3N2, Probe, 484:H3,116U (Fluorescent dye)	5'-CCATGCAGTACCAAACGGAACGA-3' (Quencher)	15
Flu B, Forward Primer, 494:B188F	(5'-3')TTTGGAGACACAATTGCCTA	16
Flu B, Reverse Primer 1, 495:B188R1	(5'-3')AAGCAKATAGAGGCACCA	17
Flu B, Reverse Primer 2 496:B188R2	(5'-3')AAGCAGATAGAGGCGCCA	18
Flu B, Probe, 497:B188L(Fluorescent dye)	5'-TCAAATTCTTTCCCACCRAAC-3' (Quencher)	19

Table 1

Having now generally described the invention, the same will be more readily understood through reference to the following examples which are provided by way of illustration, and are not intended to be limiting of the present invention.

EXAMPLES

Standard molecular biology techniques known in the art and not specifically described were generally followed as described in Sambrook and Russel, Molecular Cloning: A Laboratory Manual, Cold Springs Harbor Laboratory, New York (2001).

Example 1

4-plex detection

Fluorescent probe based real-time one-step RT-PCR was performed with the SuperScript™ III RT/Platinum® Taq Mix (catalog no. 11732-20, Invitrogen, USA) containing four primer pairs including A145F/R, H1s142F/R, H3-116F/R & B188F/R1,R2 as well as four corresponding fluorescent probes, A145-FAM, H1s142-HEX, H3-116-Cy5 & B188-TxRd. A 25-µl reaction volume containing 2.5 µl of RNA sample, each primer at a final concentration of 0.15 µM and all probes at a concentration of 0.1uM respectively in a thermal cycler, in our studies, Mx3000P QPCR System (Strategene, La Jolla, USA) was used

We ran a mixture of following samples;

(1) Swine-like H1N1, Purified RNA extracted from A/Auckland/3/2009 H1N1 swine like influenza by WHO, Australia.(-5): 10^{-5} dilution, (-6): 10^{-6} dilution, (-7); 10^{-7} dilution from the stock.

(2) H3N2, Purified RNA extracted from a patient. (-2): 10^{-2} dilution, (-3): 10^{-3} dilution.

(3) Flu B, Purified RNA extracted from virus stock obtained from ATCC, VR-786. (-5):10^{-5} dilution, (-6): 10^{-6} dilution.

(4) NTC, Water.

Results:

Results are shown in Table 2 below and Figure 1

	FluA(FAM) Threshold:1000 Gain Setting: 8X	H1sw(HEX) Threshold:1000 Gain Setting: 8X	FluB(TexRD) Threshold:600 Gain Setting: 4X	H3N2(CY5) Threshold:1000 Gain Setting: 4X
Well Name	Ct (dR)	Ct (dR)	Ct (dR)	Ct (dR)
SwH1N1 (-5)	29.37	29.29	No Ct	No Ct
SwH1N1 (-6)	32.37	32.52	No Ct	No Ct
SwH1N1 (-7)	37.21	36.65	No Ct	No Ct
FluB (-5)	No Ct	No Ct	29.65	No Ct
FluB (-6)	No Ct	No Ct	32.56	No Ct
H3N2 (-2)	26.57	No Ct	No Ct	26.94
H3N2 (-3)	30.48	No Ct	No Ct	30.75
NTC	No Ct	No Ct	No Ct	No Ct
NTC	No Ct	No Ct	No Ct	No Ct

Table 2

Example 2

H1s138 primer pair for gel electrophoresis

Monoplex one-step RT-PCR was performed with enzyme mixture, SuperScript® III Platinum® One-Step qRT-PCR Kit (Cat. No: 11732-20) containing a primer pair, H1s138 primer pair. PCR products were analysed by Ethidium bromide - agarose gel electrophoresis.

We ran a mixture of following samples;

(1) Swine-like H1N1, Purified RNA extracted from A/Auckland/3/2009 H1N1 swine like influenza by WHO, Australia. (-6): 10^{-6} dilution from the stock.

(2) Patient samples.

(3) Negative control, Water.

Results

Results are shown in Table 3 below and Figure 2. The expected length of PCR product is 138 bp.

Expected weight: 138bp

Lane	Sample	Result	Stratagene 4-plex
1	100bp ladder		
2	Neg control	Neg	
3	Swine H1 10^{-6}	Pos	
4	Patient	Neg	Neg
5	Patient	Neg	Neg
6	Patient	Pos	Swine H1
7	Patient	Pos	Swine H1
8	Patient	Pos	Swine H1
9	Patient	Pos	Swine H1
10	Swine H1 10^{-6}	Pos	
11	Patient	Neg	Neg
12	Patient	Neg	Neg
13	Patient	Pos	Swine H1
14	Patient	Pos	Swine H1
15	Patient	Pos	Swine H1
16	Patient	Pos	Swine H1
17	100bp ladder		

Table 3

Statements of invention

1. An isolated oligonucleotide comprising at least one nucleotide sequence selected from the group consisting of: SEQ ID NO:1 to SEQ ID NO:19, fragment(s), derivative(s), mutation(s), and complementary sequence(s) thereof.

2. The isolated oligonucleotide according to claim 1, wherein the oligonucleotide is capable of binding to and/or being amplified from Influenza A and/or B virus.

3. A pair of oligonucleotides comprising at least one forward primer and at least one reverse primer, wherein the forward primer comprises at least one nucleotide sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:7, SEQ ID NO:10, SEQ ID NO:13 and SEQ ID NO:16, fragment(s), derivative(s), mutation(s), and complementary sequence(s) thereof and the reverse primer comprises at least one nucleotide sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, SEQ ID NO:11, SEQ ID NO:14, SEQ ID NO:17, SEQ ID NO:18 fragment(s), derivative(s), mutation(s), and complementary sequence(s) thereof.

4. A pair of oligonucleotides comprising at least one forward primer and at least one reverse primer, wherein the forward primer comprises SEQ ID NO:1, fragment(s), derivative(s), mutation(s), or complementary sequence(s) thereof and the reverse primer comprises SEQ ID NO:2, fragment(s), derivative(s), mutation(s), or complementary sequence(s) thereof.

5. The pair of oligonucleotides according to claim 4, capable of binding to and/or being amplified from Influenza A virus.

6. A set of oligonucleotides comprising a pair of oligonucleotides according to either claim 4 or 5 and at least one probe comprising the nucleotide sequence of SEQ ID NO:3, fragment(s), derivative(s), mutation(s), or complementary sequence(s) thereof.

7. A pair of oligonucleotides comprising at least one forward primer and at least one reverse primer, wherein the forward primer comprises at least one nucleotide sequence of SEQ ID NO:4, SEQ ID NO:7, fragment(s), derivative(s), mutation(s), and complementary sequence(s) thereof and the reverse primer comprises at least one nucleotide sequence of SEQ ID NO:5 or SEQ ID NO:8, fragment(s), derivative(s), mutation(s), or complementary sequence(s) thereof.

8. The pair of oligonucleotides according to claim 7, capable of binding to and/or being amplified from Influenza A virus.

9. The pair of oligonucleotides according to claim 8, wherein the influenza A virus is H1N1 subtype of Influenza A virus.

10. The pair of oligonucleotides according to either claim 9 or 10, wherein the influenza A virus is swine origin H1N1 subtype of Influenza A virus.

11. A set of oligonucleotides comprising a pair of oligonucleotides according to any one of claims 7 to 9, and at least one probe comprising the nucleotide sequence of SEQ ID NO:6 or SEQ ID NO:9, fragment(s), derivative(s), mutation(s), or complementary sequence(s) thereof.

12. A pair of oligonucleotides comprising at least one forward primer and at least one reverse primer, wherein the forward primer comprises SEQ ID NO:10, fragment(s), derivative(s), mutation(s), or complementary sequence(s) thereof and the reverse primer comprises SEQ ID NO:11, fragment(s), derivative(s), mutation(s), or complementary sequence(s) thereof.

13. The pair of oligonucleotides according to claim 12, capable of binding to and/or being amplified from Influenza A virus.

14. The pair of oligonucleotides according to claim 13, wherein the influenza A virus is H1N1 subtype of Influenza A virus.

15. A set of oligonucleotides comprising a pair of oligonucleotides according to any one of claims 12 to 15 and at least one probe comprising the nucleotide sequence of SEQ ID NO:12, fragment(s), derivative(s), mutation(s), or complementary sequence(s) thereof.

16. A pair of oligonucleotides comprising at least one forward primer and at least one reverse primer, wherein the forward primer comprises SEQ ID NO:13, fragment(s), derivative(s), mutation(s), or complementary sequence(s) thereof and the reverse primer comprises SEQ ID NO:14, fragment(s), derivative(s), mutation(s), or complementary sequence(s) thereof.

17. The pair of oligonucleotides according to claim 16, capable of binding to and/or being amplified from Influenza A virus.

18. The pair of oligonucleotides according to claim 17, wherein the influenza A virus is H3N2 subtype of Influenza A virus.

19. A set of oligonucleotides comprising a pair of oligonucleotides according to any one of claims 16 to 18 and at least one probe comprising the nucleotide sequence of SEQ ID NO:15, fragment(s), derivative(s), mutation(s), or complementary sequence(s) thereof.

20. A pair of oligonucleotides comprising at least one forward primer and at least one reverse primer, wherein the forward primer comprises SEQ ID NO:16, fragment(s), derivative(s), mutation(s), or complementary sequence(s) thereof and the reverse primer comprises at least one of SEQ ID NO:17,

SEQ ID NO:18, fragment(s), derivative(s), mutation(s), or complementary sequence(s) thereof.

21. The pair of oligonucleotides according to claim 20, capable of binding to and/or being amplified from Influenza B virus.

22. A set of oligonucleotides comprising a pair of oligonucleotides according to either claim 20 or 21 and at least one probe comprising the nucleotide sequence of SEQ ID NO:19, fragment(s), derivative(s), mutation(s), or complementary sequence(s) thereof.

23. An amplicon amplified from Influenza A and/or B virus using at least one forward primer comprising at least one nucleotide sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:7, SEQ ID NO:10, SEQ ID NO:13 and SEQ ID NO:16, fragment(s), derivative(s), mutation(s), and complementary sequence(s) thereof and at least one reverse primer comprising the nucleotide sequence from the group consisting of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:8, SEQ ID NO:11, SEQ ID NO:14, SEQ ID NO:17, SEQ ID NO:18, fragment(s), derivative(s), mutation(s), and complementary sequence(s) thereof.

24. The amplicon according to claim 23, wherein at least one probe comprising the nucleotide sequence from the group consisting of SEQ ID NO:3, SEQ ID NO:6, SEQ ID NO:9, SEQ ID NO:12, SEQ ID NO:15 and SEQ ID NO:19, fragment(s), derivative(s), mutation(s), and complementary sequence(s) thereof is capable of binding to the amplicon.

25. A method of detecting the presence of Influenza A and/or B virus in a biological sample, the method comprising the steps of:

(a) providing at least one biological sample;

(b) contacting at least one oligonucleotide according to either claim 1 or 2, with at least one nucleic acid in the biological sample, and/or with at least one nucleic acid extracted, purified and/or amplified from the biological sample; and

(c) detecting any binding resulting from the contacting in step (b) whereby the virus is present when binding is detected.

26. A method of detecting the presence of Influenza A and/or B virus in a biological sample, the method comprising the steps of:

(a) providing at least one biological sample;

(b) contacting a set of oligonucleotides according to any one of claims 6, 11, 15, 19 or 22, with at least one nucleic acid in the biological sample, and/or with at least one nucleic acid extracted, purified and/or amplified from the biological sample; and

(c) detecting any binding resulting from the contacting in step (b) whereby the virus is present when binding is detected.

27. A kit for the detection of Influenza A and/or B virus, the kit comprising at least one oligonucleotide according to either one of claims 1 or 2.

28. A kit for the detection of Influenza A and/or B virus, the kit comprising at least one pair of oligonucleotides according to any one of claims 3 to 5, 7 to 10, 12 to 14, 16 to 18 or 20 to 21.

29. A kit for the detection of Influenza A and/or B virus, the kit comprising at least one set of oligonucleotides according to any one of claims 6, 11, 15, 19 or 22.

30. A method of detecting the presence of Influenza A and/or B virus in a sample, wherein the method is substantially as described according to the whole content of the present invention.

31. At least one oligonucleotide, fragment and/or derivative thereof, wherein the oligonucleotide is substantially as described according to the whole content of the present invention.

32. A kit for the detection of the presence of Influenza A and/or B virus in a sample and/or in a subject, wherein the kit is substantially as described according to the whole content of the present invention.