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(54) **Title**: INFLUENZA VIRUS REASSORTMENT

(57) **Abstract**: New influenza donor strains for the production of reassortant influenza A viruses are provided.

INFLUENZA VIRUS REASSORTMENT

This patent application claims priority from United States provisional patent application 61/605,922, filed March 2, 2012 and 61/685,766 filed March 23, 2012, the complete contents of which are incorporated herein by reference.

TECHNICAL FIELD

This invention is in the field of influenza A virus reassortment. Furthermore, it relates to manufacturing vaccines for protecting against influenza A viruses.

BACKGROUND ART

The most efficient protection against influenza infection is vaccination against circulating strains and it is important to produce influenza viruses for vaccine production as quickly as possible.

Wild-type influenza viruses often grow to low titres in eggs and cell culture. In order to obtain a better-growing virus strain for vaccine production it is currently common practice to reassort the circulating vaccine strain with a faster-growing high-yield donor strain. This can be achieved by co-infecting a culture host with the circulating influenza strain (the vaccine strain) and the high-yield donor strain and selecting for reassortant viruses which contain the hemagglutinin (HA) and neuraminidase (NA) segments from the vaccine strain and the other viral segments (*i.e.* those encoding PB1, PB2, PA, NP, M_1, M_2, NS_1 and NS_2) from the donor strain. Another approach is to reassort the influenza viruses by reverse genetics (see, for example references 1 and 2).

Reference 3 reports that a reassortant influenza virus containing a PB1 gene segment from A/Texas/1/77, the HA and NA segments from A/New Caledonia/20/99, a modified PA segment derived from A/Puerto Rico/8/34 and the remaining viral segments from A/Puerto Rico/8/34 shows increased growth in cells.

There are currently only a limited number of donor strains for reassorting influenza viruses for vaccine manufacture, and the strain most commonly used is the A/Puerto Rico/8/34 (A/PR/8/34) strain. However, reassortant influenza viruses comprising A/PR/8/34 backbone segments do not always grow sufficiently well to ensure efficient vaccine manufacture. Thus, there is a need in the art to provide further and improved donor strains for influenza virus reassortment.

SUMMARY OF PREFERRED EMBODIMENTS

The inventors have now surprisingly discovered that influenza viruses which comprise backbone segments from two or more influenza donor strains can grow faster in a culture host compared with reassortant influenza A viruses which contain all backbone segments from the same donor strain. In particular, the inventors have found that influenza viruses which comprise backbone segments derived from two high-yield donor strains can produce higher yield reassortants with target vaccine-relevant HA/NA genes than reassortants made with either of the two original donor strains.

In principle, all segments of closely related influenza A viruses can be specifically reassorted to produce viable viruses, but only a small fraction of these viruses will be high-growth reassortants, due to inefficient activities of the resulting viral components. The inventors have provided backbone combinations that produce the high yield strains. Reassortant influenza A viruses comprising backbone segments from two or more influenza donor strains may contain the PB1 and the PB2 viral segments from the same donor strain, in particular the A/New Caledonia/20/1999-like strain, referred to herein as the 105p30 strain. The PB1 and PB2 viral segments may have at least 95% identity or 100% identity with the sequence of SEQ ID NO: 2 and/or SEQ ID NO: 3.

Where the reassortant influenza A virus comprises backbone segments from two or three donor strains, each donor strain may provide more than one of the backbone segments of the reassortant influenza A virus, but one or two of the donor strains can also provide only a single backbone segment.

Where the reassortant influenza A virus comprises backbone segments from two, three, four or five donor strains, one or two of the donor strains may provide more than one of the backbone segments of the reassortant influenza A virus. In general the reassortant influenza A virus cannot comprise more than six backbone segments. Accordingly, for example, if one of the donor strains provides five of the viral segments, the reassortant influenza A virus can only comprise backbone segments from a total of two different donor strains.

Where a reassortant influenza A virus comprises the PB1 segment from A/Texas/1/77, it preferably does not comprise the PA, NP or M segment from A/Puerto Rico/8/34. Where a reassortant influenza A virus comprises the PA, NP or M segment from A/Puerto Rico/8/34, it preferably does not comprise the PB1 segment from A/Texas/1/77. In some embodiments, the invention does not encompass reassortant influenza A viruses which have the PB1 segment from A/Texas/1/77 and the PA, NP and M segments from A/Puerto Rico/8/34. The PB1 segment from A/Texas/1/77 may have the sequence of SEQ ID NO: 46 and the PA, NP or M segments from A/Puerto Rico/8/34 may have the sequence of SEQ ID NOs 47, 48 or 49, respectively.

The inventors have also discovered that variants of known donor strains can grow to higher viral titres compared to the original donor strain and can therefore be better donor strains for reassorting influenza viruses. Examples of such strains are PR8-X and 105p30.

Influenza A virus strains of the invention can grow to higher viral titres in MDCK cells in the same time and under the same growth conditions compared with A/Puerto Rico/8/34 and/or have a higher rescue efficiency compared with reassortant influenza strains that comprise all backbone segments from the same influenza donor strain. Further provided is a reassortant influenza A virus comprising at least one backbone viral segment from such an influenza strain.

The invention also provides a reassortant influenza A virus comprising at least one backbone viral segment from a donor strain, wherein the donor strain is selected from the group consisting of

105p30 and PR8-X. When the at least one backbone viral segment is the PA segment it may have a sequence having at least 95% or at least 99% identity with a sequence selected from the group consisting of SEQ ID NOs: 9 and 17. When the at least one backbone viral segment is the PB1 segment, it may have a sequence having at least 95% or at least 99% identity with a sequence selected from the group consisting of SEQ ID NOs 10 and 18. When the at least one backbone viral segment is the PB2 segment, it may have a sequence having at least 95% or at least 99% identity with a sequence selected from the group consisting of or SEQ ID NOs: 11 and 19. When the at least one backbone viral segment is the M segment it may have a sequence having at least 95% or at least 99% identity with a sequence selected from the group consisting of SEQ ID NOs: 13 and 21. When the at least one backbone viral segment is the NP segment it may have a sequence having at least 95% or at least 99% identity with a sequence selected from the group consisting of SEQ ID NOs: 12 and 20. When the at least one backbone viral segment is the NS segment it may have a sequence having at least 95% or at least 99% identity with a sequence selected from the group consisting of SEQ ID NOs: 14 and 22.

In embodiments where the reassortant influenza A virus comprises backbone segments from at least two influenza donor strains, at least one backbone segment may be derived from a donor strain selected from the group consisting of 105p30 and PR8-X, as discussed in the previous paragraph. Preferred reassortant influenza A viruses comprise 1, 2, 3 or 4 viral segments from the 105p30 donor strain wherein the PA segment may have at least 95% identity or 100% identity with SEQ ID NO: 17, the NP segment may have at least 95% identity or 100% identity with SEQ ID NO: 20, the M segment may have at least 95% identity or 100% identity with SEQ ID NO: 21, and/or the NS segment may have at least 95% identity or 100% identity with SEQ ID NO: 22. In some embodiments such influenza A viruses may also comprise at least one backbone viral segment from the PR8-X donor strain. Where the at least one viral segment is the PA segment it may have at least 95% identity or 100% identity with SEQ ID NO: 9. Where the at least one viral segment is the NP segment it may have at least 95% identity or 100% identity with SEQ ID NO: 12. Where the at least one viral segment is the M segment it may have at least 95% identity or 100% identity with SEQ ID NO: 13. Where the at least one viral segment is the NS segment it may have at least 95% identity or 100% identity with SEQ ID NO: 9. The inventors have shown that reassortant influenza A viruses comprising such backbone segments grow well in cell culture. In general a reassortant influenza virus will contain only one of each backbone segment. For example, when the influenza virus comprises the PA segment from 105p30 it will not at the same time comprise the PA segment of PR8-X.

In preferred embodiments, the virus comprises viral segments having at least 95% identity or 100% identity with the sequence of (a) the PB2 segment of SEQ ID NO: 19, the PB1 segment of SEQ ID: NO 18 and the NS segment of SEQ ID NO: 22; or (b) the PB2 segment of SEQ ID NO: 19, the PB1 segment of SEQ ID NO: 18 and the M segment of SEQ ID NO: 21; or (c) the PB2 segment of SEQ

ID NO: 19, the PB1 segment of SEQ ID NO: 18 and the NP segment of SEQ ID NO: 20; or (d) the PB2 segment of SEQ ID NO 19, the PB1 segment of SEQ ID NO 18 and the PA segment of SEQ ID NO 17. These embodiments are preferred because the inventors have found that such reassortant influenza A viruses grow particularly well in cell culture.

The invention provides a method of preparing the reassortant influenza A viruses of the invention. These methods comprise steps of (i) introducing into a culture host one or more expression construct(s) which encode(s) the viral segments required to produce an influenza A virus wherein the backbone viral segments are from two or more influenza strains; and (ii) culturing the culture host in order to produce reassortant virus and optionally (iii) purifying the virus obtained in step (ii).

The method may comprise the steps of (i) introducing into a culture host one or more expression construct(s) which encode(s) the viral segments required to produce an influenza A virus wherein the backbone viral segments are from two or more influenza strains and the PB1 and PB2 segments are from the same donor strain; and (ii) culturing the culture host in order to produce reassortant virus and optionally (iii) purifying the virus obtained in step (ii).

Also provided is a method of preparing a reassortant influenza A virus of the invention comprising the steps of (i) introducing into a culture host one or more expression construct(s) which encode(s) the viral segments required to produce an influenza A virus wherein the backbone viral segments are from two or more influenza strains and the HA and the PB1 segment are from different influenza strains which have the same influenza HA subtype; and (ii) culturing the culture host in order to produce reassortant virus and optionally (iii) purifying the virus obtained in step (ii).

The invention also provides a method of preparing a reassortant influenza A virus of the invention comprising steps of (i) introducing into a culture host one or more expression construct(s) which encode(s) the viral segments required to produce an influenza A virus wherein one or more backbone viral segment(s) is/are from a 105p30 and/or a PR8-X influenza strain and wherein at least one viral segment is derived from a second influenza strain; and (ii) culturing the culture host in order to produce reassortant virus and optionally (iii) purifying the virus obtained in step (ii).

The methods may further comprise steps of: (iv) infecting a culture host with the virus obtained in step (ii) or step (iii); (v) culturing the culture host from step (iv) to produce further virus; and optionally (vi) purifying the virus obtained in step (v).

The invention also provides a method for producing influenza viruses comprising steps of (a) infecting a culture host with a reassortant virus of the invention; (b) culturing the host from step (a) to produce the virus; and optionally (c) purifying the virus obtained in step (b).

The invention also provides a method of preparing a vaccine, comprising steps of (d) preparing a virus by the methods of any one of the embodiments described above and (e) preparing vaccine from the virus.

In a further embodiment, the invention provides influenza strains PR8-X and 105p30.

The invention also encompasses variant H1N1 influenza virus strains in which the M genome segment has lysine in the position corresponding to amino acid 95 of SEQ ID NO: 33 when aligned to SEQ ID NO: 33 using a pairwise alignment algorithm. The variant H1N1 influenza virus strains according to the invention may further have a HA segment which has glycine in the position corresponding to amino acid 225 of SEQ ID NO: 35 when aligned to SEQ ID NO: 35 and/or has asparagine in the position corresponding to amino acid 231 of SEQ ID NO: 35 when aligned to SEQ ID NO: 35 using a pairwise alignment algorithm. The variant H1N1 influenza virus strain may also have a NA segment which has histidine in the position corresponding to amino acid 70 of SEQ ID NO: 31 when aligned to SEQ ID NO: 31 using a pairwise alignment algorithm.

The preferred pairwise alignment algorithm is the Needleman-Wunsch global alignment algorithm [4], using default parameters (*e.g.* with Gap opening penalty = 10.0, and with Gap extension penalty = 0.5, using the EBLOSUM62 scoring matrix). This algorithm is conveniently implemented in the *needle* tool in the EMBOSS package [5].

The invention provides an expression system comprising one or more expression construct(s) comprising the vRNA encoding segments of an influenza A virus wherein the expression construct(s) encode(s) the backbone viral segments from two or more influenza donor strains. The expression construct(s) may encode the PB1 and PB2 segments from the same donor strain.

The invention also provides an expression system comprising one or more expression construct(s) comprising the vRNA encoding segments of a 105p30 or PR8-X strain wherein the expression construct(s) comprise(s) at least one backbone viral segment from the 105p30 or PR8-X, or strain. The expression construct(s) may further comprise the vRNAs which encode the PB2, NP, NS, M and PA segments from PR8-X.

The invention also provides a host cell comprising the expression systems of the invention. These host cells can express an influenza A virus from the expression construct(s) in the expression system.

Expression constructs which can be used in the expression systems of the invention are also provided. For example, the invention provides an expression construct which encodes the backbone segments of the reassortant influenza strains according to the invention on the same construct.

Donor strains

Influenza donor strains are strains which typically provide the backbone segments in a reassortant influenza virus, even though they may sometimes also provide the HA or NA segment, but not both, of the virus. Usually, however, both the HA and the NA segment in a reassortant influenza virus will be from the vaccine strain.

The inventors have surprisingly discovered that reassortant influenza A viruses comprising backbone segments from two or more influenza donor strains can grow to higher titres in cell culture compared

with reassortant influenza viruses which contain all backbone segments from the same donor strain. The inventors have shown that this effect is due to the presence of backbone segments from two donor strains and does not require the presence of viral segments with specific mutations. Particularly good results are achieved, however, with influenza A strains in which the M genome segment has lysine in the position corresponding to amino acid 95 of SEQ ID NO: 33 when aligned to SEQ ID NO: 33.

Reassortant influenza A viruses comprising the PB1 and PB2 segments from the same influenza strain (for example 105p30) are also advantageous because they showed a better rescue efficiency compared with influenza viruses in which the PB1 and PB2 segments are from different viruses. The PB1 and PB2 segments of 105p30 have the sequence of SEQ ID NOs 18 and 19, respectively.

The inventors have also shown that some influenza virus strains can grow to higher viral titres in MDCK cells in the same time and under the same growth conditions compared with A/Puerto Rico/8/34.

Variants of influenza donor strains which are derived from the donor strains of the invention or other known donor strains such A/PR/8/34 (wt PR8) can also be useful as donor strains. These donor strains can grow to higher viral titres (in the same time and under the same growth conditions) compared to the donor strain from which they are derived. For example, the inventors have surprisingly discovered that passaging the A/PR/8/34 influenza strain several times in cell culture results in a virus strain (PR8-X) which grows to much higher viral titres compared to the original A/PR8/34 strain. Likewise, the inventors have found that passaging the A/New Caledonia/20/1999 strain several times in cells results in a strain (105p30) which grows to even higher viral titres compared to the unpassaged A/New Caledonia/20/1999 strain in the same time and under the same growth conditions. Donor strain variants of the present invention will typically achieve viral titres which are at least 10%, at least 20%, at least 50%, at least 100%, at least 200%, at least 500% or at least 1000% higher under the same growth conditions and for the same time (for example 12 hours, 24 hours, 48 hours or 72 hours) compared to the viral titres obtained with the donor strain from which the variant was derived.

The segments of PR8-X have the sequences of SEQ ID NO: 11 (PB2), SEQ ID NO: 10 (PB1), SEQ ID NO: 9 (PA), SEQ ID NO: 12 (NP), SEQ ID NO: 13 (M), SEQ ID NO: 14 (NS), SEQ ID NO: 15 (HA) or SEQ ID NO: 16 (NA).

The segments of 105p30 have the sequences of SEQ ID NO: 19 (PB2), SEQ ID NO: 18 (PB1), SEQ ID NO: 17 (PA), SEQ ID NO: 20 (NP), SEQ ID NO: 21 (M), SEQ ID NO: 22 (NS), SEQ ID NO: 23 (HA) or SEQ ID NO: 24 (NA).

Influenza strains which contain one, two, three, four five, six or seven of the segments of the 105p30 or PR8-X strains can also be used as donor strains.

The invention can be practised with donor strains having a viral segment that has at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95% or at least about 99% identity to a sequence of SEQ ID NOs 11-14 or 18- 22. For example, due to the degeneracy of the genetic code, it is possible to have the same polypeptide encoded by several nucleic acids with different sequences. Thus, the invention may be practised with viral segments that encode the same polypeptides as the sequences of SEQ ID NOs 11-14 or 18-22. For example, the nucleic acid sequences of SEQ ID NOs: 3 and 28 have only 73% identity even though they encode the same viral protein.

The invention may also be practised with viral segments that encode polypeptides that have at least 80%, at least 85%, at least 90%, at least 95% or at least 99% identity to the polypeptide sequences encoded by SEQ ID NOs 11- 14 or 18- 22.

Variations in the DNA and the amino acid sequence may also stem from spontaneous mutations which can occur during passaging of the viruses. Such variant influenza strains can also be used in the invention.

Reassortant viruses

The invention provides reassortant influenza viruses which comprise backbone segments from two or more influenza donor strains. The PB1 and PB2 segments may be from the same donor strain.

The invention also provides reassortant influenza viruses comprising at least one backbone viral segment from an influenza virus strain that can grow to higher viral titres in MDCK cells in the same time and under the same growth conditions compared with A/Puerto Rico/8/34.

The invention provides reassortant influenza viruses comprising at least one backbone viral segment from the donor strains of the invention, *e.g.* a PR8-X or 105p30 strain. The reassortant influenza viruses of the invention can be reassortants between two, three or more different influenza strains provided that at least one viral segment is derived from a donor strain of the invention.

Influenza viruses are segmented negative strand RNA viruses. Influenza A and B viruses have eight segments (NP, M, NS, PA, PB1, HA and NA) whereas influenza C virus has seven. The reassortant viruses of the invention contain the backbone segments from two or more donor strains, or at least one (*i.e.* one, two, three, four, five or six) backbone viral segment from the donor strains of the invention. The backbone viral segments are those which do not encode HA or NA. Thus, backbone segments will typically encode the PB1, PB2, PA, NP, M_1, M_2, NS_1 and NS_2 polypeptides of the influenza virus. The reassortant viruses will not typically contain the segments encoding HA and NA from the donor strains even though reassortant viruses which comprise either the HA or the NA but not both from the donor strains of the invention are also envisioned.

When the reassortant viruses of the invention are reassortants comprising the backbone segments from a single donor strain, the reassortant viruses will generally include segments from the donor

strain and the vaccine strain in a ratio of 1:7, 2:6, 3:5, 4:4, 5:3, 6:2 or 7:1. Having a majority of segments from the donor strain, in particular a ratio of 6:2, is typical. When the reassortant viruses comprise backbone segments from two donor strains, the reassortant virus will generally include segments from the first donor strain, the seconds donor strain and the vaccine strain in a ratio of 1:1:6, 1:2:5, 1:3:4, 1:4:3, 1:5:2, 1:6:1, 2:1:5, 2:2:4, 2:3:3, 2:4:2, 2:5:1, 3:1:2, 3:2:1, 4:1:3, 4:2:2, 4:3:1, 5:1:2, 5:2:1 or 6:1:1.

Preferably, the reassortant viruses do not contain the HA segment of the donor strain as this encodes the main vaccine antigens of the influenza virus and should therefore come from the vaccine strain. The reassortant viruses of the invention therefore preferably have at least the HA segment and typically the HA and NA segments from the vaccine strain.

The invention also encompasses reassortant viruses which contain viral segments from more than one, for example two or three different, donor strain(s) wherein at least one viral segment, preferably not HA, is derived from the PR8-X or 105p30 influenza strains. Such reassortant influenza viruses will typically contain the HA and/or NA segment from a vaccine strain. Where the reassortants contain viral segments from more than one influenza donor strain, the further donor strain(s) can be any donor strain including the donor strains of the invention. For example, some of the viral segments may be derived from the A/PR/8/34 or AA/6/60 (A/Ann Arbor/6/60) influenza strains. Reassortants containing viral segments from the AA/6/60 strain may be advantageous, for example, where the reassortant virus is to be used in a live attenuated influenza vaccine.

The invention also encompasses reassortants which comprise viral segments from more than one vaccine strain provided that the reassortant comprises a backbone according to the present invention. For example, the reassortant influenza viruses may comprise the HA segment from one donor strain and the NA segment from a different donor strain.

The reassortant viruses of the invention can grow to higher viral titres than the wild-type vaccine strain from which some of the viral segment(s) of the reassortant virus are derived in the same time (for example 12 hours, 24 hours, 48 hours or 72 hours) and under the same growth conditions. The viral titre can be determined by standard methods known to those of skill in the art. The reassortant viruses of the invention can achieve a viral titre which is at least 10% higher, at least 20% higher, at least 50% higher, at least 100% higher, at least 200% higher, at least 500% higher, or at least 1000%higher than the viral titre of the wild type vaccine strain in the same time frame and under the same conditions.

The invention is suitable for reassorting pandemic as well as inter-pandemic (seasonal) influenza vaccine strains. The reassortant influenza strains may contain the influenza A virus HA subtypes H1, H2, H3, H4, H5, H6, H7, H8, H9, H10, H11, H12, H13, H14, H15 or H16. They may contain the influenza A virus NA subtypes N1, N2, N3, N4, N5, N6, N7, N8 or N9. Where the vaccine strain used in the reassortant influenza viruses of the invention is a seasonal influenza strain, the vaccine

strain may have a H1 or H3 subtype. In one aspect of the invention the vaccine strain is a H1N1 or H3N2 strain.

The vaccine strains for use in the invention may also be pandemic strains or potentially pandemic strains. The characteristics of an influenza strain that give it the potential to cause a pandemic outbreak are: (a) it contains a new hemagglutinin compared to the hemagglutinins in currently-circulating human strains, *i.e.* one that has not been evident in the human population for over a decade (*e.g.* H2), or has not previously been seen at all in the human population (*e.g.* H5, H6 or H9, that have generally been found only in bird populations), such that the human population will be immunologically naïve to the strain's hemagglutinin; (b) it is capable of being transmitted horizontally in the human population; and (c) it is pathogenic to humans. A vaccine strain with H5 hemagglutinin type is preferred where the reassortant virus is used in vaccines for immunizing against pandemic influenza, such as a H5N1 strain. Other possible strains include H5N3, H9N2, H2N2, H7N1 and H7N7, and any other emerging potentially pandemic strains. The invention is particularly suitable for producing reassortant viruses for use in vaccine for protecting against potential pandemic virus strains that can or have spread from a non-human animal population to humans, for example a swine-origin H1N1 influenza strain.

The reassortant influenza strain of the invention may comprise the HA segment and/or the NA segment from an A/California/4/09 strain. Thus, for instance, the HA gene segment may encode a H1 hemagglutinin which is more closely related to SEQ ID NO: 32 than to SEQ ID NO: 25 (*i.e.* has a higher degree sequence identity when compared to SEQ ID NO: 32 than to SEQ ID NO: 25 using the same algorithm and parameters). SEQ ID NOs: 32 and 25 are 80% identical. Similarly, the NA gene may encode a N1 neuraminidase which is more closely related to SEQ ID NO: 27 than to SEQ ID NO: 26. SEQ ID NOs: 27 and 26 are 82% identical.

Strains which can be used as vaccine strains include strains which are resistant to antiviral therapy (*e.g.* resistant to oseltamivir [6] and/or zanamivir), including resistant pandemic strains [7].

The choice of donor strain for use in the methods of the invention can depend on the vaccine strain which is to be reassorted. As reassortants between evolutionary distant strains might not replicate well in cell culture, it is possible that the donor strain and the vaccine strain have the same HA and/or NA subtype. In other embodiments, however, the vaccine strain and the donor strain can have different HA and/or NA subtypes, and this arrangement can facilitate selection for reassortant viruses that contain the HA and/or NA segment from the vaccine strain. Therefore, although the 105p30 and PR8-X strains contain the H1 influenza subtype these donor strains can be used for vaccine strains which do not contain the H1 influenza subtype.

Reassortants of the donor strains of the invention wherein the HA and/or NA segment has been changed to another subtype can also be used. The H1 influenza subtype of the 105p30 or PR8-X strain may be changed, for example, to a H3 or H5 subtype.

Thus, the invention encompasses an influenza A virus which comprises one, two, three, four, five, six or seven viral segments from the 105p30 or PR8-X strains of the invention and a HA segment which is not of the H1 subtype. The reassortant donor strains may further comprise an NA segment which is not of the N1 subtype. Suitable techniques for reassorting the donor strains will be evident to those of skill in the art.

The invention also encompasses reassortant donor strains which comprise at least one, at least two, at least three, at least four, at least five, at least six or at least seven viral segments from the 105p30 or PR8-X strains of the invention and a H1 HA segment which is derived from a different influenza strain.

Reassortant viruses which contain an NS segment that does not encode a functional NS protein are also within the scope of the present invention. NS1 knockout mutants are described in reference 8. These NS1-mutant virus strains are particularly suitable for preparing live attenuated influenza vaccines.

The 'second influenza strain' used in the methods of the invention is different to the donor strain which is used.

Reverse genetics

The invention is particularly suitable for producing reassortant influenza virus strains through reverse genetics techniques. In these techniques, the viruses are produced in culture hosts using an expression system.

In one aspect, the expression system may encode the PB1 and PB2 segments from the same donor strain. In this aspect of the invention, the system may encode at least one (*i.e.* one, two three or four) of the segments NP, M, NS and/or PA from another influenza donor strain.

In another aspect, the system may encode 1 or more (*e.g.* 1, 2, 3, 4, 5 or 6) genome segments from the PR8-X strain, but usually this/these will not include the PR8-X HA segment and usually will not include the PR8-X NA segment. Thus the system may encode at least one of segments NP, M, NS, PA, PB1 and/or PB2 (possibly all six) from PR8-X.

The system may encode 1 or more (*e.g.* 1, 2, 3, 4, 5 or 6) genome segments from the 105p30 strain, but usually this/these will not include the 105p30 HA segment and usually will not include the 105p30 NA segment. Thus the system may encode at least one of segments NP, M, NS, PA, PB1 and/or PB2 (possibly all six) from 105p30.

Reverse genetics for influenza A and B viruses can be practised with 12 plasmids to express the four proteins required to initiate replication and transcription (PB1, PB2, PA and nucleoprotein) and all eight viral genome segments. To reduce the number of constructs, however, a plurality of RNA polymerase I transcription cassettes (for viral RNA synthesis) can be included on a single plasmid (*e.g.* sequences encoding 1, 2, 3, 4, 5, 6, 7 or all 8 influenza vRNA segments), and a plurality of

protein-coding regions with RNA polymerase II promoters on another plasmid (*e.g.* sequences encoding 1, 2, 3, 4, 5, 6, 7 or 8 influenza mRNA transcripts) [9]. It is also possible to include one or more influenza vRNA segments under control of a pol I promoter and one or more influenza protein coding regions under control of another promoter, in particular a pol II promoter, on the same plasmid. This is preferably done by using bi-directional plasmids.

Preferred aspects of the reference 9 method involve: (a) PB1, PB2 and PA mRNA-encoding regions on a single expression construct; and (b) all 8 vRNA encoding segments on a single expression construct. Including the neuraminidase (NA) and hemagglutinin (HA) segments on one expression construct and the six other viral segments on another expression construct is particularly preferred as newly emerging influenza virus strains usually have mutations in the NA and/or HA segments. Therefore, the advantage of having the HA and/or NA segments on a separate expression construct is that only the vector comprising the HA and NA sequence needs to be replaced. Thus, in one aspect of the invention the NA and/or HA segments of the vaccine strain may be included on one expression construct and the vRNA encoding segments from the donor strain(s) of the invention, excluding the HA and/or NA segment(s), are included on a different expression construct. The invention thus provides an expression construct comprising one, two, three, four, five or six vRNA encoding backbone viral segments of a donor strain of the invention. The expression construct may not comprise HA and/or NA viral segments that produce a functional HA and/or NA protein.

Known reverse genetics systems involve expressing DNA molecules which encode desired viral RNA (vRNA) molecules from pol I promoters, bacterial RNA polymerase promoters, bacteriophage polymerase promoters, *etc.* As influenza viruses require the presence of viral polymerase to complete the life cycle, systems may also provide these proteins *e.g.* the system further comprises DNA molecules that encode viral polymerase proteins such that expression of both types of DNA leads to assembly of a complete infectious virus. It is also possible to supply the viral polymerase as a protein.

Where reverse genetics is used for the expression of influenza vRNA, it will be evident to the person skilled in the art that precise spacing of the sequence elements with reference to each other is important for the polymerase to initiate replication. It is therefore important that the DNA molecule encoding the viral RNA is positioned correctly between the pol I promoter and the termination sequence, but this positioning is well within the capabilities of those who work with reverse genetics systems.

In order to produce a recombinant virus, a cell must express all segments of the viral genome which are necessary to assemble a virion. DNA cloned into the expression constructs of the present invention preferably provides all of the viral RNA and proteins, but it is also possible to use a helper virus to provide some of the RNA and proteins, although systems which do not use a helper virus are preferred. As the influenza virus is a segmented virus, the viral genome will usually be expressed

using more than one expression construct in the methods of the invention. It is also envisioned, however, to combine one or more segments or even all segments of the viral genome on a single expression construct.

In some embodiments an expression construct will also be included which leads to expression of an accessory protein in the host cell. For instance, it can be advantageous to express a non-viral serine protease (*e.g.* trypsin) as part of a reverse genetics system.

Expression constructs

Expression constructs used in the expression systems of the invention may be uni-directional or bi-directional expression constructs. Where more than one transgene is used in the methods (whether on the same or different expression constructs) it is possible to use uni-directional and/or bi-directional expression.

As influenza viruses require a protein for infectivity, it is generally preferred to use bi-directional expression constructs as this reduces the total number of expression constructs required by the host cell. Thus, the method of the invention may utilise at least one bi-directional expression construct wherein a gene or cDNA is located between an upstream pol II promoter and a downstream non-endogenous pol I promoter. Transcription of the gene or cDNA from the pol II promoter produces capped positive-sense viral mRNA which can be translated into a protein, while transcription from the non-endogenous pol I promoter produces negative-sense vRNA. The bi-directional expression construct may be a bi-directional expression vector.

Bi-directional expression constructs contain at least two promoters which drive expression in different directions (*i.e.* both 5' to 3' and 3' to 5') from the same construct. The two promoters can be operably linked to different strands of the same double stranded DNA. Preferably, one of the promoters is a pol I promoter and at least one of the other promoters is a pol II promoter. This is useful as the pol I promoter can be used to express uncapped vRNAs while the pol II promoter can be used to transcribe mRNAs which can subsequently be translated into proteins, thus allowing simultaneous expression of RNA and protein from the same construct. Where more than one expression construct is used within an expression system, the promoters may be a mixture of endogenous and non-endogenous promoters.

The pol I and pol II promoters used in the expression constructs may be endogenous to an organism from the same taxonomic order from which the host cell is derived. Alternatively, the promoters can be derived from an organism in a different taxonomic order than the host cell. The term "order" refers to conventional taxonomic ranking, and examples of orders are primates, rodentia, carnivora, marsupialia, cetacean, *etc*. Humans and chimpanzees are in the same taxonomic order (primates), but humans and dogs are in different orders (primates *vs.* carnivora). For example, the human pol I promoter can be used to express viral segments in canine cells (e.g. MDCK cells).

The expression construct will typically include an RNA transcription termination sequence. The termination sequence may be an endogenous termination sequence or a termination sequence which is not endogenous to the host cell. Suitable termination sequences will be evident to those of skill in the art and include, but are not limited to, RNA polymerase I transcription termination sequence, RNA polymerase II transcription termination sequence, and ribozymes. Furthermore, the expression constructs may contain one or more polyadenylation signals for mRNAs, particularly at the end of a gene whose expression is controlled by a pol II promoter.

An expression system may contain at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, at least eleven or at least twelve expression constructs.

An expression construct may be a vector, such as a plasmid or other episomal construct. Such vectors will typically comprise at least one bacterial and/or eukaryotic origin of replication. Furthermore, the vector may comprise a selectable marker which allows for selection in prokaryotic and/or eukaryotic cells. Examples of such selectable markers are genes conferring resistance to antibiotics, such as ampicillin or kanamycin. The vector may further comprise one or more multiple cloning sites to facilitate cloning of a DNA sequence.

As an alternative, an expression construct may be a linear expression construct. Such linear expression constructs will typically not contain any amplification and/or selection sequences. However, linear constructs comprising such amplification and/or selection sequences are also within the scope of the present invention. Reference 10 describes a linear expression construct which describes individual linear expression constructs for each viral segment. It is also possible to include more than one, for example two, three four, five or six viral segments on the same linear expression construct. Such a system has been described, for example, in reference 11.

Expression constructs can be generated using methods known in the art. Such methods were described, for example, in reference 12. Where the expression construct is a linear expression construct, it is possible to linearise it before introduction into the host cell utilising a single restriction enzyme site. Alternatively, it is possible to excise the expression construct from a vector using at least two restriction enzyme sites. Furthermore, it is also possible to obtain a linear expression construct by amplifying it using a nucleic acid amplification technique (*e.g.* by PCR).

The expression constructs used in the systems of the invention may be non-bacterial expression constructs. This means that the construct can drive expression in a eukaryotic cell of viral RNA segments encoded therein, but it does not include components which would be required for propagation of the construct in bacteria. Thus the construct will not include a bacterial origin of replication (ori), and usually will not include a bacterial selection marker (e.g. an antibiotic resistance marker). Such expression constructs are described in reference 13 which is incorporated by reference.

The expression constructs may be prepared by chemical synthesis. The expression constructs may either be prepared entirely by chemical synthesis or in part. Suitable methods for preparing expression constructs by chemical synthesis are described, for example, in reference 13 which is incorporated by reference.

The expression constructs of the invention can be introduced into host cells using any technique known to those of skill in the art. For example, expression constructs of the invention can be introduced into host cells by employing electroporation, DEAE-dextran, calcium phosphate precipitation, liposomes, microinjection, or microparticle-bombardment.

Cells

The culture host for use in the present invention can be any eukaryotic cell that can produce the virus of interest. The invention will typically use a cell line although, for example, primary cells may be used as an alternative. The cell will typically be mammalian. Suitable mammalian cells include, but are not limited to, hamster, cattle, primate (including humans and monkeys) and dog cells. Various cell types may be used, such as kidney cells, fibroblasts, retinal cells, lung cells, *etc.* Examples of suitable hamster cells are the cell lines having the names BHK21 or HKCC. Suitable monkey cells are e.g. African green monkey cells, such as kidney cells as in the Vero cell line [14-16]. Suitable dog cells are *e.g.* kidney cells, as in the CLDK and MDCK cell lines.

Further suitable cells include, but are not limited to: CHO; 293T; BHK; MRC 5; PER.C6 [17]; FRhL2; WI-38; etc. Suitable cells are widely available e.g. from the American Type Cell Culture (ATCC) collection [18], from the Coriell Cell Repositories [19], or from the European Collection of Cell Cultures (ECACC). For example, the ATCC supplies various different Vero cells under catalogue numbers CCL 81, CCL 81.2, CRL 1586 and CRL-1587, and it supplies MDCK cells under catalogue number CCL 34. PER.C6 is available from the ECACC under deposit number 96022940.

Preferred cells for use in the invention are MDCK cells [20-22], derived from Madin Darby canine kidney. The original MDCK cells are available from the ATCC as CCL 34. It is preferred that derivatives of MDCK cells are used. Such derivatives were described, for instance, in reference 20 which discloses MDCK cells that were adapted for growth in suspension culture ('MDCK 33016' or '33016-PF', deposited as DSM ACC 2219; see also ref. 20). Furthermore, reference 23 discloses MDCK-derived cells that grow in suspension in serum free culture ('B-702', deposited as FERM BP-7449). In some embodiments, the MDCK cell line used may be tumorigenic. It is also envisioned to use non-tumorigenic MDCK cells. For example, reference 24 discloses non tumorigenic MDCK cells, including 'MDCK-S' (ATCC PTA-6500), 'MDCK-SF101' (ATCC PTA-6501), 'MDCK-SF102' (ATCC PTA-6502) and 'MDCK-SF103' (ATCC PTA-6503). Reference 25 discloses MDCK cells with high susceptibility to infection, including 'MDCK.5F1' cells (ATCC CRL 12042).

It is possible to use a mixture of more than one cell type to practise the methods of the present invention. However, it is preferred that the methods of the invention are practised with a single cell

type *e.g.* with monoclonal cells. Preferably, the cells used in the methods of the present invention are from a single cell line. Furthermore, the same cell line may be used for reassorting the virus and for any subsequent propagation of the virus.

Preferably, the cells are cultured in the absence of serum, to avoid a common source of contaminants. Various serum-free media for eukaryotic cell culture are known to the person skilled in the art (e.g. Iscove's medium, ultra CHO medium (BioWhittaker), EX-CELL (JRH Biosciences)). Furthermore, protein-free media may be used (e.g. PF-CHO (JRH Biosciences)). Otherwise, the cells for replication can also be cultured in the customary serum-containing media (e.g. MEM or DMEM medium with 0.5% to 10% of fetal calf serum).

The cells may be in adherent culture or in suspension.

Conventional reassortment

Traditionally, influenza viruses are reassorted by co-infecting a culture host, usually eggs, with a donor strain and a vaccine strain. Reassortant viruses are selected by adding antibodies with specificity for the HA and/or NA proteins of the donor strain in order to select for reassortant viruses that contain the vaccine strain's HA and/or NA proteins. Over several passages of this treatment one can select for fast growing reassortant viruses containing the vaccine strain's HA and/or NA segments.

The invention is suitable for use in these methods. It can be easier to use vaccine strains with a different HA and/or NA subtype compared to the donor strain(s) as this facilitates selection for reassortant viruses. It is also possible, however, to use vaccine strains with the same HA and/or NA subtype as the donor strain(s) and in some aspects of the invention this preferred. In this case, antibodies with preferential specificity for the HA and/or NA proteins of the donor strain(s) should be available.

Virus preparation

In one embodiment, the invention provides a method for producing influenza viruses comprising steps of (a) infecting a culture host with a reassortant virus of the invention; (b) culturing the host from step (a) to produce the virus; and optionally (c) purifying the virus obtained in step (b).

The culture host may be cells or embryonated hen eggs. Where cells are used as a culture host in this aspect of the invention, it is known that cell culture conditions (*e.g.* temperature, cell density, pH value, *etc.*) are variable over a wide range subject to the cell line and the virus employed and can be adapted to the requirements of the application. The following information therefore merely represents guidelines.

As mentioned above, cells are preferably cultured in serum-free or protein-free media.

Multiplication of the cells can be conducted in accordance with methods known to those of skill in the art. For example, the cells can be cultivated in a perfusion system using ordinary support methods

like centrifugation or filtration. Moreover, the cells can be multiplied according to the invention in a fed-batch system before infection. In the context of the present invention, a culture system is referred to as a fed-batch system in which the cells are initially cultured in a batch system and depletion of nutrients (or part of the nutrients) in the medium is compensated by controlled feeding of concentrated nutrients. It can be advantageous to adjust the pH value of the medium during multiplication of cells before infection to a value between pH 6.6 and pH 7.8 and especially between a value between pH 7.2 and pH 7.3. Culturing of cells preferably occurs at a temperature between 30 and 40°C. When culturing the infected cells (step ii), the cells are preferably cultured at a temperature of between 30 °C and 36°C or between 32 °C and 34 °C or at 33°C. This is particularly preferred, as it has been shown that incubation of infected cells in this temperature range results in production of a virus that results in improved efficacy when formulated into a vaccine [26].

Oxygen partial pressure can be adjusted during culturing before infection preferably at a value between 25% and 95% and especially at a value between 35% and 60%. The values for the oxygen partial pressure stated in the context of the invention are based on saturation of air. Infection of cells occurs at a cell density of preferably about 8-25x10^5 cells/mL in the batch system or preferably about 5-20x10^6 cells/mL in the perfusion system. The cells can be infected with a viral dose (MOI value, "multiplicity of infection"; corresponds to the number of virus units per cell at the time of infection) between 10^{-8} and 10, preferably between 0.0001 and 0.5.

Virus may be grown on cells in adherent culture or in suspension. Microcarrier cultures can be used. In some embodiments, the cells may thus be adapted for growth in suspension.

The methods according to the invention also include harvesting and isolation of viruses or the proteins generated by them. During isolation of viruses or proteins, the cells are separated from the culture medium by standard methods like separation, filtration or ultrafiltration. The viruses or the proteins are then concentrated according to methods sufficiently known to those skilled in the art, like gradient centrifugation, filtration, precipitation, chromatography, etc., and then purified. It is also preferred according to the invention that the viruses are inactivated during or after purification. Virus inactivation can occur, for example, by β-propiolactone or formaldehyde at any point within the purification process.

The culture host may be eggs. The current standard method for influenza virus growth for vaccines uses embryonated SPF hen eggs, with virus being purified from the egg contents (allantoic fluid). It is also possible to passage a virus through eggs and subsequently propagate it in cell culture and vice versa.

Vaccine

The invention utilises virus produced according to the method to produce vaccines.

Vaccines (particularly for influenza virus) are generally based either on live virus or on inactivated virus. Inactivated vaccines may be based on whole virions, 'split' virions, or on purified surface

antigens. Antigens can also be presented in the form of virosomes. The invention can be used for manufacturing any of these types of vaccine.

Where an inactivated virus is used, the vaccine may comprise whole virion, split virion, or purified surface antigens (for influenza, including hemagglutinin and, usually, also including neuraminidase). Chemical means for inactivating a virus include treatment with an effective amount of one or more of the following agents: detergents, formaldehyde, β-propiolactone, methylene blue, psoralen, carboxyfullerene (C60), binary ethylamine, acetyl ethyleneimine, or combinations thereof. Non-chemical methods of viral inactivation are known in the art, such as for example UV light or gamma irradiation.

Virions can be harvested from virus-containing fluids, e.g. allantoic fluid or cell culture supernatant, by various methods. For example, a purification process may involve zonal centrifugation using a linear sucrose gradient solution that includes detergent to disrupt the virions. Antigens may then be purified, after optional dilution, by diafiltration.

Split virions are obtained by treating purified virions with detergents (*e.g.* ethyl ether, polysorbate 80, deoxycholate, tri-*N*-butyl phosphate, Triton X-100, Triton N101, cetyltrimethylammonium bromide, Tergitol NP9, *etc.*) to produce subvirion preparations, including the 'Tween-ether' splitting process. Methods of splitting influenza viruses, for example are well known in the art *e.g.* see refs. 27-32, *etc.* Splitting of the virus is typically carried out by disrupting or fragmenting whole virus, whether infectious or non-infectious with a disrupting concentration of a splitting agent. The disruption results in a full or partial solubilisation of the virus proteins, altering the integrity of the virus. Preferred splitting agents are non-ionic and ionic (*e.g.* cationic) surfactants *e.g.* alkylglycosides, alkylthioglycosides, acyl sugars, sulphobetaines, betains, polyoxyethylenealkylethers, N,N-dialkyl-Glucamides, Hecameg, alkylphenoxy-polyethoxyethanols, NP9, quaternary ammonium compounds, sarcosyl, CTABs (cetyl trimethyl ammonium bromides), tri-*N*-butyl phosphate, Cetavlon, myristyltrimethylammonium salts, lipofectin, lipofectamine, and DOT-MA, the octyl- or nonylphenoxy polyoxyethanols (*e.g.* the Triton surfactants, such as Triton X-100 or Triton N101), polyoxyethylene sorbitan esters (the Tween surfactants), polyoxyethylene ethers, polyoxyethlene esters, *etc.* One useful splitting procedure uses the consecutive effects of sodium deoxycholate and formaldehyde, and splitting can take place during initial virion purification (e.g. in a sucrose density gradient solution). Thus a splitting process can involve clarification of the virion-containing material (to remove non-virion material), concentration of the harvested virions (*e.g.* using an adsorption method, such as $CaHPO_4$ adsorption), separation of whole virions from non-virion material, splitting of virions using a splitting agent in a density gradient centrifugation step (*e.g.* using a sucrose gradient that contains a splitting agent such as sodium deoxycholate), and then filtration (*e.g.* ultrafiltration) to remove undesired materials. Split virions can usefully be resuspended in sodium phosphate-buffered isotonic sodium chloride solution. Examples of split influenza vaccines are the BEGRIVAC™, FLUARIX™, FLUZONE™ and FLUSHIELD™ products.

Purified influenza virus surface antigen vaccines comprise the surface antigens hemagglutinin and, typically, also neuraminidase. Processes for preparing these proteins in purified form are well known in the art. The FLUVIRIN™, AGRIPPAL™ and INFLUVAC™ products are influenza subunit vaccines.

Another form of inactivated antigen is the virosome [33] (nucleic acid free viral-like liposomal particles). Virosomes can be prepared by solubilization of virus with a detergent followed by removal of the nucleocapsid and reconstitution of the membrane containing the viral glycoproteins. An alternative method for preparing virosomes involves adding viral membrane glycoproteins to excess amounts of phospholipids, to give liposomes with viral proteins in their membrane.

The methods of the invention may also be used to produce live vaccines. Such vaccines are usually prepared by purifying virions from virion-containing fluids. For example, the fluids may be clarified by centrifugation, and stabilized with buffer (*e.g.* containing sucrose, potassium phosphate, and monosodium glutamate). Various forms of influenza virus vaccine are currently available (e.g. see chapters 17 & 18 of reference 34). Live virus vaccines include MedImmune's FLUMIST™ product (trivalent live virus vaccine).

The virus may be attenuated. The virus may be temperature-sensitive. The virus may be cold-adapted. These three features are particularly useful when using live virus as an antigen.

HA is the main immunogen in current inactivated influenza vaccines, and vaccine doses are standardised by reference to HA levels, typically measured by SRID. Existing vaccines typically contain about 15μg of HA per strain, although lower doses can be used *e.g.* for children, or in pandemic situations, or when using an adjuvant. Fractional doses such as ½ (*i.e.* 7.5μg HA per strain), ¼ and $^1/_8$ have been used, as have higher doses (*e.g.* 3x or 9x doses [35,36]). Thus vaccines may include between 0.1 and 150μg of HA per influenza strain, preferably between 0.1 and 50μg *e.g.* 0.1-20μg, 0.1-15μg, 0.1-10μg, 0.1-7.5μg, 0.5-5μg, *etc.* Particular doses include *e.g.* about 45, about 30, about 15, about 10, about 7.5, about 5, about 3.8, about 3.75, about 1.9, about 1.5, *etc.* per strain.

For live vaccines, dosing is measured by median tissue culture infectious dose ($TCID_{50}$) rather than HA content, and a $TCID_{50}$ of between 10^6 and 10^8 (preferably between $10^{6.5}$-$10^{7.5}$) per strain is typical.

Influenza strains used with the invention may have a natural HA as found in a wild-type virus, or a modified HA. For instance, it is known to modify HA to remove determinants (*e.g.* hyper-basic regions around the HA1/HA2 cleavage site) that cause a virus to be highly pathogenic in avian species. The use of reverse genetics facilitates such modifications.

As well as being suitable for immunizing against inter-pandemic strains, the compositions of the invention are particularly useful for immunizing against pandemic or potentially-pandemic strains. The invention is suitable for vaccinating humans as well as non-human animals.

Other strains whose antigens can usefully be included in the compositions are strains which are resistant to antiviral therapy (*e.g.* resistant to oseltamivir [37] and/or zanamivir), including resistant pandemic strains [38].

Compositions of the invention may include antigen(s) from one or more (*e.g.* 1, 2, 3, 4 or more) influenza virus strains, including influenza A virus and/or influenza B virus provided that at least one influenza strain is a reassortant influenza strain of the invention. Compositions wherein at least two, at least three or all of the antigens are from reassortant influenza strains of the invention are also envisioned. Where a vaccine includes more than one strain of influenza, the different strains are typically grown separately and are mixed after the viruses have been harvested and antigens have been prepared. Thus a process of the invention may include the step of mixing antigens from more than one influenza strain. A trivalent vaccine is typical, including antigens from two influenza A virus strains and one influenza B virus strain. A tetravalent vaccine is also useful [39], including antigens from two influenza A virus strains and two influenza B virus strains, or three influenza A virus strains and one influenza B virus strain.

Pharmaceutical compositions

Vaccine compositions manufactured according to the invention are pharmaceutically acceptable. They usually include components in addition to the antigens *e.g.* they typically include one or more pharmaceutical carrier(s) and/or excipient(s). As described below, adjuvants may also be included. A thorough discussion of such components is available in reference 40.

Vaccine compositions will generally be in aqueous form. However, some vaccines may be in dry form, *e.g.* in the form of injectable solids or dried or polymerized preparations on a patch.

Vaccine compositions may include preservatives such as thiomersal or 2-phenoxyethanol. It is preferred, however, that the vaccine should be substantially free from (*i.e.* less than 5μg/ml) mercurial material *e.g.* thiomersal-free [31,41]. Vaccines containing no mercury are more preferred. An α-tocopherol succinate can be included as an alternative to mercurial compounds [31]. Preservative-free vaccines are particularly preferred.

To control tonicity, it is preferred to include a physiological salt, such as a sodium salt. Sodium chloride (NaCl) is preferred, which may be present at between 1 and 20 mg/ml. Other salts that may be present include potassium chloride, potassium dihydrogen phosphate, disodium phosphate dehydrate, magnesium chloride, calcium chloride, *etc.*

Vaccine compositions will generally have an osmolality of between 200 mOsm/kg and 400 mOsm/kg, preferably between 240-360 mOsm/kg, and will more preferably fall within the range of 290-310 mOsm/kg. Osmolality has previously been reported not to have an impact on pain caused by vaccination [42], but keeping osmolality in this range is nevertheless preferred.

Vaccine compositions may include one or more buffers. Typical buffers include: a phosphate buffer; a Tris buffer; a borate buffer; a succinate buffer; a histidine buffer (particularly with an aluminum hydroxide adjuvant); or a citrate buffer. Buffers will typically be included in the 5-20mM range.

The pH of a vaccine composition will generally be between 5.0 and 8.1, and more typically between 6.0 and 8.0 *e.g.* 6.5 and 7.5, or between 7.0 and 7.8. A process of the invention may therefore include a step of adjusting the pH of the bulk vaccine prior to packaging.

The vaccine composition is preferably sterile. The vaccine composition is preferably non-pyrogenic *e.g.* containing <1 EU (endotoxin unit, a standard measure) per dose, and preferably <0.1 EU per dose. The vaccine composition is preferably gluten-free.

Vaccine compositions of the invention may include detergent *e.g.* a polyoxyethylene sorbitan ester surfactant (known as 'Tweens'), an octoxynol (such as octoxynol-9 (Triton X-100) or t-octylphenoxypolyethoxyethanol), a cetyl trimethyl ammonium bromide ('CTAB'), or sodium deoxycholate, particularly for a split or surface antigen vaccine. The detergent may be present only at trace amounts. Thus the vaccine may include less than 1mg/ml of each of octoxynol-10 and polysorbate 80. Other residual components in trace amounts could be antibiotics (*e.g.* neomycin, kanamycin, polymyxin B).

A vaccine composition may include material for a single immunisation, or may include material for multiple immunisations (*i.e.* a 'multidose' kit). The inclusion of a preservative is preferred in multidose arrangements. As an alternative (or in addition) to including a preservative in multidose compositions, the compositions may be contained in a container having an aseptic adaptor for removal of material.

Influenza vaccines are typically administered in a dosage volume of about 0.5ml, although a half dose (*i.e.* about 0.25ml) may be administered to children.

Compositions and kits are preferably stored at between 2°C and 8ºC. They should not be frozen. They should ideally be kept out of direct light.

Host cell DNA

Where virus has been isolated and/or grown on a cell line, it is standard practice to minimize the amount of residual cell line DNA in the final vaccine, in order to minimize any potential oncogenic activity of the DNA.

Thus a vaccine composition prepared according to the invention preferably contains less than 10ng (preferably less than 1ng, and more preferably less than 100pg) of residual host cell DNA per dose, although trace amounts of host cell DNA may be present.

It is preferred that the average length of any residual host cell DNA is less than 500bp *e.g.* less than 400bp, less than 300bp, less than 200bp, less than 100bp, *etc.*

Contaminating DNA can be removed during vaccine preparation using standard purification procedures *e.g.* chromatography, *etc.* Removal of residual host cell DNA can be enhanced by nuclease treatment *e.g.* by using a DNase. A convenient method for reducing host cell DNA contamination is disclosed in references 43 & 44, involving a two-step treatment, first using a DNase (*e.g.* Benzonase), which may be used during viral growth, and then a cationic detergent (*e.g.* CTAB), which may be used during virion disruption. Treatment with an alkylating agent, such as β-propiolactone, can also be used to remove host cell DNA, and advantageously may also be used to inactivate virions [45].

Adjuvants

Compositions of the invention may advantageously include an adjuvant, which can function to enhance the immune responses (humoral and/or cellular) elicited in a subject who receives the composition. Preferred adjuvants comprise oil-in-water emulsions. Various such adjuvants are known, and they typically include at least one oil and at least one surfactant, with the oil(s) and surfactant(s) being biodegradable (metabolisable) and biocompatible. The oil droplets in the emulsion are generally less than 5μm in diameter, and ideally have a sub-micron diameter, with these small sizes being achieved with a microfluidiser to provide stable emulsions. Droplets with a size less than 220nm are preferred as they can be subjected to filter sterilization.

The emulsion can comprise oils such as those from an animal (such as fish) or vegetable source. Sources for vegetable oils include nuts, seeds and grains. Peanut oil, soybean oil, coconut oil, and olive oil, the most commonly available, exemplify the nut oils. Jojoba oil can be used *e.g.* obtained from the jojoba bean. Seed oils include safflower oil, cottonseed oil, sunflower seed oil, sesame seed oil and the like. In the grain group, corn oil is the most readily available, but the oil of other cereal grains such as wheat, oats, rye, rice, teff, triticale and the like may also be used. 6-10 carbon fatty acid esters of glycerol and 1,2-propanediol, while not occurring naturally in seed oils, may be prepared by hydrolysis, separation and esterification of the appropriate materials starting from the nut and seed oils. Fats and oils from mammalian milk are metabolizable and may therefore be used in the practice of this invention. The procedures for separation, purification, saponification and other means necessary for obtaining pure oils from animal sources are well known in the art. Most fish contain metabolizable oils which may be readily recovered. For example, cod liver oil, shark liver oils, and whale oil such as spermaceti exemplify several of the fish oils which may be used herein. A number of branched chain oils are synthesized biochemically in 5-carbon isoprene units and are generally referred to as terpenoids. Shark liver oil contains a branched, unsaturated terpenoids known as squalene, 2,6,10,15,19,23-hexamethyl-2,6,10,14,18,22-tetracosahexaene, which is particularly preferred herein. Squalane, the saturated analog to squalene, is also a preferred oil. Fish oils, including squalene and squalane, are readily available from commercial sources or may be obtained by methods known in the art. Another preferred oil is α-tocopherol (see below).

Mixtures of oils can be used.

Surfactants can be classified by their 'HLB' (hydrophile/lipophile balance). Preferred surfactants of the invention have a HLB of at least 10, preferably at least 15, and more preferably at least 16. The invention can be used with surfactants including, but not limited to: the polyoxyethylene sorbitan esters surfactants (commonly referred to as the Tweens), especially polysorbate 20 and polysorbate 80; copolymers of ethylene oxide (EO), propylene oxide (PO), and/or butylene oxide (BO), sold under the DOWFAX™ tradename, such as linear EO/PO block copolymers; octoxynols, which can vary in the number of repeating ethoxy (oxy-1,2-ethanediyl) groups, with octoxynol-9 (Triton X-100, or t-octylphenoxypolyethoxyethanol) being of particular interest; (octylphenoxy)polyethoxyethanol (IGEPAL CA-630/NP-40); phospholipids such as phosphatidylcholine (lecithin); nonylphenol ethoxylates, such as the Tergitol™ NP series; polyoxyethylene fatty ethers derived from lauryl, cetyl, stearyl and oleyl alcohols (known as Brij surfactants), such as triethyleneglycol monolauryl ether (Brij 30); and sorbitan esters (commonly known as the SPANs), such as sorbitan trioleate (Span 85) and sorbitan monolaurate. Non-ionic surfactants are preferred. Preferred surfactants for including in the emulsion are Tween 80 (polyoxyethylene sorbitan monooleate), Span 85 (sorbitan trioleate), lecithin and Triton X-100.

Mixtures of surfactants can be used *e.g.* Tween 80/Span 85 mixtures. A combination of a polyoxyethylene sorbitan ester such as polyoxyethylene sorbitan monooleate (Tween 80) and an octoxynol such as t-octylphenoxypolyethoxyethanol (Triton X-100) is also suitable. Another useful combination comprises laureth 9 plus a polyoxyethylene sorbitan ester and/or an octoxynol.

Preferred amounts of surfactants (% by weight) are: polyoxyethylene sorbitan esters (such as Tween 80) 0.01 to 1%, in particular about 0.1 %; octyl- or nonylphenoxy polyoxyethanols (such as Triton X-100, or other detergents in the Triton series) 0.001 to 0.1 %, in particular 0.005 to 0.02%; polyoxyethylene ethers (such as laureth 9) 0.1 to 20 %, preferably 0.1 to 10 % and in particular 0.1 to 1% or about 0.5%.

Where the vaccine contains a split virus, it is preferred that it contains free surfactant in the aqueous phase. This is advantageous as the free surfactant can exert a 'splitting effect' on the antigen, thereby disrupting any unsplit virions and/or virion aggregates that might otherwise be present. This can improve the safety of split virus vaccines [46].

Preferred emulsions have an average droplets size of <1μm *e.g.* ≤750nm, ≤500nm, ≤400nm, ≤300nm, ≤250nm, ≤220nm, ≤200nm, or smaller. These droplet sizes can conveniently be achieved by techniques such as microfluidisation.

Specific oil-in-water emulsion adjuvants useful with the invention include, but are not limited to:

- A submicron emulsion of squalene, Tween 80, and Span 85. The composition of the emulsion by volume can be about 5% squalene, about 0.5% polysorbate 80 and about 0.5% Span 85. In weight terms, these ratios become 4.3% squalene, 0.5% polysorbate 80 and 0.48% Span 85. This adjuvant is known as 'MF59' [47-49], as described in more detail in Chapter 10 of ref. 50

and chapter 12 of ref. 51. The MF59 emulsion advantageously includes citrate ions *e.g.* 10mM sodium citrate buffer.

- An emulsion comprising squalene, a tocopherol, and polysorbate 80. The emulsion may include phosphate buffered saline. These emulsions may have by volume from 2 to 10% squalene, from 2 to 10% tocopherol and from 0.3 to 3% polysorbate 80, and the weight ratio of squalene:tocopherol is preferably <1 (e.g. 0.90) as this can provide a more stable emulsion. Squalene and polysorbate 80 may be present volume ratio of about 5:2 or at a weight ratio of about 11:5. Thus the three components (squalene, tocopherol, polysorbate 80) may be present at a weight ratio of 1068:1186:485 or around 55:61:25. One such emulsion ('AS03') can be made by dissolving Tween 80 in PBS to give a 2% solution, then mixing 90ml of this solution with a mixture of (5g of DL α tocopherol and 5ml squalene), then microfluidising the mixture. The resulting emulsion may have submicron oil droplets e.g. with an average diameter of between 100 and 250nm, preferably about 180nm. The emulsion may also include a 3-de-O-acylated monophosphoryl lipid A (3d MPL). Another useful emulsion of this type may comprise, per human dose, 0.5-10 mg squalene, 0.5-11 mg tocopherol, and 0.1-4 mg polysorbate 80 [52] *e.g.* in the ratios discussed above.
- An emulsion of squalene, a tocopherol, and a Triton detergent (*e.g.* Triton X-100). The emulsion may also include a 3d-MPL (see below). The emulsion may contain a phosphate buffer.
- An emulsion comprising a polysorbate (*e.g.* polysorbate 80), a Triton detergent (*e.g.* Triton X-100) and a tocopherol (*e.g.* an α-tocopherol succinate). The emulsion may include these three components at a mass ratio of about 75:11:10 (*e.g.* 750μg/ml polysorbate 80, 110μg/ml Triton X-100 and 100μg/ml α-tocopherol succinate), and these concentrations should include any contribution of these components from antigens. The emulsion may also include squalene. The emulsion may also include a 3d-MPL (see below). The aqueous phase may contain a phosphate buffer.
- An emulsion of squalane, polysorbate 80 and poloxamer 401 ("Pluronic™ L121"). The emulsion can be formulated in phosphate buffered saline, pH 7.4. This emulsion is a useful delivery vehicle for muramyl dipeptides, and has been used with threonyl-MDP in the "SAF-1" adjuvant [53] (0.05-1% Thr-MDP, 5% squalane, 2.5% Pluronic L121 and 0.2% polysorbate 80). It can also be used without the Thr-MDP, as in the "AF" adjuvant [54] (5% squalane, 1.25% Pluronic L121 and 0.2% polysorbate 80). Microfluidisation is preferred.
- An emulsion comprising squalene, an aqueous solvent, a polyoxyethylene alkyl ether hydrophilic nonionic surfactant (*e.g.* polyoxyethylene (12) cetostearyl ether) and a hydrophobic nonionic surfactant (*e.g.* a sorbitan ester or mannide ester, such as sorbitan monoleate or 'Span 80'). The emulsion is preferably thermoreversible and/or has at least 90% of the oil droplets (by volume) with a size less than 200 nm [55]. The emulsion may also

include one or more of: alditol; a cryoprotective agent (*e.g.* a sugar, such as dodecylmaltoside and/or sucrose); and/or an alkylpolyglycoside. The emulsion may include a TLR4 agonist [56]. Such emulsions may be lyophilized.

- An emulsion of squalene, poloxamer 105 and Abil-Care [57]. The final concentration (weight) of these components in adjuvanted vaccines are 5% squalene, 4% poloxamer 105 (pluronic polyol) and 2% Abil-Care 85 (Bis-PEG/PPG-16/16 PEG/PPG-16/16 dimethicone; caprylic/capric triglyceride).

- An emulsion having from 0.5-50% of an oil, 0.1-10% of a phospholipid, and 0.05-5% of a non-ionic surfactant. As described in reference 58, preferred phospholipid components are phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, phosphatidylglycerol, phosphatidic acid, sphingomyelin and cardiolipin. Submicron droplet sizes are advantageous.

- A submicron oil-in-water emulsion of a non-metabolisable oil (such as light mineral oil) and at least one surfactant (such as lecithin, Tween 80 or Span 80). Additives may be included, such as QuilA saponin, cholesterol, a saponin-lipophile conjugate (such as GPI-0100, described in reference 59, produced by addition of aliphatic amine to desacylsaponin via the carboxyl group of glucuronic acid), dimethyidioctadecylammonium bromide and/or N,N-dioctadecyl-N,N-bis (2-hydroxyethyl)propanediamine.

- An emulsion in which a saponin (*e.g.* QuilA or QS21) and a sterol (*e.g.* a cholesterol) are associated as helical micelles [60].

- An emulsion comprising a mineral oil, a non-ionic lipophilic ethoxylated fatty alcohol, and a non-ionic hydrophilic surfactant (*e.g.* an ethoxylated fatty alcohol and/or polyoxyethylene-polyoxypropylene block copolymer) [61].

- An emulsion comprising a mineral oil, a non-ionic hydrophilic ethoxylated fatty alcohol, and a non-ionic lipophilic surfactant (*e.g.* an ethoxylated fatty alcohol and/or polyoxyethylene-polyoxypropylene block copolymer) [61].

In some embodiments an emulsion may be mixed with antigen extemporaneously, at the time of delivery, and thus the adjuvant and antigen may be kept separately in a packaged or distributed vaccine, ready for final formulation at the time of use. In other embodiments an emulsion is mixed with antigen during manufacture, and thus the composition is packaged in a liquid adjuvanted form. The antigen will generally be in an aqueous form, such that the vaccine is finally prepared by mixing two liquids. The volume ratio of the two liquids for mixing can vary (*e.g.* between 5:1 and 1:5) but is generally about 1:1. Where concentrations of components are given in the above descriptions of specific emulsions, these concentrations are typically for an undiluted composition, and the concentration after mixing with an antigen solution will thus decrease.

Packaging of vaccine compositions

Suitable containers for compositions of the invention (or kit components) include vials, syringes (*e.g.* disposable syringes), nasal sprays, *etc*. These containers should be sterile.

Where a composition/component is located in a vial, the vial is preferably made of a glass or plastic material. The vial is preferably sterilized before the composition is added to it. To avoid problems with latex-sensitive patients, vials are preferably sealed with a latex-free stopper, and the absence of latex in all packaging material is preferred. The vial may include a single dose of vaccine, or it may include more than one dose (a 'multidose' vial) *e.g.* 10 doses. Preferred vials are made of colourless glass.

A vial can have a cap (*e.g.* a Luer lock) adapted such that a pre-filled syringe can be inserted into the cap, the contents of the syringe can be expelled into the vial (*e.g.* to reconstitute lyophilised material therein), and the contents of the vial can be removed back into the syringe. After removal of the syringe from the vial, a needle can then be attached and the composition can be administered to a patient. The cap is preferably located inside a seal or cover, such that the seal or cover has to be removed before the cap can be accessed. A vial may have a cap that permits aseptic removal of its contents, particularly for multidose vials.

Where a component is packaged into a syringe, the syringe may have a needle attached to it. If a needle is not attached, a separate needle may be supplied with the syringe for assembly and use. Such a needle may be sheathed. Safety needles are preferred. 1-inch 23-gauge, 1-inch 25-gauge and 5/8-inch 25-gauge needles are typical. Syringes may be provided with peel-off labels on which the lot number, influenza season and expiration date of the contents may be printed, to facilitate record keeping. The plunger in the syringe preferably has a stopper to prevent the plunger from being accidentally removed during aspiration. The syringes may have a latex rubber cap and/or plunger. Disposable syringes contain a single dose of vaccine. The syringe will generally have a tip cap to seal the tip prior to attachment of a needle, and the tip cap is preferably made of a butyl rubber. If the syringe and needle are packaged separately then the needle is preferably fitted with a butyl rubber shield. Preferred syringes are those marketed under the trade name "Tip-Lok"™.

Containers may be marked to show a half-dose volume *e.g.* to facilitate delivery to children. For instance, a syringe containing a 0.5ml dose may have a mark showing a 0.25ml volume.

Where a glass container (*e.g.* a syringe or a vial) is used, then it is preferred to use a container made from a borosilicate glass rather than from a soda lime glass.

A kit or composition may be packaged (*e.g.* in the same box) with a leaflet including details of the vaccine *e.g.* instructions for administration, details of the antigens within the vaccine, *etc*. The instructions may also contain warnings *e.g.* to keep a solution of adrenaline readily available in case of anaphylactic reaction following vaccination, *etc*.

Methods of treatment, and administration of the vaccine

The invention provides a vaccine manufactured according to the invention. These vaccine compositions are suitable for administration to human or non-human animal subjects, such as pigs or birds, and the invention provides a method of raising an immune response in a subject, comprising the step of administering a composition of the invention to the subject. The invention also provides a composition of the invention for use as a medicament, and provides the use of a composition of the invention for the manufacture of a medicament for raising an immune response in a subject.

The immune response raised by these methods and uses will generally include an antibody response, preferably a protective antibody response. Methods for assessing antibody responses, neutralising capability and protection after influenza virus vaccination are well known in the art. Human studies have shown that antibody titers against hemagglutinin of human influenza virus are correlated with protection (a serum sample hemagglutination-inhibition titer of about 30–40 gives around 50% protection from infection by a homologous virus) [62]. Antibody responses are typically measured by hemagglutination inhibition, by microneutralisation, by single radial immunodiffusion (SRID), and/or by single radial hemolysis (SRH). These assay techniques are well known in the art.

Compositions of the invention can be administered in various ways. The most preferred immunisation route is by intramuscular injection (*e.g.* into the arm or leg), but other available routes include subcutaneous injection, intranasal [63-65], oral [66], intradermal [67,68], transcutaneous, transdermal [69], *etc.*

Vaccines prepared according to the invention may be used to treat both children and adults. Influenza vaccines are currently recommended for use in pediatric and adult immunisation, from the age of 6 months. Thus a human subject may be less than 1 year old, 1-5 years old, 5-15 years old, 15-55 years old, or at least 55 years old. Preferred subjects for receiving the vaccines are the elderly (*e.g.* ≥50 years old, ≥60 years old, and preferably ≥65 years), the young (*e.g.* ≤5 years old), hospitalised subjects, healthcare workers, armed service and military personnel, pregnant women, the chronically ill, immunodeficient subjects, subjects who have taken an antiviral compound (*e.g.* an oseltamivir or zanamivir compound; see below) in the 7 days prior to receiving the vaccine, people with egg allergies and people travelling abroad. The vaccines are not suitable solely for these groups, however, and may be used more generally in a population. For pandemic strains, administration to all age groups is preferred.

Preferred compositions of the invention satisfy 1, 2 or 3 of the CPMP criteria for efficacy. In adults (18-60 years), these criteria are: (1) ≥70% seroprotection; (2) ≥40% seroconversion; and/or (3) a GMT increase of ≥2.5-fold. In elderly (>60 years), these criteria are: (1) ≥60% seroprotection; (2) ≥30% seroconversion; and/or (3) a GMT increase of ≥2-fold. These criteria are based on open label studies with at least 50 patients.

Treatment can be by a single dose schedule or a multiple dose schedule. Multiple doses may be used in a primary immunisation schedule and/or in a booster immunisation schedule. In a multiple dose schedule the various doses may be given by the same or different routes *e.g.* a parenteral prime and mucosal boost, a mucosal prime and parenteral boost, *etc.* Administration of more than one dose (typically two doses) is particularly useful in immunologically naïve patients *e.g.* for people who have never received an influenza vaccine before, or for vaccinating against a new HA subtype (as in a pandemic outbreak). Multiple doses will typically be administered at least 1 week apart (*e.g.* about 2 weeks, about 3 weeks, about 4 weeks, about 6 weeks, about 8 weeks, about 10 weeks, about 12 weeks, about 16 weeks, *etc.*).

Vaccines produced by the invention may be administered to patients at substantially the same time as (*e.g.* during the same medical consultation or visit to a healthcare professional or vaccination centre) other vaccines *e.g.* at substantially the same time as a measles vaccine, a mumps vaccine, a rubella vaccine, a MMR vaccine, a varicella vaccine, a MMRV vaccine, a diphtheria vaccine, a tetanus vaccine, a pertussis vaccine, a DTP vaccine, a conjugated *H.influenzae* type b vaccine, an inactivated poliovirus vaccine, a hepatitis B virus vaccine, a meningococcal conjugate vaccine (such as a tetravalent A-C-W135-Y vaccine), a respiratory syncytial virus vaccine, a pneumococcal conjugate vaccine, *etc.* Administration at substantially the same time as a pneumococcal vaccine and/or a meningococcal vaccine is particularly useful in elderly patients.

Similarly, vaccines of the invention may be administered to patients at substantially the same time as (*e.g.* during the same medical consultation or visit to a healthcare professional) an antiviral compound, and in particular an antiviral compound active against influenza virus (*e.g.* oseltamivir and/or zanamivir). These antivirals include neuraminidase inhibitors, such as a (3R,4R,5S)-4-acetylamino-5-amino-3(1-ethylpropoxy)-1-cyclohexene-1-carboxylic acid or 5-(acetylamino)-4-[(aminoiminomethyl)-amino]-2,6-anhydro-3,4,5-trideoxy-D-glycero-D-galactonon-2-enonic acid, including esters thereof (*e.g.* the ethyl esters) and salts thereof (*e.g.* the phosphate salts). A preferred antiviral is (3R,4R,5S)-4-acetylamino-5-amino-3(1-ethylpropoxy)-1-cyclohexene-1-carboxylic acid, ethyl ester, phosphate (1:1), also known as oseltamivir phosphate (TAMIFLU™).

General

The term "comprising" encompasses "including" as well as "consisting" *e.g.* a composition "comprising" X may consist exclusively of X or may include something additional *e.g.* X + Y.

The word "substantially" does not exclude "completely" *e.g.* a composition which is "substantially free" from Y may be completely free from Y. Where necessary, the word "substantially" may be omitted from the definition of the invention.

The term "about" in relation to a numerical value *x* is optional and means, for example, $x \pm 10\%$.

Unless specifically stated, a process comprising a step of mixing two or more components does not require any specific order of mixing. Thus components can be mixed in any order. Where there are

three components then two components can be combined with each other, and then the combination may be combined with the third component, *etc.*

The various steps of the methods may be carried out at the same or different times, in the same or different geographical locations, *e.g.* countries, and by the same or different people or entities.

Where animal (and particularly bovine) materials are used in the culture of cells, they should be obtained from sources that are free from transmissible spongiform encephalopathies (TSEs), and in particular free from bovine spongiform encephalopathy (BSE). Overall, it is preferred to culture cells in the total absence of animal-derived materials.

Where a compound is administered to the body as part of a composition then that compound may alternatively be replaced by a suitable prodrug.

References to a percentage sequence identity between two amino acid sequences means that, when aligned, that percentage of amino acids are the same in comparing the two sequences. This alignment and the percent homology or sequence identity can be determined using software programs known in the art, for example those described in section 7.7.18 of reference 70. A preferred alignment is determined by the Smith-Waterman homology search algorithm using an affine gap search with a gap open penalty of 12 and a gap extension penalty of 2, BLOSUM matrix of 62. The Smith-Waterman homology search algorithm is taught in reference 71.

References to a percentage sequence identity between two nucleic acid sequences mean that, when aligned, that percentage of bases are the same in comparing the two sequences. This alignment and the percent homology or sequence identity can be determined using software programs known in the art, for example those described in section 7.7.18 of reference 70. A preferred alignment program is GCG Gap (Genetics Computer Group, Wisconsin, Suite Version 10.1), preferably using default parameters, which are as follows: open gap = 3; extend gap = 1.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 illustrates virus titers (by Focus-Formation assay (FFA); Figure 1A) and HA titers (by Red Blood Cell Hemagglutination assay; Figure 1B) at different times post-infection of wt PR8 and PR8-X viruses grown in MDCK cells. The solid line in Figure 1A and hatched columns in Figure 1B represent results with wild-type PR8. The dotted line in Figure 1A and empty columns in Figure 1B represent results with wild-type PR8-X. The x-axis shows the hours post infection and the y-axis in figures 1A and 1B shows the virus titer (IU/ml) and HA titre, respectively.

Figure 2 illustrates virus titers (by FFA; Figure 2A) and HA titers (by Red Blood Cell Hemagglutination assay; Figure 2B) at different times post-infection of reverse genetics derived PR8 and PR8-X viruses grown in MDCK cells. The solid line in Figure 2A and hatched columns in Figure 2B represent results with PR8. The dotted line in Figure 2A and empty columns in Figure 2B

represent results with RG-derived PR8-X. The x-axis shows the hours post infection and the y-axis in figures 2A and 2B shows the virus titer (IU/ml) and HA titre, respectively.

Figure 3 compares virus titers (by FFA; Figure 3A) and HA titers (by Red Blood Cell Hemagglutination assay; Figure 3B) at different times post-infection in MDCK cells of reverse genetics-derived 6:2 reassortant viruses made with either PR8 or PR8-X backbone segments which contain the HA and NA segments from PR8-X. The solid line in Figure 3A and hatched columns in Figure 3B represent results with the PR8 backbone. The dotted line in Figure 3A and empty columns in Figure 3B represent results with the PR8-X backbone. The x-axis shows the hours post infection and the y-axis in figures 3A and 3B shows the virus titer (IU/ml) and HA titre, respectively.

Figure 4 compares virus titers by FFA (Figure 4A) and HA titers (by Red Blood Cell Hemagglutination assay; Figure 4B) at different times post-infection in MDCK cells of reverse genetics-derived 6:2 reassortant viruses made with either wt PR8 or PR8-X backbone segments which contain the HA and NA segments from a pandemic H1 strain (strain 1). The solid line in Figure 4A and hatched columns in Figure 4B represent results with the wt PR8 backbone. The dotted line in Figure 4A and empty columns in Figure 4B represent results with the PR8-X backbone. The x-axis shows the hours post infection and the y-axis in figures 4A and 4B shows the virus titer (IU/ml) and HA titre, respectively.

Figure 5 compares virus titers by a focus-formation assay (FFA) (Figure 5A) and HA titers (Figure 5B) at different times post-infection in MDCK cells of reverse genetics-derived 6:2 reassortant viruses made with either PR8 or PR8-X backbone segments which contain the HA and NA segments from 105p30. The solid line in Figure 5A and hatched columns in Figure 5B represent results with the wt PR8 backbone. The dotted line in Figure 5A and empty columns in Figure 5B represent results with the PR8-X backbone. The x-axis shows the hours post infection and the y-axis shows the virus titer (IU/ml).

Figure 6 illustrates virus titers by a focus-formation assay (FFA) at different times post-infection of wild-type PR8-X and 105p30 viruses (Figure 6A) or reverse genetics-derived PR8-X and 105p30 viruses (Figure 6B) grown in MDCK cells. In Figures 6A and 6B, the solid lines represent results with 105p30. The dotted lines represent results with PR8-X. The x-axis shows the hours post infection and the y-axis in figures 6A and 6B shows the virus titer (IU/ml) and HA titre, respectively.

Figure 7 shows the growth characteristics of reassortant viruses containing the backbone segments of the wt PR8 strain (line with triangles) or 105p30 strain (line with squares) and the HA and NA segments of a pandemic H1 influenza strain (strain 2). The x-axis in figures 7A and 7B indicates the hours post infection. The y-axis in Figure 7A shows the titreLog10 in FFU per mL. The y-axis in Figure 7B shows the titre log10 in virus particles per mL.

Figure 8 compares virus titers by a focus-formation assay (FFA) at different times post-infection in MDCK cells of reverse genetics-derived 6:2 reassortant viruses made with either 105p30 or PR8-X

backbone segments which contain the HA and NA segments from (A) a H1 strain (strain 1) or (B) a pandemic H1 strain (strain 2). The solid lines represent results with the 105p30 backbone. The dotted lines represent results with the PR8-X backbone. The x-axis shows the hours post infection and the y-axis shows the virus titer (IU/ml).

Figure 9 compares virus titers by a focus-formation assay (FFA) at different times post-infection in MDCK cells of reverse genetics-derived 6:2 reassortant viruses made with either the #17, #19, or PR8-X backbone in combination with the HA and NA segments from (A) a pandemic H1 strain (strain 3) or (B) a H3 (strain 1). In Figures 9A and 9B, the dotted lines with the circle markers represent results with the #17 backbone. The solid lines with diamond markers represent results with the #19 backbone. The dotted lines with square markers represent results with the PR8-X backbone. The x-axis shows the hours post infection and the y-axis shows the virus titer (IU/ml).

Figure 10 compares virus titers by a focus-formation assay (FFA) at different times post-infection in MDCK cells of a panel of different reverse genetics-derived 6:2 reassortant viruses made with either the chimeric #19 or PR8-X backbone plus the HA and NA segments from the following strains: (A) a pandemic H1 strain (strain 2), (B) a pandemic H1 strain (strain 4), (C) a H1 strain (strain 2), (D) a H1 strain (strain 3), or (E) a H3 strain (strain 2). In Figures 10A-E, the solid lines with the triangle markers represent results with the #19 backbone. The dotted lines with square markers represent results with the PR8-X backbone. The x-axis shows the hours post infection and the y-axis shows the virus titer (IU/ml).

Figure 11 compares HA yields (by lectin-capture ELISA) at 60hr post-infection in MDCK cells of different 6:2 reassortant viruses made with either the chimeric #19 (empty columns) or PR8-X backbone (solid columns) plus the HA and NA segments from the following strains: (A) a pandemic H1 strain (strain 2), (B) a pandemic H1 strain (strain 4), (C) a H3 strain (strain 1), or (D) a H3 strain (strain 2). Corresponding 6:2 reassortant viruses made by classical reassortment ("classical") with the wt PR8 backbone were included as controls (hatched columns). The y-axis shows the HA content in µg per mL.

Figure 12 shows the growth curves of reassortant influenza viruses comprising backbones 17, 18, 19 and 20 (as shown in table 1; line with diamonds, squares, triangles and crosses, respectively), a control comprising the same HA and NA segments from a H3 influenza strain (strain 1) but all backbone segments from PR8-X (line with circles) and the equivalent wildtype strain (line with plus sign). The x axis indicates the hours post infection (hpi) and the y-axis shows IU/mL.

Figure 13 shows the growth curve of reassortant influenza viruses comprising backbones 17 and 19 (line with diamonds and triangles, respectively) and the HA segments from a H3 influenza strain (strain 3), a control comprising the same HA and NA segments but all backbone segments from PR8-X (line with plus sign) and the equivalent wildtype strain (line with circles).

Figure 14 shows the results of a FFA (14(A) and 14 (C)) and HA-ELISA (14(B) and 14 (D)) assay using reassortant influenza viruses comprising backbone 19 (open box), PR8-X backbone (hatched box) and the wildtype influenza virus (dotted box) . Figures 14(A) and 14(B) show the results with a H1 influenza strain (strain 2) and figures 14(C) and (D) show the results with a H3 influenza virus strain. The y axis in figures 14(A) and (C) indicates the virus titre in IU/mL and the y axis in figures 14(B) and 14 (D) indicates HA in µg/mL.

Figure 15 is an alignment of the M1 viral segment of A/New Caledonia/20/99 (SEQ ID NO: 33) and 105p30 (SEQ ID NO: 45).

MODES FOR CARRYING OUT THE INVENTION

Development of new donor strains

In order to provide high-growth donor strains, the donor strain A/Puerto Rico/8/34 is passaged in MDCK 33016 cells five times. Using this method, the inventors were able to obtain the strain PR8-X which shows improved growth characteristics compared with the original strain.

The 105p30 influenza donor strain was provided by isolating an A/New Caledonia/20/1999 influenza virus from a clinical isolate in MDCK 33016 cells and passaging the virus 30 times. The resulting strain has a M segment with lysine in the position corresponding to amino acid 95 of SEQ ID NO: 33 when aligned to SEQ ID NO: 33.

Growth characteristics of wt PR8 and PR8-X viruses

In order to compare the growth characteristics of PR8-X and wt PR8 donor strains, the viral titre of these virus strains is measured in MDCK cells by focus-forming assays and hemagglutination assays.

Focus-Forming Assays (FFA)

For the FFA, uninfected MDCK cells are plated at a density of $1.8x10^4$ cells/well in 96 well plates in 100 µl of DMEM with 10% FCS. The next day, medium is aspirated and cells are infected with viruses in a volume of 50µl (viruses diluted in DMEM + 1% FCS). The cells are incubated at 37°C until the next day.

At several time points after infection, the medium is aspirated and the cells washed once with PBS. 50µl of ice-cold 50%/50% acetone-methanol is added to each well followed by incubation at -20°C for 30 minutes. The acetone mix is aspirated and the cells washed once with PBST (PBS + 0.1% Tween). 50µl of 2% BSA in PBS is added to each well followed by incubation at room temperature (RT) for 30 minutes. 50µl of a 1:6000 dilution of anti-NP is added in blocking buffer followed by incubation at RT for 1 hours. The antibody solution is aspirated and the cells washed three times with PBST. Secondary antibody (goat anti mouse) is added at a dilution 1:2000 in 50µl blocking buffer and the plate is incubated at RT for 1 hours. The antibody solution is aspirated and the cells washed three times with PBST. 50µl of KPL True Blue is added to each well and incubated for 10 minutes.

The reaction is stopped by aspirating the True-Blue and washing once with dH_2O. The water is aspirated and the cells are left to dry.

The results (Figure 1) show that the PR8-X strain can grow to higher titres in the same time frame compared to the wt PR8 strain from which it is derived.

Growth characteristics of reassortant viruses containing PR8-X or wt PR8 backbones

In order to test the suitability of the PR8-X strain as a donor strain for virus reassortment, reassortant viruses are produced by reverse genetics which contain the HA and NA proteins from a pandemic H1 strain and the other viral segments from either PR8-X or PR8. The viral titres of these reassortant viruses are determined by FFA and HA assays as described above. The results are shown in Figure 4.

The results indicate that reassortant viruses which contain viral segments from PR8-X grow faster in MDCK cells compared to reassortant viruses containing viral segments from the PR8/34 strain.

Growth characteristics of 105p30 strain compared with PR8-X

MDCK cells are infected with 105p30 and PR8-X at a moi of 10^{-3} and samples are taken at several time points after infection. The titre is determined by a FFA assay. The results show that 105p30 grows even faster in MDCK cells compared to PR8-X (Figure 6).

Growth characteristics of reassortant viruses containing 105p30 or wt PR8 backbones

In order to test the suitability of the 105p30 strain as a donor strain for virus reassortment, reverse genetics is used to produce reassortant viruses that contain the HA and NA segments from a pandemic H1 influenza strain and the backbone segments either from the 105p30 or the wt PR8 strain. MDCK cells are infected with the reassortant viruses at a moi of 10^{-3} and samples are taken 1 hour, 12 hours, 36 hours and 60 hours after infection. The titres are determined either by focus-forming assays or by determining the virus particles by real-time detection PCR. The reassortant viruses that contain the backbone segments from the 105p30 strain grow faster than the viruses that are reassorted with the backbone segments of the wt PR8 strain. This shows that the 105p30 strain is a good donor strain for producing fast-growing reassortant viruses (Figure 7).

Rescue of influenza viruses using backbone segments from two donor strains

The rescue efficiency of reassortant influenza viruses containing the HA and NA segments from a H3 influenza virus and backbone segments from the 105p30 and the PR8-X donor strains is tested in MDCK cells. The reassortant influenza viruses contain backbone segments of the 105p30 and the PR8-X donor strains, as indicated in the following table:

Table 1

Backbone #	**PB1**	**PB2**	**PA**	**NP**	**M**	**NS**
1	**PR8-X**	**PR8-X**	**PR8-X**	*105p30*	*105p30*	*105p30*

2	**PR8-X**	**PR8-X**	*105p30*	**PR8-X**	*105p30*	*105p30*
3	**PR8-X**	**PR8-X**	*105p30*	*105p30*	**PR8-X**	*105p30*
4	**PR8-X**	**PR8-X**	*105p30*	*105p30*	*105p30*	**PR8-X**
5	**PR8-X**	*105p30*	**PR8-X**	**PR8-X**	*105p30*	*105p30*
6	**PR8-X**	*105p30*	**PR8-X**	*105p30*	**PR8-X**	105p30
7	**PR8-X**	*105p30*	**PR8-X**	*105p30*	*105p30*	**PR8-X**
8	**PR8-X**	*105p30*	*105p30*	**PR8-X**	**PR8-X**	*105p30*
9	**PR8-X**	*105p30*	*105p30*	**PR8-X**	*105p30*	**PR8-X**
10	**PR8-X**	*105p30*	*105p30*	*105p30*	**PR8-X**	**PR8-X**
11	*105p30*	**PR8-X**	**PR8-X**	**PR8-X**	*105p30*	*105p30*
12	*105p30*	**PR8-X**	**PR8-X**	*105p30*	**PR8-X**	*105p30*
13	*105p30*	**PR8-X**	**PR8-X**	*105p30*	*105p30*	**PR8-X**
14	*105p30*	**PR8-X**	*105p30*	**PR8-X**	*105p30*	**PR8-X**
15	*105p30*	**PR8-X**	*105p30*	**PR8-X**	**PR8-X**	*105p30*
16	*105p30*	**PR8-X**	*105p30*	*105p30*	**PR8-X**	**PR8-X**
17	*105p30*	*105p30*	**PR8-X**	**PR8-X**	**PR8-X**	*105p30*
18	*105p30*	*105p30*	**PR8-X**	**PR8-X**	*105p30*	**PR8-X**
19	*105p30*	*105p30*	**PR8-X**	*105p30*	**PR8-X**	**PR8-X**
20	*105p30*	*105p30*	*105p30*	**PR8-X**	**PR8-X**	**PR8-X**

Reassortant influenza viruses which contain a backbone according to number 3, 4, 10, 11, 14 and 16-20 are rescuable. Influenza viruses which contain backbones number 3, 4, 10, 11 or 16 achieve viral titres of less than 10^2 IU/mL. Influenza viruses containing backbone numbers 17 and 18 achieve viral titres between 10^2 and 10^6 IU/mL and influenza viruses having backbone numbers 19 and 20 even achieve titres of more than 10^6 IU/mL.

These data show that influenza viruses in which the PB1 and PB2 segments come from the same influenza donor strain can show a higher rescue efficiency compared with influenza viruses in which these segments come from different influenza donor strains.

Growth characteristics of reassortant influenza viruses containing backbone segments from two donor strains

Reassortant influenza strains are created which contain backbone numbers 17, 18, 19 and 20 (as shown in table 1 above) and the HA and NA segments from a H3 influenza strain (strain 1). As controls, the equivalent wildtype H3 influenza virus, and a reassortant influenza virus comprising the same HA and NA segments and all backbone segments from PR8-X are used.

Furthermore, reassortant influenza strains are produced which contain backbone numbers 17 and 19 and the HA and NA segments from either a second H3 influenza (strain 1) virus or a pandemic H1 influenza virus (strain 3). As controls for the H3 strain, the equivalent wildtype H3 (strain 2) influenza virus, and a reassortant influenza virus comprising the same HA and NA segments and all backbone segments from PR8-X is used. For the pandemic H1 influenza virus a reassortant influenza virus comprising the same HA and NA segments and all backbone segments from PR8-X is used.

The reassortant influenza viruses and the control viruses are grown in MDCK cells and the viral titre is measured by FFA at different time points. For the reassortant H3 viruses (strain 1) containing backbones 17, 19 and 20, and the H3 influenza viruses (strain 3) containing backbones 17 and 19, the influenza viruses containing backbone segments from two donor strains grow to higher titres compared with the wildtype virus and the reassortant virus which contains backbone segments from only a single donor strain (see figures 11 and 12).

For the pandemic H1 influenza virus, the reassortant influenza strains containing backbones 17 and 19 grow to higher titres compared with the control which contained all backbone segments from PR8-X (see figure 9).

The data show that reassortant influenza viruses which contain backbone segments from two different donor strains can show improved growth rates compared with reassortant influenza viruses which contain backbone segments from only a single donor strain.

The experiments were also repeated using reassortant influenza viruses which contain backbone 19 or the backbone segments from PR8-X in combination with the HA and NA segments from four different H1 strains or a H3 strain. The results are shown in Figure 10.

Reassortant influenza viruses with backbone segments from two different donor strains give higher yields

To test whether reassortant influenza viruses containing backbone segments from two different influenza donor strains can also provide higher yields, the HA yield of the reassortant strains is tested by HA-ELISA. To this end, the same reassortant influenza viruses as described above containing backbone # 19 and the HA/NA segments of the H3 (strain 2) and H1 influenza strains are used. As controls, the equivalent wildtype influenza viruses and reassortant influenza viruses comprising the

same HA and NA segments and all backbone segments from PR8-X are used. In addition, the viral titres are confirmed with a FFA assay.

The results confirm that the reassortant influenza strains which contain backbone segments from two different donor strains can grow to higher yields compared with influenza viruses which contained all backbones from PR8-X (see figures 13 (A) and (C)). Furthermore, reassortant influenza viruses comprising backbone segments from two donor strains also give higher HA yields (see figures 13(B) and (D)).

These data show that reassortant influenza viruses which contain backbone segments from two donor strains give higher yields compared with reassortant influenza viruses which contain backbone segments from only a single donor strains.

It will be understood that the invention has been described by way of example only and modifications may be made whilst remaining within the scope and spirit of the invention.

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THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A reassortant influenza A virus comprising backbone viral segments that are all from the same donor strain, wherein the reassortant influenza A virus is selected from:
   (i) a reassortant influenza A virus comprising backbone viral segments PA, PB1, PB2, NP, M and NS comprising nucleotide sequences set forth in SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21 and SEQ ID NO: 22 respectively;
   (ii) a reassortant influenza A virus comprising backbone viral segments PA, PB1, PB2, NP, M and NS comprising nucleotide sequences having at least 95% identity to nucleotide sequences set forth in SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21 and SEQ ID NO: 22 respectively;
   (iii) a reassortant influenza A virus comprising backbone viral segments PA, PB1, PB2, NP, M and NS comprising nucleotide sequences set forth in SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13 and SEQ ID NO: 14 respectively;
   (iv) a reassortant influenza A virus comprising backbone viral segments PA, PB1, PB2, NP, M and NS comprising nucleotide sequence having at least 95% identity to the nucleotide sequences set forth in SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13 and SEQ ID NO: 14 respectively, with the proviso that at least one of the backbone viral segments comprises a sequence which has 100% identity over its entire length to a sequence set forth in SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12 or SEQ ID NO: 14; and
   (v) a reassortant influenza A virus comprising backbone viral segments PA, PB1, PB2, NP, M and NS comprising nucleotide sequences encoding a viral backbone polypeptide having the same amino acid sequence as a polypeptide encoded by the backbone viral segments of any one of (i) to (iii).

2. A reassortant influenza A virus comprising the following backbone viral segments:
   (i) backbone viral segments comprising the nucleotide sequences set forth in SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, and SEQ ID NO: 14; or
   (ii) backbone viral segments comprising the nucleotide sequences set forth in SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, and SEQ ID NO: 22.

3. The reassortant influenza virus according to claim 1 or 2, wherein the reassortant influenza A virus grows to a higher viral titre than influenza strain A/Puerto Rico/8/34 when grown in MDCK cells for the same time and under the same culture conditions.

4. A reassortant influenza A virus that grows to a higher viral titre than influenza strain A/Puerto Rico/8/34 when grown in MDCK cells for the same time and under the same culture conditions,

said reassortant influenza A virus comprising backbone viral segments from two or more donor strains, wherein:

(a) each of two or three or four or five of said backbone viral segments comprises a nucleotide sequence selected from the group consisting of:
  (i) a sequence selected from the group consisting of: SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, and SEQ ID NO: 14; and
  (ii) a sequence encoding a viral backbone polypeptide having the same amino acid sequence as a polypeptide encoded by SEQ ID NO: 10 or SEQ ID NO: 11; or

(b) each of one or more of said backbone viral segments comprises a nucleotide sequence selected from the group consisting of:
  (i) a sequence selected from the group consisting of: SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, and SEQ ID NO: 14; and
  (ii) a sequence encoding a viral backbone polypeptide having the same amino acid sequence as a polypeptide encoded by SEQ ID NO: 10 or SEQ ID NO: 11.

5. The reassortant influenza A virus of claim 4, wherein the virus comprises at least one backbone viral segment comprising a sequence selected from the group consisting of:
(i) a nucleotide sequence selected from the group consisting of SEQ ID NOs 17-22;
(ii) a sequence having at least 95% identity to a sequence at (i); and
(iii) a sequence encoding a viral backbone polypeptide having the same amino acid sequence as a polypeptide encoded by a sequence at (i).

6. The reassortant influenza A virus of claim 5, wherein at least one backbone viral segment has a nucleotide sequence of SEQ ID NO: 17 or SEQ ID NO: 20.

7. The reassortant influenza A virus according to any one of claims 4 to 6, wherein the reassortant influenza A virus comprises backbone viral segment PB1 and backbone viral segment PB2 from the same donor strain.

8. The reassortant influenza A virus of claim 7, wherein:
(a) the PB1 viral segment comprises a nucleotide sequences selected from the group consisting of:
  (i) a sequence set forth in SEQ ID NO: 18;
  (ii) a sequence having at least 95% identity to SEQ ID NO: 18; and
  (iii) a sequence encoding a polypeptide having the same amino acid sequence as the polypeptide encoded by SEQ ID NO: 18; and
(b) the PB2 viral segment comprises a nucleotide sequences selected from the group consisting of:
  (i) a sequence set forth in SEQ ID NO: 19;
  (ii) a sequence having at least 95% identity to SEQ ID NO: 19; and

(iii) a sequence encoding a polypeptide having the same amino acid sequence as the polypeptide encoded by SEQ ID NO: 19.

9. The reassortant influenza A virus according to any one of claims 1 to 8, wherein the virus comprises the PB2 segment of SEQ ID NO: 19, the PB1 segment of SEQ ID NO: 18 and the NP segment of SEQ ID NO: 20.

10. The reassortant influenza A virus according to any one of claims 1 to 9, wherein the virus has an HA segment from a pandemic influenza strain.

11. A method of preparing a reassortant influenza A virus comprising steps of:
(i) introducing into a culture host one or more expression construct(s) which encode(s) backbone viral segments required to produce the reassortant influenza A virus according to any one of claims 1 to 10; and
(ii) culturing the culture host in order to produce reassortant virus.

12. The method according to claim 11, wherein at least one expression construct comprises a sequence selected from the group consisting of:
(i) a sequence selected from the group consisting of: SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, and SEQ ID NO: 22;
(ii) a sequence having at least 95% identity over its entire length to a sequence at (i); and
(iii) a sequence encoding a viral backbone polypeptide having the same amino acid sequence as a polypeptide encoded by a sequence at (i).

13. The method of claim 11 or 12, further comprising purifying the reassortant virus obtained in step (ii).

14. The method according to any one of claims 11 to 13, wherein the backbone viral segments are from two or more donor strains and wherein a backbone viral segment encoding influenza virus haemagglutinin (HA) is from a donor strain other than 105p30 or PR8-X .

15. A method for producing influenza viruses comprising steps of:
(a) infecting a culture host with the reassortant influenza virus according to any one of claims 1 to 10;
(b) culturing the host from step (a) to produce the virus; and optionally
(c) purifying the virus obtained in step (b).

16. A method of preparing a vaccine comprising steps of:
(a) preparing a reassortant influenza A virus by performing the method according to any one of claims 11 to 14; and
(b) preparing vaccine from the virus.

17. The method of claim 15 or 16, wherein the culture host is an embryonated hen egg or a mammalian cell.

18. The method of claim 17, wherein the mammalian cell is an MDCK, Vero or PerC6 cell.

19. The method of claim 18, wherein the cell grows adherently or in suspension.

20. The method of claim 19 or 20, wherein the MDCK cell is cell line MDCK 33016 (DSM ACC2219).

21. The method according to any one of claims 16 to 20, wherein step (b) comprises inactivating the virus.

22. The method according to any one of claims 16 to 21, wherein the vaccine is a whole virion vaccine, a split virion vaccine, a surface antigen vaccine, or a virosomal vaccine.

23. The method according to any one of claims 16 to 22, wherein the vaccine contains less than 10ng of residual host cell DNA per dose.

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Novartis AG

By patent attorneys for the applicant:

FB Rice

FIG. 1(A)

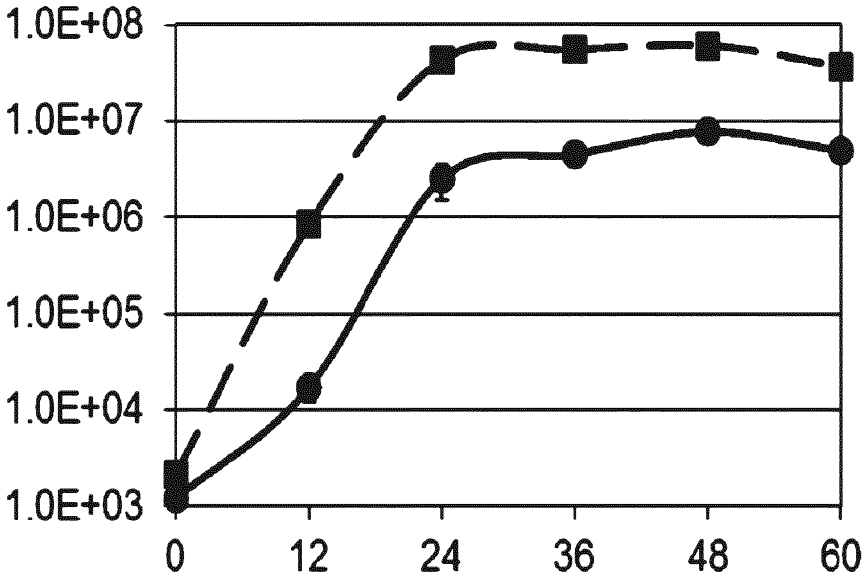
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FIG. 1(B)

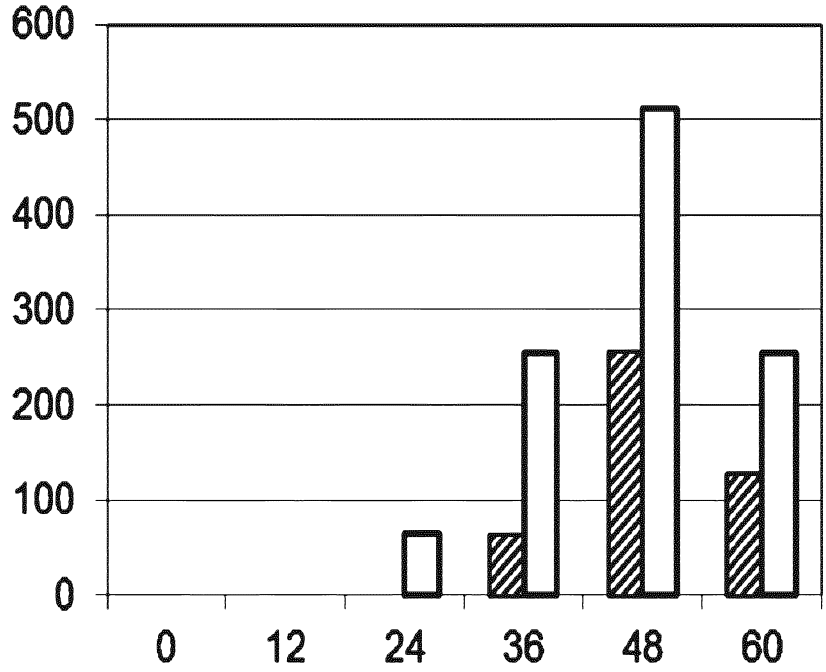
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FIG. 2(A)

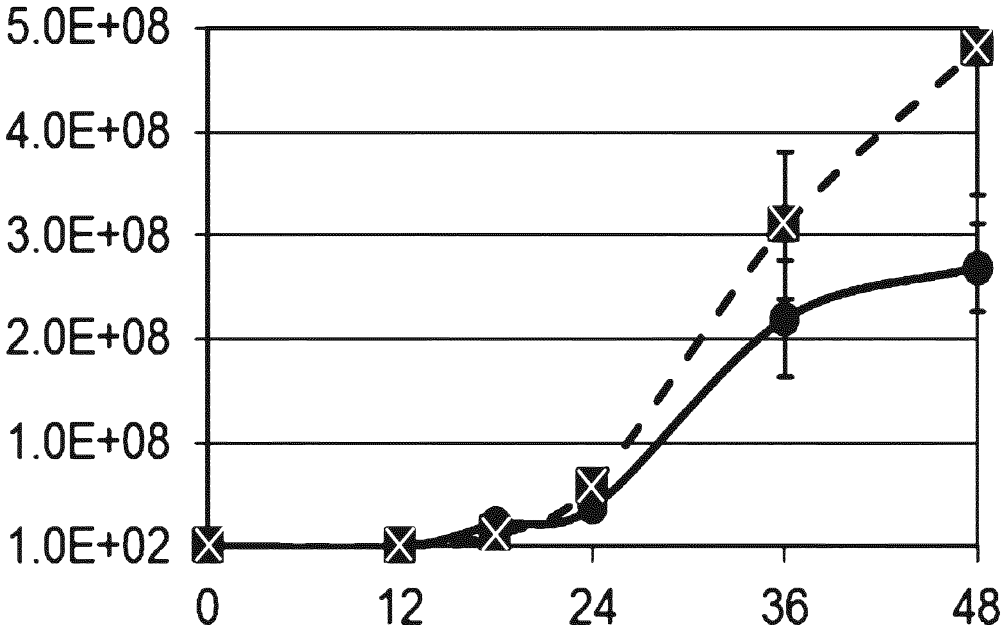
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FIG. 2(B)

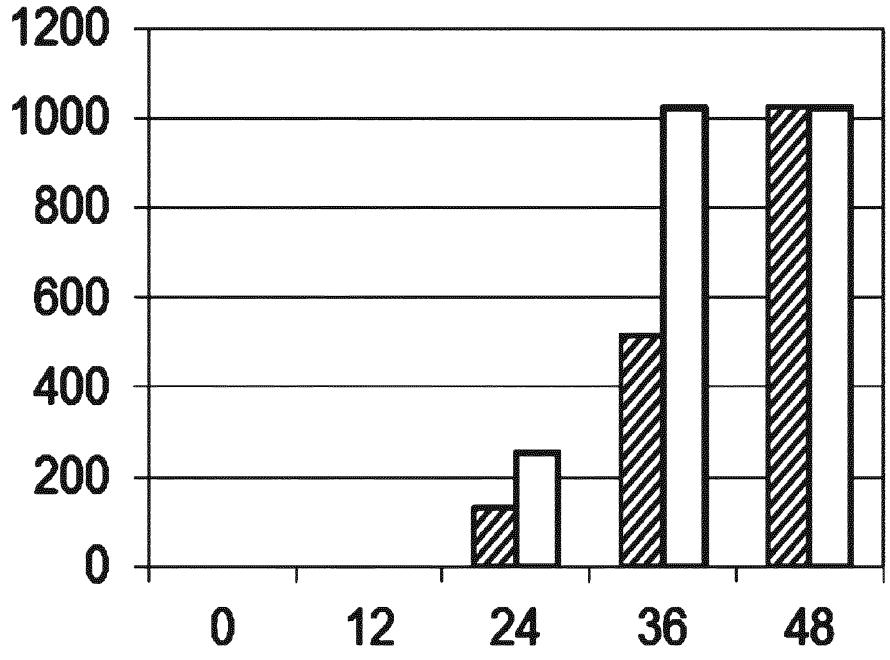
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FIG. 3(A)

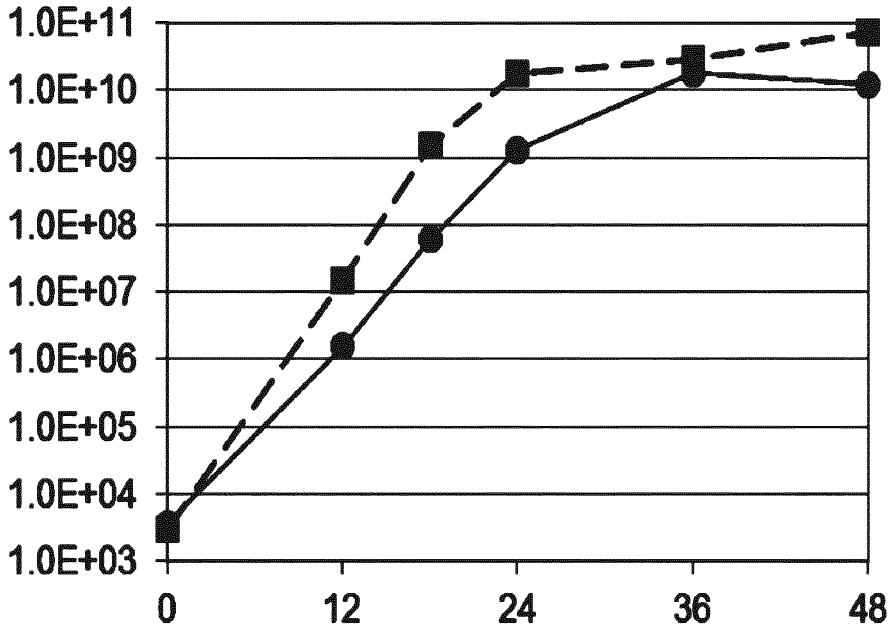
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FIG. 3(B)

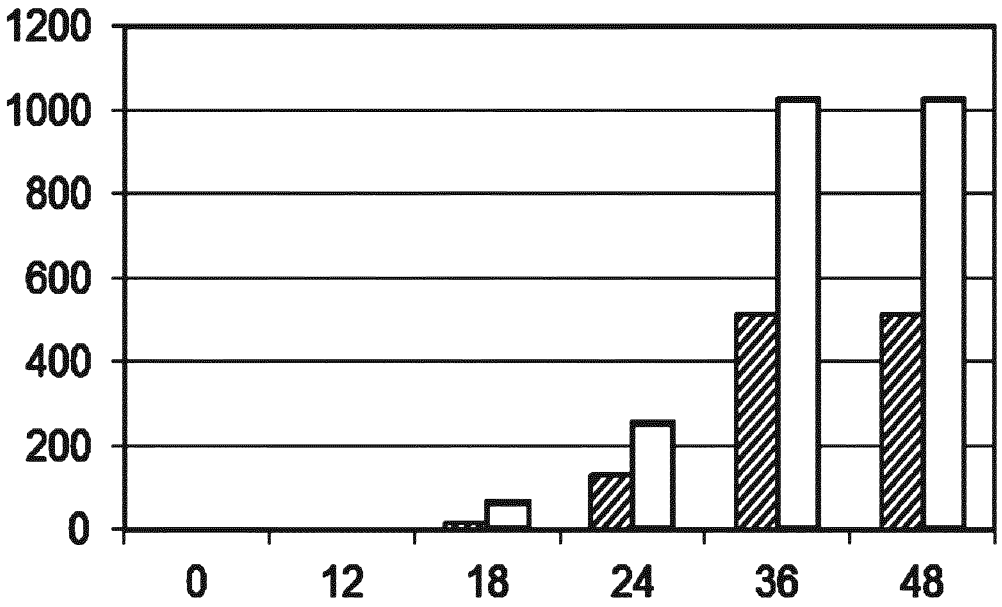
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FIG. 4(A)

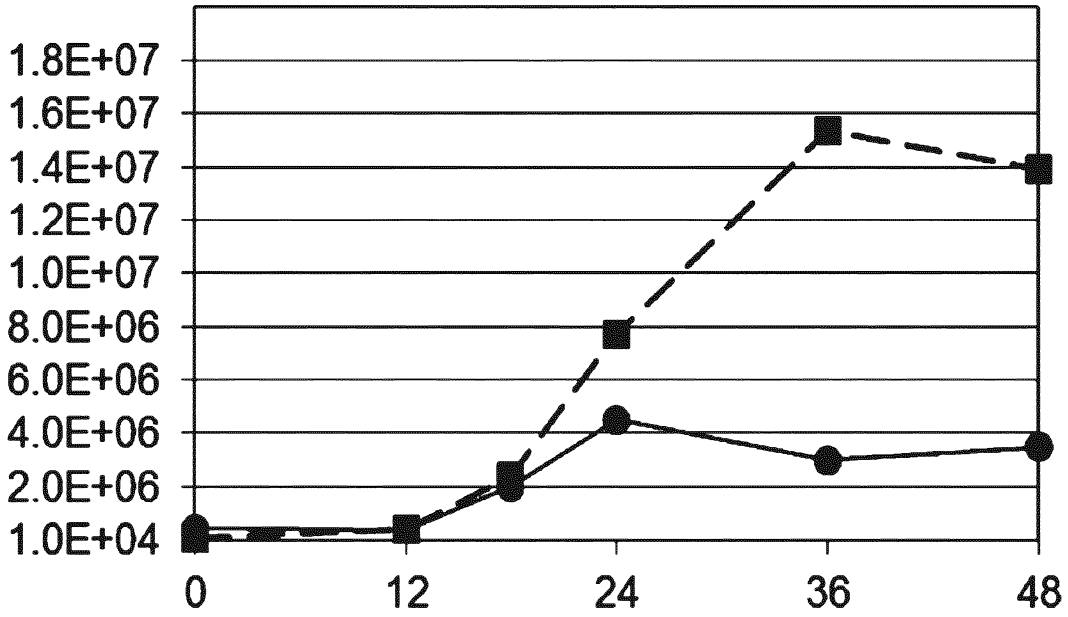
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FIG. 4(B)

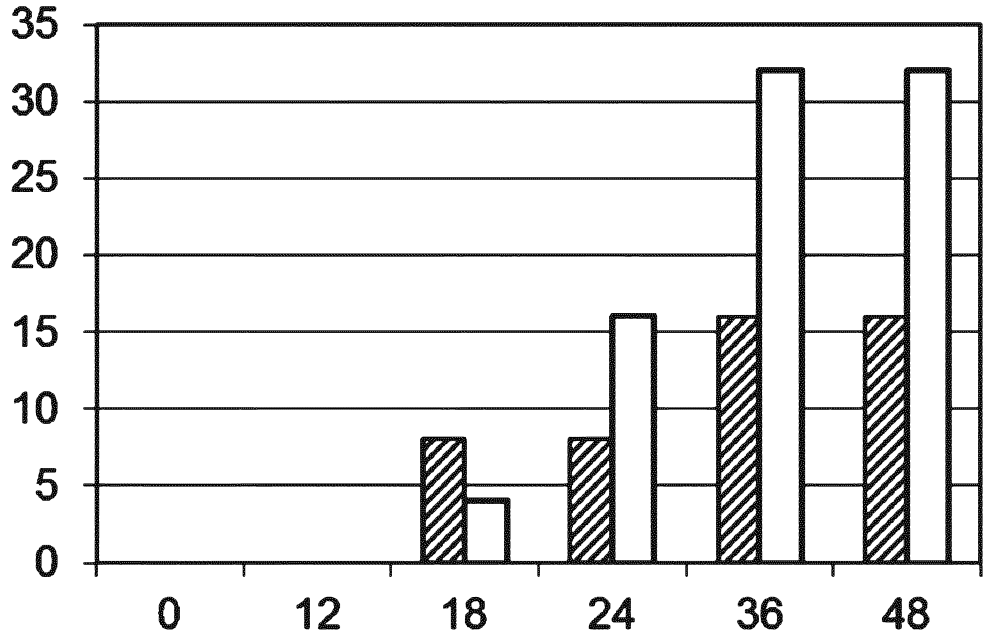
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FIG. 5(A)

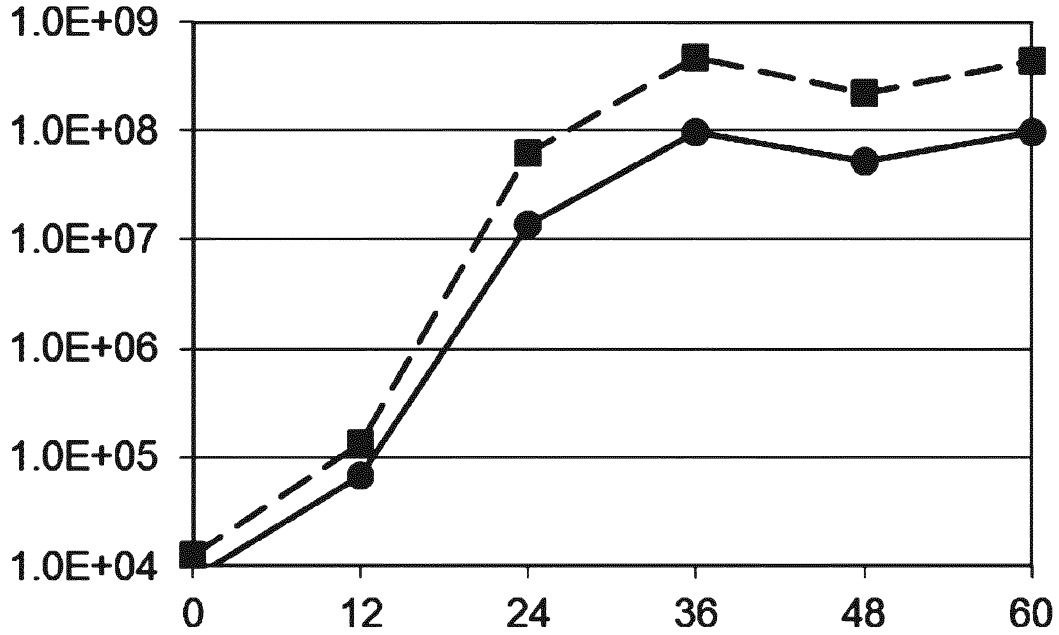
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FIG. 5(B)

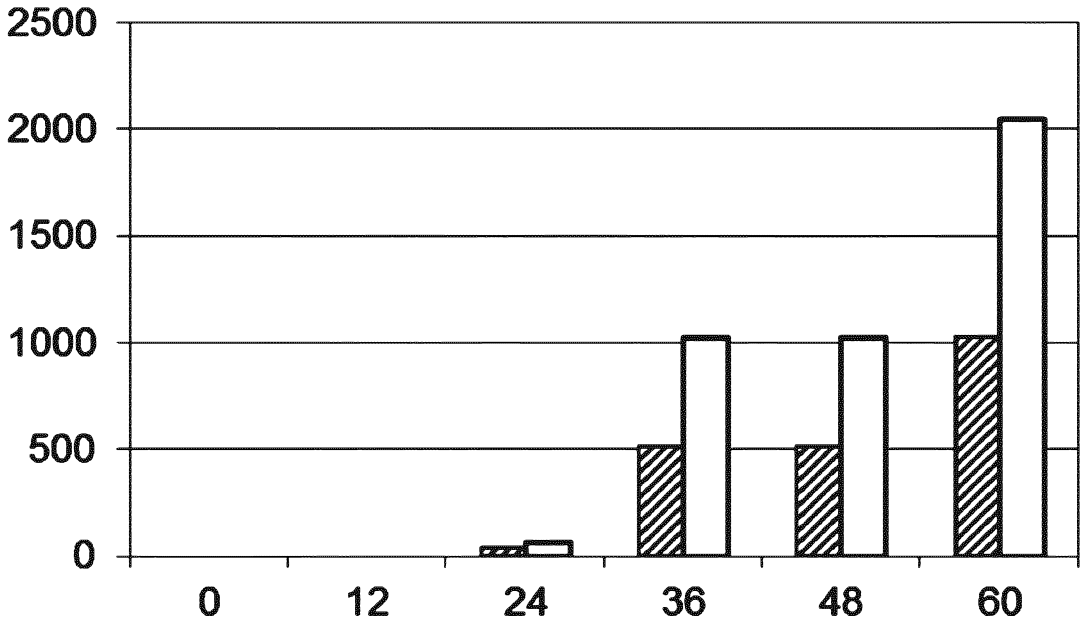
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FIG. 6(A)

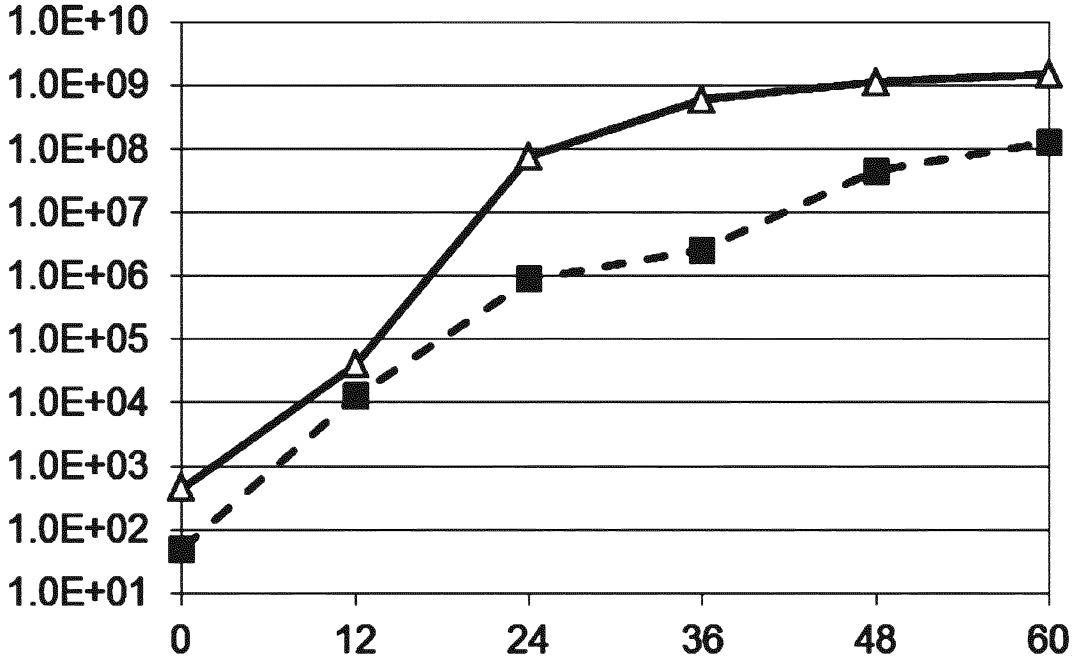
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FIG. 6(B)

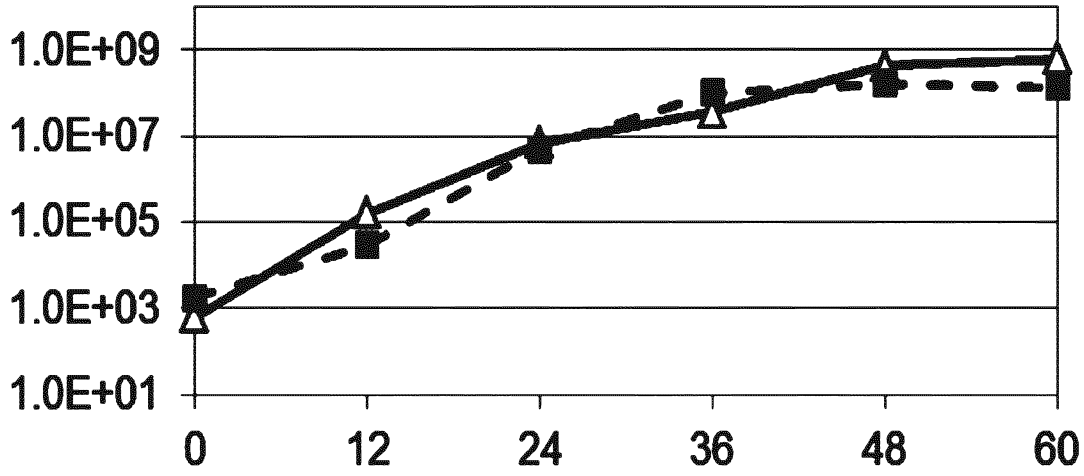
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FIG. 7(A)

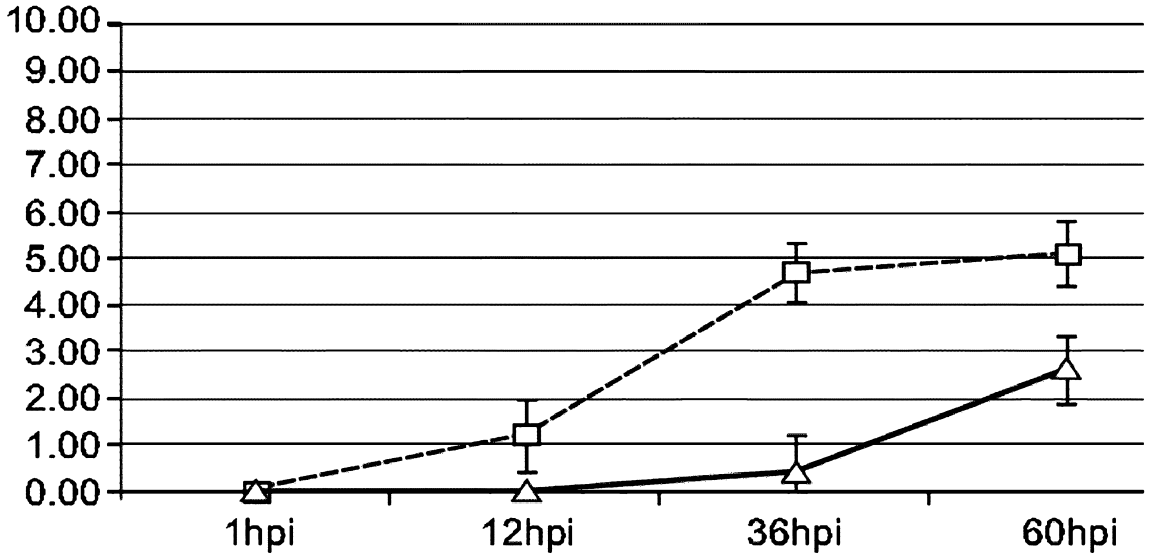
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FIG. 7(B)

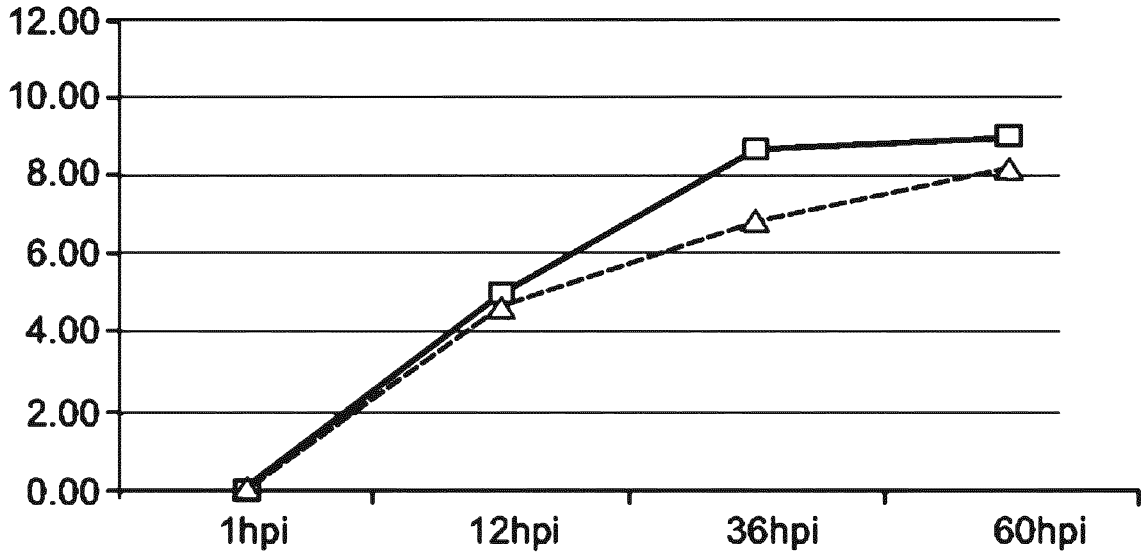
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FIG. 8(A)

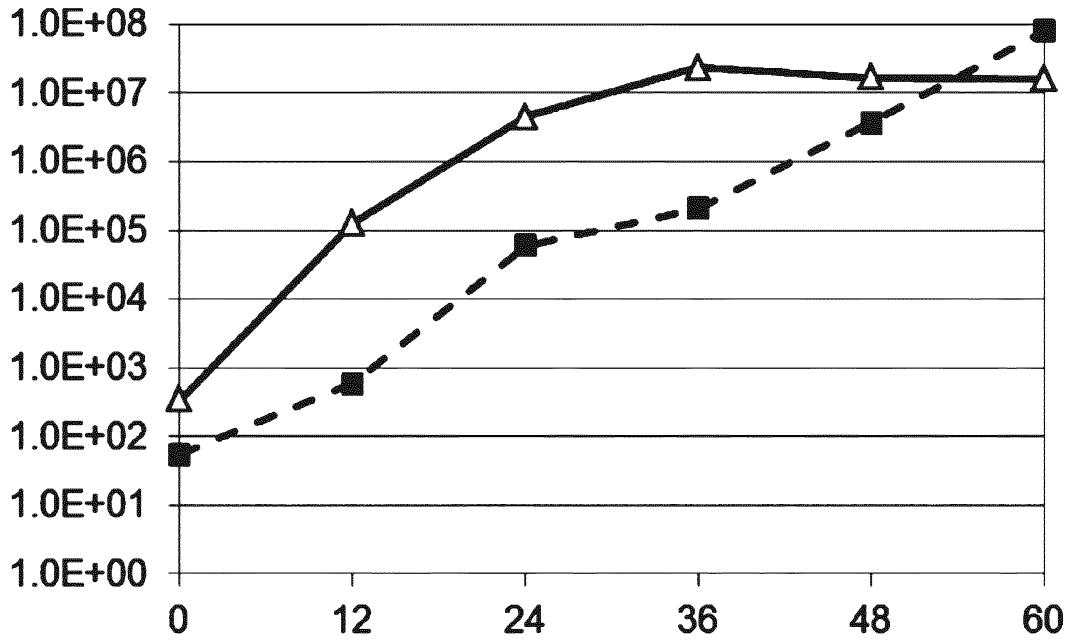
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FIG. 8(B)

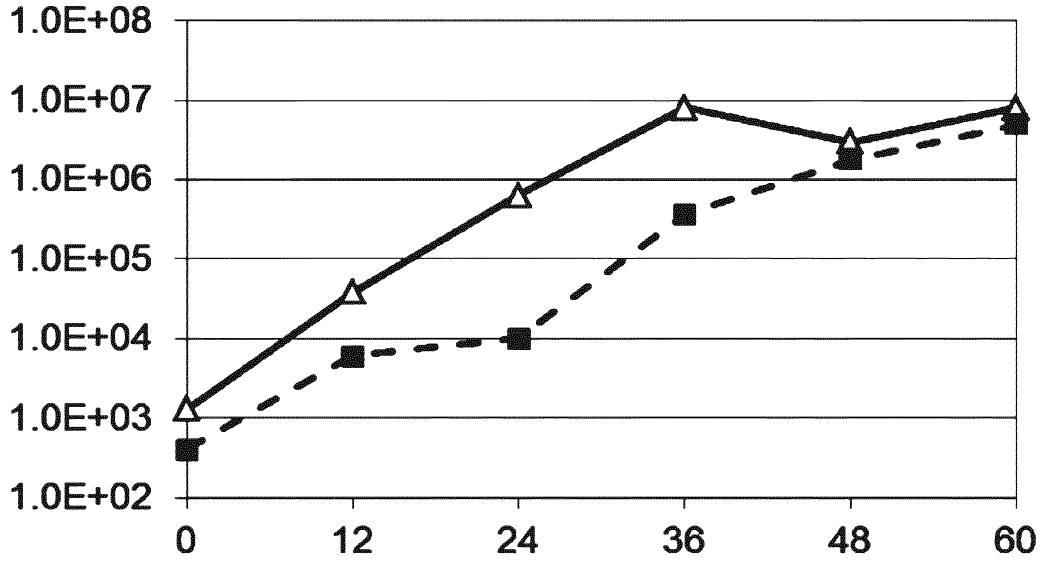
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FIG. 9(A)

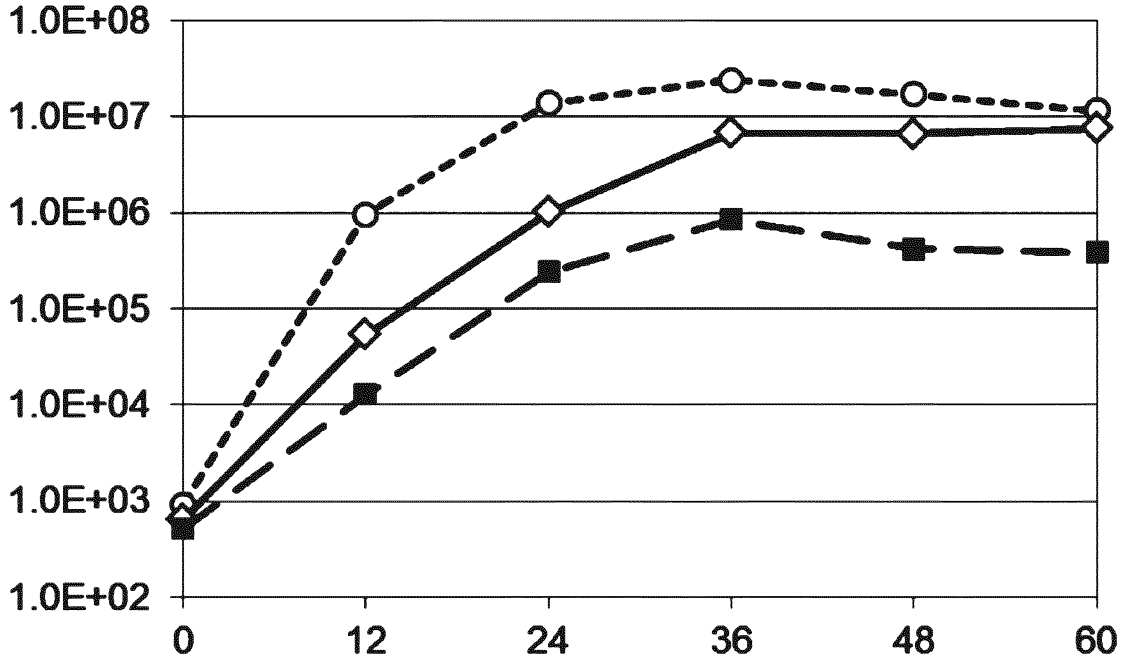
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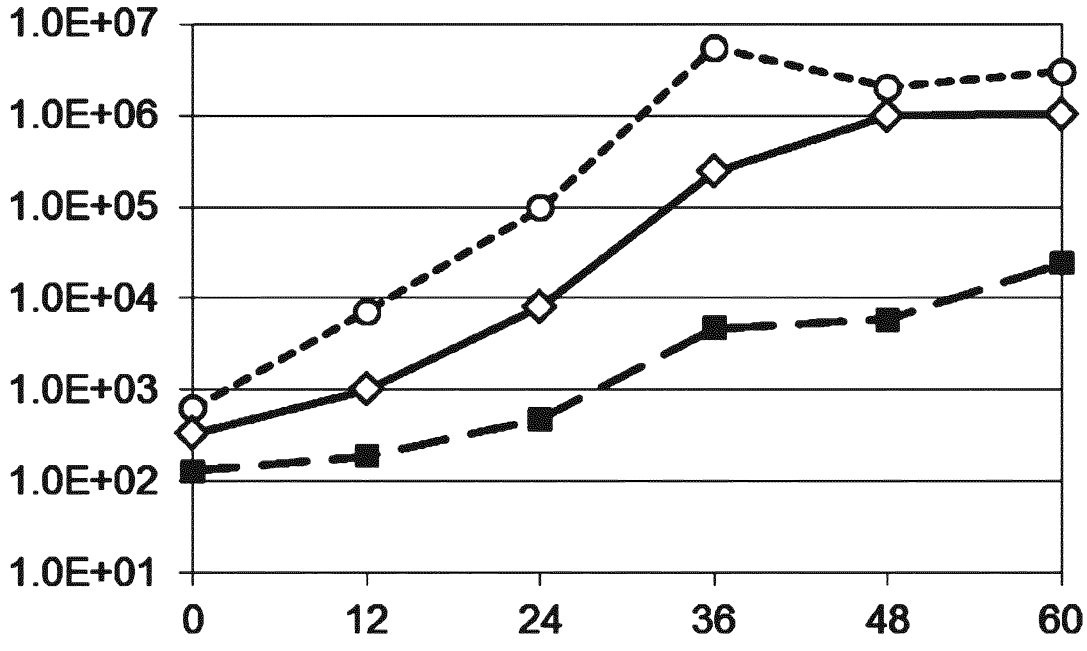
FIG. 9(B)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60

FIG. 10(A)

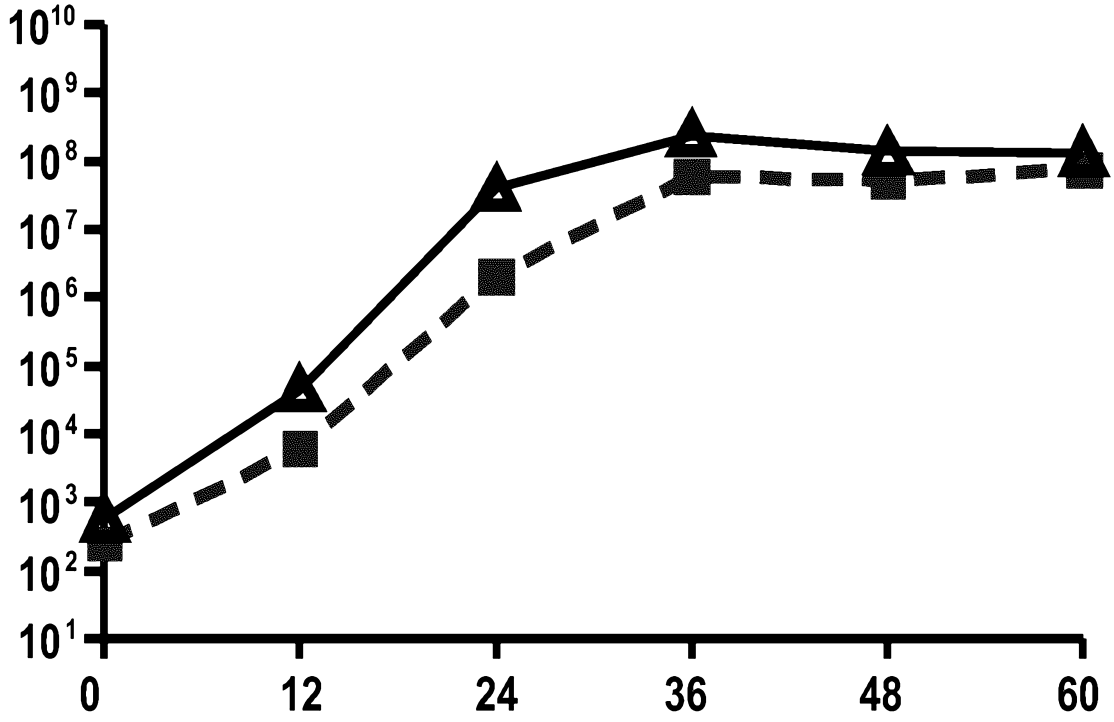


FIG. 10(B)

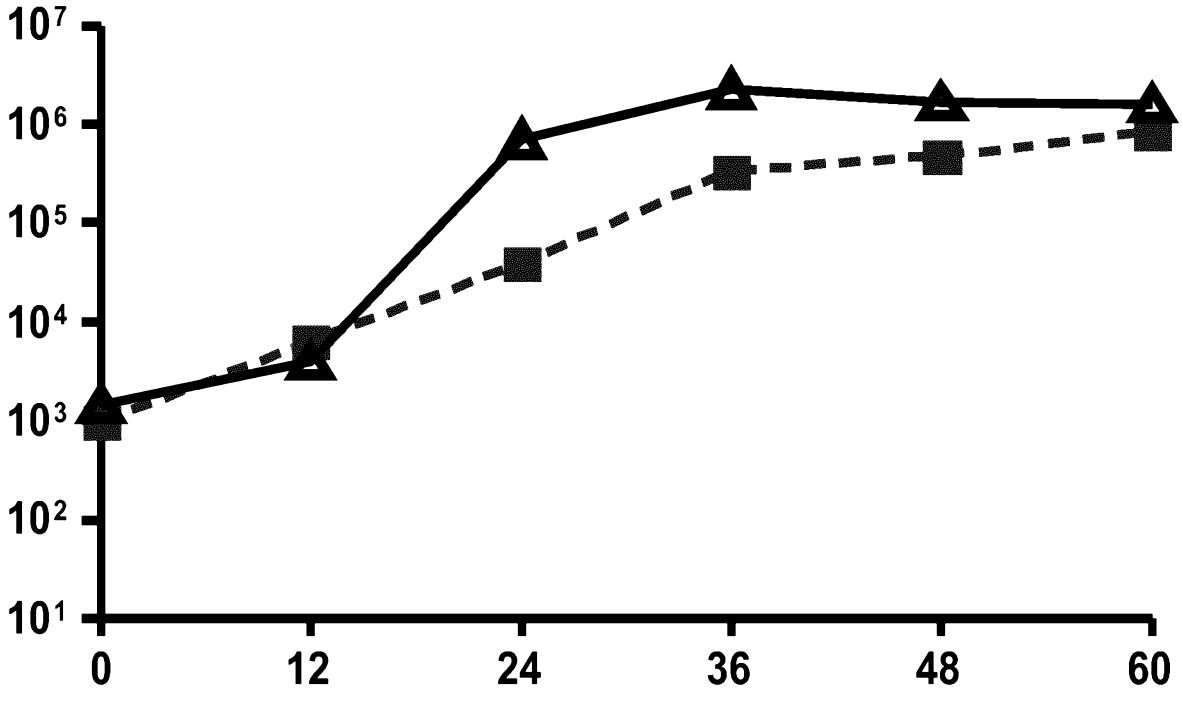


FIG.10(C)

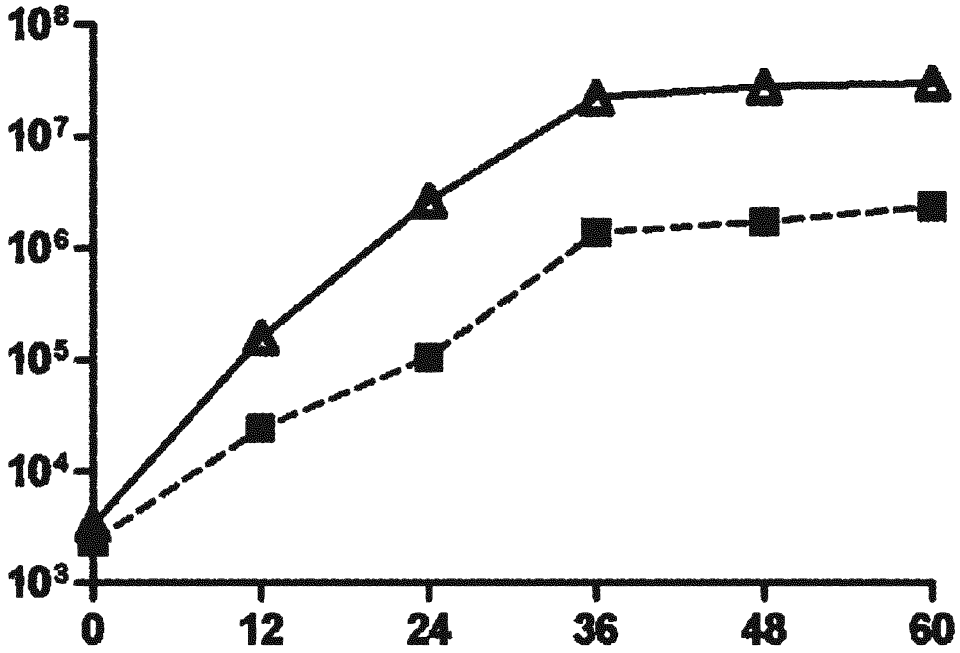


FIG. 10(D)

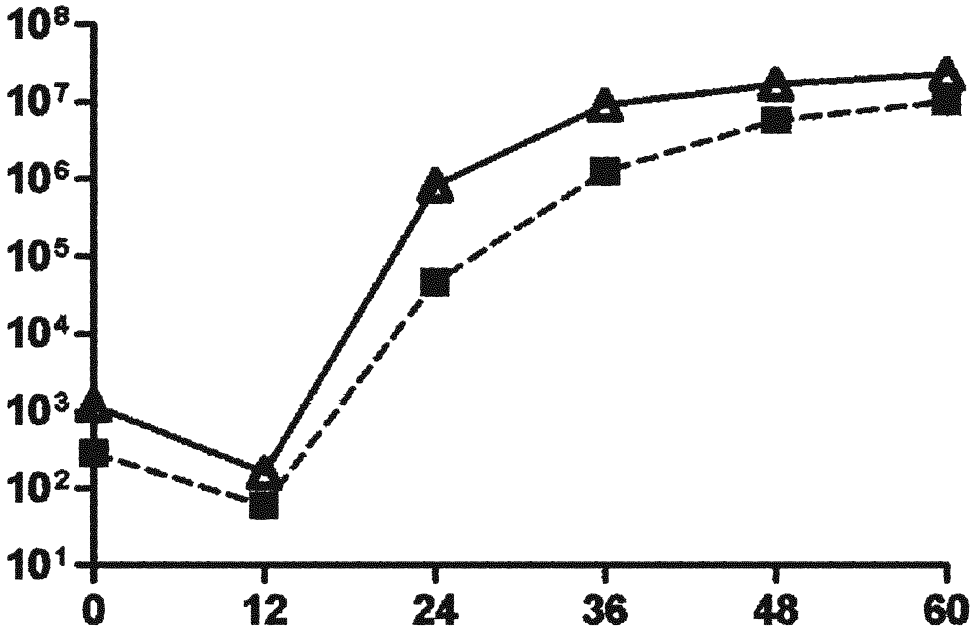


FIG. 10(E)

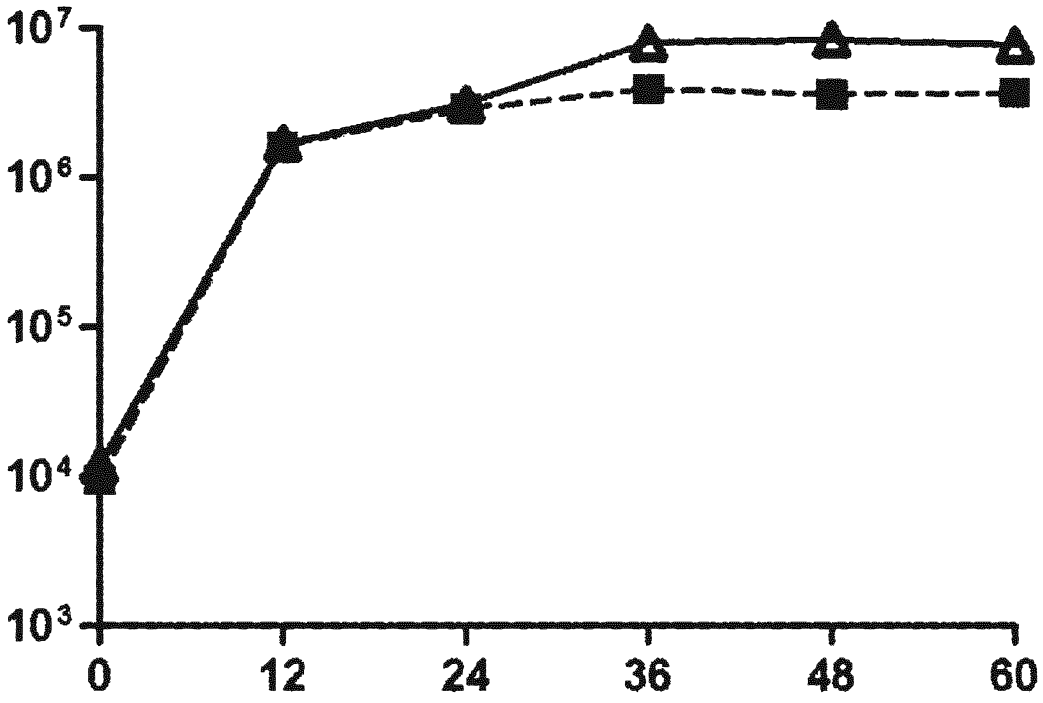


FIG. 11(A)

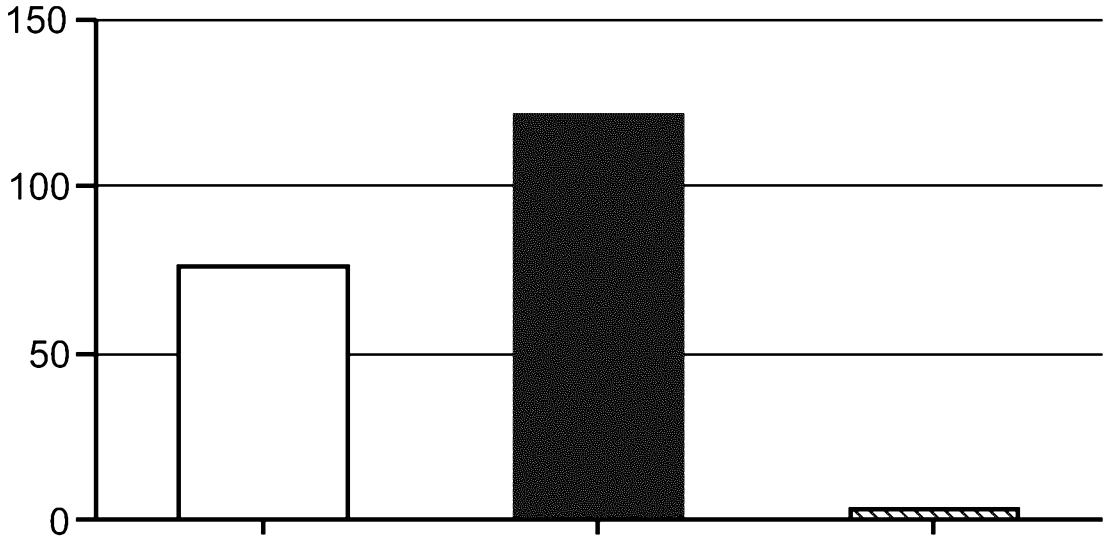


FIG. 11(B)

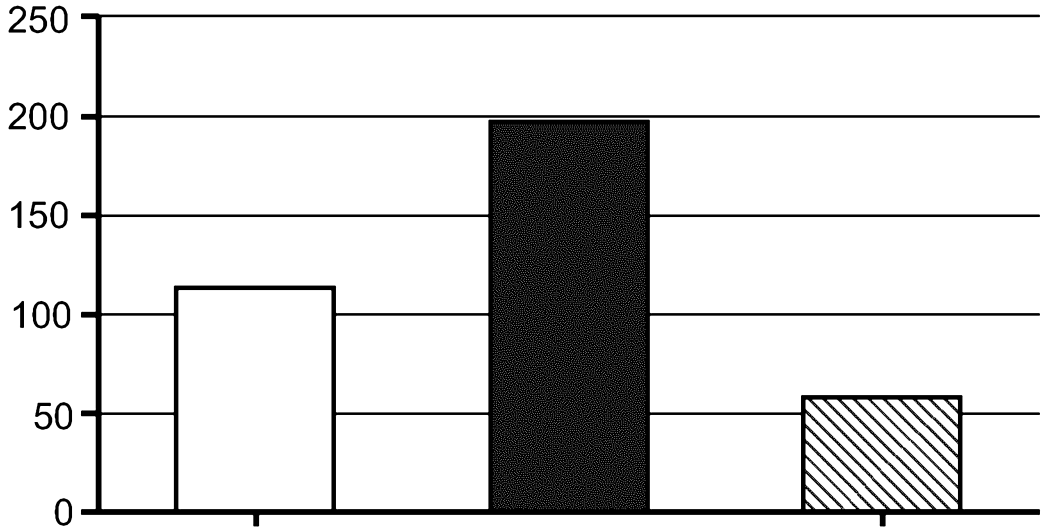


FIG. 11(C)

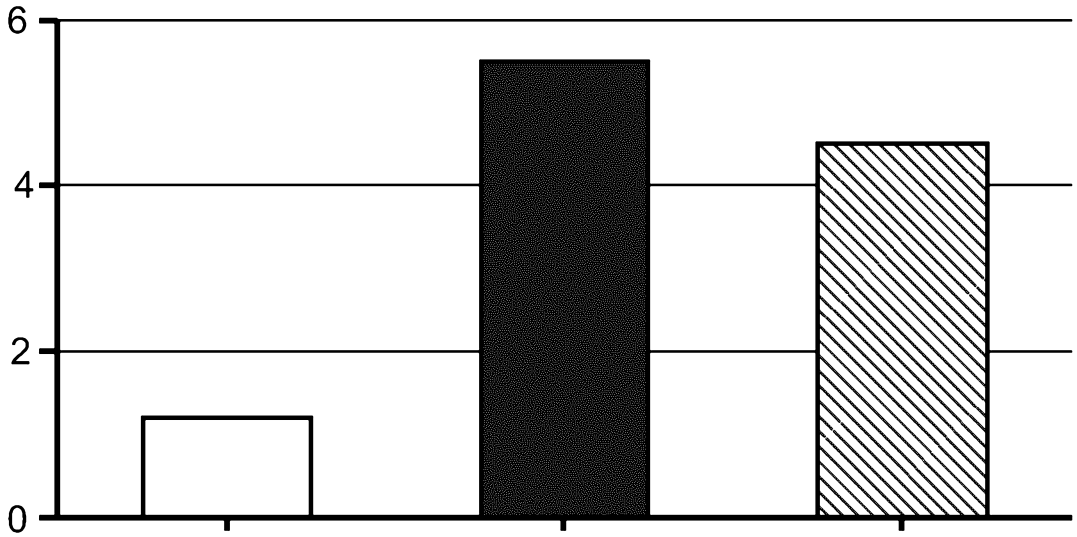


FIG. 11(D)

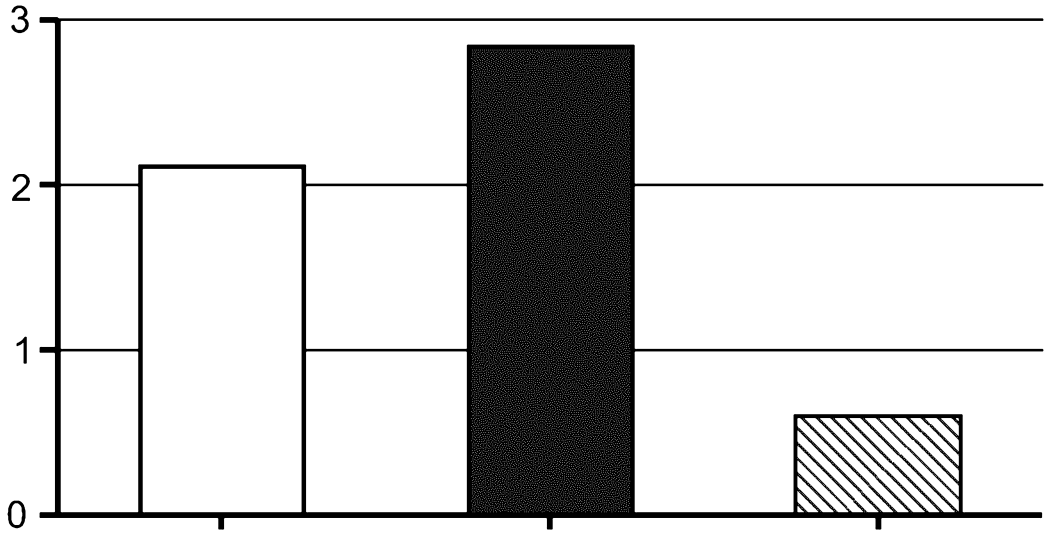
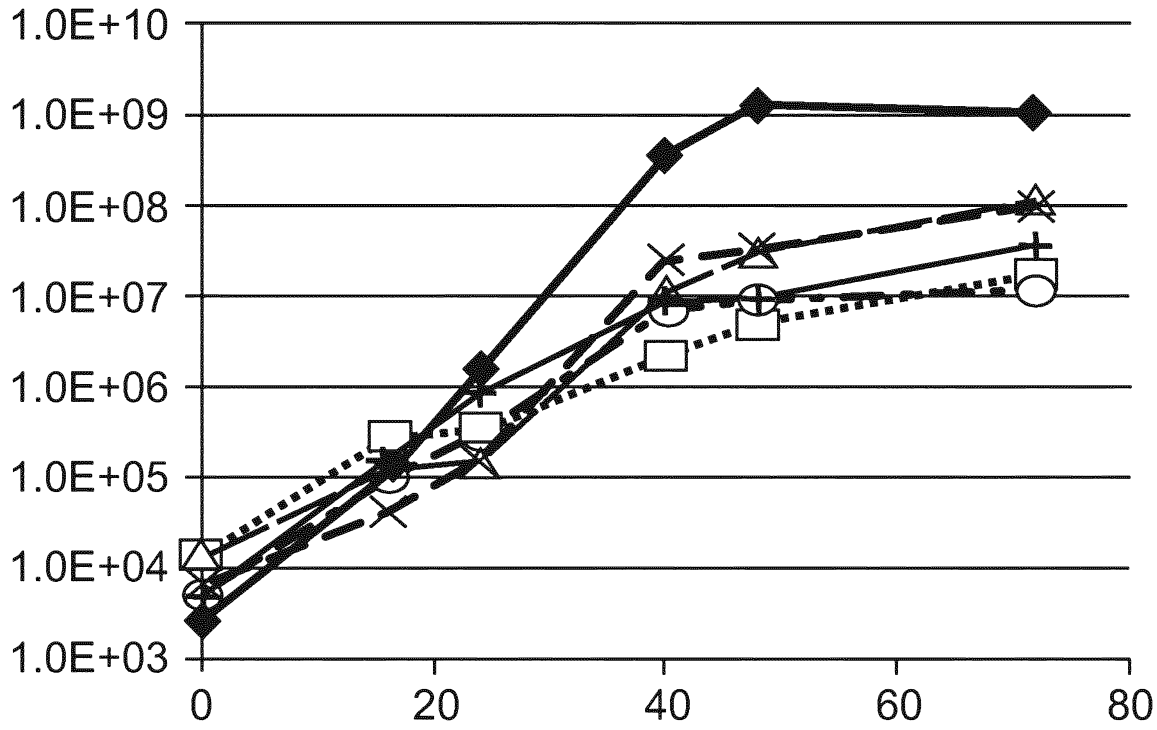


FIG. 12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60
80

FIG. 13

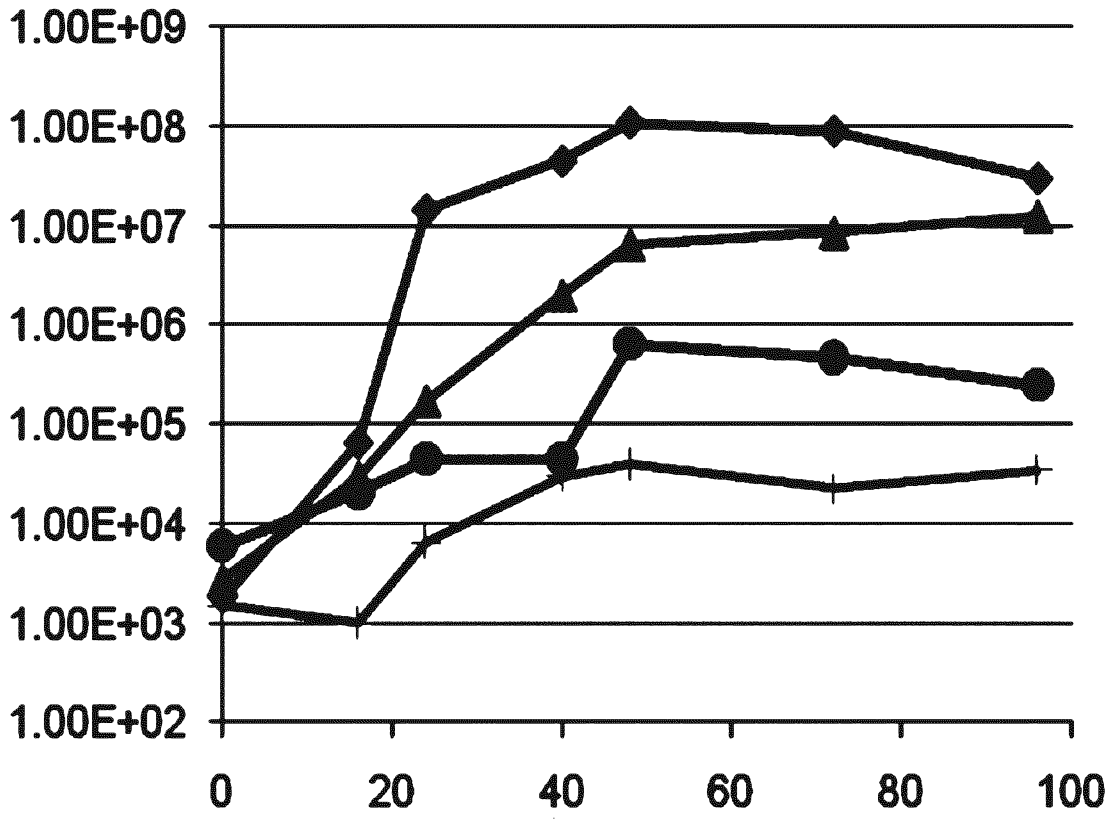
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FIG. 14(A)

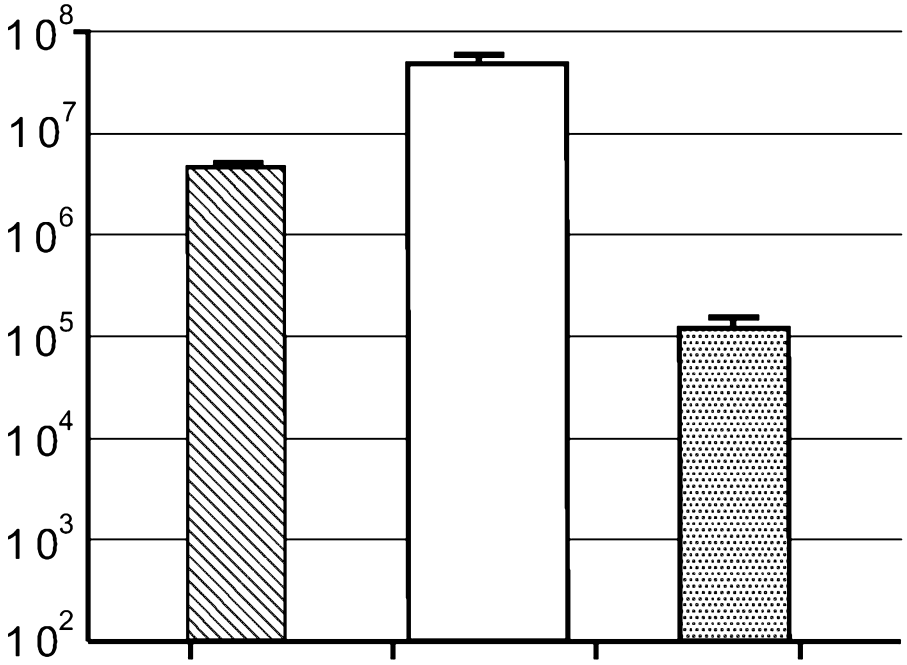


FIG. 14(B)

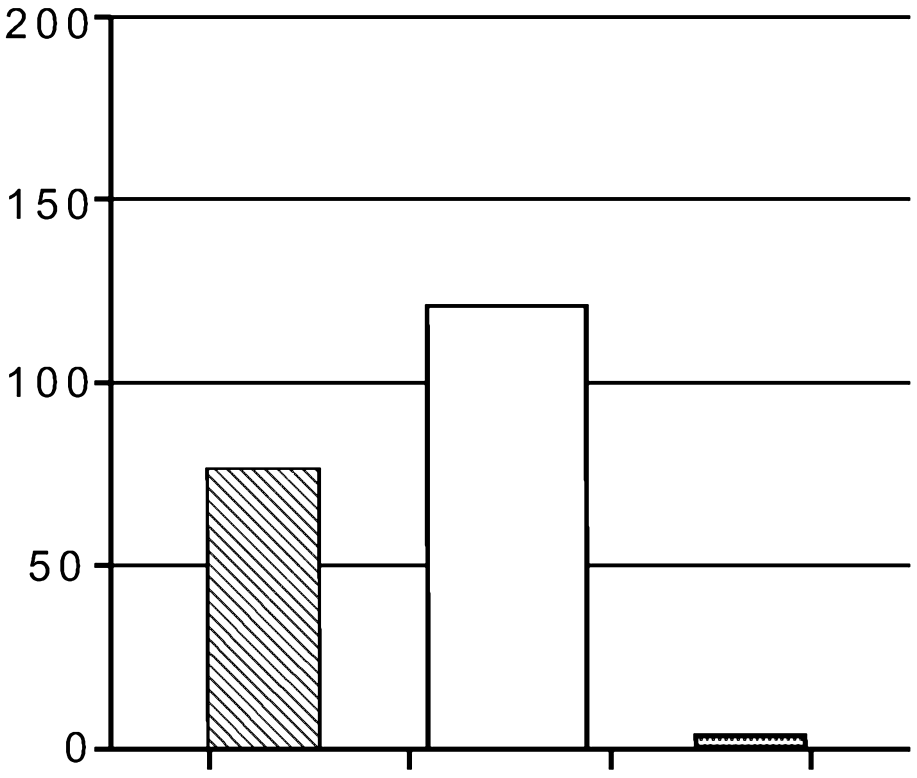


FIG. 14(C)

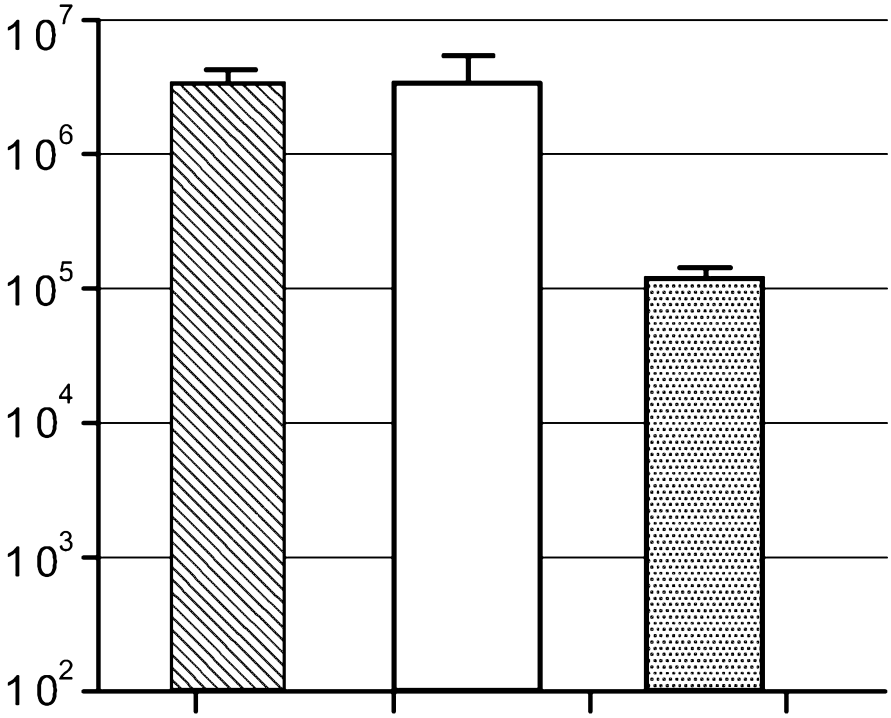
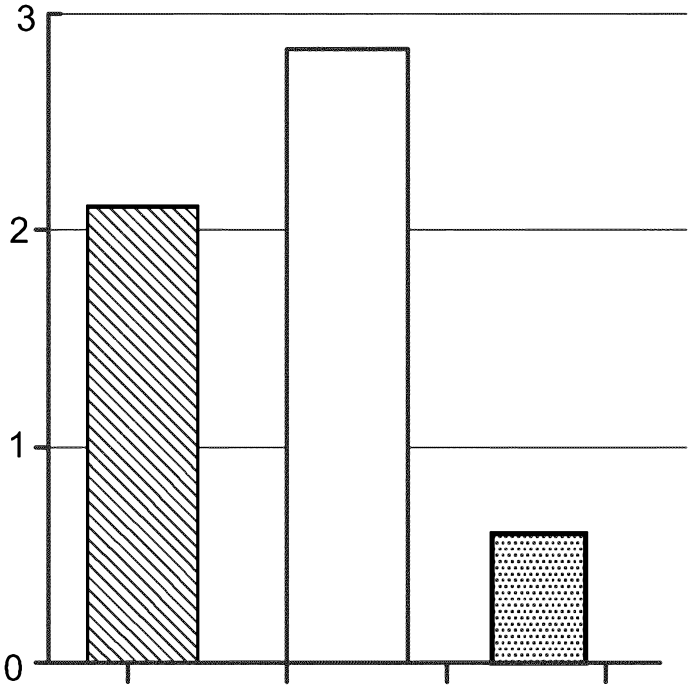


FIG. 14(D)



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A/NC/20/66     51 ilspltkgilgfvftltvpserglqrrrfvqnalngngdpnnmdkavkly  100

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FIG. 15

2013205478 29 Apr 2013

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<210> 4
<211> 1527
<212> DNA
<213> Influenza A virus

<400> 4
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tgattggtgg aattgggcga ttctacatcc aaatgtgcac cgagcttaaa ctcaatgatt 180
atgagggacg actgatccag aacagcttga caatagagag aatggtgctc tctgcttttg 240
atgagaggag gaataaatat ctggaagaac atcccagcgc ggggaaagat cctaagaaaa 300
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atgcagagga gtacgacaat taatgaa 1527

<210> 5

<211> 984
<212> DNA
<213> Influenza A virus

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ggctctcatg gaatggctaa agacaagacc aatcctgtca cctctgacta aggggatttt 180
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<210> 6
<211> 844
<212> DNA
<213> Influenza A virus

<400> 6
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aagtctctaa agggaagagg cagcactctc ggtctgaaca tcgaaacagc cacttgtgtt 180
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gactggttca tgctcatgcc caagcagaaa gtggctggcc ctctttgtgt cagaatggac 360
caggcgataa tggataagaa catcatactg aaagcgaatt tcagtgtgat ttttgaccgg 420
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<210> 7
<211> 1728
<212> DNA
<213> Influenza A virus

<400> 7
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agaatgtgac agtgacacac tctgtcaacc tacttgagga cagtcacaat ggaaaactat 180
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caccaaatcc tgagaatgga acatgttacc cagggtattt cgccgactat gaggaactga 360
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ctaacatagg gaaccaaagg gccctctatc atacagaaaa tgcttatgtc tctgtagtgt 660
cttcacatta tagcagaaga ttcaccccag aaatagccaa aagacccaaa gtaagagatc 720

aggaaggaag aatcaactac tactggactc tgctggaacc tggggataca ataatatttg 780
aggcaaatgg aaatctaata gcgccatggt atgcttttgc actgagtaga ggctttggat 840
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<210> 8
<211> 1414
<212> DNA
<213> Influenza A virus

<400> 8
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tggaagtcaa aaccacactg gagtatgcaa ccaaagaatc atcacatatg aaaacagcac 180
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<210> 9
<211> 2233
<212> DNA
<213> Influenza A virus

<400> 9
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gaaaaggcca ataaaattaa atctgagaaa acacacatcc acattttctc gttcactggg 480

gaagaaatgg ccacaaaggc agactacact ctcgatgaag aaagcagggc taggatcaaa 540
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ccttgtttct act 2233

<210> 10
<211> 2341
<212> DNA
<213> Influenza A virus

<400> 10
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<210> 11
<211> 2341
<212> DNA
<213> Influenza A virus

<400> 11
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<211> 1565
<212> DNA
<213> Influenza A virus

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ctact 1565

<210> 13
<211> 1027
<212> DNA
<213> Influenza A virus

<400> 13
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ttctact 1027

<210> 14
<211> 890
<212> DNA
<213> Influenza A virus

<400> 14
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<210> 15
<211> 1775
<212> DNA
<213> Influenza A virus

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<210> 16
<211> 1413
<212> DNA
<213> Influenza A virus

<400> 16
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ttagccattc aattcaaact ggaagtcaaa accatactgg aatatgcaac caaaacatca 180

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<210> 17
<211> 2220
<212> DNA
<213> Influenza A virus

<400> 17
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<210> 18
<211> 2341
<212> DNA
<213> Influenza A virus

<400> 18
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<210> 19
<211> 2341
<212> DNA
<213> Influenza A virus

<400> 19
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acaactcaga taataaaact tcttcccttt gcagccgctc caccaaagca aagtagaatg 1920
caatttctcgt cattaactgt gaatgtgagg ggatcaggaa tgagaatact tgtaaggggt 1980
aattctccag tattcaacta caacaagacc actaagagac tcacaatcct cggaaaggat 2040
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aggggattcc tcattctagg caaagaagat agaagatatg ggccagcatt aagcatcagt 2160
gaattgagca accttgcgaa aggggagaaa gctaatgtgc taattgggca aggggatgta 2220
gtgttggtaa tgaaacgaaa acgggactct agcatactta ctgacagcca gacagcgacc 2280
aaaagaattc ggatggccat caattaattt cgaataattt aaaaacgacc ttgtttctac 2340
t 2341

<210> 20
<211> 1565
<212> DNA
<213> Influenza A virus

<400> 20
agcaaaagca gggtagataa tcactcactg agtgacatca aagtcatggc gtcccaaggc 60
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agagcatccg tcggaagaat gattgggggа attgggcgat tctacatcca aatgtgcacc 180
gagcttaagc tcaatgatta tgagggacga ctgatccaga acagcttaac aatagagaga 240
atggtgcttt ctgcttttga tgagaggaga aataaatatc tggaagaaca tcccagcgca 300
gggaaagatc ctaagaaaac tggaggaccc atatacaaga gagtagatgg aaagtgggtg 360
agggaactcg tcctttatga caaagaagaa ataaggcgga tttggcgcca agccaacaat 420
ggtgatgatg caacagctgg tttgactcac attatgatct ggcattctaa tttgaatgat 480
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ttgatgcaag gttcaactct ccctagaaga tctggagcag caggcgctgc agtcaaagga 600
gttgggacaa tggtattgga gttaatcagg atgatcaaac gtgggatcaa cgaccgaaac 660
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ctcaaaggaa aatttcaaac agctgcacaa aaagcaatga tggatcaagt gagagaaagc 780
cggaacccag gaaatgctga gatcgaagat ctcacttttc tggcacggtc tgcactcata 840
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gcttcaaatg aaaacatgga tgctattgtc tcaagtactc ttgaactgag aagcagatac 1200
tgggccataa gaaccagaag tggagggaac accaatcaac aaagggcctc tgcgggccaa 1260
atcagcacac aacctacgtt ttctgtgcag agaaacctcc catttgacaa aacaaccatc 1320
atggcagcat tcactgggaa tacagaggga agaacatcag acatgcgggc agaaatcata 1380

aagatgatgg aaagtgcaag accagaagaa gtgtccttcc agggacgggg agtctttgag 1440
ctctcggacg aaagggcaac gaacccgatc gtgccctcct ttgacatgag taatgaagga 1500
tcttatttct tcggagacaa tgcagaggag tacgacaatt aatgaaaaat acccttgttt 1560
ctact 1565

<210> 21
<211> 1027
<212> DNA
<213> Influenza A virus

<400> 21
agcaaaagca ggtagatatt gaaagatgag tcttctaacc gaggtcgaaa cgtacgttct 60
ctctatcgtc ccatcaggcc ccctcaaagc cgagatcgca cagagacttg aagatgtatt 120
tgctggaaag aataccgatc ttgaggctct catggaatgg ctaaagacaa gaccaatcct 180
gtcacctctg actaaggggga ttttaggatt tgtgttcacg ctcaccgtgc ccagtgagcg 240
aggactgcag cgtagacgct ttgtccaaaa tgcccttaat gggaatgggg atccaaataa 300
tatggacaag gctgtcaaac tgtatcgaaa gcttaagagg gagataacat tccatggggc 360
caaagaaata gcactcagtt attctgctgg agcacttgcc agttgtatgg gactcatata 420
caacaggatg ggggctgtga ccaccgaatc agcatttggc cttatatgtg caacctgtga 480
acagattgcc gactcccagc ataagtctca taggcaaatg gtaacaacaa ccaatccatt 540
aataagacat gagaacagaa tggttctggc cagcactaca gctaaggcta tggagcaaat 600
ggctggatcg agtgaacaag cagctgaggc catggaggtt gctagtcagg ccaggcagat 660
ggtgcaggca atgagagcca ttgggactca tcctagctct agcactggtc tgaaaaatga 720
tctccttgaa aatttgcagg cctatcagaa acgaatgggg gtgcagatgc aacgattcaa 780
gtgatcctct tgttgttgcc gcaagtataa ttgggattgt gcacctgata ttgtggatta 840
ttgatcgcct tttttccaaa agcatttatc gtatttttaa acacggttta aaaagagggc 900
cttctacgga aggagtaccg gagtctatga gggaagaata tcgagaggaa cagcagaatg 960
ctgtggatgc tgacgatggt cattttgtca gcatagagct agagtaaaaa actaccttgt 1020
ttctact 1027

<210> 22
<211> 889
<212> DNA
<213> Influenza A virus

<400> 22
agcaaaagca gggtggcaaa gacataatgg attcccacac tgtgtcaagc tttcaggtag 60
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tccttgatcg gcttcgccga gatcagaagt ctctaaaggg acgaggcaac actctcggtc 180
tgaacatcga aacagccact tgtgttggaa agcaaatagt agagaggatt ctgaaagaag 240
aatccgatga gacatttaga atgaccatgg cctccgcact tgcttcgcgg tacctaactg 300
acatgactgt tgaagaaatg tcaagggact ggttcatgct catgcccaag cagaaagtgg 360
ctggccctct ttgtgtcaga atggaccagg cgataatgga taagaacatc atactgaaag 420
cgaacttcag tgtgattttt gaccggttgg agaatctgac attactaagg gctttcaccg 480
aagagggagc aattgttggc gaaatttcac cattgccttc ttttccagga catactaatg 540
aggatgtcaa aaatgcaatt ggggtcctca tcgggggact tgaatggaat gataacacag 600
ttcgagtctc tgaagctcta cagagattcg cttggagaag cagtaatgag actgggggac 660
ctccattcac tacaacacag aaacggaaaa tggcgggaac aattaggtca gaagtttgaa 720
gaaataagat ggctgattga agaagtgagg cataaattga agacgacaga gagtagtttt 780
gaacaaataa catttatgca agcattacag ctattgtttg aagtggaaca agagattaga 840
acgttctcgt ttcagcttat ttaatgataa aaacaccctt gtttctact 889

<210> 23
<211> 1775
<212> DNA
<213> Influenza A virus

<400> 23
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gtacatttac agctacatat gcagacacaa tatgtatagg ctaccatgcc aacaactcaa 120
ccgacactgt tgacacagta cttgagaaga atgtaacagt gacacactct gtcaacctac 180
ttgaggacag tcacaatgga aaactatgtc tactaaaagg aatagcccca ctacaattgg 240
gtaattgcag cgttgccgga tggatcttag gaaacccaga atgcgaatta ctgatttcca 300

aggaatcatg gtcctacatt gtagaaacac caaatcctga gaatggaaca tgttacccag 360
ggtatttcgc cgactatgag gaactgaggg agcaattgag ttcagtatct tcatttgaaa 420
ggttcgaaat attccccaaa gagagctcat ggcccaacca caccgtaacc ggagtatcag 480
catcatgctc ccataacggg aaaagcagtt tttacagaaa tttgctatgg ctgacgggga 540
agaatggttt gtacccaaac ctgagcaagt cctatgcaaa caacaaagag aaagaagtcc 600
ttgtactatg gggtgttcat cacccgccta acatagggga ccaaagggcc ctctatcata 660
cagaaaatgc ttatgtctct gtagtgtctt cacattatag cagaagattc accccagaaa 720
tagccaaaag acccaaggtg agagaccagg aaggaagaat caactactac tggactctgc 780
tggaacccgg ggatacaata atatttgagg caaatggaaa tctaatagcg ccaaggtatg 840
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gcaaagaatt caacaaattg gaaagaagga tggaaaactt aaataaaaaa gttgatgatg 1320
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agaataatgc caaagaaata ggaaacgggt gttttgaatt ctatcacaag tgtaacgatg 1500
aatgcatgga gagtgtgaaa aatggaactt atgactatcc aaaatattcc gaagaatcaa 1560
agttaaacag agagaaaatt gatggagtga aattggaatc aatgggagtc tatcagattc 1620
tggcgatcta ctcaacagtc gccagttccc tggttctttt ggtctccctg ggggcaatca 1680
gcttctggat gtgttccaat gggtctttgc agtgtagaat atgcatctaa gaccagaatt 1740
tcagaaatat aaggaaaaac acccttgttt ctact 1775

<210> 24
<211> 1462
<212> DNA
<213> Influenza A virus

<400> 24
agcaaaagca ggagtttaaa atgaatccaa atcaaaaaat aataaccatt ggatcaatca 60
gtatagcaat cggaataatt agtctaatgt tgcaaatagg aaatattatt tcaatatggg 120
ctagtcactc aatccaaact ggaagtcaaa accacactgg aatatgcaac caaaaaatca 180
tcacatatga aaacagcacc tgggtgaatc acacatatgt taatattaac aacactaatg 240
ttgttgctgg aaaggacaaa acttcagtga cactggccgg caattcatct ctttgtccta 300
tcagtggatg ggctatatac acaaaagaca acagcataag aattggctcc aaaggagatg 360
tttttgtcat aagagaacct ttcatatcat gttctcactt ggaatgcaga acctttttttc 420
tgacccaagg tgctctatta aatgacaaac attcaaatgg aaccgttaag gacagaagtc 480
cttatagggc cttaatgagc tgtcctctag gtgaagcccc gtcaccatac aattcaaagt 540
ttgaatcagt tgcatggtca gcaagcgcat gccatgatgg caagggctgg ttaacaatcg 600
gaatttctgg tccagacaat ggagctgtgg ctgtactaaa atacaacgga ataataactg 660
aaaccataaa aagttgggaa aagcgaatat tgagaacaca agagtctgaa tgtgtttgtg 720
tgaacgggtc atgtttcacc ataatgaccg atggcccgag taatggggcc gcctcgtaca 780
aaatcttcaa gatcgaaaag gggaaggtta ctaaatcaac agagttgaat gcacccaatt 840
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acaactggca tggttcaaat cgaccttggg tatcttttaa tcaaaacttg gattatcaaa 960
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atacaacaat ctggactagt gggagcagca tttctttttg tggcgttgat agtgatactg 1380
caaattggtc ttggccagac ggtgctgagt tgccgttcac cattgacaag tagctcgttg 1440
aaaaaaactc cttgtttcta ct 1462

<210> 25
<211> 566
<212> PRT
<213> Influenza A virus

<400> 25

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Met Lys Ala Lys Leu Leu Val Leu Leu Cys Ala Leu Ser Ala Thr Asp
1               5                  10                  15

Ala Asp Thr Ile Cys Ile Gly Tyr His Ala Asn Asn Ser Thr Asp Thr
            20                  25                  30

Val Asp Thr Val Leu Glu Lys Asn Val Thr Val Thr His Ser Val Asn
        35                  40                  45

Leu Leu Glu Asp Asn His Asn Gly Lys Leu Cys Lys Leu Lys Gly Ile
    50                  55                  60

Ala Pro Leu Gln Leu Gly Lys Cys Ser Ile Ala Gly Trp Ile Leu Gly
65                  70                  75                  80

Asn Pro Glu Cys Glu Ser Leu Phe Ser Lys Lys Ser Trp Ser Tyr Ile
                85                  90                  95

Ala Glu Thr Pro Asn Ser Glu Asn Gly Thr Cys Tyr Pro Gly Tyr Phe
            100                 105                 110

Ala Asp Tyr Glu Glu Leu Arg Glu Gln Leu Ser Ser Val Ser Ser Phe
        115                 120                 125

Glu Arg Phe Glu Ile Phe Pro Lys Glu Ser Ser Trp Pro Lys His Asn
    130                 135                 140

Val Thr Lys Gly Val Thr Ala Ala Cys Ser His Lys Gly Lys Ser Ser
145                 150                 155                 160

Phe Tyr Arg Asn Leu Leu Trp Leu Thr Glu Lys Asn Gly Ser Tyr Pro
                165                 170                 175

Asn Leu Ser Lys Ser Tyr Val Asn Asn Lys Glu Lys Glu Val Leu Val
            180                 185                 190

Leu Trp Gly Val His His Pro Ser Asn Ile Glu Asp Gln Lys Thr Ile
        195                 200                 205

Tyr Arg Lys Glu Asn Ala Tyr Val Ser Val Val Ser Ser His Tyr Asn
    210                 215                 220

Arg Arg Phe Thr Pro Glu Ile Ala Lys Arg Pro Lys Val Arg Asn Gln
225                 230                 235                 240

Glu Gly Arg Ile Asn Tyr Tyr Trp Thr Leu Leu Glu Pro Gly Asp Thr
                245                 250                 255

Ile Ile Phe Glu Ala Asn Gly Asn Leu Ile Ala Pro Trp Tyr Ala Phe
            260                 265                 270

Ala Leu Ser Arg Gly Phe Gly Ser Gly Ile Ile Thr Ser Asn Ala Ser
        275                 280                 285

Met Asp Glu Cys Asp Ala Lys Cys Gln Thr Pro Gln Gly Ala Ile Asn
    290                 295                 300

Ser Ser Leu Pro Phe Gln Asn Val His Pro Val Thr Ile Gly Glu Cys
305                 310                 315                 320

Pro Lys Tyr Val Arg Ser Thr Lys Leu Arg Met Val Thr Gly Leu Arg
                325                 330                 335
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Asn Ile Pro Ser Ile Gln Ser Arg Gly Leu Phe Gly Ala Ile Ala Gly
            340                 345                 350

Phe Ile Glu Gly Gly Trp Thr Gly Met Ile Asp Gly Trp Tyr Gly Tyr
        355                 360                 365

His His Gln Asn Glu Gln Gly Ser Gly Tyr Ala Ala Asp Gln Lys Ser
    370                 375                 380

Thr Gln Asn Ala Ile Asn Gly Ile Thr Asn Lys Val Asn Ser Ile Ile
385                 390                 395                 400

Glu Lys Met Asn Thr Gln Phe Thr Ala Val Gly Lys Glu Phe Asn Lys
                405                 410                 415

Leu Glu Lys Arg Met Glu Asn Leu Asn Lys Lys Val Asp Asp Gly Phe
            420                 425                 430

Leu Asp Ile Trp Thr Tyr Asn Ala Glu Leu Leu Val Leu Leu Glu Asn
        435                 440                 445

Glu Arg Thr Leu Asp Phe His Asp Ser Asn Val Lys Asn Leu Tyr Glu
    450                 455                 460

Lys Val Lys Ser Gln Leu Lys Asn Asn Ala Lys Glu Ile Gly Asn Gly
465                 470                 475                 480

Cys Phe Glu Phe Tyr His Lys Cys Asn Asn Glu Cys Met Glu Ser Val
                485                 490                 495

Lys Asn Gly Thr Tyr Asp Tyr Pro Lys Tyr Ser Glu Glu Ser Lys Leu
            500                 505                 510

Asn Arg Glu Lys Ile Asp Gly Val Lys Leu Glu Ser Met Gly Val Tyr
        515                 520                 525

Gln Ile Leu Ala Ile Tyr Ser Thr Val Ala Ser Ser Leu Val Leu Leu
    530                 535                 540

Val Ser Leu Gly Ala Ile Ser Phe Trp Met Cys Ser Asn Gly Ser Leu
545                 550                 555                 560

Gln Cys Arg Ile Cys Ile
                565

<210>   26
<211>   470
<212>   PRT
<213>   Influenza A virus

<400>   26
Met Asn Pro Asn Gln Lys Ile Ile Thr Ile Gly Ser Ile Cys Met Thr
1               5                   10                  15

Ile Gly Ile Ile Ser Leu Ile Leu Gln Ile Gly Asn Ile Ile Ser Ile
            20                  25                  30

Trp Val Ser His Ser Ile Gln Thr Gly Ser Gln Asn His Thr Gly Ile
        35                  40                  45

Cys Asn Gln Arg Ile Ile Thr Tyr Glu Asn Ser Thr Trp Val Asn Gln
    50                  55                  60
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Thr Tyr Val Asn Ile Asn Asn Thr Asn Val Val Ala Gly Lys Asp Thr
65                  70                  75                  80

Thr Ser Val Thr Leu Ala Gly Asn Ser Ser Leu Cys Pro Ile Arg Gly
                85                  90                  95

Trp Ala Ile Tyr Ser Lys Asp Asn Ser Ile Arg Ile Gly Ser Lys Gly
            100                 105                 110

Asp Val Phe Val Ile Arg Glu Pro Phe Ile Ser Cys Ser His Leu Glu
        115                 120                 125

Cys Arg Thr Phe Phe Leu Thr Gln Gly Ala Leu Leu Asn Asp Lys His
    130                 135                 140

Ser Asn Gly Thr Val Lys Asp Arg Ser Pro Tyr Arg Ala Leu Met Ser
145                 150                 155                 160

Cys Pro Ile Gly Glu Ala Pro Ser Pro Tyr Asn Ser Arg Phe Glu Ser
                165                 170                 175

Val Ala Trp Ser Ala Ser Ala Cys His Asp Gly Met Gly Trp Leu Thr
            180                 185                 190

Ile Gly Ile Ser Gly Pro Asp Asp Gly Ala Val Ala Val Leu Lys Tyr
        195                 200                 205

Asn Gly Ile Ile Thr Glu Thr Ile Lys Ser Trp Arg Lys Arg Ile Leu
    210                 215                 220

Arg Thr Gln Glu Ser Glu Cys Val Cys Val Asn Gly Ser Cys Phe Thr
225                 230                 235                 240

Ile Met Thr Asp Gly Pro Ser Asn Gly Pro Ala Ser Tyr Arg Ile Phe
                245                 250                 255

Lys Ile Glu Lys Gly Lys Ile Thr Lys Ser Ile Glu Leu Asp Ala Pro
            260                 265                 270

Asn Ser His Tyr Glu Glu Cys Ser Cys Tyr Pro Asp Thr Gly Thr Val
        275                 280                 285

Met Cys Val Cys Arg Asp Asn Trp His Gly Ser Asn Arg Pro Trp Val
    290                 295                 300

Ser Phe Asn Gln Asn Leu Asp Tyr Gln Ile Gly Tyr Ile Cys Ser Gly
305                 310                 315                 320

Val Phe Gly Asp Asn Pro Arg Pro Lys Asp Gly Lys Gly Ser Cys Asp
                325                 330                 335

Pro Val Thr Val Asp Gly Ala Asp Gly Val Lys Gly Phe Ser Tyr Arg
            340                 345                 350

Tyr Gly Asn Gly Val Trp Ile Gly Arg Thr Lys Ser Asn Ser Ser Arg
        355                 360                 365

Lys Gly Phe Glu Met Ile Trp Asp Pro Asn Gly Trp Thr Asp Thr Asp
    370                 375                 380

Ser Asn Phe Leu Val Lys Gln Asp Val Val Ala Met Thr Asp Trp Ser
385                 390                 395                 400
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Gly Tyr Ser Gly Ser Phe Val Gln His Pro Glu Leu Thr Gly Leu Asp
                405                 410                 415

Cys Met Arg Pro Cys Phe Trp Val Glu Leu Val Arg Gly Arg Pro Arg
            420                 425                 430

Glu Gly Thr Thr Val Trp Thr Ser Gly Ser Ser Ile Ser Phe Cys Gly
        435                 440                 445

Val Asn Ser Asp Thr Ala Asn Trp Ser Trp Pro Asp Gly Ala Glu Leu
    450                 455                 460

Pro Phe Thr Ile Asp Lys
465                 470

<210>   27
<211>   469
<212>   PRT
<213>   Influenza A virus

<400>   27
Met Asn Pro Asn Gln Lys Ile Ile Thr Ile Gly Ser Val Cys Met Thr
1               5                   10                  15

Ile Gly Met Ala Asn Leu Ile Leu Gln Ile Gly Asn Ile Ile Ser Ile
            20                  25                  30

Trp Ile Ser His Ser Ile Gln Leu Gly Asn Gln Asn Gln Ile Glu Thr
        35                  40                  45

Cys Asn Gln Ser Val Ile Thr Tyr Glu Asn Asn Thr Trp Val Asn Gln
    50                  55                  60

Thr Tyr Val Asn Ile Ser Asn Thr Asn Phe Ala Ala Gly Gln Ser Val
65                  70                  75                  80

Val Ser Val Lys Leu Ala Gly Asn Ser Ser Leu Cys Pro Val Ser Gly
                85                  90                  95

Trp Ala Ile Tyr Ser Lys Asp Asn Ser Val Arg Ile Gly Ser Lys Gly
            100                 105                 110

Asp Val Phe Val Ile Arg Glu Pro Phe Ile Ser Cys Ser Pro Leu Glu
        115                 120                 125

Cys Arg Thr Phe Phe Leu Thr Gln Gly Ala Leu Leu Asn Asp Lys His
    130                 135                 140

Ser Asn Gly Thr Ile Lys Asp Arg Ser Pro Tyr Arg Thr Leu Met Ser
145                 150                 155                 160

Cys Pro Ile Gly Glu Val Pro Ser Pro Tyr Asn Ser Arg Phe Glu Ser
                165                 170                 175

Val Ala Trp Ser Ala Ser Ala Cys His Asp Gly Ile Asn Trp Leu Thr
            180                 185                 190

Ile Gly Ile Ser Gly Pro Asp Asn Gly Ala Val Ala Val Leu Lys Tyr
        195                 200                 205

Asn Gly Ile Ile Thr Asp Thr Ile Lys Ser Trp Arg Asn Asn Ile Leu
    210                 215                 220
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Arg Thr Gln Glu Ser Glu Cys Ala Cys Val Asn Gly Ser Cys Phe Thr
225                 230                 235                 240

Val Met Thr Asp Gly Pro Ser Asn Gly Gln Ala Ser Tyr Lys Ile Phe
                245                 250                 255

Arg Ile Glu Lys Gly Lys Ile Val Lys Ser Val Glu Met Asn Ala Pro
            260                 265                 270

Asn Tyr His Tyr Glu Glu Cys Ser Cys Tyr Pro Asp Ser Ser Glu Ile
        275                 280                 285

Thr Cys Val Cys Arg Asp Asn Trp His Gly Ser Asn Arg Pro Trp Val
    290                 295                 300

Ser Phe Asn Gln Asn Leu Glu Tyr Gln Ile Gly Tyr Ile Cys Ser Gly
305                 310                 315                 320

Ile Phe Gly Asp Asn Pro Arg Pro Asn Asp Lys Thr Gly Ser Cys Gly
                325                 330                 335

Pro Val Ser Ser Asn Gly Ala Asn Gly Val Lys Gly Phe Ser Phe Lys
            340                 345                 350

Tyr Gly Asn Gly Val Trp Ile Gly Arg Thr Lys Ser Ile Ser Ser Arg
        355                 360                 365

Asn Gly Phe Glu Met Ile Trp Asp Pro Asn Gly Trp Thr Gly Thr Asp
    370                 375                 380

Asn Asn Phe Ser Ile Lys Gln Asp Ile Val Gly Ile Asn Glu Trp Ser
385                 390                 395                 400

Gly Tyr Ser Gly Ser Phe Val Gln His Pro Glu Leu Thr Gly Leu Asp
                405                 410                 415

Cys Ile Arg Pro Cys Phe Trp Val Glu Leu Ile Arg Gly Arg Pro Lys
            420                 425                 430

Glu Asn Thr Ile Trp Thr Ser Gly Ser Ser Ile Ser Phe Cys Gly Val
        435                 440                 445

Asn Ser Asp Thr Val Gly Trp Ser Trp Pro Asp Gly Ala Glu Leu Pro
    450                 455                 460

Phe Thr Ile Asp Lys
465
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<210> 28
<211> 2277
<212> DNA
<213> Influenza A virus

<400> 28

```
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accaaaacca ccgtggatca tatggcgatt attaaaaaat ataccagcgg ccgccaggaa  120
aaaaacccga gcctgcgcat gaaatggatg atggcgatga aatatccgat taccgcggat  180
aaacgcatta ccgaaatgat tccggaacgc aacgaacagg gccagaccct gtggagcaaa  240
gtgaacgatg cgggcagcga tcgcgtgatg attagcccgc tggcggtgac ctggtggaac  300
cgcaacggcc cggtggcgag caccattcat tatccgaaaa tttataaaac ctattttgaa  360
aaagtggaac gcctgaaaca tggcaccttt ggccccggtgc attttcgcaa ccaggtgaaa  420
attcgccgcc gcgtggatat taacccgggc catgcggatc tgagcgcgaa agaagcgcag  480
```

```
gatgtgatta tggaagtggt gtttccgaac gaagtgggcg cgcgcattct gaccagcgaa  540
agccagctga ccattaccaa agaaaaaaaa gaagaactgc agaactgcaa aattagcccg  600
ctgatggtgg cgtatatgct ggaacgcgaa ctggtgcgca aaacccgctt tctgccggtg  660
gcgggcggca ccagcagcgt gtatattgaa gtgctgcatc tgacccaggg cacctgctgg  720
gaacagatgt ataccccggg cggcgaagtg cgcaacgatg atgtggatca gagcctgatt  780
attgcggcgc gcaacattgt gcgccgcgcg gcggtgagcg cggatccgct ggcgagcctg  840
ctggaaatgt gccatagcac ccagattggc ggcacccgca tggtggatat tctgcgccag  900
aacccgaccg aagaacaggc ggtggatatt tgcaaagcgg cgatgggcct gcgcattagc  960
agcagcttta gctttggcgg ctttaccttt aaacgcacca gcggcagcag cgtgaaacgc 1020
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gaagaattta ccatggtggg caaacgcgcg accgcgattc tgcgcaaagc gacccgccgc 1140
ctgattcagc tgattgtgag cggccgcgat gaacagagca ttgtggaagc gattgtggtg 1200
gcgatggtgt ttagccagga agattgcatg gtgaaagcgg tgcgcggcga tctgaacttt 1260
gtgaaccgcg cgaaccagcg cctgaacccg atgcatcagc tgctgcgcca ttttcagaaa 1320
gatgcgaaag tgctgtttct gaactggggc attgaaccga ttgataacgt gatgggcatg 1380
attggcattc tgccggatat gaccccgagc accgaaatga gcatgcgcgg cgtgcgcgtg 1440
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tttgcggcgg cgccgccgaa acagagccgc atgcagttta gcagcctgac cgtgaacgtg 1920
cgcggcagcg gcatgcgcat tctggtgcgc ggcaacagcc cggtgtttaa ctataacaaa 1980
accaccaaac gcctgaccgt gctgggcaaa gatgcgggca ccctgaccga agatccggat 2040
gaaggcaccg cgggcgtgga aagcgcggtg ctgcgcggct ttctgattct gggcaaagaa 2100
gatcgccgct atggcccggc gctgagcatt aacgaactga gcaacctggc gaaaggcgaa 2160
aaagcgaacg tgctgattgg ccagggcgat gtggtgctgg tgatgaaacg caaacgcgat 2220
agcagcattc tgaccgatag ccagaccgcg accaaacgca ttcgcatggc gattaac     2277
```

<210> 29
<211> 716
<212> PRT
<213> Influenza A virus

<400> 29

```
Met Glu Asp Phe Val Arg Gln Cys Phe Asn Pro Met Ile Val Glu Leu
1               5                  10                  15

Ala Glu Lys Ala Met Lys Glu Tyr Gly Glu Asp Pro Lys Ile Glu Thr
            20                  25                  30

Asn Lys Phe Ala Ala Ile Cys Thr His Leu Glu Val Cys Phe Met Tyr
        35                  40                  45

Ser Asp Phe His Phe Ile Asp Glu Arg Gly Glu Ser Ile Ile Val Glu
    50                  55                  60

Ser Gly Asp Pro Asn Ala Leu Leu Lys His Arg Phe Glu Ile Ile Glu
65                  70                  75                  80

Gly Arg Asp Arg Ile Met Ala Trp Thr Val Val Asn Ser Ile Cys Asn
                85                  90                  95

Thr Thr Gly Val Glu Lys Pro Lys Phe Leu Pro Asp Leu Tyr Asp Tyr
            100                 105                 110

Lys Glu Asn Arg Phe Ile Glu Ile Gly Val Thr Arg Arg Glu Val His
        115                 120                 125

Ile Tyr Tyr Leu Glu Lys Ala Asn Lys Ile Lys Ser Glu Lys Thr His
    130                 135                 140
```

```
Ile His Ile Phe Ser Phe Thr Gly Glu Glu Met Ala Thr Lys Ala Asp
145                 150                 155                 160

Tyr Thr Leu Asp Glu Glu Ser Arg Ala Arg Ile Lys Thr Arg Leu Phe
                165                 170                 175

Thr Ile Arg Gln Glu Met Ala Ser Arg Ser Leu Trp Asp Ser Phe Arg
            180                 185                 190

Gln Ser Glu Arg Gly Glu Glu Thr Ile Glu Glu Lys Phe Glu Ile Thr
        195                 200                 205

Gly Thr Met Arg Lys Leu Ala Asp Gln Ser Leu Pro Pro Asn Phe Pro
    210                 215                 220

Ser Leu Glu Asn Phe Arg Ala Tyr Val Asp Gly Phe Glu Pro Asn Gly
225                 230                 235                 240

Cys Ile Glu Gly Lys Leu Ser Gln Met Ser Lys Glu Val Asn Ala Lys
                245                 250                 255

Ile Glu Pro Phe Leu Arg Thr Thr Pro Arg Pro Leu Arg Leu Pro Asp
            260                 265                 270

Gly Pro Leu Cys His Gln Arg Ser Lys Phe Leu Leu Met Asp Ala Leu
        275                 280                 285

Lys Leu Ser Ile Glu Asp Pro Ser His Glu Gly Glu Gly Ile Pro Leu
    290                 295                 300

Tyr Asp Ala Ile Lys Cys Met Lys Thr Phe Phe Gly Trp Lys Glu Pro
305                 310                 315                 320

Asn Ile Val Lys Pro His Glu Lys Gly Ile Asn Pro Asn Tyr Leu Met
                325                 330                 335

Ala Trp Lys Gln Val Leu Ala Glu Leu Gln Asp Ile Glu Asn Glu Glu
            340                 345                 350

Lys Ile Pro Arg Thr Lys Asn Met Lys Arg Thr Ser Gln Leu Lys Trp
        355                 360                 365

Ala Leu Gly Glu Asn Met Ala Pro Glu Lys Val Asp Phe Asp Asp Cys
    370                 375                 380

Lys Asp Val Gly Asp Leu Lys Gln Tyr Asp Ser Asp Glu Pro Glu Pro
385                 390                 395                 400

Arg Ser Leu Ala Ser Trp Val Gln Asn Glu Phe Asn Lys Ala Cys Glu
                405                 410                 415

Leu Thr Asp Ser Ser Trp Ile Glu Leu Asp Glu Ile Gly Glu Asp Val
            420                 425                 430

Ala Pro Ile Glu His Ile Ala Ser Met Arg Arg Asn Tyr Phe Thr Ala
        435                 440                 445

Glu Val Ser His Cys Arg Ala Thr Glu Tyr Ile Met Lys Gly Val Tyr
    450                 455                 460

Ile Asn Thr Ala Leu Leu Asn Ala Ser Cys Ala Ala Met Asp Asp Phe
465                 470                 475                 480
```

```
Gln Leu Ile Pro Met Ile Ser Lys Cys Arg Thr Lys Glu Gly Arg Arg
                485                 490                 495

Lys Thr Asn Leu Tyr Gly Phe Ile Ile Lys Gly Arg Ser His Leu Arg
            500                 505                 510

Asn Asp Thr Asp Val Val Asn Phe Val Ser Met Glu Phe Ser Leu Thr
        515                 520                 525

Asp Pro Arg Leu Glu Pro His Lys Trp Glu Lys Tyr Cys Val Leu Glu
    530                 535                 540

Ile Gly Asp Met Leu Leu Arg Thr Ala Ile Gly Gln Val Ser Arg Pro
545                 550                 555                 560

Met Phe Leu Tyr Val Arg Thr Asn Gly Thr Ser Lys Ile Lys Met Lys
                565                 570                 575

Trp Gly Met Glu Met Arg Arg Cys Leu Leu Gln Ser Leu Gln Gln Ile
            580                 585                 590

Glu Ser Met Ile Glu Ala Glu Ser Ser Val Lys Glu Lys Asp Met Thr
        595                 600                 605

Lys Glu Phe Phe Glu Asn Lys Ser Glu Thr Trp Pro Ile Gly Glu Ser
    610                 615                 620

Pro Arg Gly Val Glu Glu Gly Ser Ile Gly Lys Val Cys Arg Thr Leu
625                 630                 635                 640

Leu Ala Lys Ser Val Phe Asn Ser Leu Tyr Ala Ser Pro Gln Leu Glu
                645                 650                 655

Gly Phe Ser Ala Glu Ser Arg Lys Leu Leu Leu Ile Val Gln Ala Leu
            660                 665                 670

Arg Asp Asn Leu Glu Pro Gly Thr Phe Asp Leu Gly Gly Leu Tyr Glu
        675                 680                 685

Ala Ile Glu Glu Cys Leu Ile Asn Asp Pro Trp Val Leu Leu Asn Ala
    690                 695                 700

Ser Trp Phe Asn Ser Phe Leu Thr His Ala Leu Lys
705                 710                 715

<210>   30
<211>   757
<212>   PRT
<213>   Influenza A virus

<400>   30
Met Asp Val Asn Pro Thr Leu Leu Phe Leu Lys Val Pro Ala Gln Asn
1               5                   10                  15

Ala Ile Ser Thr Thr Phe Pro Tyr Thr Gly Asp Pro Pro Tyr Ser His
            20                  25                  30

Gly Thr Gly Thr Gly Tyr Thr Met Asp Thr Val Asn Arg Thr His Gln
        35                  40                  45

Tyr Ser Glu Arg Gly Arg Trp Thr Lys Asn Thr Glu Thr Gly Ala Pro
    50                  55                  60
```

```
Gln Leu Asn Pro Ile Asp Gly Pro Leu Pro Lys Asp Asn Glu Pro Ser
65                  70                  75                  80

Gly Tyr Ala Gln Thr Asp Cys Val Leu Glu Ala Met Ala Phe Leu Glu
                85                  90                  95

Glu Ser His Pro Gly Ile Phe Glu Asn Ser Cys Ile Glu Thr Met Glu
            100                 105                 110

Val Val Gln Gln Thr Arg Val Asp Lys Leu Thr Gln Gly Arg Gln Thr
        115                 120                 125

Tyr Asp Trp Thr Leu Asn Arg Asn Gln Pro Ala Ala Thr Ala Leu Ala
    130                 135                 140

Asn Thr Ile Glu Val Phe Arg Ser Asn Gly Leu Ile Ala Asn Glu Ser
145                 150                 155                 160

Gly Arg Leu Ile Asp Phe Leu Lys Asp Val Met Glu Ser Met Asp Arg
                165                 170                 175

Asp Glu Val Glu Val Thr Thr His Phe Gln Arg Lys Arg Arg Val Arg
            180                 185                 190

Asp Asn Val Thr Lys Lys Met Val Thr Gln Arg Thr Ile Gly Lys Lys
        195                 200                 205

Lys His Lys Leu Asp Lys Arg Ser Tyr Leu Ile Arg Ala Leu Thr Leu
    210                 215                 220

Asn Thr Met Thr Lys Asp Ala Glu Arg Gly Lys Leu Lys Arg Arg Ala
225                 230                 235                 240

Ile Ala Thr Pro Gly Met Gln Ile Arg Gly Phe Val Tyr Phe Val Glu
                245                 250                 255

Thr Leu Ala Arg Ser Ile Cys Glu Lys Leu Glu Gln Ser Gly Leu Pro
            260                 265                 270

Val Gly Gly Asn Glu Lys Lys Ala Lys Leu Ala Asn Val Val Arg Lys
        275                 280                 285

Met Met Thr Asn Ser Gln Asp Thr Glu Ile Ser Phe Thr Ile Thr Gly
    290                 295                 300

Asp Asn Thr Lys Trp Asn Glu Asn Gln Asn Pro Arg Met Phe Leu Ala
305                 310                 315                 320

Met Ile Thr Tyr Ile Thr Lys Asn Gln Pro Glu Trp Phe Arg Asn Ile
                325                 330                 335

Leu Ser Ile Ala Pro Ile Met Phe Ser Asn Lys Met Ala Arg Leu Gly
            340                 345                 350

Lys Gly Tyr Met Phe Glu Ser Lys Ser Met Lys Leu Arg Thr Gln Ile
        355                 360                 365

Pro Ala Glu Met Leu Ala Asn Ile Asp Leu Lys Tyr Phe Asn Asp Ser
    370                 375                 380

Thr Lys Arg Lys Ile Glu Lys Ile Arg Pro Leu Leu Ile Asp Gly Thr
385                 390                 395                 400
```

```
Ala Ser Leu Ser Pro Gly Met Met Met Gly Met Phe Asn Met Leu Ser
                405                 410                 415

Thr Val Leu Gly Val Ser Ile Leu Asn Leu Gly Gln Lys Arg Tyr Thr
            420                 425                 430

Lys Thr Thr Tyr Trp Trp Asp Gly Leu Gln Ser Ser Asp Asp Phe Ala
        435                 440                 445

Leu Ile Val Asn Ala Pro Asn Tyr Ala Gly Ile Gln Ala Gly Val Asp
    450                 455                 460

Arg Phe Tyr Arg Thr Cys Lys Leu Leu Gly Ile Asn Met Ser Lys Lys
465                 470                 475                 480

Lys Ser Tyr Ile Asn Arg Thr Gly Thr Phe Glu Phe Thr Ser Phe Phe
                485                 490                 495

Tyr Arg Tyr Gly Phe Val Ala Asn Phe Ser Met Glu Leu Pro Ser Phe
            500                 505                 510

Gly Val Ser Gly Val Asn Glu Ser Ala Asp Met Ser Ile Gly Val Thr
        515                 520                 525

Val Ile Lys Asn Asn Met Ile Asn Asn Asp Leu Gly Pro Ala Thr Ala
    530                 535                 540

Gln Met Ala Leu Gln Leu Phe Ile Lys Asp Tyr Arg Tyr Thr Tyr Arg
545                 550                 555                 560

Cys His Arg Gly Asp Thr Gln Ile Gln Thr Arg Arg Ser Phe Glu Ile
                565                 570                 575

Lys Lys Leu Trp Asp Gln Thr Arg Ser Lys Ala Gly Leu Leu Val Ser
            580                 585                 590

Asp Gly Gly Pro Asn Leu Tyr Asn Ile Arg Asn Leu His Ile Pro Glu
        595                 600                 605

Val Cys Leu Lys Trp Glu Leu Met Asp Glu Asp Tyr Gln Gly Arg Leu
    610                 615                 620

Cys Asn Pro Ser Asn Pro Phe Val Ser His Lys Glu Ile Glu Ser Val
625                 630                 635                 640

Asn Asn Ala Val Met Met Pro Ala His Gly Pro Ala Lys Asn Met Glu
                645                 650                 655

Tyr Asp Ala Val Ala Thr Thr His Ser Trp Val Pro Lys Arg Asn Arg
            660                 665                 670

Ser Ile Leu Asn Thr Ser Gln Arg Gly Ile Leu Glu Asp Glu Gln Met
        675                 680                 685

Tyr Gln Arg Cys Cys Asn Leu Phe Glu Lys Phe Phe Pro Ser Ser Ser
    690                 695                 700

Tyr Arg Arg Pro Val Gly Ile Ser Ser Met Val Glu Ala Met Val Ser
705                 710                 715                 720

Arg Ala Arg Ile Asp Ala Arg Ile Asp Phe Glu Ser Gly Arg Ile Lys
                725                 730                 735
```

Lys Glu Glu Phe Ala Glu Ile Met Lys Thr Cys Ser Thr Ile Glu Asp
740 745 750

Leu Arg Arg Gln Lys
755

<210> 31
<211> 759
<212> PRT
<213> Influenza A virus

<400> 31
Met Glu Arg Ile Lys Glu Leu Arg Asn Leu Met Ser Gln Ser Arg Thr
1 5 10 15

Arg Glu Ile Leu Thr Lys Thr Thr Val Asp His Met Ala Ile Ile Lys
20 25 30

Lys Tyr Thr Ser Gly Arg Gln Glu Lys Asn Pro Ser Leu Arg Met Lys
35 40 45

Trp Met Met Ala Met Lys Tyr Pro Ile Thr Ala Asp Lys Arg Ile Thr
50 55 60

Glu Met Ile Pro Glu Arg Asn Glu Gln Gly Gln Thr Leu Trp Ser Lys
65 70 75 80

Val Asn Asp Ala Gly Ser Asp Arg Val Met Ile Ser Pro Leu Ala Val
85 90 95

Thr Trp Trp Asn Arg Asn Gly Pro Val Ala Ser Thr Ile His Tyr Pro
100 105 110

Lys Ile Tyr Lys Thr Tyr Phe Glu Lys Val Glu Arg Leu Lys His Gly
115 120 125

Thr Phe Gly Pro Val His Phe Arg Asn Gln Val Lys Ile Arg Arg Arg
130 135 140

Val Asp Ile Asn Pro Gly His Ala Asp Leu Ser Ala Lys Glu Ala Gln
145 150 155 160

Asp Val Ile Met Glu Val Val Phe Pro Asn Glu Val Gly Ala Arg Ile
165 170 175

Leu Thr Ser Glu Ser Gln Leu Thr Ile Thr Lys Glu Lys Lys Glu Glu
180 185 190

Leu Gln Asn Cys Lys Ile Ser Pro Leu Met Val Ala Tyr Met Leu Glu
195 200 205

Arg Glu Leu Val Arg Lys Thr Arg Phe Leu Pro Val Ala Gly Gly Thr
210 215 220

Ser Ser Val Tyr Ile Glu Val Leu His Leu Thr Gln Gly Thr Cys Trp
225 230 235 240

Glu Gln Met Tyr Thr Pro Gly Gly Glu Val Arg Asn Asp Asp Val Asp
245 250 255

Gln Ser Leu Ile Ile Ala Ala Arg Asn Ile Val Arg Arg Ala Ala Val
260 265 270

```
Ser Ala Asp Pro Leu Ala Ser Leu Leu Glu Met Cys His Ser Thr Gln
        275                 280                 285

Ile Gly Gly Thr Arg Met Val Asp Ile Leu Arg Gln Asn Pro Thr Glu
    290                 295                 300

Glu Gln Ala Val Asp Ile Cys Lys Ala Ala Met Gly Leu Arg Ile Ser
305                 310                 315                 320

Ser Ser Phe Ser Phe Gly Gly Phe Thr Phe Lys Arg Thr Ser Gly Ser
                325                 330                 335

Ser Val Lys Arg Glu Glu Glu Val Leu Thr Gly Asn Leu Gln Thr Leu
            340                 345                 350

Lys Leu Thr Val His Glu Gly Tyr Glu Glu Phe Thr Met Val Gly Lys
        355                 360                 365

Arg Ala Thr Ala Ile Leu Arg Lys Ala Thr Arg Arg Leu Ile Gln Leu
    370                 375                 380

Ile Val Ser Gly Arg Asp Glu Gln Ser Ile Val Glu Ala Ile Val Val
385                 390                 395                 400

Ala Met Val Phe Ser Gln Glu Asp Cys Met Val Lys Ala Val Arg Gly
                405                 410                 415

Asp Leu Asn Phe Val Asn Arg Ala Asn Gln Arg Leu Asn Pro Met His
            420                 425                 430

Gln Leu Leu Arg His Phe Gln Lys Asp Ala Lys Val Leu Phe Leu Asn
        435                 440                 445

Trp Gly Ile Glu Pro Ile Asp Asn Val Met Gly Met Ile Gly Ile Leu
    450                 455                 460

Pro Asp Met Thr Pro Ser Thr Glu Met Ser Met Arg Gly Val Arg Val
465                 470                 475                 480

Ser Lys Met Gly Val Asp Glu Tyr Ser Asn Ala Glu Arg Val Val Val
                485                 490                 495

Ser Ile Asp Arg Phe Leu Arg Val Arg Asp Gln Arg Gly Asn Val Leu
            500                 505                 510

Leu Ser Pro Glu Glu Val Ser Glu Thr Gln Gly Thr Glu Lys Leu Thr
        515                 520                 525

Ile Thr Tyr Ser Ser Ser Met Met Trp Glu Ile Asn Gly Pro Glu Ser
    530                 535                 540

Val Leu Ile Asn Thr Tyr Gln Trp Ile Ile Arg Asn Trp Glu Thr Val
545                 550                 555                 560

Lys Ile Gln Trp Ser Gln Asn Pro Thr Met Leu Tyr Asn Lys Met Glu
                565                 570                 575

Phe Glu Pro Phe Gln Ser Leu Val Pro Lys Ala Ile Arg Gly Gln Tyr
            580                 585                 590

Ser Gly Phe Val Arg Thr Leu Phe Gln Gln Met Arg Asp Val Leu Gly
        595                 600                 605
```

```
Thr Phe Asp Thr Thr Gln Ile Ile Lys Leu Leu Pro Phe Ala Ala Ala
    610                 615                 620

Pro Pro Lys Gln Ser Arg Met Gln Phe Ser Ser Leu Thr Val Asn Val
625                 630                 635                 640

Arg Gly Ser Gly Met Arg Ile Leu Val Arg Gly Asn Ser Pro Val Phe
                645                 650                 655

Asn Tyr Asn Lys Thr Thr Lys Arg Leu Thr Val Leu Gly Lys Asp Ala
            660                 665                 670

Gly Thr Leu Thr Glu Asp Pro Asp Glu Gly Thr Ala Gly Val Glu Ser
        675                 680                 685

Ala Val Leu Arg Gly Phe Leu Ile Leu Gly Lys Glu Asp Arg Arg Tyr
    690                 695                 700

Gly Pro Ala Leu Ser Ile Asn Glu Leu Ser Asn Leu Ala Lys Gly Glu
705                 710                 715                 720

Lys Ala Asn Val Leu Ile Gly Gln Gly Asp Val Val Leu Val Met Lys
                725                 730                 735

Arg Lys Arg Asp Ser Ser Ile Leu Thr Asp Ser Gln Thr Ala Thr Lys
            740                 745                 750

Arg Ile Arg Met Ala Ile Asn
        755

<210>   32
<211>   498
<212>   PRT
<213>   Influenza A virus

<400>   32
Met Ala Ser Gln Gly Thr Lys Arg Ser Tyr Glu Gln Met Glu Thr Asp
1               5                   10                  15

Gly Glu Arg Gln Asn Ala Thr Glu Ile Arg Ala Ser Val Gly Arg Met
            20                  25                  30

Ile Gly Gly Ile Gly Arg Phe Tyr Ile Gln Met Cys Thr Glu Leu Lys
        35                  40                  45

Leu Asn Asp Tyr Glu Gly Arg Leu Ile Gln Asn Ser Leu Thr Ile Glu
    50                  55                  60

Arg Met Val Leu Ser Ala Phe Asp Glu Arg Arg Asn Lys Tyr Leu Glu
65                  70                  75                  80

Glu His Pro Ser Ala Gly Lys Asp Pro Lys Lys Thr Gly Gly Pro Ile
                85                  90                  95

Tyr Lys Arg Val Asp Gly Lys Trp Val Arg Glu Leu Val Leu Tyr Asp
            100                 105                 110

Lys Glu Glu Ile Arg Arg Ile Trp Arg Gln Ala Asn Asn Gly Asp Asp
        115                 120                 125

Ala Thr Ala Gly Leu Thr His Ile Met Ile Trp His Ser Asn Leu Asn
    130                 135                 140
```

```
Asp Thr Thr Tyr Gln Arg Thr Arg Ala Leu Val Arg Thr Gly Met Asp
145                 150                 155                 160

Pro Arg Met Cys Ser Leu Met Gln Gly Ser Thr Leu Pro Arg Arg Ser
                165                 170                 175

Gly Ala Ala Gly Ala Ala Val Lys Gly Val Gly Thr Met Val Leu Glu
            180                 185                 190

Leu Ile Arg Met Ile Lys Arg Gly Ile Asn Asp Arg Asn Phe Trp Arg
        195                 200                 205

Gly Glu Asn Gly Arg Lys Thr Arg Ile Ala Tyr Glu Arg Met Cys Asn
    210                 215                 220

Ile Leu Lys Gly Lys Phe Gln Thr Ala Ala Gln Lys Ala Met Met Asp
225                 230                 235                 240

Gln Val Arg Glu Ser Arg Asn Pro Gly Asn Ala Glu Ile Glu Asp Leu
                245                 250                 255

Thr Phe Leu Ala Arg Ser Ala Leu Ile Leu Arg Gly Ser Val Ala His
            260                 265                 270

Lys Ser Cys Leu Pro Ala Cys Val Tyr Gly Pro Ala Val Ala Ser Gly
        275                 280                 285

Tyr Asp Phe Glu Lys Glu Gly Tyr Ser Leu Val Gly Val Asp Pro Phe
    290                 295                 300

Lys Leu Leu Gln Thr Ser Gln Val Tyr Ser Leu Ile Arg Pro Asn Glu
305                 310                 315                 320

Asn Pro Ala His Lys Ser Gln Leu Val Trp Met Ala Cys Asn Ser Ala
                325                 330                 335

Ala Phe Glu Asp Leu Arg Val Ser Ser Phe Ile Arg Gly Thr Arg Val
            340                 345                 350

Leu Pro Arg Gly Lys Leu Ser Thr Arg Gly Val Gln Ile Ala Ser Asn
        355                 360                 365

Glu Asn Met Asp Ala Ile Val Ser Ser Thr Leu Glu Leu Arg Ser Arg
    370                 375                 380

Tyr Trp Ala Ile Arg Thr Arg Ser Gly Gly Asn Thr Asn Gln Gln Arg
385                 390                 395                 400

Ala Ser Ala Gly Gln Ile Ser Thr Gln Pro Thr Phe Ser Val Gln Arg
                405                 410                 415

Asn Leu Pro Phe Asp Lys Thr Thr Ile Met Ala Ala Phe Thr Gly Asn
            420                 425                 430

Thr Glu Gly Arg Thr Ser Asp Met Arg Ala Glu Ile Ile Lys Met Met
        435                 440                 445

Glu Ser Ala Arg Pro Glu Glu Val Ser Phe Gln Gly Arg Gly Val Phe
    450                 455                 460

Glu Leu Ser Asp Glu Arg Ala Thr Asn Pro Ile Val Pro Ser Phe Asp
465                 470                 475                 480
```

```
Met Ser Asn Glu Gly Ser Tyr Phe Phe Gly Asp Asn Ala Glu Glu Tyr
                485                 490                 495

Asp Asn


<210>   33
<211>   252
<212>   PRT
<213>   Influenza A virus

<400>   33
Met Ser Leu Leu Thr Glu Val Glu Thr Tyr Val Leu Ser Ile Val Pro
1               5                   10                  15

Ser Gly Pro Leu Lys Ala Glu Ile Ala Gln Arg Leu Glu Asn Val Phe
            20                  25                  30

Ala Gly Lys Asn Thr Asp Leu Glu Ala Leu Met Glu Trp Leu Lys Thr
        35                  40                  45

Arg Pro Ile Leu Ser Pro Leu Thr Lys Gly Ile Leu Gly Phe Val Phe
    50                  55                  60

Thr Leu Thr Val Pro Ser Glu Arg Gly Leu Gln Arg Arg Arg Phe Val
65                  70                  75                  80

Gln Asn Ala Leu Asn Gly Asn Gly Asp Pro Asn Asn Met Asp Arg Ala
                85                  90                  95

Val Lys Leu Tyr Arg Lys Leu Lys Arg Glu Ile Thr Phe His Gly Ala
            100                 105                 110

Lys Glu Ile Ala Leu Ser Tyr Ser Ala Gly Ala Leu Ala Ser Cys Met
        115                 120                 125

Gly Leu Ile Tyr Asn Arg Met Gly Ala Val Thr Thr Glu Ser Ala Phe
    130                 135                 140

Gly Leu Ile Cys Ala Thr Cys Glu Gln Ile Ala Asp Ser Gln His Lys
145                 150                 155                 160

Ser His Arg Gln Met Val Thr Thr Thr Asn Pro Leu Ile Arg His Glu
                165                 170                 175

Asn Arg Met Val Leu Ala Ser Thr Thr Ala Lys Ala Met Glu Gln Met
            180                 185                 190

Ala Gly Ser Ser Glu Gln Ala Ala Glu Ala Met Glu Val Ala Ser Gln
        195                 200                 205

Ala Arg Gln Met Val Gln Ala Met Arg Ala Ile Gly Thr His Pro Ser
    210                 215                 220

Ser Ser Thr Gly Leu Lys Asn Asp Leu Leu Glu Asn Leu Gln Ala Tyr
225                 230                 235                 240

Gln Lys Arg Met Gly Val Gln Met Gln Arg Phe Lys
                245                 250

<210>   34
<211>   470
```

<212> PRT
<213> Influenza A virus

<400> 34

```
Met Asn Pro Asn Gln Lys Ile Ile Thr Ile Gly Ser Ile Ser Ile Ala
1               5                  10                  15

Ile Gly Ile Ile Ser Leu Met Leu Gln Ile Gly Asn Ile Ile Ser Ile
            20                  25                  30

Trp Ala Ser His Ser Ile Gln Thr Gly Ser Gln Asn His Thr Gly Val
        35                  40                  45

Cys Asn Gln Arg Ile Ile Thr Tyr Glu Asn Ser Thr Trp Val Asn His
    50                  55                  60

Thr Tyr Val Asn Ile Asn Asn Thr Asn Val Val Ala Gly Lys Asp Lys
65                  70                  75                  80

Thr Ser Val Thr Leu Ala Gly Asn Ser Ser Leu Cys Ser Ile Ser Gly
                85                  90                  95

Trp Ala Ile Tyr Thr Lys Asp Asn Ser Ile Arg Ile Gly Ser Lys Gly
            100                 105                 110

Asp Val Phe Val Ile Arg Glu Pro Phe Ile Ser Cys Ser His Leu Glu
        115                 120                 125

Cys Arg Thr Phe Phe Leu Thr Gln Gly Ala Leu Leu Asn Asp Lys His
    130                 135                 140

Ser Asn Gly Thr Val Lys Asp Arg Ser Pro Tyr Arg Ala Leu Met Ser
145                 150                 155                 160

Cys Pro Leu Gly Glu Ala Pro Ser Pro Tyr Asn Ser Lys Phe Glu Ser
                165                 170                 175

Val Ala Trp Ser Ala Ser Ala Cys His Asp Gly Met Gly Trp Leu Thr
            180                 185                 190

Ile Gly Ile Ser Gly Pro Asp Asn Gly Ala Val Ala Val Leu Lys Tyr
        195                 200                 205

Asn Gly Ile Ile Thr Glu Thr Ile Lys Ser Trp Lys Lys Arg Ile Leu
    210                 215                 220

Arg Thr Gln Glu Ser Glu Cys Val Cys Val Asn Gly Ser Cys Phe Thr
225                 230                 235                 240

Ile Met Thr Asp Gly Pro Ser Asn Gly Ala Ala Ser Tyr Lys Ile Phe
                245                 250                 255

Lys Ile Glu Lys Gly Lys Val Thr Lys Ser Ile Glu Leu Asn Ala Pro
            260                 265                 270

Asn Phe His Tyr Glu Glu Cys Ser Cys Tyr Pro Asp Thr Gly Thr Val
        275                 280                 285

Met Cys Val Cys Arg Asp Asn Trp His Gly Ser Asn Arg Pro Trp Val
    290                 295                 300

Ser Phe Asn Gln Asn Leu Asp Tyr Gln Ile Gly Tyr Ile Cys Ser Gly
305                 310                 315                 320
```

Val Phe Gly Asp Asn Pro Arg Pro Lys Asp Gly Glu Gly Ser Cys Asn
325 330 335

Pro Val Thr Val Asp Gly Ala Asp Gly Val Lys Gly Phe Ser Tyr Lys
340 345 350

Tyr Gly Asn Gly Val Trp Ile Gly Arg Thr Lys Ser Asn Arg Leu Arg
355 360 365

Lys Gly Phe Glu Met Ile Trp Asp Pro Asn Gly Trp Thr Asp Thr Asp
370 375 380

Ser Asp Phe Ser Val Lys Gln Asp Val Val Ala Ile Thr Asp Trp Ser
385 390 395 400

Gly Tyr Ser Gly Ser Phe Val Gln His Pro Glu Leu Thr Gly Leu Asp
405 410 415

Cys Ile Arg Pro Cys Phe Trp Val Glu Leu Val Arg Gly Leu Pro Arg
420 425 430

Glu Asn Thr Thr Ile Trp Thr Ser Gly Ser Ser Ile Ser Phe Cys Gly
435 440 445

Val Asn Ser Asp Thr Ala Asn Trp Ser Trp Pro Asp Gly Ala Glu Leu
450 455 460

Pro Phe Thr Ile Asp Lys
465 470

<210> 35
<211> 716
<212> PRT
<213> Influenza A virus

<400> 35
Met Glu Asp Phe Val Arg Gln Cys Phe Asn Pro Met Ile Val Glu Leu
1 5 10 15

Ala Glu Lys Ala Met Lys Glu Tyr Gly Glu Asp Leu Lys Ile Glu Thr
20 25 30

Asn Lys Phe Ala Ala Ile Cys Thr His Leu Glu Val Cys Phe Met Tyr
35 40 45

Ser Asp Phe His Phe Ile Asn Glu Gln Gly Glu Ser Ile Val Val Glu
50 55 60

Leu Asp Asp Pro Asn Ala Leu Leu Lys His Arg Phe Glu Ile Ile Glu
65 70 75 80

Gly Arg Asp Arg Thr Met Ala Trp Thr Val Val Asn Ser Ile Cys Asn
85 90 95

Thr Thr Gly Ala Gly Lys Pro Lys Phe Leu Pro Asp Leu Tyr Asp Tyr
100 105 110

Lys Glu Asn Arg Phe Ile Glu Ile Gly Val Thr Arg Arg Glu Val His
115 120 125

Ile Tyr Tyr Leu Glu Lys Ala Asn Lys Ile Lys Ser Glu Asn Thr His
130 135 140

```
Ile His Ile Phe Ser Phe Thr Gly Glu Glu Met Ala Thr Lys Ala Asp
145                 150                 155                 160

Tyr Thr Leu Asp Glu Glu Ser Arg Ala Arg Ile Lys Thr Arg Leu Phe
                165                 170                 175

Thr Ile Arg Gln Glu Met Ala Asn Arg Gly Leu Trp Asp Ser Phe Arg
            180                 185                 190

Gln Ser Glu Arg Gly Glu Glu Thr Ile Glu Glu Lys Phe Glu Ile Thr
        195                 200                 205

Gly Thr Met Arg Arg Leu Ala Asp Gln Ser Leu Pro Pro Asn Phe Ser
    210                 215                 220

Cys Leu Glu Asn Phe Arg Ala Tyr Val Asp Gly Phe Glu Pro Asn Gly
225                 230                 235                 240

Cys Ile Glu Gly Lys Leu Ser Gln Met Ser Lys Glu Val Asn Ala Gln
                245                 250                 255

Ile Glu Pro Phe Leu Lys Thr Thr Pro Arg Pro Ile Lys Leu Pro Asn
            260                 265                 270

Gly Pro Pro Cys Tyr Gln Arg Ser Lys Phe Leu Leu Met Asp Ala Leu
        275                 280                 285

Lys Leu Ser Ile Glu Asp Pro Ser His Glu Gly Glu Gly Ile Pro Leu
    290                 295                 300

Tyr Asp Ala Ile Lys Cys Met Lys Thr Phe Phe Gly Trp Lys Glu Pro
305                 310                 315                 320

Tyr Ile Val Lys Pro His Glu Lys Gly Ile Asn Ser Asn Tyr Leu Leu
                325                 330                 335

Ser Trp Lys Gln Val Leu Ser Glu Leu Gln Asp Ile Glu Asn Glu Glu
            340                 345                 350

Lys Ile Pro Arg Thr Lys Asn Met Lys Lys Thr Ser Gln Leu Lys Trp
        355                 360                 365

Ala Leu Gly Glu Asn Met Ala Pro Glu Lys Val Asp Phe Glu Asn Cys
    370                 375                 380

Arg Asp Ile Ser Asp Leu Lys Gln Tyr Asp Ser Asp Glu Pro Glu Leu
385                 390                 395                 400

Arg Ser Leu Ser Ser Trp Ile Gln Asn Glu Phe Asn Lys Ala Cys Glu
                405                 410                 415

Leu Thr Asp Ser Val Trp Ile Glu Leu Asp Glu Ile Gly Glu Asp Val
            420                 425                 430

Ala Pro Ile Glu His Ile Ala Ser Met Arg Arg Asn Tyr Phe Thr Ala
        435                 440                 445

Glu Val Ser His Cys Arg Ala Thr Glu Tyr Ile Met Lys Gly Val Tyr
    450                 455                 460

Ile Asn Thr Ala Leu Leu Asn Ala Ser Cys Ala Ala Met Asp Asp Phe
465                 470                 475                 480
```

```
Gln Leu Ile Pro Met Ile Ser Lys Cys Arg Thr Lys Glu Gly Arg Arg
                485                 490                 495

Lys Thr Asn Leu Tyr Gly Phe Ile Ile Lys Gly Arg Ser His Leu Arg
            500                 505                 510

Asn Asp Thr Asp Val Val Asn Phe Val Ser Met Glu Phe Ser Leu Thr
        515                 520                 525

Asp Pro Arg Leu Glu Pro His Lys Trp Glu Lys Tyr Cys Val Leu Glu
    530                 535                 540

Ile Gly Asp Met Leu Leu Arg Ser Ala Ile Gly Gln Ile Ser Arg Pro
545                 550                 555                 560

Met Phe Leu Tyr Val Arg Thr Asn Gly Thr Ser Lys Val Lys Met Lys
                565                 570                 575

Trp Gly Met Glu Met Arg Arg Cys Leu Leu Gln Ser Leu Gln Gln Ile
            580                 585                 590

Glu Ser Met Ile Glu Ala Glu Ser Ser Val Lys Glu Lys Asp Met Thr
        595                 600                 605

Lys Glu Phe Phe Glu Asn Lys Ser Glu Ala Trp Pro Ile Gly Glu Ser
    610                 615                 620

Pro Lys Gly Val Glu Glu Gly Ser Ile Gly Lys Val Cys Arg Thr Leu
625                 630                 635                 640

Leu Ala Lys Ser Val Phe Asn Ser Leu Tyr Ala Ser Pro Gln Leu Glu
                645                 650                 655

Gly Phe Ser Ala Glu Ser Arg Lys Leu Leu Leu Val Val Gln Ala Leu
            660                 665                 670

Arg Asp Asn Leu Glu Pro Gly Thr Phe Asp Leu Gly Gly Leu Tyr Glu
        675                 680                 685

Ala Ile Glu Glu Cys Leu Ile Asn Asp Pro Trp Val Leu Leu Asn Ala
    690                 695                 700

Ser Trp Phe Asn Ser Phe Leu Thr His Ala Leu Lys
705                 710                 715

<210>   36
<211>   757
<212>   PRT
<213>   Influenza A virus

<400>   36
Met Asp Val Asn Pro Thr Leu Leu Phe Leu Lys Val Pro Ala Gln Asn
1               5                   10                  15

Ala Ile Ser Thr Thr Phe Pro Tyr Thr Gly Asp Pro Pro Tyr Ser His
            20                  25                  30

Gly Thr Gly Thr Gly Tyr Thr Met Asp Thr Val Asn Arg Thr His Gln
        35                  40                  45

Tyr Ser Glu Lys Gly Lys Trp Thr Thr Asn Thr Glu Thr Gly Ala Pro
    50                  55                  60
```

```
Gln Leu Asn Pro Ile Asp Gly Pro Leu Pro Glu Asp Asn Glu Pro Ser
65                  70                  75                  80

Gly Tyr Ala Gln Thr Asp Cys Val Leu Glu Ala Met Ala Phe Leu Glu
                85                  90                  95

Glu Ser His Pro Gly Ile Phe Glu Asn Ser Cys Leu Glu Thr Met Glu
            100                 105                 110

Ala Val Gln Gln Thr Arg Val Asp Arg Leu Thr Gln Gly Arg Gln Thr
        115                 120                 125

Tyr Asp Trp Thr Leu Asn Arg Asn Gln Pro Ala Ala Thr Ala Leu Ala
    130                 135                 140

Asn Thr Ile Glu Val Phe Arg Ser Asn Gly Leu Thr Ala Asn Glu Ser
145                 150                 155                 160

Gly Arg Leu Ile Asp Phe Leu Lys Asp Val Met Glu Ser Met Asp Lys
                165                 170                 175

Glu Glu Met Glu Ile Thr Thr His Phe Gln Arg Lys Arg Arg Val Arg
            180                 185                 190

Asp Asn Met Thr Lys Lys Met Val Thr Gln Arg Thr Ile Gly Lys Lys
        195                 200                 205

Lys Gln Arg Val Asn Lys Arg Gly Tyr Leu Ile Arg Ala Leu Thr Leu
    210                 215                 220

Asn Thr Met Thr Lys Asp Ala Glu Arg Gly Lys Leu Lys Arg Arg Ala
225                 230                 235                 240

Ile Ala Thr Pro Gly Met Gln Ile Arg Gly Phe Val Tyr Phe Val Glu
                245                 250                 255

Thr Leu Ala Arg Ser Ile Cys Glu Lys Leu Glu Gln Ser Gly Leu Pro
            260                 265                 270

Val Gly Gly Asn Glu Lys Lys Ala Lys Leu Ala Asn Val Val Arg Lys
        275                 280                 285

Met Met Thr Asn Ser Gln Asp Thr Glu Leu Ser Phe Thr Ile Thr Gly
    290                 295                 300

Asp Asn Thr Lys Trp Asn Glu Asn Gln Asn Pro Arg Met Phe Leu Ala
305                 310                 315                 320

Met Ile Thr Tyr Ile Thr Lys Asn Gln Pro Glu Trp Phe Arg Asn Ile
                325                 330                 335

Leu Ser Ile Ala Pro Ile Met Phe Ser Asn Lys Met Ala Arg Leu Gly
            340                 345                 350

Lys Gly Tyr Met Phe Glu Ser Lys Arg Met Lys Leu Arg Thr Gln Ile
        355                 360                 365

Pro Ala Glu Met Leu Ala Ser Ile Asp Leu Lys Tyr Phe Asn Glu Ser
    370                 375                 380

Thr Arg Lys Lys Ile Glu Lys Ile Arg Pro Leu Leu Ile Asp Gly Thr
385                 390                 395                 400
```

```
Ala Ser Leu Ser Pro Gly Met Met Met Gly Met Phe Asn Met Leu Ser
                405                 410                 415

Thr Val Leu Gly Val Ser Ile Leu Asn Leu Gly Gln Lys Lys Tyr Thr
            420                 425                 430

Lys Thr Thr Tyr Trp Trp Asp Gly Leu Gln Ser Ser Asp Asp Phe Ala
        435                 440                 445

Leu Ile Val Asn Ala Pro Asn His Glu Gly Ile Gln Ala Gly Val Asn
    450                 455                 460

Arg Phe Tyr Arg Thr Cys Lys Leu Val Gly Ile Asn Met Ser Lys Lys
465                 470                 475                 480

Lys Ser Tyr Ile Asn Lys Thr Gly Thr Phe Glu Phe Thr Ser Phe Phe
                485                 490                 495

Tyr Arg Tyr Gly Phe Val Ala Asn Phe Ser Met Glu Leu Pro Ser Phe
            500                 505                 510

Gly Val Ser Gly Ile Asn Glu Ser Ala Asp Met Ser Ile Gly Val Thr
        515                 520                 525

Val Ile Lys Asn Asn Met Ile Asn Asn Asp Leu Gly Pro Ala Thr Ala
    530                 535                 540

Gln Met Ala Leu Gln Leu Phe Ile Lys Asp Tyr Arg Tyr Thr Tyr Arg
545                 550                 555                 560

Cys His Arg Gly Asp Thr Gln Ile Gln Thr Arg Arg Ser Phe Glu Leu
                565                 570                 575

Lys Lys Leu Trp Asp Gln Thr Gln Ser Arg Ala Gly Leu Leu Val Ser
            580                 585                 590

Asp Gly Gly Pro Asn Leu Tyr Asn Ile Arg Asn Leu His Ile Pro Glu
        595                 600                 605

Val Cys Leu Lys Trp Glu Leu Met Asp Glu Asn Tyr Arg Gly Arg Leu
    610                 615                 620

Cys Asn Pro Leu Asn Pro Phe Val Ser His Lys Glu Ile Glu Ser Val
625                 630                 635                 640

Asn Asn Ala Val Val Met Pro Ala His Gly Pro Ala Lys Ser Met Glu
                645                 650                 655

Tyr Asp Ala Val Ala Thr Thr His Ser Trp Ile Pro Lys Arg Asn Arg
            660                 665                 670

Ser Ile Leu Asn Thr Ser Gln Arg Gly Ile Leu Glu Asp Glu Gln Met
        675                 680                 685

Tyr Gln Lys Cys Cys Asn Leu Phe Glu Lys Phe Phe Pro Ser Ser Ser
    690                 695                 700

Tyr Arg Arg Pro Ile Gly Ile Ser Ser Met Val Glu Ala Met Val Ser
705                 710                 715                 720

Arg Ala Arg Ile Asp Ala Arg Ile Asp Phe Glu Ser Gly Arg Ile Lys
                725                 730                 735
```

```
Lys Glu Glu Phe Ser Glu Ile Met Lys Ile Cys Ser Thr Ile Glu Glu
            740                 745                 750

Leu Arg Arg Gln Arg
        755

<210>   37
<211>   759
<212>   PRT
<213>   Influenza A virus

<400>   37
Met Glu Arg Ile Lys Glu Leu Arg Asn Leu Met Ser Gln Ser Arg Thr
1               5                   10                  15

Arg Glu Ile Leu Thr Lys Thr Thr Val Asp His Met Ala Ile Ile Lys
            20                  25                  30

Lys Tyr Thr Ser Gly Arg Gln Glu Lys Asn Pro Ser Leu Arg Met Lys
        35                  40                  45

Trp Met Met Ala Met Lys Tyr Pro Ile Thr Ala Asp Lys Arg Ile Thr
    50                  55                  60

Glu Met Val Pro Glu Arg Asn Glu Gln Gly Gln Thr Leu Trp Ser Lys
65                  70                  75                  80

Met Ser Asp Ala Gly Ser Asp Arg Val Met Val Ser Pro Leu Ala Val
                85                  90                  95

Thr Trp Trp Asn Arg Asn Gly Pro Val Thr Ser Thr Val His Tyr Pro
            100                 105                 110

Lys Val Tyr Lys Thr Tyr Phe Asp Lys Val Glu Arg Leu Lys His Gly
        115                 120                 125

Thr Phe Gly Pro Val His Phe Arg Asn Gln Val Lys Ile Arg Arg Arg
    130                 135                 140

Val Asp Ile Asn Pro Gly His Ala Asp Leu Ser Ala Lys Glu Ala Gln
145                 150                 155                 160

Asp Val Ile Met Glu Val Val Phe Pro Asn Glu Val Gly Ala Arg Ile
                165                 170                 175

Leu Thr Ser Glu Ser Gln Leu Thr Ile Thr Lys Glu Lys Lys Glu Glu
            180                 185                 190

Leu Arg Asp Cys Lys Ile Ser Pro Leu Met Val Ala Tyr Met Leu Glu
        195                 200                 205

Arg Glu Leu Val Arg Lys Thr Arg Phe Leu Pro Val Ala Gly Gly Thr
    210                 215                 220

Ser Ser Ile Tyr Ile Glu Val Leu His Leu Thr Gln Gly Thr Cys Trp
225                 230                 235                 240

Glu Gln Met Tyr Thr Pro Gly Gly Glu Val Arg Asn Asp Asp Val Asp
                245                 250                 255

Gln Ser Leu Ile Ile Ala Ala Arg Asn Ile Val Arg Arg Ala Ala Val
            260                 265                 270
```

```
Ser Ala Asp Pro Leu Ala Ser Leu Leu Glu Met Cys His Ser Thr Gln
        275                 280                 285

Ile Gly Gly Thr Arg Met Val Asp Ile Leu Arg Gln Asn Pro Thr Glu
    290                 295                 300

Glu Gln Ala Val Asp Ile Cys Lys Ala Ala Met Gly Leu Arg Ile Ser
305                 310                 315                 320

Ser Ser Phe Ser Phe Gly Gly Phe Thr Phe Lys Arg Thr Ser Gly Ser
                325                 330                 335

Ser Val Lys Lys Glu Glu Glu Val Leu Thr Gly Asn Leu Gln Thr Leu
            340                 345                 350

Lys Ile Arg Val His Glu Gly Tyr Glu Glu Phe Thr Met Val Gly Lys
        355                 360                 365

Arg Ala Thr Ala Ile Leu Arg Lys Ala Thr Arg Arg Leu Val Gln Leu
    370                 375                 380

Ile Val Ser Gly Arg Asp Glu Gln Ser Ile Ala Glu Ala Ile Ile Val
385                 390                 395                 400

Ala Met Val Phe Ser Gln Glu Asp Cys Met Ile Lys Ala Val Arg Gly
                405                 410                 415

Asp Leu Asn Phe Val Asn Arg Ala Asn Gln Arg Leu Asn Pro Met His
            420                 425                 430

Gln Leu Leu Arg His Phe Gln Lys Asp Ala Lys Val Leu Phe Gln Asn
        435                 440                 445

Trp Gly Ile Glu His Ile Asp Ser Val Met Gly Met Val Gly Val Leu
    450                 455                 460

Pro Asp Met Thr Pro Ser Thr Glu Met Ser Met Arg Gly Ile Arg Val
465                 470                 475                 480

Ser Lys Met Gly Val Asp Glu Tyr Ser Ser Thr Glu Arg Val Val Val
                485                 490                 495

Ser Ile Asp Arg Phe Leu Arg Val Arg Asp Gln Arg Gly Asn Val Leu
            500                 505                 510

Leu Ser Pro Glu Glu Val Ser Glu Thr Gln Gly Thr Glu Arg Leu Thr
        515                 520                 525

Ile Thr Tyr Ser Ser Ser Met Met Trp Glu Ile Asn Gly Pro Glu Ser
    530                 535                 540

Val Leu Val Asn Thr Tyr Gln Trp Ile Ile Arg Asn Trp Glu Ala Val
545                 550                 555                 560

Lys Ile Gln Trp Ser Gln Asn Pro Ala Met Leu Tyr Asn Lys Met Glu
                565                 570                 575

Phe Glu Pro Phe Gln Ser Leu Val Pro Lys Ala Ile Arg Ser Gln Tyr
            580                 585                 590

Ser Gly Phe Val Arg Thr Leu Phe Gln Gln Met Arg Asp Val Leu Gly
        595                 600                 605
```

```
Thr Phe Asp Thr Thr Gln Ile Ile Lys Leu Leu Pro Phe Ala Ala Ala
    610                 615                 620

Pro Pro Lys Gln Ser Arg Met Gln Phe Ser Ser Leu Thr Val Asn Val
625                 630                 635                 640

Arg Gly Ser Gly Met Arg Ile Leu Val Arg Gly Asn Ser Pro Val Phe
                645                 650                 655

Asn Tyr Asn Lys Thr Thr Lys Arg Leu Thr Ile Leu Gly Lys Asp Ala
            660                 665                 670

Gly Thr Leu Ile Glu Asp Pro Asp Glu Ser Thr Ser Gly Val Glu Ser
        675                 680                 685

Ala Val Leu Arg Gly Phe Leu Ile Ile Gly Lys Glu Asp Arg Arg Tyr
    690                 695                 700

Gly Pro Ala Leu Ser Ile Asn Glu Leu Ser Asn Leu Ala Lys Gly Glu
705                 710                 715                 720

Lys Ala Asn Val Leu Ile Gly Gln Gly Asp Val Val Leu Val Met Lys
                725                 730                 735

Arg Lys Arg Asp Ser Ser Ile Leu Thr Asp Ser Gln Thr Ala Thr Lys
            740                 745                 750

Arg Ile Arg Met Ala Ile Asn
        755

<210>   38
<211>   498
<212>   PRT
<213>   Influenza A virus

<400>   38
Met Ala Ser Gln Gly Thr Lys Arg Ser Tyr Glu Gln Met Glu Thr Asp
1               5                   10                  15

Gly Asp Arg Gln Asn Ala Thr Glu Ile Arg Ala Ser Val Gly Lys Met
            20                  25                  30

Ile Asp Gly Ile Gly Arg Phe Tyr Ile Gln Met Cys Thr Glu Leu Lys
        35                  40                  45

Leu Ser Asp Tyr Glu Gly Arg Leu Ile Gln Asn Ser Leu Thr Ile Glu
    50                  55                  60

Lys Met Val Leu Ser Ala Phe Asp Glu Arg Arg Asn Lys Tyr Leu Glu
65                  70                  75                  80

Glu His Pro Ser Ala Gly Lys Asp Pro Lys Lys Thr Gly Gly Pro Ile
                85                  90                  95

Tyr Arg Arg Val Asp Gly Lys Trp Met Arg Glu Leu Val Leu Tyr Asp
            100                 105                 110

Lys Glu Glu Ile Arg Arg Ile Trp Arg Gln Ala Asn Asn Gly Glu Asp
        115                 120                 125

Ala Thr Ala Gly Leu Thr His Ile Met Ile Trp His Ser Asn Leu Asn
    130                 135                 140
```

```
Asp Ala Thr Tyr Gln Arg Thr Arg Ala Leu Val Arg Thr Gly Met Asp
145                 150                 155                 160

Pro Arg Met Cys Ser Leu Met Gln Gly Ser Thr Leu Pro Arg Arg Ser
                165                 170                 175

Gly Ala Ala Gly Ala Ala Val Lys Gly Ile Gly Thr Met Val Met Glu
            180                 185                 190

Leu Ile Arg Met Val Lys Arg Gly Ile Asn Asp Arg Asn Phe Trp Arg
        195                 200                 205

Gly Glu Asn Gly Arg Lys Thr Arg Ser Ala Tyr Glu Arg Met Cys Asn
    210                 215                 220

Ile Leu Lys Gly Lys Phe Gln Thr Ala Ala Gln Arg Ala Met Val Asp
225                 230                 235                 240

Gln Val Arg Glu Ser Arg Asn Pro Gly Asn Ala Glu Ile Glu Asp Leu
                245                 250                 255

Ile Phe Leu Ala Arg Ser Ala Leu Ile Leu Arg Gly Ser Val Ala His
            260                 265                 270

Lys Ser Cys Leu Pro Ala Cys Val Tyr Gly Pro Ala Val Ser Ser Gly
        275                 280                 285

Tyr Asn Phe Glu Lys Glu Gly Tyr Ser Leu Val Gly Ile Asp Pro Phe
    290                 295                 300

Lys Leu Leu Gln Asn Ser Gln Val Tyr Ser Leu Ile Arg Pro Asn Glu
305                 310                 315                 320

Asn Pro Ala His Lys Ser Gln Leu Val Trp Met Ala Cys His Ser Ala
                325                 330                 335

Ala Phe Glu Asp Leu Arg Leu Leu Ser Phe Ile Arg Gly Thr Lys Val
            340                 345                 350

Ser Pro Arg Gly Lys Leu Ser Thr Arg Gly Val Gln Ile Ala Ser Asn
        355                 360                 365

Glu Asn Met Asp Asn Met Gly Ser Gly Thr Leu Glu Leu Arg Ser Gly
    370                 375                 380

Tyr Trp Ala Ile Arg Thr Arg Ser Gly Gly Asn Thr Asn Gln Gln Arg
385                 390                 395                 400

Ala Ser Ala Gly Gln Thr Ser Val Gln Pro Thr Phe Ser Val Gln Arg
                405                 410                 415

Asn Leu Pro Phe Glu Lys Ser Thr Ile Met Ala Ala Phe Thr Gly Asn
            420                 425                 430

Thr Glu Gly Arg Thr Ser Asp Met Arg Ala Glu Ile Ile Arg Met Met
        435                 440                 445

Glu Gly Ala Lys Pro Glu Glu Val Ser Phe Arg Gly Arg Gly Val Phe
    450                 455                 460

Glu Leu Ser Asp Glu Lys Ala Thr Asn Pro Ile Val Pro Ser Phe Asp
465                 470                 475                 480
```

```
Met Ser Asn Glu Gly Ser Tyr Phe Phe Gly Asp Asn Ala Glu Glu Tyr
                485                 490                 495

Asp Asn


<210>   39
<211>   252
<212>   PRT
<213>   Influenza A virus

<400>   39
Met Ser Leu Leu Thr Glu Val Glu Thr Tyr Val Leu Ser Ile Val Pro
1               5                   10                  15

Ser Gly Pro Leu Lys Ala Glu Ile Ala Gln Arg Leu Glu Asp Val Phe
            20                  25                  30

Ala Gly Lys Asn Thr Asp Leu Glu Ala Leu Met Glu Trp Leu Lys Thr
        35                  40                  45

Arg Pro Ile Leu Ser Pro Leu Thr Lys Gly Ile Leu Gly Phe Val Phe
    50                  55                  60

Thr Leu Thr Val Pro Ser Glu Arg Gly Leu Gln Arg Arg Arg Phe Val
65                  70                  75                  80

Gln Asn Ala Leu Asn Gly Asn Gly Asp Pro Asn Asn Met Asp Lys Ala
                85                  90                  95

Val Lys Leu Tyr Arg Lys Leu Lys Arg Glu Ile Thr Phe His Gly Ala
            100                 105                 110

Lys Glu Ile Ala Leu Ser Tyr Ser Ala Gly Ala Leu Ala Ser Cys Met
        115                 120                 125

Gly Leu Ile Tyr Asn Arg Met Gly Ala Val Thr Thr Glu Val Ala Phe
    130                 135                 140

Gly Leu Val Cys Ala Thr Cys Glu Gln Ile Ala Asp Ser Gln His Arg
145                 150                 155                 160

Ser His Arg Gln Met Val Ala Thr Thr Asn Pro Leu Ile Arg His Glu
                165                 170                 175

Asn Arg Met Val Leu Ala Ser Thr Thr Ala Lys Ala Met Glu Gln Met
            180                 185                 190

Ala Gly Ser Ser Glu Gln Ala Ala Glu Ala Met Glu Ile Ala Ser Gln
        195                 200                 205

Ala Arg Gln Met Val Gln Ala Met Arg Ala Ile Gly Thr His Pro Ser
    210                 215                 220

Ser Ser Thr Gly Leu Arg Asp Asp Leu Leu Glu Asn Leu Gln Thr Tyr
225                 230                 235                 240

Gln Lys Arg Met Gly Val Gln Met Gln Arg Phe Lys
                245                 250

<210>   40
<211>   97
```

<212> PRT
<213> Influenza A virus

<400> 40
Met Ser Leu Leu Thr Glu Val Glu Thr Pro Ile Arg Asn Glu Trp Gly
1 5 10 15

Cys Arg Cys Asn Asp Ser Ser Asp Pro Leu Val Val Ala Ala Asn Ile
20 25 30

Ile Gly Ile Leu His Leu Ile Leu Trp Ile Leu Asp Arg Leu Phe Phe
35 40 45

Lys Cys Val Tyr Arg Leu Phe Lys His Gly Leu Lys Arg Gly Pro Ser
50 55 60

Thr Glu Gly Val Pro Glu Ser Met Arg Glu Glu Tyr Arg Lys Glu Gln
65 70 75 80

Gln Asn Ala Val Asp Ala Asp Asp Ser His Phe Val Ser Ile Glu Leu
85 90 95

Glu

<210> 41
<211> 846
<212> DNA
<213> Influenza A virus

<400> 41
aatggattcc aacactgtgt caagtttcca ggtagattgc tttctttggc atatccggaa 60
acaagttgta gaccaagaac tgagtgatgc cccattcctt gatcggcttc gccgagatca 120
gaggtcccta aggggaagag gcaatactct cggtctagac atcaaagcag ccacccatgt 180
tggaaagcaa attgtagaaa agattctgaa agaagaatct gatgaggcac ttaaaatgac 240
catggtctcc acacctgctt cgcgatacat aactgacatg actattgagg aattgtcaag 300
aaactggttc atgctaatgc ccaagcagaa agtggaagga cctctttgca tcagaatgga 360
ccaggcaatc atggagaaaa acatcatgtt gaaagcgaat ttcagtgtga tttctgaccg 420
actagagacc atagtattac taagggcttt caccgaagag ggagcaattg ttggcgaaat 480
ctcaccattg ccttcttttc caggacatac tattgaggat gtcaaaaatg caattggggt 540
cctcatcgga ggacttgaat ggaatgataa cacagttcga gtctctaaaa atctacagag 600
attcgcttgg agaagcagta atgagaatgg gggacctcca cttactccaa aacagaaacg 660
gaaaatggcg agaacagcta ggtcaaaagt ttgaagagat aagatggctg attgaagaag 720
tgagacacag actaaaaaca actgaaaata gctttgaaca aataacattc atgcaagcat 780
tacaactgct gtttgaagtg gaacaggaga taagaacttt ctcatttcag cttatttaat 840
gataaa 846

<210> 42
<211> 566
<212> PRT
<213> Influenza A virus

<400> 42
Met Lys Thr Ile Ile Ala Leu Ser Tyr Ile Leu Cys Leu Val Phe Ala
1 5 10 15

Gln Lys Leu Pro Gly Asn Asp Asn Ser Thr Ala Thr Leu Cys Leu Gly
20 25 30

His His Ala Val Pro Asn Gly Thr Ile Val Lys Thr Ile Thr Asn Asp
35 40 45

Gln Ile Glu Val Thr Asn Ala Thr Glu Leu Val Gln Ser Ser Ser Thr

```
    50                  55                  60

Gly Gly Ile Cys Asp Ser Pro His Gln Ile Leu Asp Gly Glu Asn Cys
65                  70                  75                  80

Thr Leu Ile Asp Ala Leu Leu Gly Asp Pro Gln Cys Asp Gly Phe Gln
                85                  90                  95

Asn Lys Lys Trp Asp Leu Phe Val Glu Arg Ser Lys Ala Tyr Ser Asn
            100                 105                 110

Cys Tyr Pro Tyr Asp Val Pro Asp Tyr Ala Ser Leu Arg Ser Leu Val
        115                 120                 125

Ala Ser Ser Gly Thr Leu Glu Phe Asn Asp Glu Ser Phe Asn Trp Thr
    130                 135                 140

Gly Val Thr Gln Asn Gly Thr Ser Ser Ser Cys Lys Arg Arg Ser Asn
145                 150                 155                 160

Asn Ser Phe Phe Ser Arg Leu Asn Trp Leu Thr His Leu Lys Phe Lys
                165                 170                 175

Tyr Pro Ala Leu Asn Val Thr Met Pro Asn Asn Glu Lys Phe Asp Lys
            180                 185                 190

Leu Tyr Ile Trp Gly Val His His Pro Val Thr Asp Asn Asp Gln Ile
        195                 200                 205

Phe Leu Tyr Ala Gln Ala Ser Gly Arg Ile Thr Val Ser Thr Lys Arg
    210                 215                 220

Ser Gln Gln Thr Val Ile Pro Asn Ile Gly Ser Arg Pro Arg Ile Arg
225                 230                 235                 240

Asn Ile Pro Ser Arg Ile Ser Ile Tyr Trp Thr Ile Val Lys Pro Gly
                245                 250                 255

Asp Ile Leu Leu Ile Asn Ser Thr Gly Asn Leu Ile Ala Pro Arg Gly
            260                 265                 270

Tyr Phe Lys Ile Arg Ser Gly Lys Ser Ser Ile Met Arg Ser Asp Ala
        275                 280                 285

Pro Ile Gly Lys Cys Asn Ser Glu Cys Ile Thr Pro Asn Gly Ser Ile
    290                 295                 300

Pro Asn Asp Lys Pro Phe Gln Asn Val Asn Arg Ile Thr Tyr Gly Ala
305                 310                 315                 320

Cys Pro Arg Tyr Val Lys Gln Asn Thr Leu Lys Leu Ala Thr Gly Met
                325                 330                 335

Arg Asn Val Pro Glu Lys Gln Thr Arg Gly Ile Phe Gly Ala Ile Ala
            340                 345                 350

Gly Phe Ile Glu Asn Gly Trp Glu Gly Met Val Asp Gly Trp Tyr Gly
        355                 360                 365

Phe Arg His Gln Asn Ser Glu Gly Ile Gly Gln Ala Ala Asp Leu Lys
    370                 375                 380

Ser Thr Gln Ala Ala Ile Asn Gln Ile Asn Gly Lys Leu Asn Arg Leu
```

```
385                 390                 395                 400

Ile Gly Lys Thr Asn Glu Lys Phe His Gln Ile Glu Lys Glu Phe Ser
                405                 410                 415

Glu Val Glu Gly Arg Ile Gln Asp Leu Glu Lys Tyr Val Glu Asp Thr
            420                 425                 430

Lys Ile Asp Leu Trp Ser Tyr Asn Ala Glu Leu Leu Val Ala Leu Glu
        435                 440                 445

Asn Gln His Thr Ile Asp Leu Thr Asp Ser Glu Met Asn Lys Leu Phe
    450                 455                 460

Glu Arg Thr Lys Lys Gln Leu Arg Glu Asn Ala Glu Asp Met Gly Asn
465                 470                 475                 480

Gly Cys Phe Lys Ile Tyr His Lys Cys Asp Asn Ala Cys Ile Gly Ser
                485                 490                 495

Ile Arg Asn Gly Thr Tyr Asp His Asp Val Tyr Arg Asp Glu Ala Leu
            500                 505                 510

Asn Asn Arg Phe Gln Ile Lys Gly Val Glu Leu Lys Ser Gly Tyr Lys
        515                 520                 525

Asp Trp Ile Leu Trp Ile Ser Phe Ala Ile Ser Cys Phe Leu Leu Cys
    530                 535                 540

Val Ala Leu Leu Gly Phe Ile Met Trp Ala Cys Gln Lys Gly Asn Ile
545                 550                 555                 560

Arg Cys Asn Ile Cys Ile
                565

<210>   43
<211>   469
<212>   PRT
<213>   Influenza A virus

<400>   43
Met Asn Pro Asn Gln Lys Ile Ile Thr Ile Gly Ser Val Ser Leu Thr
1               5                   10                  15

Ile Ser Thr Ile Cys Phe Phe Met Gln Ile Ala Ile Leu Ile Thr Thr
            20                  25                  30

Val Thr Leu His Phe Lys Gln Tyr Glu Phe Asn Ser Pro Pro Asn Asn
        35                  40                  45

Gln Val Met Leu Cys Glu Pro Thr Ile Ile Glu Arg Asn Ile Thr Glu
    50                  55                  60

Ile Val Tyr Leu Thr Asn Thr Thr Ile Glu Lys Glu Ile Cys Pro Lys
65                  70                  75                  80

Leu Ala Glu Tyr Arg Asn Trp Ser Lys Pro Gln Cys Asn Ile Thr Gly
                85                  90                  95

Phe Ala Pro Phe Ser Lys Asp Asn Ser Ile Arg Leu Ser Ala Gly Gly
            100                 105                 110

Asp Ile Trp Val Thr Arg Glu Pro Tyr Val Ser Cys Asp Pro Asp Lys
```

```
        115                 120                 125

Cys Tyr Gln Phe Ala Leu Gly Gln Gly Thr Thr Leu Asn Asn Val His
    130                 135                 140

Ser Asn Asp Thr Val His Asp Arg Thr Pro Tyr Arg Thr Leu Leu Met
145                 150                 155                 160

Asn Glu Leu Gly Val Pro Phe His Leu Gly Thr Lys Gln Val Cys Ile
                165                 170                 175

Ala Trp Ser Ser Ser Ser Cys His Asp Gly Lys Ala Trp Leu His Val
            180                 185                 190

Cys Val Thr Gly Asp Asp Lys Asn Ala Thr Ala Ser Phe Ile Tyr Asn
        195                 200                 205

Gly Arg Leu Val Asp Ser Ile Val Ser Trp Ser Lys Glu Ile Leu Arg
    210                 215                 220

Thr Gln Glu Ser Glu Cys Val Cys Ile Asn Gly Thr Cys Thr Val Val
225                 230                 235                 240

Met Thr Asp Gly Ser Ala Ser Gly Lys Ala Asp Thr Lys Ile Leu Phe
                245                 250                 255

Ile Glu Glu Gly Lys Ile Val His Thr Ser Thr Leu Ser Gly Ser Ala
            260                 265                 270

Gln His Val Glu Glu Cys Ser Cys Tyr Pro Arg Tyr Leu Gly Val Arg
        275                 280                 285

Cys Val Cys Arg Asp Asn Trp Lys Gly Ser Asn Arg Pro Ile Val Asp
    290                 295                 300

Ile Asn Ile Lys Asp Tyr Ser Ile Val Ser Ser Tyr Val Cys Ser Gly
305                 310                 315                 320

Leu Val Gly Asp Thr Pro Arg Lys Asn Asp Ser Ser Ser Ser Ser His
                325                 330                 335

Cys Leu Asp Pro Asn Asn Glu Glu Gly Gly His Gly Val Lys Gly Trp
            340                 345                 350

Ala Phe Asp Asp Gly Asn Asp Val Trp Met Gly Arg Thr Ile Ser Glu
        355                 360                 365

Lys Leu Arg Ser Gly Tyr Glu Thr Phe Lys Val Ile Glu Gly Trp Ser
    370                 375                 380

Asn Pro Asn Ser Lys Leu Gln Ile Asn Arg Gln Val Ile Val Asp Arg
385                 390                 395                 400

Gly Asn Arg Ser Gly Tyr Ser Gly Ile Phe Ser Val Glu Gly Lys Ser
                405                 410                 415

Cys Ile Asn Arg Cys Phe Tyr Val Glu Leu Ile Arg Gly Arg Lys Glu
            420                 425                 430

Glu Thr Glu Val Leu Trp Thr Ser Asn Ser Ile Val Val Phe Cys Gly
        435                 440                 445

Thr Ser Gly Thr Tyr Gly Thr Gly Ser Trp Pro Asp Gly Ala Asp Ile
```

```
    450                 455                 460

Asn Leu Met Pro Ile
465

<210>   44
<211>   716
<212>   PRT
<213>   Influenza A virus

<400>   44
Met Glu Asp Phe Val Arg Gln Cys Phe Asn Pro Met Ile Val Glu Leu
1               5                   10                  15

Ala Glu Lys Ala Met Lys Glu Tyr Gly Glu Asp Pro Lys Ile Glu Thr
            20                  25                  30

Asn Lys Phe Ala Ala Ile Cys Thr His Leu Glu Val Cys Phe Met Tyr
        35                  40                  45

Ser Asp Phe His Phe Ile Asp Glu Arg Gly Glu Ser Ile Ile Val Glu
    50                  55                  60

Ser Gly Asp Pro Asn Ala Leu Leu Lys His Arg Phe Glu Ile Ile Glu
65                  70                  75                  80

Gly Arg Asp Arg Ile Met Ala Trp Thr Val Ile Asn Ser Ile Cys Asn
                85                  90                  95

Thr Thr Gly Val Glu Lys Pro Lys Phe Leu Pro Asp Leu Tyr Asp Tyr
            100                 105                 110

Lys Glu Asn Arg Phe Ile Glu Ile Gly Val Thr Arg Arg Glu Val His
        115                 120                 125

Ile Tyr Tyr Leu Glu Lys Ala Asn Lys Ile Lys Ser Glu Lys Thr His
    130                 135                 140

Ile His Ile Phe Ser Phe Thr Gly Glu Glu Met Ala Thr Lys Ala Asp
145                 150                 155                 160

Tyr Thr Leu Asp Glu Glu Ser Arg Ala Arg Ile Lys Thr Arg Leu Phe
                165                 170                 175

Thr Ile Arg Gln Glu Met Ala Ser Lys Ser Leu Trp Asp Ser Phe Arg
            180                 185                 190

Gln Ser Glu Arg Gly Glu Glu Thr Ile Glu Glu Lys Phe Glu Ile Thr
        195                 200                 205

Gly Thr Met Arg Lys Leu Ala Asp Gln Ser Leu Pro Pro Asn Phe Pro
    210                 215                 220

Ser Leu Glu Asn Phe Arg Ala Tyr Val Asp Gly Phe Glu Pro Asn Gly
225                 230                 235                 240

Cys Ile Glu Gly Lys Leu Ser Gln Met Ser Lys Glu Val Asn Ala Lys
                245                 250                 255

Ile Glu Pro Phe Leu Arg Thr Thr Pro Arg Pro Leu Arg Leu Pro Asp
            260                 265                 270

Gly Pro Leu Cys His Gln Arg Ser Lys Phe Leu Leu Met Asp Ala Leu
```

```
        275                 280                 285

Lys Leu Ser Ile Glu Asp Pro Ser His Glu Gly Glu Gly Ile Pro Leu
    290                 295                 300

Tyr Asp Ala Ile Lys Cys Met Lys Thr Phe Phe Gly Trp Lys Glu Pro
305                 310                 315                 320

Asn Ile Val Lys Pro His Glu Lys Gly Ile Asn Pro Asn Tyr Leu Met
                325                 330                 335

Ala Trp Lys Gln Val Leu Ala Glu Leu Gln Asp Ile Glu Asn Glu Glu
            340                 345                 350

Lys Ile Pro Arg Thr Lys Asn Met Lys Arg Thr Ser Gln Leu Lys Trp
        355                 360                 365

Ala Leu Gly Glu Asn Met Ala Pro Glu Lys Val Asp Phe Asp Asp Cys
    370                 375                 380

Lys Asp Val Gly Asp Leu Lys Gln Tyr Asp Ser Asp Glu Pro Glu Pro
385                 390                 395                 400

Arg Ser Leu Ala Ser Trp Val Gln Asn Glu Phe Asn Lys Ala Cys Glu
                405                 410                 415

Leu Thr Asp Ser Ser Trp Ile Glu Leu Asp Glu Ile Gly Glu Asp Val
            420                 425                 430

Ala Pro Ile Glu His Ile Ala Ser Met Arg Arg Asn Tyr Phe Thr Ala
        435                 440                 445

Glu Val Ser His Cys Arg Ala Thr Glu Tyr Ile Met Lys Gly Val Tyr
    450                 455                 460

Ile Asn Thr Ala Leu Leu Asn Ala Ser Cys Ala Ala Met Asp Asp Phe
465                 470                 475                 480

Gln Leu Ile Pro Met Ile Ser Lys Cys Arg Thr Lys Glu Gly Arg Arg
                485                 490                 495

Lys Thr Asn Leu Tyr Gly Phe Ile Ile Lys Gly Arg Ser His Leu Arg
            500                 505                 510

Asn Asp Thr Asp Val Val Asn Phe Val Ser Met Glu Phe Ser Leu Thr
        515                 520                 525

Asp Pro Arg Leu Glu Pro His Lys Trp Glu Lys Tyr Cys Val Leu Glu
    530                 535                 540

Ile Gly Asp Met Leu Leu Arg Thr Ala Ile Gly Gln Val Ser Arg Pro
545                 550                 555                 560

Met Phe Leu Tyr Val Arg Thr Asn Gly Thr Ser Lys Ile Lys Met Lys
                565                 570                 575

Trp Gly Met Glu Met Arg Arg Cys Leu Leu Gln Ser Leu Gln Gln Ile
            580                 585                 590

Glu Ser Met Ile Glu Ala Glu Ser Ser Val Lys Glu Lys Asp Met Thr
        595                 600                 605

Lys Glu Phe Phe Glu Asn Lys Ser Glu Thr Trp Pro Ile Gly Glu Ser
```

```
    610                 615                 620

Pro Arg Gly Val Glu Glu Gly Ser Ile Gly Lys Val Cys Arg Thr Leu
625                 630                 635                 640

Leu Ala Lys Ser Val Phe Asn Ser Leu Tyr Ala Ser Pro Gln Leu Glu
                645                 650                 655

Gly Phe Ser Ala Glu Ser Arg Lys Leu Leu Leu Ile Val Gln Ala Leu
            660                 665                 670

Arg Asp Asn Leu Glu Pro Gly Thr Phe Asp Leu Gly Gly Leu Tyr Glu
        675                 680                 685

Ala Ile Glu Glu Cys Leu Ile Asn Asp Pro Trp Val Leu Leu Asn Ala
    690                 695                 700

Ser Trp Phe Asn Ser Phe Leu Thr His Ala Leu Lys
705                 710                 715

<210>   45
<211>   252
<212>   PRT
<213>   Influenza A virus

<400>   45
Met Ser Leu Leu Thr Glu Val Glu Thr Tyr Val Leu Ser Ile Val Pro
1               5                   10                  15

Ser Gly Pro Leu Lys Ala Glu Ile Ala Gln Arg Leu Glu Asn Val Phe
            20                  25                  30

Ala Gly Lys Asn Thr Asp Leu Glu Ala Leu Met Glu Trp Leu Lys Thr
        35                  40                  45

Arg Pro Ile Leu Ser Pro Leu Thr Lys Gly Ile Leu Gly Phe Val Phe
    50                  55                  60

Thr Leu Thr Val Pro Ser Glu Arg Gly Leu Gln Arg Arg Arg Phe Val
65                  70                  75                  80

Gln Asn Ala Leu Asn Gly Asn Gly Asp Pro Asn Asn Met Asp Lys Ala
                85                  90                  95

Val Lys Leu Tyr Arg Lys Leu Lys Arg Glu Ile Thr Phe His Gly Ala
            100                 105                 110

Lys Glu Ile Ala Leu Ser Tyr Ser Ala Gly Ala Leu Ala Ser Cys Met
        115                 120                 125

Gly Leu Ile Tyr Asn Arg Met Gly Ala Val Thr Thr Glu Ser Ala Phe
    130                 135                 140

Gly Leu Ile Cys Ala Thr Cys Glu Gln Ile Ala Asp Ser Gln His Lys
145                 150                 155                 160

Ser His Arg Gln Met Val Thr Thr Thr Asn Pro Leu Ile Arg His Glu
                165                 170                 175

Asn Arg Met Val Leu Ala Ser Thr Thr Ala Lys Ala Met Glu Gln Met
            180                 185                 190

Ala Gly Ser Ser Glu Gln Ala Ala Glu Ala Met Glu Val Ala Ser Gln
```

```
        195                 200                 205

Ala Arg Gln Met Val Gln Ala Met Arg Ala Ile Gly Thr His Pro Ser
    210                 215                 220

Ser Ser Thr Gly Leu Lys Asn Asp Leu Leu Glu Asn Leu Gln Ala Tyr
225                 230                 235                 240

Gln Lys Arg Met Gly Val Gln Met Gln Arg Phe Lys
                245                 250

<210>   46
<211>   758
<212>   PRT
<213>   Influenza A virus

<400>   46
Met Asp Val Asn Pro Thr Leu Leu Phe Leu Lys Ile Pro Ala Gln Asn
1               5                   10                  15

Ala Ile Ser Thr Thr Phe Pro Tyr Thr Gly Asp Pro Pro Tyr Ser His
            20                  25                  30

Gly Thr Gly Thr Gly Tyr Thr Met Asp Thr Val Asn Arg Thr His Gln
        35                  40                  45

Tyr Ser Glu Lys Gly Lys Trp Thr Thr Asn Thr Glu Thr Gly Ala Pro
    50                  55                  60

Gln Leu Asn Pro Ile Asp Gly Pro Leu Pro Glu Asp Asn Glu Pro Ser
65                  70                  75                  80

Gly Tyr Ala Gln Thr Asp Cys Val Leu Glu Ala Met Ala Phe Leu Glu
                85                  90                  95

Glu Ser His Pro Gly Ile Phe Glu Asn Ser Cys Leu Glu Thr Met Glu
            100                 105                 110

Val Val Gln Gln Thr Arg Val Asp Arg Leu Thr Gln Gly Arg Gln Thr
        115                 120                 125

Tyr Asp Trp Thr Leu Asn Arg Asn Gln Pro Ala Ala Thr Ala Leu Ala
    130                 135                 140

Asn Thr Ile Glu Val Phe Arg Ser Asn Gly Leu Thr Ala Asn Glu Ser
145                 150                 155                 160

Gly Arg Leu Ile Asp Phe Leu Lys Asp Val Met Glu Ser Met Asp Lys
                165                 170                 175

Glu Glu Ile Glu Ile Thr Thr His Phe Gln Arg Lys Arg Arg Val Arg
            180                 185                 190

Asp Asn Met Thr Lys Lys Met Val Thr Gln Arg Thr Ile Gly Lys Lys
        195                 200                 205

Lys Gln Arg Val Asn Lys Arg Ser Tyr Leu Ile Arg Ala Leu Thr Leu
    210                 215                 220

Asn Thr Met Thr Lys Asp Ala Glu Arg Gly Lys Leu Lys Arg Arg Ala
225                 230                 235                 240

Ile Ala Thr Pro Gly Met Gln Ile Arg Gly Phe Val Tyr Phe Val Glu
```

```
                245                 250                 255

Thr Leu Ala Arg Ser Ile Cys Glu Lys Leu Glu Gln Ser Gly Leu Pro
            260                 265                 270

Val Gly Gly Asn Glu Lys Lys Ala Lys Leu Ala Asn Val Val Arg Lys
        275                 280                 285

Met Met Thr Asn Ser Gln Asp Thr Glu Leu Ser Phe Thr Ile Thr Gly
    290                 295                 300

Asp Asn Thr Lys Trp Asn Glu Asn Gln Asn Pro Arg Met Phe Leu Ala
305                 310                 315                 320

Met Ile Thr Tyr Ile Thr Lys Asn Gln Pro Glu Trp Phe Arg Asn Ile
                325                 330                 335

Leu Ser Ile Ala Pro Ile Met Phe Ser Asn Lys Met Ala Arg Leu Gly
            340                 345                 350

Lys Gly Tyr Met Phe Glu Ser Lys Arg Met Lys Leu Arg Thr Gln Ile
        355                 360                 365

Pro Ala Glu Met Leu Ala Ser Ile Asp Leu Lys Tyr Phe Asn Glu Ser
    370                 375                 380

Thr Arg Lys Lys Ile Glu Lys Ile Arg Pro Leu Leu Ile Asp Gly Thr
385                 390                 395                 400

Ala Ser Leu Ser Pro Gly Met Met Met Gly Met Phe Asn Met Leu Ser
                405                 410                 415

Thr Val Leu Gly Val Ser Ile Leu Asn Leu Gly Gln Lys Lys Tyr Thr
            420                 425                 430

Lys Thr Thr Tyr Trp Trp Asp Gly Leu Gln Ser Ser Asp Asp Phe Ala
        435                 440                 445

Leu Ile Val Asn Ala Pro Asn His Glu Gly Ile Gln Ala Gly Val Asp
    450                 455                 460

Arg Phe Tyr Arg Thr Cys Lys Leu Val Gly Ile Asn Met Ser Lys Lys
465                 470                 475                 480

Lys Ser Tyr Ile Asn Arg Thr Gly Thr Phe Glu Phe Thr Ser Phe Phe
                485                 490                 495

Tyr Arg Tyr Gly Phe Val Ala Asn Phe Ser Met Glu Leu Pro Ser Phe
            500                 505                 510

Gly Val Ser Gly Ile Asn Glu Ser Ala Asp Met Ser Ile Gly Val Thr
        515                 520                 525

Val Ile Lys Asn Asn Met Ile Asn Asn Asp Leu Gly Pro Ala Thr Ala
    530                 535                 540

Gln Met Ala Leu Gln Leu Phe Ile Lys Asp Tyr Arg Tyr Thr Tyr Arg
545                 550                 555                 560

Cys His Arg Gly Asp Thr Gln Ile Gln Thr Arg Arg Ser Phe Glu Leu
                565                 570                 575

Lys Lys Leu Trp Glu Gln Thr Arg Ser Lys Ala Gly Leu Leu Val Ser
```

```
            580                 585                 590

Asp Gly Gly Pro Asn Leu Tyr Asn Ile Arg Asn Leu His Ile Pro Glu
        595                 600                 605

Val Cys Leu Lys Trp Glu Leu Met Asp Glu Asp Tyr Gln Gly Arg Leu
    610                 615                 620

Cys Asn Pro Leu Asn Pro Phe Val Ser His Lys Glu Ile Glu Ser Val
625                 630                 635                 640

Asn Asn Ala Val Val Met Pro Ala His Gly Pro Ala Lys Ser Met Glu
                645                 650                 655

Tyr Asp Ala Val Ala Thr Thr His Ser Trp Ile Pro Lys Arg Asn Arg
            660                 665                 670

Ser Ile Leu Asn Thr Ser Gln Arg Gly Ile Leu Glu Asp Glu Gln Met
        675                 680                 685

Tyr Gln Lys Cys Cys Asn Leu Phe Glu Lys Phe Phe Pro Ser Ser Ser
    690                 695                 700

Tyr Arg Arg Pro Val Gly Ile Ser Ser Met Val Glu Ala Met Val Ser
705                 710                 715                 720

Arg Ala Arg Ile Asp Ala Arg Ile Asp Phe Glu Ser Gly Arg Ile Lys
                725                 730                 735

Lys Glu Glu Phe Ser Glu Ile Met Lys Ile Cys Ser Thr Ile Glu Glu
            740                 745                 750

Leu Arg Arg Gln Lys Gln
        755

<210>   47
<211>   716
<212>   PRT
<213>   Influenza A virus

<400>   47
Met Glu Asp Phe Val Arg Gln Cys Phe Asn Pro Met Ile Val Glu Leu
1               5                   10                  15

Ala Glu Lys Thr Met Lys Glu Tyr Gly Glu Asp Leu Lys Ile Glu Thr
            20                  25                  30

Asn Lys Phe Ala Ala Ile Cys Thr His Leu Glu Val Cys Phe Met Tyr
        35                  40                  45

Ser Asp Phe His Phe Ile Asn Glu Gln Gly Glu Ser Ile Ile Val Glu
    50                  55                  60

Leu Gly Asp Pro Asn Ala Leu Leu Lys His Arg Phe Glu Ile Ile Glu
65                  70                  75                  80

Gly Arg Asp Arg Thr Met Ala Trp Thr Val Val Asn Ser Ile Cys Asn
                85                  90                  95

Thr Thr Gly Ala Glu Lys Pro Lys Phe Leu Pro Asp Leu Tyr Asp Tyr
            100                 105                 110

Lys Glu Asn Arg Phe Ile Glu Ile Gly Val Thr Arg Arg Glu Val His
```

```
        115                 120                 125

Ile Tyr Tyr Leu Glu Lys Ala Asn Lys Ile Lys Ser Glu Lys Thr His
    130                 135                 140

Ile His Ile Phe Ser Phe Thr Gly Glu Glu Met Ala Thr Lys Ala Asp
145                 150                 155                 160

Tyr Thr Leu Asp Glu Glu Ser Arg Ala Arg Ile Lys Thr Arg Leu Phe
                165                 170                 175

Thr Ile Arg Gln Glu Met Ala Ser Arg Gly Leu Trp Asp Ser Phe Arg
            180                 185                 190

Gln Ser Glu Arg Gly Glu Glu Thr Ile Glu Glu Arg Phe Glu Ile Thr
        195                 200                 205

Gly Thr Met Arg Lys Leu Ala Asp Gln Ser Leu Pro Pro Asn Phe Ser
    210                 215                 220

Ser Leu Glu Asn Phe Arg Ala Tyr Val Asp Gly Phe Glu Pro Asn Gly
225                 230                 235                 240

Tyr Ile Glu Gly Lys Leu Ser Gln Met Ser Lys Glu Val Asn Ala Arg
                245                 250                 255

Ile Glu Pro Phe Leu Lys Thr Thr Pro Arg Pro Leu Arg Leu Pro Asn
            260                 265                 270

Gly Pro Pro Cys Ser Gln Arg Ser Lys Phe Leu Leu Met Asp Ala Leu
        275                 280                 285

Lys Leu Ser Ile Glu Asp Pro Ser His Glu Gly Glu Gly Ile Pro Leu
    290                 295                 300

Tyr Asp Ala Ile Lys Cys Met Arg Thr Phe Phe Gly Trp Lys Glu Pro
305                 310                 315                 320

Asn Val Val Lys Pro His Glu Lys Gly Ile Asn Pro Asn Tyr Leu Leu
                325                 330                 335

Ser Trp Lys Gln Val Leu Ala Glu Leu Gln Asp Ile Glu Asn Glu Glu
            340                 345                 350

Lys Ile Pro Lys Thr Lys Asn Met Lys Lys Thr Ser Gln Leu Lys Trp
        355                 360                 365

Ala Leu Gly Glu Asn Met Ala Pro Glu Lys Val Asp Phe Asp Asp Cys
    370                 375                 380

Lys Asp Val Gly Asp Leu Lys Gln Tyr Asp Ser Asp Glu Pro Glu Leu
385                 390                 395                 400

Arg Ser Leu Ala Ser Trp Ile Gln Asn Glu Phe Asn Lys Ala Cys Glu
                405                 410                 415

Leu Thr Asp Ser Ser Trp Ile Glu Leu Asp Glu Ile Gly Glu Asp Val
            420                 425                 430

Ala Pro Ile Glu His Ile Ala Ser Met Arg Arg Asn Tyr Phe Thr Ser
        435                 440                 445

Glu Val Ser His Cys Arg Ala Thr Glu Tyr Ile Met Lys Gly Val Tyr
```

```
    450                 455                 460

Ile Asn Thr Ala Leu Leu Asn Ala Ser Cys Ala Ala Met Asp Asp Phe
465                 470                 475                 480

Gln Leu Ile Pro Met Ile Ser Lys Cys Arg Thr Lys Glu Gly Arg Arg
                485                 490                 495

Lys Thr Asn Leu Tyr Gly Phe Ile Ile Lys Gly Arg Ser His Leu Arg
            500                 505                 510

Asn Asp Thr Asp Val Val Asn Phe Val Ser Met Glu Phe Ser Leu Thr
        515                 520                 525

Asp Pro Arg Leu Glu Pro His Lys Trp Glu Lys Tyr Cys Val Leu Glu
    530                 535                 540

Ile Gly Asp Met Leu Ile Arg Ser Ala Ile Gly Gln Val Ser Arg Pro
545                 550                 555                 560

Met Phe Leu Tyr Val Arg Thr Asn Gly Thr Ser Lys Ile Lys Met Lys
                565                 570                 575

Trp Gly Met Glu Met Arg Arg Cys Leu Leu Gln Ser Leu Gln Gln Ile
            580                 585                 590

Glu Ser Met Ile Glu Ala Glu Ser Ser Val Lys Glu Lys Asp Met Thr
        595                 600                 605

Lys Glu Phe Phe Glu Asn Lys Ser Glu Thr Trp Pro Ile Gly Glu Ser
    610                 615                 620

Pro Lys Gly Val Glu Glu Ser Ser Ile Gly Lys Val Cys Arg Thr Leu
625                 630                 635                 640

Leu Ala Lys Ser Val Phe Asn Ser Leu Tyr Ala Ser Pro Gln Leu Glu
                645                 650                 655

Gly Phe Ser Ala Glu Ser Arg Lys Leu Leu Leu Ile Val Gln Ala Leu
            660                 665                 670

Arg Asp Asn Leu Glu Pro Gly Thr Phe Asp Leu Gly Gly Leu Tyr Glu
        675                 680                 685

Ala Ile Glu Glu Cys Leu Ile Asn Asp Pro Trp Val Leu Leu Asn Ala
    690                 695                 700

Ser Trp Phe Asn Ser Phe Leu Thr His Ala Leu Ser
705                 710                 715

<210>   48
<211>   326
<212>   PRT
<213>   Influenza A virus

<400>   48
Met Ala Ser Gln Gly Thr Lys Arg Ser Tyr Glu Gln Met Glu Thr Asp
1               5                   10                  15

Gly Glu Arg Gln Asn Ala Thr Glu Ile Arg Ala Ser Val Gly Lys Met
            20                  25                  30

Ile Gly Gly Ile Gly Arg Phe Tyr Ile Gln Met Cys Thr Glu Leu Lys
```

```
        35                  40                  45

Leu Ser Asp Tyr Glu Gly Arg Leu Ile Gln Asn Ser Leu Thr Ile Glu
    50                  55                  60

Arg Met Val Leu Ser Ala Phe Asp Glu Arg Arg Asn Lys Tyr Leu Glu
65                  70                  75                  80

Glu His Pro Ser Ala Gly Lys Asp Pro Lys Lys Thr Gly Gly Pro Ile
                85                  90                  95

Tyr Arg Arg Val Asn Gly Lys Trp Met Arg Glu Leu Ile Leu Tyr Asp
            100                 105                 110

Lys Glu Glu Ile Arg Arg Ile Trp Arg Gln Ala Asn Asn Gly Asp Asp
        115                 120                 125

Ala Thr Ala Gly Leu Thr His Met Met Ile Trp His Ser Asn Leu Asn
    130                 135                 140

Asp Ala Thr Tyr Gln Arg Thr Arg Ala Leu Val Arg Thr Gly Met Asp
145                 150                 155                 160

Pro Arg Met Cys Ser Leu Met Gln Gly Ser Thr Leu Pro Arg Arg Ser
                165                 170                 175

Gly Ala Ala Gly Ala Ala Val Lys Gly Val Gly Thr Met Val Met Glu
            180                 185                 190

Leu Val Arg Met Ile Lys Arg Gly Ile Asn Asp Arg Asn Phe Trp Arg
        195                 200                 205

Gly Glu Asn Gly Arg Lys Thr Arg Ile Ala Tyr Glu Arg Met Cys Asn
    210                 215                 220

Ile Leu Lys Gly Lys Phe Gln Thr Ala Ala Gln Lys Ala Met Met Asp
225                 230                 235                 240

Gln Val Arg Glu Ser Arg Asp Pro Gly Asn Ala Glu Phe Glu Asp Leu
                245                 250                 255

Thr Phe Leu Ala Arg Ser Ala Leu Ile Leu Arg Gly Ser Val Ala His
            260                 265                 270

Lys Ser Cys Leu Pro Ala Cys Val Tyr Gly Pro Ala Val Ala Ser Gly
        275                 280                 285

Tyr Asp Phe Glu Arg Glu Gly Tyr Ser Leu Val Gly Ile Asp Pro Phe
    290                 295                 300

Arg Leu Leu Gln Asn Ser Gln Val Tyr Ser Leu Ile Arg Pro Asn Glu
305                 310                 315                 320

Asn Pro Ala His Lys Ser
                325

<210>   49
<211>   252
<212>   PRT
<213>   Influenza A virus

<400>   49
Met Ser Leu Leu Thr Glu Val Glu Thr Tyr Val Leu Ser Ile Ile Pro
```

```
1               5                   10                  15

Ser Gly Pro Leu Lys Ala Glu Ile Ala Gln Arg Leu Glu Asp Val Phe
            20                  25                  30

Ala Gly Lys Asn Thr Asp Leu Glu Val Leu Met Glu Trp Leu Lys Thr
        35                  40                  45

Arg Pro Ile Leu Ser Pro Leu Thr Lys Gly Ile Leu Gly Phe Val Phe
    50                  55                  60

Thr Leu Thr Val Pro Ser Glu Arg Gly Leu Gln Arg Arg Arg Phe Val
65                  70                  75                  80

Gln Asn Ala Leu Asn Gly Asn Gly Asp Pro Asn Asn Met Asp Lys Ala
                85                  90                  95

Val Lys Leu Tyr Arg Lys Leu Lys Arg Glu Ile Thr Phe His Gly Ala
            100                 105                 110

Lys Glu Ile Ser Leu Ser Tyr Ser Ala Gly Ala Leu Ala Ser Cys Met
        115                 120                 125

Gly Leu Ile Tyr Asn Arg Met Gly Ala Val Thr Thr Glu Val Ala Phe
    130                 135                 140

Gly Leu Val Cys Ala Thr Cys Glu Gln Ile Ala Asp Ser Gln His Arg
145                 150                 155                 160

Ser His Arg Gln Met Val Thr Thr Thr Asn Pro Leu Ile Arg His Glu
                165                 170                 175

Asn Arg Met Val Leu Ala Ser Thr Thr Ala Lys Ala Met Glu Gln Met
            180                 185                 190

Ala Gly Ser Ser Glu Gln Ala Ala Glu Ala Met Glu Val Ala Ser Gln
        195                 200                 205

Ala Arg Gln Met Val Gln Ala Met Arg Thr Ile Gly Thr His Pro Ser
    210                 215                 220

Ser Ser Ala Gly Leu Lys Asn Asp Leu Leu Glu Asn Leu Gln Ala Tyr
225                 230                 235                 240

Gln Lys Arg Met Gly Val Gln Met Gln Arg Phe Lys
                245                 250

<210>   50
<211>   566
<212>   PRT
<213>   Influenza A virus

<400>   50
Met Lys Ala Ile Leu Val Val Leu Leu Tyr Thr Phe Ala Thr Ala Asn
1               5                   10                  15

Ala Asp Thr Leu Cys Ile Gly Tyr His Ala Asn Asn Ser Thr Asp Thr
            20                  25                  30

Val Asp Thr Val Leu Glu Lys Asn Val Thr Val Thr His Ser Val Asn
        35                  40                  45

Leu Leu Glu Asp Lys His Asn Gly Lys Leu Cys Lys Leu Arg Gly Val
```

```
    50                  55                  60

Ala Pro Leu His Leu Gly Lys Cys Asn Ile Ala Gly Trp Ile Leu Gly
65                  70                  75                  80

Asn Pro Glu Cys Glu Ser Leu Ser Thr Ala Ser Ser Trp Ser Tyr Ile
                85                  90                  95

Val Glu Thr Pro Ser Ser Asp Asn Gly Thr Cys Tyr Pro Gly Asp Phe
            100                 105                 110

Ile Asp Tyr Glu Glu Leu Arg Glu Gln Leu Ser Ser Val Ser Ser Phe
        115                 120                 125

Glu Arg Phe Glu Ile Phe Pro Lys Thr Ser Ser Trp Pro Asn His Asp
    130                 135                 140

Ser Asn Lys Gly Val Thr Ala Ala Cys Pro His Ala Gly Ala Lys Ser
145                 150                 155                 160

Phe Tyr Lys Asn Leu Ile Trp Leu Val Lys Lys Gly Asn Ser Tyr Pro
                165                 170                 175

Lys Leu Ser Lys Ser Tyr Ile Asn Asp Lys Gly Lys Glu Val Leu Val
            180                 185                 190

Leu Trp Gly Ile His His Pro Ser Thr Ser Ala Asp Gln Gln Ser Leu
        195                 200                 205

Tyr Gln Asn Ala Asp Thr Tyr Val Phe Val Gly Ser Ser Arg Tyr Ser
    210                 215                 220

Lys Lys Phe Lys Pro Glu Ile Ala Ile Arg Pro Lys Val Arg Asp Gln
225                 230                 235                 240

Glu Gly Arg Met Asn Tyr Tyr Trp Thr Leu Val Glu Pro Gly Asp Lys
                245                 250                 255

Ile Thr Phe Glu Ala Thr Gly Asn Leu Val Val Pro Arg Tyr Ala Phe
            260                 265                 270

Ala Met Glu Arg Asn Ala Gly Ser Gly Ile Ile Ile Ser Asp Thr Pro
        275                 280                 285

Val His Asp Cys Asn Thr Thr Cys Gln Thr Pro Lys Gly Ala Ile Asn
    290                 295                 300

Thr Ser Leu Pro Phe Gln Asn Ile His Pro Ile Thr Ile Gly Lys Cys
305                 310                 315                 320

Pro Lys Tyr Val Lys Ser Thr Lys Leu Arg Leu Ala Thr Gly Leu Arg
                325                 330                 335

Asn Ile Pro Ser Ile Gln Ser Arg Gly Leu Phe Gly Ala Ile Ala Gly
            340                 345                 350

Phe Ile Glu Gly Gly Trp Thr Gly Met Val Asp Gly Trp Tyr Gly Tyr
        355                 360                 365

His His Gln Asn Glu Gln Gly Ser Gly Tyr Ala Ala Asp Leu Lys Ser
    370                 375                 380

Thr Gln Asn Ala Ile Asp Glu Ile Thr Asn Lys Val Asn Ser Val Ile
```

```
385                 390                 395                 400

Glu Lys Met Asn Thr Gln Phe Thr Ala Val Gly Lys Glu Phe Asn His
                405                 410                 415

Leu Glu Lys Arg Ile Glu Asn Leu Asn Lys Lys Val Asp Asp Gly Phe
            420                 425                 430

Leu Asp Ile Trp Thr Tyr Asn Ala Glu Leu Leu Val Leu Leu Glu Asn
        435                 440                 445

Glu Arg Thr Leu Asp Tyr His Asp Ser Asn Val Lys Asn Leu Tyr Glu
    450                 455                 460

Lys Val Arg Ser Gln Leu Lys Asn Asn Ala Lys Glu Ile Gly Asn Gly
465                 470                 475                 480

Cys Phe Glu Phe Tyr His Lys Cys Asp Asn Thr Cys Met Glu Ser Val
                485                 490                 495

Lys Asn Gly Thr Tyr Asp Tyr Pro Lys Tyr Ser Glu Glu Ala Lys Leu
            500                 505                 510

Asn Arg Glu Glu Ile Asp Gly Val Lys Leu Glu Ser Thr Arg Ile Tyr
        515                 520                 525

Gln Ile Leu Ala Ile Tyr Ser Thr Val Ala Ser Ser Leu Val Leu Val
    530                 535                 540

Val Ser Leu Gly Ala Ile Ser Phe Trp Met Cys Ser Asn Gly Ser Leu
545                 550                 555                 560

Gln Cys Arg Ile Cys Ile
                565
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