others, discuss development of cell culture lines that are capable of producing vaccine quality virus. See also Kistner et al., (Vaccine 16:960–8, 1998), which discloses VERO cells adapted for improved viral growth and vaccine production are described, and Ehrlich et al, (New Eng J Med 35:2573-84, 2008), which described, *inter alia*, a formalin-inactivated H5N1 whole virus vaccine grown in Vero cells.

[0012] Thus, there remains a need in the art to produce a pandemic or seasonal influenza vaccine having increased antigenicity compared to previous vaccines, that can elicit a good immune response in a host without causing infection, and which exhibits robust growth in mammalian cell culture.

SUMMARY ~~OF THE INVENTION~~

[0013] The present ~~invention~~disclosure provides for production of an improved vaccine against influenza virus, wherein the vaccine comprises a reassortant virus having a hemagglutinin gene and a neuraminidase gene from the same viral influenza A subtype or influenza B strain and internal genes from a different influenza A subtype or influenza B strain. In one aspect, the HA and NA genes and the internal genes are derived from a highly pathogenic H5N1 strain of influenza A.

[0014] In one ~~aspect~~embodiment, the ~~invention~~disclosure provides a reassortant influenza virus comprising: internal gene segments (PB1, PB2, PA, NP, M, and NS) from a first influenza virus A subtype and hemagglutinin (HA) and neuraminidase (NA) genes from the influenza virus subtype from which the internal genes are derived, the internal genes derived from a first strain of an influenza A subtype and the HA and NA genes derived from a second strain in the influenza A subtype. It is further contemplated that the NS gene comprises an NS1, an NS2 or an NS1 and NS2 gene.

[0015] In one embodiment, the ~~invention~~disclosure contemplates a purified reassortant influenza virus comprising internal gene segments (PB1, PB2, PA, NP, M, and NS) of an H5N1 strain of influenza A and hemagglutinin (HA) and neuraminidase (NA) genes from an H5N1 influenza A virus, the HA and NA genes derived from the same viral strain. In a related embodiment, the HA and NA genes are from a first H5N1 clade and the internal genes are from a second H5N1 clade. In a further embodiment, the HA and NA genes and the internal genes are derived from the same clade. It is further contemplated that the NS gene comprises an NS1, an NS2 or an NS1 and NS2 gene.

[0016] In another embodiment, the ~~invention~~disclosure comtemplates a reassortant influenza virus comprising, internal gene segments PB1, PB2, PA, M, NP and NS from a first influenza virus B strain and hemagglutinin (HA) and neuraminidase (NA) genes from a second influenza B strain, wherein the virus is characterized by the ability to propagate in mammalian cell culture.

[0017] In a further embodiment, the HA gene of the reassortant virus is modified to produce an attenuated virus. In one embodiment, the HA gene is modified at a polybasic cleavage site. In a still further embodiment, the modification at the polybasic cleavage site in the HA gene is mutation from **RE***RRRK***KR** (SEQ ID NO: 13)→ **TETR** (SEQ ID NO: 14).

[0018] In another embodiment, the virus is characterized by the ability to propagate in mammalian cell culture. In a still further embodiment, the mammalian cells are selected from the group consisting of MRC-5, MRC-9, Lederle 130, Chang liver and WI-38; U937, Vero, CV-1, IMR-90 and IMR-91, MDCK, MDBK, HEK, H9, CEM and CD4-expressing HUT78, PerC6, BHK-21 cells, BSC and LLC-MK2. In a related embodiment, the mammalian cell culture is a Vero cell culture.

[0019] In certain embodiments, the influenza A subtype comprises any combination of H1 to H16 and N1 to N9, including H1N1, H2N1, H3N1, H4N1, H5N1, H6N1, H7N1, H8N1, H9N1, H10N1, H11N1, H12N1, H13N1, H14N1, H15N1, H16N1; H1N2, H2N2, H3N2, H4N2, H5N2, H6N2, H7N2, H8N2, H9N2, H10N2, H11N2, H12N2, H13N2, H14N2, H15N2, H16N2; H1N3, H2N3, H3N3, H4N3, H5N3, H6N3, H7N3, H8N3, H9N3, H10N3, H11N3, H12N3, H13N3, H14N3, H15N3, H16N3; H1N4, H2N4, H3N4, H4N4, H5N4, H6N4, H7N4, H8N4, H9N4, H10N4, H11N4, H12N4, H13N4, H14N4, H15N4, H16N4; H1N5, H2N5, H3N5, H4N5, H5N5, H6N5, H7N5, H8N5, H9N5, H10N5, H11N5, H12N5, H13N5, H14N5, H15N5, H16N5; H1N6, H2N6, H3N6, H4N6, H5N6, H6N6, H7N6, H8N6, H9N6, H10N6, H11N6, H12N6, H13N6, H14N6, H15N6, H16N6; H1N7, H2N7, H3N7, H4N7, H5N7, H6N7, H7N7, H8N7, H9N7, H10N7, H11N7, H12N7, H13N7, H14N7, H15N7, H16N7; H1N8, H2N8, H3N8, H4N8, H5N8, H6N8, H7N8, H8N8, H9N8, H10N8, H11N8, H12N8, H13N8, H14N8, H15N8, H16N8; H1N9, H2N9, H3N9, H4N9, H5N9, H6N9, H7N9, H8N9, H9N9, H10N9, H11N9, H12N9, H13N9, H14N9, H15N9, and H16N9. In some embodiments the influenza A subtype is H5N1.

[0020] In one embodiment, the internal genes of the reassortant virus are derived from an H5N1 virus known in the art and those described in further detail below in the Detailed

Description. In a related embodiment, said internal genes are derived from the H5N1 strain selected from the group consisting of A/Vietnam/1203/2004, A/Hong Kong/213/03, A/Indonesia/5/05, A/Hong Kong/156/97, A/turkey/Turkey/01/2005, A/Anhui/1/05, and A/Cambodia/R0405050/2007, A/chicken/Nakorn-Patom/Thailand/CU-K2/04, A/chicken/Vietnam/C58/04, A/quail/Vietnam/36/04, MDk/JX/1653/05, MDk/1657/JX/05, BH goose/QH/65/05, Gs/GD/1/96-like, Dk/Vietnam/568/05, Gs/GX/345/05, MDk/JX/1701/05, MDk/JX/2136/05, MDk/JX/2295/05, MDk/JX/2300/05, Dk/GX/351/04, Dk/GX/380/04, Dk/ST/4610/03, Ck/MYS/5858/04, Ck/Salatiga/BBVet-I/05, and Dk/VNM/S654/05, A/chicken/Vietnam/C58/05, A/Muscovy Duck/Vietnam/453/2004, A/duck/Singapore/3/97, A/HK/156/97, and A/Hong Kong/156/1996. In a specific embodiment, he internal genes are from A/Vietnam/1203/2004.

[0021] In another embodiment, the HA and NA genes of the reassortant virus are derived from an H5N1 virus known in the art and those described in further detail below. In a further embodiment, said HA and NA genes are derived from an H5N1 strain selected from the group consisting of A/Vietnam/1203/2004, A/Hong Kong/213/03, A/Indonesia/5/05, A/Hong Kong/156/97, A/turkey/Turkey/01/2005, A/Anhui/1/05, and A/Cambodia/R0405050/2007, A/chicken/Nakorn-Patom/Thailand/CU-K2/04, A/chicken/Vietnam/C58/04, A/quail/Vietnam/36/04, MDk/JX/1653/05, MDk/1657/JX/05, BH goose/QH/65/05, Gs/GD/1/96-like, Dk/Vietnam/568/05, Gs/GX/345/05, MDk/JX/1701/05, MDk/JX/2136/05, MDk/JX/2295/05, MDk/JX/2300/05, Dk/GX/351/04, Dk/GX/380/04, Dk/ST/4610/03, Ck/MYS/5858/04, Ck/Salatiga/BBVet-I/05, and Dk/VNM/S654/05, A/chicken/Vietnam/C58/05, A/Muscovy Duck/Vietnam/453/2004, A/duck/Singapore/3/97, A/HK/156/97, and A/Hong Kong/156/1996.

[0022] In certain embodiments, the internal genes and the HA and NA genes are derived from the same virus strain. In other embodiments, the internal genes and the HA and NA genes are derived from different virus strains.

[0023] In an embodiment, the HA and NA genes are from the H5N1 strain A/Vietnam/1203/2004. In another embodiment, the HA and NA genes are from the H5N1 strain A/Indonesia/5/05.

[0023a] In one aspect, there is provided an antigenic reassortant influenza virus composition comprising,

(i) internal gene segments PB1, PB2, PA, M, NP and NS from a first H5N1 influenza virus A strain and (ii) hemagglutinin (HA) and neuraminidase (NA) genes from a second H5N1 influenza virus A strain, the HA and NA genes being from the same viral strain, wherein said HA gene is modified to produce an attenuated virus.

[0024] In another ~~aspect~~embodiment, the ~~invention~~disclosure provides an antigenic reassortant influenza virus composition comprising: internal gene segments (PB1, PB2, PA, NP, M, and NS) from a first influenza virus A subtype and hemagglutinin (HA) and neuraminidase (NA) genes from

the influenza virus subtype from which the internal genes are derived, the internal genes derived from a first strain of an influenza A subtype and the HA and NA genes derived from a second strain in the influenza A subtype. It is further contemplated that the NS gene comprises an NS1, an NS2 or an NS1 and NS2 gene.

[0025] In one embodiment, the antigenic reassortant influenza A virus composition, comprises internal gene segments (PB1, PB2, PA, NP, M, and NS) derived from an H5N1 strain of influenza A and hemagglutinin (HA) and neuraminidase (NA) genes derived from an H5N1 influenza A virus, the HA and NA genes derived from the same viral strain. It is further contemplated that the NS gene comprises an NS1, an NS2 or an NS1 and NS2 gene.

[0026] In a further embodiment, the ~~invention~~disclosure provides an antigenic reassortant influenza virus composition comprising, internal gene segments PB1, PB2, PA, M, NP and NS from a first influenza virus B strain and hemagglutinin (HA) and neuraminidase (NA) genes from a second influenza B strain, wherein the virus is characterized by the ability to propagate in mammalian cell culture.

[0027] In one embodiment, the HA gene of the compositions is modified to produce an attenuated virus. In a related embodiment, the HA gene is modified at a polybasic cleavage site.

[0028] In a further embodiment, the influenza virus is characterized by the ability to propagate in mammalian cell culture.

[0029] In one embodiment, the internal genes of the antigenic composition are derived from an H5N1 virus known in the art and those described in further detail below In a related embodiment, said internal genes are derived from the H5N1 strain selected from the group consisting of A/Vietnam/1203/2004, A/Hong Kong/213/03, A/Indonesia/5/05, A/chicken/Nakorn-Patom/Thailand/CU-K2/04, A/chicken/Vietnam/C58/04, A/quail/Vietnam/36/04, MDk/JX/1653/05, MDk/1657/JX/05, BH goose/QH/65/05, Gs/GD/1/96-like, Dk/Vietnam/568/05, Gs/GX/345/05, MDk/JX/1701/05, MDk/JX/2136/05, MDk/JX/2295/05, MDk/JX/2300/05, Dk/GX/351/04, Dk/GX/380/04, Dk/ST/4610/03, Ck/MYS/5858/04, Ck/Salatiga/BBVet-I/05, and Dk/VNM/S654/05, A/chicken/Vietnam/C58/05, A/Muscovy Duck/Vietnam/453/2004, A/duck/Singapore/3/97, A/HK/156/97, and A/Hong Kong/156/1996. In a specific embodiment, the internal genes are from A/Vietnam/1203/2004.

[0030] In another embodiment, the HA and NA genes of the antigenic composition are derived from an H5N1 virus known in the art and those described in further detail below. In a further embodiment, said HA and NA genes are derived from an H5N1 strain selected from the group consisting of A/Vietnam/1203/2004, A/Hong Kong/213/03, A/Indonesia/5/05, A/Hong Kong/156/97, A/turkey/Turkey/01/2005, A/Anhui/1/05, and A/Cambodia/R0405050/2007, A/chicken/Nakorn-Patom/Thailand/CU-K2/04, A/chicken/Vietnam/C58/04, A/quail/Vietnam/36/04, MDk/JX/1653/05, MDk/1657/JX/05, BH goose/QH/65/05, Gs/GD/1/96-like, Dk/Vietnam/568/05, Gs/GX/345/05, MDk/JX/1701/05, MDk/JX/2136/05, MDk/JX/2295/05, MDk/JX/2300/05, Dk/GX/351/04, Dk/GX/380/04, Dk/ST/4610/03, Ck/MYS/5858/04, Ck/Salatiga/BBVet-I/05, and Dk/VNM/S654/05, A/chicken/Vietnam/C58/05, A/Muscovy Duck/Vietnam/453/2004, A/duck/Singapore/3/97, A/HK/156/97, and A/Hong Kong/156/1996.

[0031] In certain embodiments, the internal genes and the HA and NA genes of the antigenic composition are derived from the same virus strain. In other embodiments, the internal genes and the HA and NA genes are derived from different virus strains.

[0032] In an embodiment, the HA and NA genes of the antigenic composition are from the H5N1 strain A/Vietnam/1203/2004. In another embodiment, the HA and NA genes are from the H5N1 strain A/Indonesia/5/05.

[0033] In a still further embodiment, the modification at the polybasic cleavage site in the HA gene is mutation from **RE***RRRK***KR** (SEQ ID NO: 13)→ **TETR** (SEQ ID NO: 14).

[0034] In another embodiment, the antigenic composition further comprises a pharmaceutically acceptable carrier.

[0034a] In one aspect, there is provided a vaccine comprising a reassortant influenza A virus, the virus comprising: (i) a polynucleotide encoding for PB1; a polynucleotide encoding for PA, a polynucleotide encoding for PB2, a polynucleotide encoding for M, a polynucleotide encoding for NP, a polynucleotide encoding for NS, the polynucleotides for PB1, PA, PB2, M, NP and NS derived from a first H5N1 influenza virus, and (ii) a polynucleotide encoding for surface protein HA and a polynucleotide encoding for surface protein NA, each of HA and NA derived from a second H5N1 influenza virus, the polynucleotides being operatively linked to allow packaging of the reassorted polynucleotides into a virion, wherein said HA gene is modified to produce an attenuated virus.

[0035] In one ~~aspect~~embodiment, the ~~invention~~disclosure contemplates a vaccine comprising a reassortant influenza virus, the virus comprising: i) a polynucleotide encoding for surface protein HA and a polynucleotide encoding for surface protein NA, each of HA and NA derived from an first strain of an influenza virus A subtype; a polynucleotide encoding for PB1; a polynucleotide encoding for PA, a polynucleotide encoding for PB2, a polynucleotide encoding for M, a polynucleotide encoding for NS (NS1 and/or NS2), a polynucleotide encoding for NP, the polynucleotides for PB1, PB2, PA, NP, M, and NS derived from a second strain of an influenza A virus subtype, wherein the polynucleotides are operatively linked to allow packaging of the reassorted polynucleotides into a virion.

[0036] In a related embodiment, the ~~invention~~disclosure provides a vaccine comprising a reassortant influenza A virus, the virus comprising: a polynucleotide encoding for surface protein HA and a polynucleotide encoding for surface protein NA, each of HA and NA derived from an H5N1 influenza virus; a polynucleotide encoding for PB1; a polynucleotide encoding for PA, a polynucleotide encoding for PB2, a polynucleotide encoding for M, a polynucleotide encoding for NS1 (NS1 and/or NS2, a polynucleotide encoding for NP, the polynucleotides for PB1, PA, PB2, M, NP, and NS) derived from an H5N1 influenza virus, the polynucleotides being operatively linked to allow packaging of the reassorted polynucleotides into a virion.

[0037] In a related embodiment, the ~~invention~~disclosure contemplates a vaccine comprising a reassortant influenza virus, the virus comprising: a polynucleotide encoding for surface protein HA and a polynucleotide encoding for surface protein NA, each of HA and NA derived from an first strain of an influenza B virus; a polynucleotide encoding for PB1; a polynucleotide encoding for PA, a polynucleotide encoding for PB2, a polynucleotide encoding for M, a polynucleotide encoding for NP, a polynucleotide encoding for NS, the polynucleotides for PB1, PA, PB2, M, NP and NS derived from a second strain of an influenza B virus, wherein the polynucleotides are operatively linked to allow packaging of the reassorted polynucleotides into a virion, and wherein the virus is characterized by the ability to propagate in mammalian cell culture.

[0038] In one embodiment, the HA gene of the compositions is modified to produce an attenuated virus. In a related embodiment, the HA gene is modified at a polybasic cleavage site.

[0039] In one embodiment, the internal genes in the vaccine are derived from an H5N1 virus known in the art and those described in further detail below. In a related embodiment, said internal genes are derived from the H5N1 strain A/Vietnam/1203/2004.

[0040] In another embodiment, the HA and NA genes in the vaccine are derived from an H5N1 virus known in the art and those described in further detail below. In a further embodiment, said HA and NA genes are derived from an H5N1 strain selected from the group consisting of A/Vietnam/1203/2004, A/Hong Kong/213/03, A/Indonesia/5/05, A/Hong Kong/156/97, A/turkey/Turkey/01/2005, A/Anhui/1/05, and A/Cambodia/R0405050/2007, A/chicken/Nakorn-Patom/Thailand/CU-K2/04, A/chicken/Vietnam/C58/04, A/quail/Vietnam/36/04, MDk/JX/1653/05, MDk/1657/JX/05, BH goose/QH/65/05,

100 μg HA as necessary. Accordingly single vaccine dosages include those having about 1 μg, about 2 μg, about 3 μg, about 4 μg, about 5 μg, about 6 μg, about 7 μg, about 8 μg, about 9 μg, about 10 μg, about 11 μg, about 12 μg, about 13 μg, about 14 μg, about 15 μg, about 16 μg, about 17 μg, about 18 μg, about 19 μg, about 20 μg, about 21 μg, about 22 μg, about 23 μg, about 24 μg, about 25 μg, about 30 μg, about 35 μg, about 40 μg, about 45 μg, about 50 μg, about 55 μg, about 60 μg, about 65 μg, about 70 μg, about 75 μg, about 80 μg, about 85 μg, about 90 μg, about 95 μg, about 100 μg, and more than 100 μg hemagglutinin provided in single or multiple dosages at the same or different amount of hemagglutinin.

[0047] In another ~~aspect~~embodiment, the ~~invention~~disclosure provides a method for eliciting an immune response to at least one pandemic influenza virus strain in a subject, comprising administering a virus, antigenic composition or a vaccine as described herein in an amount effective to protect the subject against infection of at least one H5N1 influenza virus strain. In a related ~~aspect~~embodiment, the ~~invention~~disclosure contemplates a method for eliciting an immune response to at least one seasonal influenza virus strain in a subject, comprising administering a virus, antigenic composition or a vaccine as described herein in an amount effective to protect the subject against infection of at least one influenza A and/or influenza B virus strain.

[0047a] In one aspect, there is provided the antigenic composition as defined above or the vaccine as defined above for use in eliciting an immune response to at least one pandemic influenza virus strain in a subject in an amount effective to protect the subject against infection of at least one H5N1 influenza virus strain.

[0047b] In one aspect, there is provided the use of the antigenic composition as defined above or the vaccine as defined above in the manufacture of a medicament for eliciting an immune response to at least one pandemic influenza virus strain in a subject, wherein the medicament is in an amount effective to protect the subject against infection of at least one H5N1 influenza virus strain.

[0048] In a further ~~aspect~~embodiment, the ~~invention~~disclosure contemplates a method for preventing infection of a subject by an H5N1 influenza virus comprising administering to the subject an effective amount of a virus, antigenic composition or a vaccine as described herein. In another ~~aspect~~embodiment, the ~~invention~~disclosure contemplates a method for preventing infection of a subject by an influenza A or influenza B virus comprising administering to the subject an effective amount of a virus, antigenic composition or a vaccine as described herein.

[0048a] In one aspect, there is provided the antigenic composition as defined above or the vaccine as defined above for use in preventing infection of a subject by an influenza virus.

[0048b] In one aspect, there is provided the use of the antigenic composition as defined above or the vaccine as defined above in the manufacture of a medicament for preventing infection of a subject by an influenza virus.

[0049] In one embodiment, the vaccine useful in the methods of the ~~invention~~disclosure comprises an HA content from 1 μg to 75 μg HA. In another embodiment, the vaccine comprises an HA content of 1 μg to 30 μg per vaccine. In related embodiments, the vaccine dose is administered at 1 μg, at 3 μg, at 5 μg, at 7.5 μg, at 10 μg, at 12.5 μg, at 15 μg, at 20 μg, at 25 μg, at 30 μg HA, or in any amount up to 100 μg HA as necessary. Accordingly single vaccine dosages include those having about 1 μg, about 2 μg, about 3 μg, about 4 μg, about 5 μg, about 6 μg, about 7 μg, about 8 μg, about 9 μg, about 10 μg, about 11 μg, about 12 μg, about 13 μg, about 14 μg, about 15 μg, about 16 μg, about 17 μg, about 18 μg, about 19 μg, about 20 μg, about 21 μg, about 22 μg, about 23 μg, about 24 μg, about 25 μg, about 30 μg, about 35 μg, about 40 μg, about 45 μg, about 50 μg, about 55 μg, about 60 μg, about 65 μg, about 70 μg, about 75 μg, about 80 μg, about 85 μg, about 90 μg, about 95 μg, about 100 μg,

and more than 100 μg hemagglutinin provided in single or multiple dosages at the same or different amount of hemagglutinin.

[0049a] In one aspect, there is provided a method of making a vaccine comprising a reassortant influenza virus comprising, internal gene segments PB1, PB2, PA, M, NP and NS of a first H5N1 strain of influenza A and the hemagglutinin (HA) and neuraminidase (NA) genes from a second H5N1 influenza A virus, the HA and NA genes derived from the same viral strain and the HA gene modified at a polybasic cleavage site to produce an attenuated HA gene, the method comprising transfecting the virus in ex vivo mammalian cells under conditions suitable for growth of the reassortant virus.

[0050] In another ~~aspect~~embodiment, the ~~invention~~disclosure provides a method of making a vaccine comprising a reassortant influenza virus comprising internal gene segments (PB1, PB2, PA, NP, M, and NS) of an H5N1 strain of influenza A and the hemagglutinin (HA) and neuraminidase (NA) genes from an H5N1 influenza A virus, the HA and NA genes derived from the same viral strain and the HA gene modified at a polybasic cleavage site to produce an attenuated HA gene, the method comprising transfecting the virus in mammalian cells under conditions suitable for growth of the reassortant virus. In a related embodiment, the method of making a vaccine described herein is useful to make a vaccine comprising a seasonal influenza strain of the influenza A or influenza B type.

[0051] In one embodiment, the mammalian cells are selected from the group consisting of MRC-5, MRC-9, Lederle 130, Chang liver and WI-38; U937, Vero, CV-1, IMR-90 and IMR-91, MDCK, MDBK, HEK, H9, CEM and CD4-expressing HUT78, PerC6, BHK-21 cells, BSC and LLC-MK2. In a related embodiment, the mammalian cells are Vero cells.

[0052] It is further contemplated that, in some embodiments, the virus and compositions described above lack all six internal genes of influenza. In one embodiment, the reassortant virus comprises two, three, four or five internal genes of the influenza virus. For example, in one embodiment, the reassortant virus and/or compositions thereof and/or vaccines lack all or part of the NS1 gene.

[0053] In still another ~~aspect~~embodiment, it is contemplated that the ~~invention~~disclosure is carried out using any influenza A and influenza B virus strain. In one embodiment, the reassortant virus may have any combination of eight influenza genes from an influenza A. In a related embodiment, the reassortant virus may have any combination of eight influenza genes from an influenza B virus.

[0054] Excluded from the present ~~invention~~disclosure are any viruses (Influenza A and influenza B) previously disclosed or produced, e.g., using backbones such as A/Puerto Rico/8/34 (H1N1), A/Ann Arbor/6/60 (H2N2), A/Leningrad/134/17/57 (H2N2) and B/Ann Arbor/1/66, having internal genes from one strain of a first subtype and the HA and NA genes from a different strain of the same subtype, in any prior publications referenced herein, including but not limited to: U.S. Patents 4,552,758; 7,037,707; 7,601,356; 7,566,458; 7,527,800; 7,510,719; 7,504,109; 7,465,456; and 7,459,162; U.S. Patent Publication Nos. 20090297554,

20090246225, 20090208527, 20090175909, 20090175908, 20090175907, 20090136530, 20080069821, 20080057081, 20060252132, 20060153872, 20060110406, 20050158342, 20050042229 and 20070172929; International Patent Publication Nos.; WO 2008/157583, WO 2008/021959, WO 2007/048089, WO 2006/098901, WO 2006/063053, WO 2006/041819, WO 2005/116260, WO 2005/116258, WO 2005/115448, WO 2005/062820, WO 2003/091401 and any viruses identified therein as useful for the FLUMIST™ vaccine, which may contain internal genes from one influenza A subtype strain and HA and NA genes of a different strain of the same subtype in a recombinant virus. All such documents are incorporated by reference herein in their entirety.

[0055] In an embodiment, the present ~~invention~~disclosure also excludes naturally-occurring reasortant viruses that comprise a backbone of a first strain of an influenza subtype, and HA and NA genes from a second strain of the same influenza subtype. The term "naturally-occurring reassortant virus" as used herein refers to a reassortant virus that recombines in a natural environment, e.g., viral host, without intervention from an outside source.

[0056] In a further embodiment, the ~~invention~~disclosure excludes reassortant viruses having one or more modified internal genes from a first influenza A or B subtype as a backbone, e.g., mutated A/Puerto Rico/8/34 (H1N1), A/Ann Arbor/6/60 (H2N2), or B/Ann Arbor/1/66, and HA and NA genes from the same strain of influenza virus, e.g., as exemplified in U.S. Patent Publication No. 20070172929. The ~~invention~~disclosure contemplates, however, that a virus comprising modified internal genes of the backbone strain of an influenza subtype and HA and NA genes from the same subtype, but different strains, are included within the scope of the ~~invention~~disclosure.

[0057] In still another embodiment, the ~~invention~~disclosure excludes reassortant viruses generated using cold adapted viruses as the backbone/donor strain, e.g., A/Ann Arbor/6/60 (H2N2), A/Leningrad/134/17/57 (H2N2), B/Ann Arbor/1/66, and others known in the art, in which the internal backbone genes are from a first subtype and the HA and NA genes are from a different strain of the same subtype.