



US007572620B2

(12) **United States Patent**
Olsen et al.

(10) **Patent No.:** **US 7,572,620 B2**
(45) **Date of Patent:** **Aug. 11, 2009**

(54) **H3 EQUINE INFLUENZA A VIRUS**

(75) Inventors: **Christopher W. Olsen**, Madison, WI (US); **Gabriele A. Landolt**, Madison, WI (US); **Alexander I. Karasin**, Madison, WI (US)

(73) Assignee: **Wisconsin Alumni Research Foundation**, Madison, WI (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 486 days.

(21) Appl. No.: **11/033,248**

(22) Filed: **Jan. 11, 2005**

(65) **Prior Publication Data**

US 2006/0153871 A1 Jul. 13, 2006

(51) **Int. Cl.**
C12N 7/00 (2006.01)

(52) **U.S. Cl.** **435/235.1**; 435/5; 424/206.1; 424/209.1

(58) **Field of Classification Search** 435/235.1; 424/206.1, 209.1

See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

5,925,359	A	7/1999	Van Woensel et al.
6,406,843	B1	6/2002	Skeeles et al.
2004/0146530	A1	7/2004	Sharma
2004/0223976	A1	11/2004	Bianchi et al.
2007/0253981	A1	11/2007	Dubovi

OTHER PUBLICATIONS

Daly et al. *J Gen Virol* vol. 77 pp. 661-671, 1996.*
 "Regional Reports of Outbreaks Diagnosed and Domestic Vaccination Policies", *Proceedings of the Fourth International Meeting of OIE and WHO Experts on Control of Equine Influenza*, (Havemeyer Foundation Monograph Series No. 7), Mumford, J. A., et al., Editors, R & W Publications Limited,(2003), 6-14.
 "Session 3: Vaccine Strain Selection Scheme", *Proceedings of the Fourth International Meeting of OIE and Who Experts on Control of Equine Influenza*, (Havemeyer Foundation Monograph Series No. 7), Mumford, J. A., et al., Editors, R & W Publications Limited,(2003), 21-29.
 "Session 4: Vaccines", *Proceedings of the Fourth International Meeting of OIE and WHO Experts on Control of Equine Influenza*, (Havemeyer Foundation Monograph Series No. 7), Mumford, J. A., Editors, et al., R & W Publications Limited,(2003), 31-44.
 "Session 6: International Movement and Disease Control", *Proceedings of the Fourth International Meeting of OIE and WHO Experts on Control of Equine Influenza*, (Havemeyer Foundation Monograph Series No. 7), Mumford, J. A., et al., Editors, R & W Publications Limited,(2003), 55-60.
 "Session 7: The Way Ahead", *Proceedings of the Fourth International Meeting of OIE and WHO Experts on Control of Equine Influenza*, (Havemeyer Foundation Monograph Series No. 7), Mumford, J. A., et al., Editors, R & W Publications Limited,(2003), 61-65.

"UF Researchers: Equine Influenza Virus Likely Involved in Recent Respiratory Disease Outbreak in Racing Greyhounds", *UF News*, <http://www.napa.ufl.edu/2004news/racedogflu.htm>,(Observed Sep. 20, 2004), 2 pgs.
 "University of Pittsburgh Researchers Develop Virus for First Intranasal Equine Influenza Vaccine", *UPMC, University of Pittsburgh News Bureau*, (Nov. 23, 1999), 1-3.
 Barnett, D. C., "Vigilance and Vaccination: The Best Defenses Against Costly Equine Influenza", (published prior to Jan. 11, 2005), 4 pgs.
 Bridgen, A., "Rescue of a Segmented Negative-Strand RNA Virus Entirely From Cloned Complementary DNAs", *Proc. Natl. Acad. Sci. USA*, 93, (1996), 15400-15404.
 Castrucci, M. R., et al., "Attenuation of influenza a Virus by Insertion of a Foreign Epitope into the Neuraminidase", *Journal of Virology*, 66(8), (Aug. 1992), 4647-4653.
 Castrucci, M. E., et al., "Reverse Genetics System for Generation of an Influenza A Virus Mutant Containing a Deletion of the Carboxyl-Terminal Residue of M2 Protein", *Journal of Virology*, 69(5), (1995), 2725-2728.
 Conzelmann, K.-K., et al., "Genetic Engineering of Animal RNA Viruses", *Trends in Microbiology*, 4(10), (1996), 386-393.
 Conzelmann, K.-K., "Genetic Manipulation of Non-Segmented Negative-Strand RNA Viruses", *Journal of General Virology*, 77, (1996), 381-389.
 Conzelmann, K.-K., "Nonsegmented Negative-Strand RNA Viruses: Genetics and Manipulation of Viral Genomes", *Annu. Rev. Genet.*, 32, (1998), 123-162.
 Conzelmann, K.-K., "Rescue of Synthetic Genomic RNA Analogs of Rabies Virus by Plasmid-Encoded Proteins", *Journal of Virology*, 68(2), (1994),713-719.
 Crawford, P. C., et al., "Transmission of Equine Influenza Virus to Dogs", *Science*, 310, (2005), 482-485.
 Daly, J. M., et al., "Influenza Infections", *In: Equine Respiratory Diseases*, Lekeux, P., Editor, International Veterinary Information Services,(Nov. 13, 2001), 8 pgs.
 Dubovi, E. J., et al., "Isolation of Equine Influenza Virus From Racing Greyhounds With Fatal Hemorrhagic Pneumonia", *Proceedings of the 47th Annual Conference of the American Association of Veterinary Laboratory Diagnosticians*, (Oct. 2004), p. 158.
 Enami, M., "An Influenza Virus Containing Nine Different RNA Segments", *Virology*, 185(1), (1991),291-298.
 Enami, M., "High-Efficiency Formation of Influenza Virus Transfectants", *Journal of Virology*, 65(5), (1991),2711-2713.
 Filaroski, P. D., "Equine Flu Hits Jacksonville Greyhounds", *The Business Journal of Jacksonville*, Apr. 22, 2004, <http://jacksonville.bizjournals.com/jacksonville/stories/2004/04/19/daily33.html>,(observed Apr. 23, 2004) ,2 pgs.
 Fodor, E., "Rescue of Influenza A Virus from Recombinant DNA", *Journal of Virology*, 73(11), (Nov. 1999), 9679-9682.
 Goto, H., "Mutations Affecting the Sensitivity of the Influenza Virus Neuraminidase to 4-Guanidino-2, 4-dideoxy 2, 3-dehydro-N-acetylneuraminic Acid", *Virology*, 238, (1997), 265-272.
 Hatta, M., et al., "Molecular Basis for High Virulence of Hong Kong H5N1", *Science*, 293(5536), (Sep. 7, 2001), 1840-1842.

(Continued)

Primary Examiner—Bruce Campell
Assistant Examiner—Myron G Hill
 (74) *Attorney, Agent, or Firm*—Schwegman, Lundberg & Woessner, P.A.

(57) **ABSTRACT**

The invention provides an isolated H3 equine influenza A virus, as well as methods of preparing and using the virus, and genes or proteins thereof.

33 Claims, 13 Drawing Sheets

OTHER PUBLICATIONS

- Horimoto, T., et al., "Reverse Genetics Provides Direct Evidence for a Correction of Hemagglutinin Cleavability and Virulence of an Avian Influenza A Virus", *Journal of Virology*, 68(5), (May 1994), 3120-3128.
- Huddlestone, J. A., et al., "The Sequence of the Nucleoprotein Gene of Human Influenza A Virus, Strain A/NT/60/68", *Nucleic Acids Research*, 10(3), (1982), 1029-1038.
- Kendal, A. P., et al., "Further Studies of the Neuraminidase Content of Inactivated Influenza Vaccines and the Neuraminidase Antibody Responses After Vaccination of Immunology Primed and Unprimed Populations", *Infection and Immunity*, 29(3), (Sep. 1980), 966-971.
- Kovesdi, I., et al., "Adenoviral Vectors for Gene Transfer", *Current Opinion in Biotechnology*, 8(5), (Oct. 1997), 583-589.
- Lai, A. C., et al., "Alternative Circulation of Recent Equine-2 Influenza Viruses (H3N8) From Two Distinct Lineages in the United States", *Virus Research*, 100(2), (2004), 159-164.
- Lai, A., "Introduction; Genetic Analysis Based on Nucleotide Sequence of the HA and Other Genes", *Proceedings of the Fourth International Meeting of OIE and WHO Experts on Control of Equine Influenza*, (Havemeyer Foundation Monograph Series No. 7), Mumford, J. A., et al., Editors, R & W Publications Limited, (2003), 16-19.
- Landolt, G., et al., "Growth Characteristics of Influenza A Viruses in Primary Canine Respiratory Cells", *Proceedings of the 85th Annual Meeting of the Research Workers in Animal Diseases (CRWAD)*, (Abstract No. P92), (2004), p. 104.
- Lawson, N. D., "Recombinant Vesicular Stomatitis Viruses From DNA", *Proc. Natl. Acad. Sci. USA*, 92(10), (1995), 4477-4481.
- Li, S., et al., "Electroporation of Influenza Virus Ribonucleoprotein Complexes for Rescue of the Nucleoprotein and Matrix Genes", *Virus Research*, 37(2), (Jul. 1995), 153-161.
- Luytjes, W., "Amplification, Expression, and Packaging of a Foreign Gene by Influenza Virus", *Cell*, 59(6), (1989), 1107-1113.
- MacAllister, DVM, C., et al., "OSU—Equine Vaccination Programs", *Oklahoma Cooperative Extension Fact Sheet No. F-9119*, (prior to Jan. 11, 2005), 4 pgs.
- Mena, I., "Rescue of a Synthetic Choramphenicol Acetyltransferase RNA Into Influenza Virus-Like Particles Obtained From Recombinant Plasmids", *Journal of Virology*, 70(8), (Aug. 1996), 5016-5024.
- Mumford, J. A., "OIE Standards", *Proceedings of the Fourth International Meeting of OIE and WHO Experts on Control of Equine Influenza*, (Havemeyer Foundation Monograph Series No. 7), Mumford, J. A., Editors, et al., R & W Publications Limited, (2003), 46-53.
- Munoz, F. M., et al., "Current Research on Influenza and Other Respiratory Viruses: II International Symposium", *Antiviral Research*, 46(2), (May 2000), 91-124.
- Nagai, Y., "Paramyxovirus Replication and Pathogenesis. Reverse Genetics Transforms Understanding", *Reviews in Medical Virology*, 9(2), (1999), 83-99.
- Neumann, G., et al., "A Decade After the Generation of a Negative-Sense RNA Virus From Cloned cDNA—What Have We Learned?", *Journal of General Virology*, 83(11), (Nov. 2002), 2635-2662.
- Neumann, G., et al., "Generation of Influenza A Viruses Entirely From Cloned cDNAs", *Proc. Natl. Acad. Sci. USA*, 96(16), (1999), 9345-9350.
- Neumann, G., et al., "Nuclear Import and Export of Influenza Virus Nucleoprotein", *Journal of Virology*, 71(12), (Dec. 1997), 9690-9700.
- Neumann, G., et al., "Reverse Genetics of Influenza Virus", *Virology*, 287(2), (Sep. 2001), 243-250.
- Neumann, G. et al., "RNA Polymerase I-Mediated Expression of Influenza Viral RNA Molecules", *Virology*, 202(1), (Jul 1994), 477-479.
- Niwa, H., et al., "Efficient Selection for High-Expression Transfectants With a Novel Eukaryotic Factor", *Gene*, 108(2), (1991), 193-199.
- Olsen, C. W., et al., "Immunogenicity and Efficacy of Baculovirus-Expressed and DNA-Based Equine Influenza Virus Hemagglutinin Vaccines in Mice", *Vaccine*, 15(10), (1997), 1149-1156.
- Park, A. W., et al., "The Effects of Strain Heterology on the Epidemiology of Equine Influenza in a Vaccinated Population", *Proc. R. Soc. Lond. B.*, 271, (2004), 1547-1555.
- Parks, C. L., et al., "Enhanced Measles Virus cDNA Rescue and Gene Expression After Heat Shock", *Journal of Virology*, 73(5), (May 1999), 3560-3566.
- Peek, S. F., et al., "Acute Respiratory Distress Syndrome and Fatal Interstitial Pneumonia Associated With Equine Influenza in a Neonatal Foal", *Journal of Veterinary Internal Medicine*, 18, (2004), 132-134.
- Pekosz, A., "Commentary—Reverse Genetics of Negative-Strand RNA Viruses: Cloning the Circle", *Proc. Natl. Acad. Sci. USA*, 96, (1999), 8804-8806.
- Perez, D. R., et al., "The Matrix 1 Protein of Influenza A Virus Inhibits the Transcriptase Activity of a Model Influenza Reporter Genome in Vivo", *Virology*, 249(1), (1998), 52-61.
- Pleschka, S., "A Plasmid-Based Reverse Genetics System for Influenza A Virus", *Journal of Virology*, 70(6), (Jun. 1996), 4188-4192.
- Powell, D. W., "Overview of Equine Influenza From the American Perspective", *Proceedings of the Fourth International Meeting of OIE and WHO Experts on Control of Equine Influenza*, (Havemeyer Foundation Monograph Series No. 7), Mumford, J. A., et al., Editors, R & W Publications Limited, (2003), 2-5.
- Radecke, F., "Rescue of Measles Viruses from Cloned DNA", *The EMBO Journal*, 14(23), (1995), 5773-5784.
- Roberts, A., et al., "Recovery of Negative-Strand RNA Virus from Plasmid DNAs: A Positive Approach Revitalizes a Negative Field", *Virology*, 247(1), (1998), 1-6.
- Rose, J. K., "Positive Strands to the Rescue Again: A Segmented Negative-Strand RNA Virus Derived From Cloned cDNAs", *Proc. Natl. Acad. Sci. USA*, 93(26), (Dec. 24, 1996), 14998-15000.
- Schnell, M. J., "Infectious Rabies Viruses From Cloned cDNA", *The EMBO Journal*, 13(18), (1994), 4195-4203.
- Suzuki, Y., et al., "Origin and Evolution of Influenza Virus Hemagglutinin Genes", *Mol. Biol. Evol.*, 19(4), (2002), 501-509.
- Townsend, H. G., et al., "Comparative Efficacy of Commercial Vaccines in Five Horses: Serologic Responses and Protection After Influenza Challenge", *Proceedings, 49th Annual Conference of the American Association of Equine Practitioners*, (2003), 3 pgs.
- Wilson, W. D., "Equine Influenza", *Vet. Clin. North Am. Equine Pract.*, 9(2), (Abstract Only), (1993), 257-282.

* cited by examiner

HAmino

MKTTIILLTHWAYSQNPISGNNTATLCLGHHA VANGTLVKTISDDQIEVTNATE
LVQSISMGKICNNSYRILDGRNCTLIDAMLGDPHCDAFQYENWDLFIERSAFAFN
CYPYDIPDYASLRSIVASSGTLEFTAEGFTWTGVTQNGRSGACKRGSADSFFSRL
NWLTKSGSSYPTLNVTMPNNKNFDKLYIWGIHHPSSNQEQTCLYIQESGRVTVST
KRSQQTIIPNIGSRPWVRGQSGRISYWTIVKPGDILMINSNGNLVAPRGYFKLKT
GKSSVMRSDVPIDICVSECITPNGSISNDKPFQNVNKVTYGKCPKYIRQNTLKLAT
GMRNVPEKQIRGIFGAIAGFIENGWEGMVDGWYGFYQNSEGTGQAADLKSTQ
AAIDQINGKLN RVIERTNEKFHQIEKEFSEVERRIQDLEKYVEDTKIDLWSYNAEL
LVALENQHTIDL TDAEMNKLFEKTRRQLRENAEDMGGGCFKIYHKCDNACIGSI
RNGTYDHYIYRDEALNNRFQIKGVELKSGYKDWILWISFAISCFLICVLLGFIM
WACQKGNIRCNICI

SEQ ID NO:1

FIG. 1A

NAmino

MNPNQKIIAIGFASLGILINVLHVSVIIVTVLVLNNNRDLDLNCCKGTIIREYNETVR
VEKITQWYNTSTIKYIERPSNEYYMNNTEPLCEAQQGFAPFSKDNIGIRIGSRGHVFN
IREPFVSCSPSECRFFLTQGSLLNDKHSNGTVKDRSPYRTLMSVKIGQSPNVYQA
RFESVAWSATAACHDGKKWMTVGVTPDNQALAVVNYGGVPVDIINSWAGDILR
TQESSCTCIKGDCYWVMTDGPANRQAKYRIFKAKDGRVIGQTDISFNGGHIECS
CYPNEGKVEICRDNWTGTNRPILVISSDLSYTVGYLCAGIPTDTPRGEDSQFTGS
CTSPLGNKGYGVKGFGRQGTDVWAGRTISRTRSGFEIIRNGWTQNSKDQIR
RQVIIDDPNWSGYSGSFTLPVELTKKGCLVPCFWVEMIRGKPEETTIWTSSSSIVM
CGVDHKLASWSWHDGAILPFDIDKM

SEQ ID NO:2

FIG. 1B

PB1amino

MDVNPTLLFLKVPQAQNAISTTFPYTGDPPYSHGTGTGYTMDTVNRTHQYSEK GK
WTTNTEIGAPQLNPIDGPLEDNPSGYAQTDCVLEAMAFLEESHPIGIFENSCL
MEVIQQTRVDKLTQGRQTYDWTLNRNQPAAATALANTIEVFRSNGLTSNESGR
LMDFLKDVMEISMNKEEMEITTHFORKRRVRDNMTKRMVTQRTIGKKKQRLNR
KSYLIRTLTLNTMTKDAERGKLRRAIATPGMQIRGFVYFVETLARRICEKLEQ
SGLPVGGNEKKAKLANVVRKMMTNSQDTELSFTITGDNTKWNENQNPRIFLA
MITYITRNQPEWFRNVLSLAPIMFSNKMARLGKGYMFESKSMKLRTOIPAGML
ASIDLKYFNDPTKKKIEKIRPLLVDGTASLSPGMMMGMFNMLSTVLGVSILNL
GQRKYTKTTYWWDGLQSSDDFALIVNAPNHEGIQAGVDRFYRTCKLVGINMS
KKSINRTGTFEFTSFFYRYGFVANFSMELPSFGVSGINESADMSIGVTVIK
NNMINNDLGPATAQMALQLFIKDYRYTYRCHRGDTOIQRRSFELKKLWEQTR
SKTGLLVSDGGPNLYNIRNLHIPEVCLKWELMDEDYKGRLCNPLNPFVSHKEI
ESVNSAVVMPAHGPAKSMEYDAVATTHSWIPKRNRSILNTSQRGILEDEQMY
QKCCNLFEKFFPSSSYRPPVGISSMVEAMVSRARIDARIDFESGRICKDEF
AEIMKICSTIEELRQK

SEQ ID NO:3

FIG. 1C

PB2amino

MERIKELRDLMLQSRTREILTKTTVDHMAIKKYTSGRQEKNPALRMKWMMAM
KYPITADKRIMEMIPERNEQQQTLWSKTNDAGSDRVMVSPLAVTWWNRNGPTT
STIHYPKVYKTYFEKVERLKHGTFGPVHFRNQVKIRRRVDVNPGHADLSAKEAQ
DVIMEVVPNEVGARILTSESQLTITKEKKEELQDCKIAPLMVAYMLERELVRKT
RFLPVAGGTSSVYIEVLHLTQGTCEWQMYTPGGEVRNDDIDQSLIIAARNIVRA
TVSADPLASLLEMCHSTQIGGIRMVDILKQNPTEEQAVDICKAAMGLRISSSFSG
GFTFKRTSGSSVKREEEMLTGNLQTLKIRVHEGYEEFTMVGRRATAILRKATRRL
IQLIVSGRDEQSLAEAIIVAMVFSQEDCMIKAVRGDLNFVNRRANQRLNPMHQLLR
HFQKDAKVLFQNWGIEPIDNVMGMIGILPDMTPSTEMSLRGVVRVSKMGVDEYSS
TERVVVSIDRFLRVRDQRGNILLSPEEVSETQGTEKLTIIYSSSMMWEINGPESVL
VNTYQWIIRNWEIVKIQWSQDPTMLYNKIEFEPFQSLVPRATRSQYSGFVRTLFQ
QMRDVLGTFDTAQIIKLLPFAAAPPEQSRMQFSSLTVNVRGSGMRILVRGNPSPF
NYNKATKRLTVLGKDAGALTEDPDEGTAGVESAVLRGFLILGKENKRYGPALSI
NELSKLAKGEKANVLIQGDVVLVMKRKRDRSSILTDSQTATKRIRMAIN

SEQ ID NO:4

FIG. 1D

PAmino

MEDFVRQCFNPMIVELAEKAMKEYGEDPKIETNKFAAICTHLEVCFMYSDFHFIN
ELSESVVIESGDPNALLKHRFEIIEGRDRMTAWTVVNSICNTTRAEEKPKFLPDLYD
YKENRFVEIGVTRREVHIYYLEKANKIKSEKTHIHIFSFTGEEMATKADYTLDEES
RARIKTRLFTIRQEMASRGLWDSFRQSERGEETIEERFEITGTMRKLANYSLPPNF
SSLENFRVYVDGFEPNGCIESKLSQMSKEVNARIEPFSKTTTPRPLKMPGGPPCHQR
SKFLLMDALKLSIEDPSHEGEGIPLYDAIKCMKTFFGWKEPSIVKPHEKGINPNYL
QTWKQVLAELQDLENEEKDPKTKNMKKTSQLKWALSENMAPEKVDFEDCKDIS
DLKQYDSDEPETRSLASWIQSEFNKACELTDSSWIELDEIGEDVAPIEYIASMRRN
YFTAEVSHCRATEYIMKGVYINTALLNASCAAMDEFQLIPMISKCRTKEGRRKTN
LYGFIVKGRSHLRNDTDVNVFVSMESLTDPRFEPHKWEKYCVLEIGDMLLRTA
VGQVSRPMFLYVRTNGTSKIKMKWGMEMRRCLLQSLQQIESMIEAESSVKEKD
MTKEFFENKSETWPIGESPKGVEEGSIGKVCRTLLAKSVFNLSYASPLQLEGFSAES
RKLLLIVQALRDNLEPGTFDIGGLYESIEECLINDPWVLLNASWFNSFLTHALK

SEQ ID NO:5

FIG. 1E

NP amino

MASQGTKRSYEQMETDGERQNATEIRASVGRMVGGIGRFYVQMCTELKLNDEH
GRLIQNSITIERMVLSAFDERRNKYLEEHPSAGKDPKKTGGPIYRRKD GKWMREL
ILHDKEEIMRIWRQANNGEDATAGLTHMMIWHSNLNDTTYQRTRALVRTGMDP
RMCSLMQGSTLPRRSGAAGAAVKGVGTMMELIRMIKRGINDRNFWRGNGR
RTRIAYERM CNILKGFQTA AQRAMMDQVREGRNPGNAEIEDLIFLARSALILRG
SVAHKSLPACVYGLAVTSGYDFEKEGYSLVGIDPFKLLQNSQIFSLIRPKENPAH
KSQLVWMACHSAAFEDLRVLNFIRGTKVIPRGQLTTRGVQIASNENMETIDSSTL
ELRSKYWAIRTRSGGNTSQQRASAGQISVQPTFSVQRNLPFERATIMAAFTGNTE
GRTSDMRTEIIRMMENAKSEDVSEFQGRGVFELSDEKATNPVPSFDMSNEGSYFF
GDNAEEFDS

SEQ ID NO:6

FIG. 1F

M1amino

MSLLTEVETYVLSIVPSGPLKAEIAQRLEDVFAGKNTDLEALMEWLKTRPILSPLT
KGILGFVFTLTVPSERGLQRRRFVQNALSGNGDPNNMDRAVKLYRKLKREITFH
GAKEVALSYSTGALASCMGLIYNRMGTVTTEVAFGLVCATCEQIADSQHRSHRQ
MVTTTNPLIRHENRMVLA STTAKAMEQ MAGSSEQAAEAMEV ASRARQMVQAM
RTIGTHPSSAGLKDDLLENLQAYQKRMGVQMQRFK

SEQ ID NO:7

FIG. 1G

NS1amino

MDSNTVSSFQVDCFLWHVRKRFADQELGDAPFLDRLRRDQKSLRGRGSTLGLDI
ETATHAGKQIVEQILEKESDEALKMTIASVPTSRYLTDMTLDEM SRDWFMLMPK
QKVTGSLCIRMDQAIMDKNILKANFSVIFERLET LILLRAFTEEGAVVGEISPLPSL
PGHTNEDVKNAIGVLI GGLKWNDNTVRISETLQRFAWRSSHENGRPSFPSKQKR
KMERTIKPKI

SEQ ID NO:8

FIG. 1H

HA

TCATGAAGACAACCATTATTTTGATACTACTGACCCATTGGGCTTACAGTCAA
AACCCAATCAGTGGCAACAACACAGCCACATTGTGTCTGGGACACCATGCAG
TAGCAAATGGAACATTGGTAAAAACAATAAGTGATGATCAAATTGAGGTGAC
AAATGCTACAGAATTAGTTCAAAGCATTTC AATGGGGAAAATATGCAACAAC
TCATATAGAATTCTAGATGGAAGAAATTGCACATTAATAGATGCAATGCTAG
GAGACCCCCACTGTGACGCCTTTCAGTATGAGAATTGGGACCTCTTTATAGAA
AGAAGCAGCGCTTTCAGCAATTGCTACCCATATGACATCCCTGACTATGCATC
GCTCCGATCCATTGTAGCATCCTCAGGAACATTGGAATTCACAGCAGAGGGA
TTCACATGGACAGGTGTCACTCAAACGGAAGAAGTGGAGCCTGCAAAGG
GGATCAGCCGATAGTTTCTTTAGCCGACTGAATTGGCTAACAAAATCTGGAA
GCTCTTACCCACATTGAATGTGACAATGCCTAACAAATAAAAATTTGACAA
GCTATACATCTGGGGGATTCATACCCGAGCTCAAATCAAGAGCAGACAAAA
TTGTACATCCAAGAATCAGGACGAGTAACAGTCTCAACAAAAAGAAGTCAAC
AAACAATAATCCCTAACATCGGATCTAGACCGTGGGTGAGAGGTCAATCAGG
TAGGATAAGCATATACTGGACCATTGTA AACCTGGAGATATCCTAATGATA
AACAGTAATGGCAACTTAGTTGCACCGCGGGGATATTTTAAATTGAAAACAG
GGAAAAGCTCTGTAATGAGATCAGATGTACCCATAGACATTTGTGTGTCTGA
ATGTATTACACCAAATGGAAGCATCTCCAACGACAAGCCATTCCAAAATGTG
AACAAAGTTACATATGGAAAATGCCCAAGTATATCAGGCAAAACACTTTAA
AGCTGGCCACTGGGATGAGGAATGTACCAGAAAAGCAAATCAGAGGAATCT
TTGGAGCAATAGCGGGATTCATCGAAAACGGCTGGGAAGGAATGGTTGATGG
GTGGTATGGGTTCCGATATCAA AACTCTGAAGGAACAGGGCAAGCTGCAGAT
CTAAAGAGCACTCAAGCAGCCATCGACCAGATTAATGGAAAGTTAAACAGA
GTGATTGAAAGAACCAATGAGAAATTCATCAAATAGAGAAGGAATTTCTCAG
AAGTAGAAAGAAGAATTCAGGACTTGGAGAAATATGTAGAAGACACCAAAA
TAGACCTATGGTCTACAATGCAGAATTGCTGGTGGCTCTAGAAAATCAACA
TACAATTGACTTAACAGATGCAGAAATGAATAAATTATTTGAGAAGACTAGA
CGCCAGTTAAGAGAAAACGCAGAAGACATGGGAGGTGGATGTTTCAAGATTT
ACCACAAATGTGATAATGCATGCATTGGATCAATAAGAAATGGGACATATGA
CCATTACATATACAGAGATGAAGCATTAAACAACCGATTT CAGATCAAAGGT
GTAGAGTTGAAATCAGGCTACAAAGATTGGATACTGTGGATTT CATTCCGCA
TATCATGCTTCTTAATTTGCGTTGTTCTATTGGGTTTCATTATGTGGGCTTGCC
AAAAAGGCAACATCAGATGCAACATTTGCATTTGAG

SEQ ID NO:9

FIG. 11

NA

ATGAATCCAAATCAAAGATAATAGCAATTGGATTTGCATCATTGGGGATAT
TAATCATTAATGTCATTCTCCATGTAGTCAGCATTATAGTAACAGTACTGGTC
CTCAATAACAATAGAACAGATCTGAACTGCAAAGGGACGATCATAAGAGAG
TACAATGAAACAGTAAGAGTAGAAAAAATTACTCAATGGTATAATACCAGTA
CAATTAAGTACATAGAGAGACCTTCAAATGAATACTACATGAACAACACTGA
ACCACTTTGTGAGGCCCAAGGCTTTGCACCATTTTCAAAGATAATGGAATAC
GAATTGGGTCGAGAGGCCATGTTTTGTGATAAGAGAACCCTTTTGTATCATGT
TCGCCCTCAGAATGTAGAACCTTTTCCCTCACACAGGGCTCATTACTCAATGA
CAAACATTCTAACGGCACAGTAAAGGACCGAAGTCCGTATAGGACTTTGATG
AGTGTCAAATAGGGCAATCACCTAATGTATATCAAGCTAGGTTTGAATCGG
TGGCATGGTCAGCAACAGCATGCCATGATGGAAAAAATGGATGACAGTTGG
AGTCACAGGGCCCGACAATCAAGCAATTGCAGTAGTGA ACTATGGAGGTGTT
CCGGTTGATATTATTAATTCATGGGCAGGGGATATTTTAAGAACCCAAGAAT
CATCATGCACCTGCATTAAAGGAGACTGTTATTGGGTAATGACTGATGGACC
GGCAAATAGGCAAGCTAAATATAGGATATTCAAAGCAAAGATGGAAGAGT
AATTGGACAGACTGATATAAGTTTCAATGGGGGACACATAGAGGAGTGTCT
TGTTACCCCAATGAAGGGAAGGTGGAATGCATATGCAGGGACAATTGGACTG
GAACAAATAGACCAATTCTGGTAATATCTTCTGATCTATCGTACACAGTTGGA
TATTTGTGTGCTGGCATTCCCCTGACACTCCTAGGGGAGAGGATAGTCAATT
CACAGGCTCATGTACAAGTCCTTTGGGAAATAAAGGATACGGTGTA AAAGGT
TTCGGGTTTCGACAAGGAACTGACGTATGGGCCGGAAGGACAATTAGTAGGA
CTTCAAGATCAGGATTCGAAATAATAAAAATCAGGAATGGTTGGACACAGAA
CAGTAAAGACCAAATCAGGAGGCAAGTGATTATCGATGACCCAAATTGGTCA
GGATATAGCGGTTCTTTACATTGCCGTTGAACTAACAAAAAAGGGATGTT
TGGTCCCCTGTTTCTGGGTTGAAATGATTAGAGGTAAACCTGAAGAAACAAC
AATATGGACCTCTAGCAGCTCCATTGTGATGTGTGGAGTAGATCATAAAATT
GCCAGTTGGTCATGGCAGATGGAGCTATTCTTCCCTTTGACATCGATAAGAT
GTAA

SEQ ID NO:10

FIG. 1J

PB1

ATGGATGTCAATCCGACTCTACTTTTCTTAAAGGTGCCAGCGCAAATGCTAT
AAGCACAACATTTCTTATACTGGAGATCCTCCCTACAGTCATGGAACAGGG
ACAGGATACACCATGGATACTGTCAACAGAACACACCAATATTCAGAAAAAG
GGAAATGGACAACAAACACTGAGATTGGAGCACCACAACCTTAATCCAATCGA
TGGACCACTTCTGAAGACAATGAACCAAGTGGGTACGCCCAAACAGATTGT
GTATTGGAAGCAATGGCTTTCTTGAAGAATCCCATCCCGGAATCTTTGAAAA
TTCGTGTCTTGAAACGATGGAGGTGATTCAGCAGACAAGAGTGGACAAACTA
ACACAAGGCCGACAAACTTATGATTGGACCTTGAATAGGAATCAACCTGCCG
CAACAGCACTTGCTAATACGATTGAAGTATTCAGATCAAATGGTCTGACTTCC
AATGAATCGGGGAGATTGATGGACTTCTCAAAGATGTCATGGAGTCCATGA
ACAAGGAAGAAATGGAAATAACAACACACTTCCAACGGAAGAGAAGAGTAA
GAGACAACATGACAAAGAGAATGGTAACACAGAGAACCATAGGGAAGAAAA
AACCAACGATTAACAGAAAGAGCTATCTAATCAGAACATTAACCCTAAACAC
AATGACCAAGGACGCTGAGAGAGGGAAATTGAAACGACGAGCAATCGCTAC
CCCAGGGATGCAGATAAGAGGGTTTGTATATTTTGTGAAACACTAGCCCGA
AGAATATGTGAAAAGCTTGAACAATCAGGATTGCCAGTTGGCGGTAATGAGA
AAAAGGCCAAACTGGCTAATGTCGTGAGAAAAATGATGACTAATTCCTAAGA
CACTGAACTCTCCTTACCATCACTGGGGACAATACCAAATGGAATGAAAAT
CAGAACCCACGCATATTCCTGGCAATGATCACATACATAACTAGAAACCAGC
CAGAATGGTTCAGAAATGTTCTAAGCATTGCACCGATTATGTTCTCAAATAAA
ATGGCAAGACTGGGGAAAGGATATATGTTTGAAGCAAAGTATGAAATTG
AGAACTCAAATACCAGCAGGAATGCTTGAAGCATTGACCTGAAATATTTCA
ATGATCCAACAAAAAAGAAAATTGAAAAGATACGACCACTTCTGGTTGACGG
GACTGCTTCACTGAGTCCTGGCATGATGATGGGAATGTTCAACATGTTGAGC
ACTGTGCTAGGTGTATCCATATTAACCTGGGCCAGAGGAAATACACAAAGA
CCACATACTGGTGGGATGGTCTGCAATCATCCGATGACTTTGCTTTGATAGTG
AATGCGCCTAATCATGAAGGAATACAAGCTGGAGTAGACAGATTCTATAGGA
CTTGCAAACCTGGTTCGGGATCAACATGAGCAAAAAGAAGTCCTACATAAATAG
AACTGGAACATTGGAATTCACAAGCTTTTTCTACCGGTATGGTTTTGAGCCA
ATTTGAGCATGGAACCTACCAGTTTTGGGGTTTTCCGGAATAAATGAATCTGCA
GACATGAGCATTGGAGTGACAGTCATCAAAAACAACATGATAAATAATGATC
TCGGTCTGCCACGGCACAAATGGCACTCCAACCTTTCATTAAGGATTATCGG
TACACATAACCGGTGCCATAGAGGTGATACCCAGATACAAACCAGAAGATCTT
TTGAGTTGAAGAACTGTGGGAACAGACTCGATCAAAGACTGGTCTACTGGT
ATCAGATGGGGGTCCAAACCTATATAACATCAGAAACCTACACATCCCGGAA
GTCTGTTTAAAATGGGAGCTAATGGATGAAGATTATAAGGGGAGGCTATGCA
ATCCATTGAATCCTTTTCGTTAGTCACAAAGAAATTGAATCAGTCAACAGTGCA
GTAGTAATGCCTGCGCATGGCCCTGCCAAAAGCATGGAGTATGATGCTGTTG
CAACAACACATTCTTGGATCCCCAAGAGGAACCGGTCCATATTGAACACAAG
CCAAAGGGGAATACTCGAAGATGAGCAGATGTATCAGAAATGCTGCAACCTG
TTTAAAAAATTCTTCCCAGCAGCTCATAACAGAAGACCAGTCGGGATTTCTAG
TATGGTTGAGGCCATGGTGTCCAGGGCCCGCATTGATGCACGAATTGACTTC
GAATCTGGACGGATAAAGAAGGATGAGTTCGCTGAGATCATGAAGATCTGTT
CCACCATTGAAGAGCTCAGACGGCAAAAATAAGTGA

SEQ ID NO:11

FIG. 1K

PB2

ATGGAGAGAATAAAAAGAACTGAGAGATCTGATGTTACAATCCCGCACCCGCG
AGATACTAACAAAACTACTGTGGACCACATGGCCATAATCAAGAAATACAC
ATCAGGAAGACAAGAGAAGAACCCTGCACTTAGGATGAAATGGATGATGGC
AATGAAATAACCAATTACAGCAGATAAGAGGATAATGGAGATGATTCCTGAG
AGAAATGAACAGGGACAAACCCTTTGGAGCAAACGAACGATGCTGGCTCA
GACCGCGTAATGGTATCACCTCTGGCAGTGACATGGTGGAATAGGAATGGAC
CAACAACAAGCACAATTCATTATCCAAAAGTCTACAAAACCTTATTTTGAAAA
GGTTGAAAGATTGAAACACGGAACCTTTGGCCCGTTCATTTTAGGAATCAA
GTCAAGATAAGACGAAGAGTTGATGTAAACCCTGGTCACGCGGACCTCAGTG
CCAAAGAAGCACAAGATGTGATCATGGAAGTTGTTTTCCCAAATGAAGTGGG
AGCCAGAATTCTAACATCGGAATCACAATAACCAAAGAGAAAAA
GGAAGAACTTCAGGACTGCAAATTGCTCCCTTGATGGTAGCATAACATGCTA
GAAAGAGAGTTGGTCCGAAAAACAAGGTTCCCTCCAGTAGCAGGCGGAACA
AGCAGTGTATACATTGAAGTGTTCATCTGACTCAGGGAACATGCTGGGAGC
AATGTACACCCCAGGAGGAGAAGTTAGAAACGATGATATTGATCAAAGTTT
AATTATTGCAGCCCGGAACATAGTGAGAAGAGCAACAGTATCAGCAGATCCA
CTAGCATCCCTACTGGAAATGTGCCACAGTACACAGATTGGTGGAATAAGGA
TGGTAGACATCCTTAAGCAGAATCCAACAGAGGAACAAGCTGTGGATATATG
CAAAGCAGCAATGGGATTGAGAATTAGCTCATCATTTCAGCTTTGGTGGATT
ACCTTCAAGAGAACAAGTGGATCATCAGTCAAGAGAGAAGAAGAATGCTT
ACGGGCAACCTTCAAACATTGAAAATAAGAGTGCATGAGGGCTATGAAGAAT
TCACAATGGTCGGAAGAAGAGCAACAGCCATTCTCAGAAAGGCAACCAGAA
GATTGATTCAATTGATAGTAAGTGGGAGAGATGAACAGTCAATTGCTGAAGC
AATAATTGTAGCCATGGTGTTCGCAAGAAGATTGCATGATAAAAGCAGTT
CGAGGCGATTTGAACTTTGTTAATAGAGCAAATCAGCGCTTGAACCCCATGC
ATCAACTCTTGAGGCATTTCCAAAAGGATGCAAAGTGTCTTTCCAAAATTG
GGGATTGAACCCATCGACAATGTAATGGGAATGATTGGAATATTGCCTGAC
ATGACCCAAGCACCGAGATGTCATTGAGAGGAGTGAGAGTCAGCAAAATG
GGAGTGGATGAGTACTCCAGCACTGAGAGAGTGGTGGTGAGCATTGACCGTT
TTTTAAGAGTTCGGGATCAAAGGGGAAACATACTACTGTCCCCTGAAGAAGT
CAGTGAACACAAAGGAACGGAAAAGCTGACAATAATTTATTCGTATCAATG
ATGTGGGAGATTAATGGTCCCGAATCAGTGTTGGTCAATACTTATCAATGGAT
CATCAGGAACTGGGAAATTGTAAAATTTCAGTGGTCACAGGACCCCAACAATG
TTATAAATAAGATAGAATTTGAGCCATTCCAATCCCTGGTCCCTAGGGCTAC
CAGAAGCCAATACAGCGGTTTCGTAAGAACCCTGTTTCAGCAAATGCGAGAT
GTACTTGGAACATTTGATACTGCTCAAATAATAAACTCCTCCCTTTTGCCGC
TGCTCCTCCGGAACAGAGTAGGATGCAGTTCTCTTCTTTGACTGTTAATGLAA
GAGGTTCCGGGAATGAGGATACTTGTAAAGAGGCAATTCCCAGTGTTCAACTA
CAATAAAGCCACTAAAAGGCTCACAGTCCTCGGAAAGGATGCAGGTGCGCTT
ACTGAGGACCCAGATGAAGGTACGGCTGGAGTAGAATCTGCTGTTCTAAGAG
GGTTTCTCATTTTAGGTAAAGAAAATAAGAGATATGGCCCAGCACTAAGCAT
CAATGAACTAAGCAAACCTTGCAAAGGGGAGAAAGCCAATGACTAATTGG
GCAAGGGGACGTAGTGTGGTAATGAAACGGAAACGTGACTCTAGCATACTT
ACTGACAGCCAGACAGCGACCAAAAGGATTCGGATGGCCATCAATTAGT

SEQ ID NO:12

FIG. 1L

PA

ATGGAAGACTTTGTGCGACAATGCTTCAATCCAATGATCGTCGAGCTTGCGG
AAAAGGCAATGAAAGAATATGGAGAGGACCCGAAAATCGAAACAAACAAAT
TTGCAGCAATATGCACTCACTTGGAAAGTCTGCTTCATGTACTCGGATTTCCAC
TTTATTAATGAACTGAGTGAGTCAGTGGTCATAGAGTCTGGTGACCCAAATG
CTCTTTTGAACACAGATTTGAAATCATTGAGGGGAGAGATCGAACAATGGC
ATGGACAGTAGTAAACAGCATCTGCAACACCACAAGAGCTGAAAAACCTAA
ATTTCTTCCAGATTTATACGACTATAAGGAGAACAGATTTGTTGAAATTGGTG
TGACAAGGAGAGAAGTTCACATATACTACCTGGAGAAGGCCAACAAAATAA
AGTCTGAGAAAACACATATCCACATTTTCTCATTACAGGAGAGGAAATGGC
TACAAAAGCGGACTATACTCTTGATGAAGAGAGTAGAGCCAGGATCAAGACC
AGACTATTCACTATAAGACAAGAAATGGCCAGTAGAGGCCTCTGGGATTCTT
TTCGTCAGTCCGAGAGAGGGCGAAGAGACAATTGAAGAAAGATTTGAAATCAC
AGGGACGATGCGCAAGCTTGCCAATTACAGTCTCCACCGAACTTCTCCAGC
CTTGAAAATTTTAGAGTCTATGTGGATGGATTGGAACCGAACGGCTGCATTG
AGAGTAAGCTTTCTCAAATGTCCAAAGAAGTAAATGCCAGAATCGAACCAT
TTCAAAGACAACACCCCGACCACTCAAATGCCAGGTGGTCCACCTGCCAT
CAGCGATCTAAATTCCTGCTAATGGATGCTCTGAAACTGAGCATTGAGGACC
CAAGTCACGAGGGAGAGGGAATACCACTATATGATGCCATCAAATGCATGAA
AACTTTCTTTGGATGGAAAGAGCCCAGTATTGTTAAACCACATGAAAAGGGT
ATAAACCCGAACTATCTCCAACTTGGAAGCAAGTATTAGCAGAATTACAAG
ACCTTGAGAACGAAGAAAAGGACCCCAAGACCAAGAATATGAAAAAACAA
GCCAATTGAAATGGGCACTTAGTGAAAATATGGCACCAGAGAAAGTGGATTT
TGAGGATTGTAAAGACATCAGTGATTTAAAACAGTATGACAGTGATGAGCCA
GAAACAAGGTCTCTTGCAAGTTGGATTCAAAGTGAGTTC AACAAAGCTTGTG
AACTGACAGATTCAAGCTGGATAGAGCTCGATGAAATTGGGGAGGATGTTGC
CCCAATAGAATACATTGCGAGCATGAGGAGAAATTATTTTACTGCTGAGGTT
TCCCATTGTAGAGCAACAGAATATATAATGAAGGGAGTGTACATCAACACTG
CTCTACTCAATGCATCCTGTGCTGCGATGGATGAATTCCAATTAATTCCGATG
ATAAGTAAATGCAGGACCAAAGAAGGGAGAAGGAAGACAAATTTATATGGA
TTCATAGTAAAGGGAAGGTCCCATTAAAGAAATGATACTGACGTGGTGAAC
TTGTAAGTATGGAATTTTCTCTCACTGATCCAAGATTTGAGCCACACAAATGG
GAAAAATACTGCGTTCTAGAAATTGGAGACATGCTTCTAAGAACTGCTGTAG
GTCAAGTGTCAAGACCCATGTTTTTGTATGTAAGGACAAATGGAACCTCTAA
AATTA AAATGAAATGGGGAATGGAAATGAGGCGCTGCCTCCTCAGTCTCTG
CAACAGATTGAAAGCATGATCGAAGCTGAGTCCTCAGTCAAAGAAAAGGAC
ATGACCAAAGAATTTTTTGGAGAACAATCAGAGACATGGCCTATAGGAGAGT
CCCCAAAGGAGTGGAAAGAGGGCTCAATCGGGAAGGTTTGCAGGACCTTATT
AGCAAATCTGTGTTTAAACAGTTTGTATGCATCTCCACAACCTGGAAGGGTTT
CAGCTGAATCTAGGAAATTA CTCTCATTGTT CAGGCTCTTAGGGATAACCTG
GAACCTGGAACCTTTGATATTGGGGGGTTATATGAATCAATTGAGGAGTGCC
TGATTAATGATCCCTGGGTTTTGCTTAATGCATCTTGGTTCAACTCCTTCCTTA
CACATGCACTGAAGTAGTTGTGGCAATGCTACTATTTGCTATCCATACTGTCC
AAAAAAGTACCTTGTTTCTACT

SEQ ID NO:13

FIG. 1M

NP

ATGGCGTCTCAAGGCACCAAACGATCCTATGAACAGATGGAAACTGATGGGG
AACGCCAGAATGCAACTGAAATCAGAGCATCTGTCCGAAGGATGGTGGGAG
GAATCGGCCGGTTTTATGTTTCAGATGTGTACTGAGCTTAAACTAAACGACCAT
GAAGGGCGGCTGATTCAGAACAGCATAACAATAGAAAGGATGGTACTTTCCG
CATTCGACGAAAGAAGAAACAAGTATCTCGAGGAGCATCCCAGTGCTGGGA
AAGACCCTAAGAAAACAGGAGGCCCGATATACAGAAGGAAAGATGGGAAAT
GGATGAGGGAACTCATCCTCCATGATAAAGAAGAAATCATGAGAATCTGGCG
TCAGGCCAACAAATGGTGAAGACGCTACTGCTGGTCTTACTCATATGATGATCT
GGCACTCCAATCTCAATGACACCACATAACAAAGAACAAGGGCTCTTGTTCCG
GACTGGGATGGATCCCAGAATGTGCTCTCTGATGCAAGGCTCAACCTCCCA
CGGAGATCTGGAGCCGCTGGTGCTGCAGTAAAAGGTGTTGGAACAATGGTAA
TGAAACTCATCAGAATGATCAAACGCGGAATAAATGATCGGAATTTCTGGAG
AGGTGAAAATGGTTCGAAGAACCAGAATTGCTTATGAAAGAATGTGCAATATC
CTCAAAGGGAAATTTTCAGACAGCAGCACAACGGGCTATGATGGACCAGGTG
AGGGAAGGCCGCAATCCTGGAAACGCTGAGATTGAGGATCTCATTTCCTGG
CACGATCAGCACTTATTTTGAGAGGATCAGTAGCCATAAATCATGCCTACCT
GCCTGTGTTTATGGCCTTGCAGTAAACAGTGGGTATGACTTTGAGAAGGAAG
GATACTCTCTGGTTGGAATTGATCCTTTCAAACACTACTCCAGAACAGTCAAATT
TTCAGTCTAATCAGACCAAAGAAAACCCAGCACACAAGAGCCAGTTGGTGT
GGATGGCATGCCATTCTGCAGCATTGAGGACCTGAGAGTTTTAAATTTTATT
AGAGGAACCAAAGTAATCCCAAGAGGACAGTTAACAACAGAGGAGTTCAA
ATAGCTTCAAATGAAAACATGGAGACAATAGATTCTAGCACACTTGAACCTGA
GAAGCAAATATTGGGCAATAAGGACCAGAAGCGGAGGAAACACCAGTCAAC
AGAGAGCATCTGCAGGACAGATAAGTGTGCAACCTACTTTCTCAGTACAGAG
AAATCTTCCCTTTGAGAGAGCAACCATTATGGCTGCATTCACTGGTAACACTG
AAGGGAGGACTTCCGACATGAGAACGGAAATCATAAGGATGATGGAAAATG
CCAAATCAGAAGATGTGTCTTTCCAGGGGGCGGGGAGTCTTCGAGCTCTCGGA
CGAAAAGGCAACGAACCCGATCGTGCCTTCCTTTGACATGAGCAATGAAGGG
TCTTATTTCTTCGGAGACAATGCTGAGGAGTTTGACAGTTAAA

SEQ ID NO:14

FIG. 1N

M

ATGAGTCTTCTAACCGAGGTCGAAACGTACGTTCTCTCTATCGTACCATCAGG
CCCCCTCAAAGCCGAGATCGCGCAGAGACTTGAAGATGTCTTTGCAGGGAAG
AACACCGATCTTGAGGCACTCATGGAATGGCTAAAGACAAGACCAATCCTGT
CACCTCTGACTAAAGGGATTTTAGGATTTGTATTACGCTCACCGTGCCCAGT
GAGCGAGGACTGCAGCGTAGACGCTTTGTCCAAAATGCCCTTAGTGGAACG
GAGATCCAAACAACATGGACAGAGCAGTAAAAGTGTACAGGAAGCTTAAAA
GAGAAATAACATTCCATGGGGCAAAGAGGTGGCACTCAGCTATTCCACTGG
TGCCTAGCCAGCTGCATGGGACTCATATACAACAGAATGGGAAGTGTTACA
ACCGAAGTGGCATTGTCCTGGTATGCGCCACATGTGAACAGATTGCTGATT
CCCAGCATCGGTCTCACAGGCAGATGGTGACAACAACCAACCCATTAATCAG
ACATGAAAACAGAATGGTATTAGCCAGTACCACGGCTAAAGCCATGGAACA
GATGGCAGGATCGAGTGAGCAGGCAGCAGAGGCCATGGAGGTTGCTAGTAG
GGCTAGGCAGATGGTACAGGCAATGAGAACCATTGGGACCCACCCTAGCTCC
AGTGCCGGTTTGAAAGATGATCTCCTTGAAAATTTACAGGCCTACCAGAAAC
GGATGGGAGTGCAAATGCAGCGATTCAAGTGATCCTCTCGTTATTGCAGCAA
GTATCATTGGGATCTTGCACTTGATATTGTGGATTCTTGATCGTCTTTCTTCA
AATTCATTTATCGTCGCCTTAAATACGGGTTGAAAAGAGGGCCTTCTACGGA
AGGAGTACCTGAGTCTATGAGGGAAGAATATCGGCAGGAACAGCAGAATGC
TGTGGATGTTGACGATGGTCATTTTGTCAACATAGAGCTGGAGTAA

SEQ ID NO:15

FIG. 10

NS

ATGGATTCCAACACTGTGTCAAGCTTTCAGGTAGACTGTTTTCTTTGGCATGT
CCGCAAACGATTTCGCAGACCAAGAAGTGGGTGATGCCCCATTCCCTTGACCGG
CTTCGCCGAGACCAGAAGTCCCTAAGGGGAAGAGGTAGCACTCTTGGTCTGG
ACATCGAAACAGCCACTCATGCAGGAAAGCAGATAGTGGAGCAGATTCTGG
AAAAGGAATCAGATGAGGCACTTAAAATGACCATTGCCTCTGTTCTACTTC
ACGCTACTTAACTGACATGACTCTTGATGAGATGTCAAGAGACTGGTTCATGC
TCATGCCCAAGCAAAAAGTAACAGGCTCCCTATGTATAAGAATGGACCAGGC
AATCATGGATAAGAACATCATACTTAAAGCAAACCTTTAGTGTGATTTTCGAA
AGGCTGGAAACACTAATACTACTTAGAGCCTTCACCGAAGAAGGAGCAGTCG
TTGGCGAAATTTACCATTACCTTCTCTTCCAGGACATACTAATGAGGATGTC
AAAAATGCAATTGGGGTCCTCATCGGAGGACTTAAATGGAATGATAATACGG
TTAGAATCTCTGAAACTCTACAGAGATTCGCTTGGAGAAGCAGTCATGAGAA
TGGGAGACCTTCATTCCCTCAAAGCAGAAACGAAAAATGGAGAGAAACAATT
AAGCCAAAAATTTGAAGAAATAAGATGGTTGATTGAAGAAGTGCAGACATAG
ATTGAAAAATACAGAAAATAGTTTTGAACAAATAACATTTATGCAAGCCTTA
CAACTATTGCTTGAAGTAGAACAAGAGATAAGAACTTTCTCGTTTCAGCTTAT
TTAA

SEQ ID NO:16

FIG. 1P

M2amino

MSLLTEVETPTRNGWECKCSDSSDPLVIAASIIIGILHLILWILDRLFFKFIYRRLKY
GLKRGPSSTEGVPESMREEYRQEQQNAVDVDDGHFVNIELE

SEQ ID NO:17

FIG. 1Q

NS2amino

MDSNTVSSFQLMRMSKMQLGSSSEDLNGMIIRLESKLYRDSLGEAVMRMGDL
HSLQSRNEKWREQLSQKFEEIRWLIEBVRHRLKNTENSFEQITFMQALQLLLEVE
QEIRTFQSLI

SEQ ID NO:18

FIG. 1R

M K T T I I L I L L T H W A Y S Q N P I S G N N T A T L C L A/Equine/WI/1/03
M K T T I I L I L L T H W A Y S Q N P I S G N N T A T L C L A/Equine/New York/99

G H H A V A N G T L V K T I S D D Q I E V T N A T E L V Q S A/Equine/WI/1/03
G H H A V A N G T L V K T I S D D Q I E V T N A T E L V Q S A/Equine/New York/99

I S M G K I C N N S Y R I L D G R N C T L I D A M L G D P H A/Equine/WI/1/03
I S M G K I C N N S Y R I L D G R N C T L I D A M L G D P H A/Equine/New York/99

C D A F Q Y E N W D L F I E R S S A F S N C Y P Y D I P D Y A/Equine/WI/1/03
C D V F Q Y E N W D L F I E R S S A F S N C Y P Y D I P D Y A/Equine/New York/99

A S L R S I V A S S G T L E F T A E G F T W T G V T Q N G R A/Equine/WI/1/03
A S L R S I V A S S G T L E F T A E G F T W T G V T Q N G R A/Equine/New York/99

S G A C K R G S A D S F F S R L N W L T K S G S S Y P T L N A/Equine/WI/1/03
S G A C K R G S A D S F F S R L N W L T K S G N S Y P T L N A/Equine/New York/99

V T M P N N K N F D K L Y I W G I H H P S S N Q E Q T K L Y A/Equine/WI/1/03
V T M P N N K N F D K L Y I W G I H H P S S N Q E Q T K L Y A/Equine/New York/99

I Q E S G R V T V S T K R S Q Q T I I P N I G S R P W V R G A/Equine/WI/1/03
I Q E S G R V T V S T K R S Q Q T I I P N I G S R P W V R G A/Equine/New York/99

Q S G R I S I Y W T I V K P G D I L M I N S N G N L V A P R A/Equine/WI/1/03
Q S G R I S I Y W T I V K P G D I L M I N S N G N L V A P R A/Equine/New York/99

G Y F K L K T G K S S V M R S D V P I D I C V S E C I T P N A/Equine/WI/1/03
G Y F K L K T G K S S V M R S D A P I D I C V S E C I T P N A/Equine/New York/99

G S I S N D K P F Q N V N K V T Y G K C P K Y I R Q N T L K A/Equine/WI/1/03
G S I S N D K P F Q N V N K V T Y G K C P K Y I R Q N T L K A/Equine/New York/99

L A T G M R N V P E K Q I R A/Equine/WI/1/03
L A T G M R N V P E K Q I R A/Equine/New York/99

FIG. 2

H3 EQUINE INFLUENZA A VIRUS

STATEMENT OF GOVERNMENT RIGHTS

The invention was made, at least in part, with a grant from the Government of the United States of America (grant 2001-35204-10184 from the United States Department of Agriculture). The Government may have certain rights to the invention.

BACKGROUND

Influenza is a major respiratory disease in some mammals including horses and is responsible for substantial morbidity and economic losses each year. In addition, influenza virus infections can cause severe systemic disease in some avian species, leading to death. The segmented nature of the influenza virus genome allows for reassortment of segments during virus replication in cells infected with two or more influenza viruses. The reassortment of segments, combined with genetic mutation and drift, can give rise to a myriad of divergent strains of influenza virus over time. The new strains exhibit antigenic variation in their hemagglutinin (HA) and/or neuraminidase (NA) proteins, and in particular the gene coding for the HA protein has a high rate of variability. The predominant current practice for the prevention of flu is vaccination. Most commonly, whole virus vaccines are used. As the influenza HA protein is the major target antigen for the protective immune responses of a host to the virus and is highly variable, the isolation of influenza virus and the identification and characterization of the HA antigen in viruses associated with recent outbreaks is important for vaccine production. Based on prevalence and prediction, a vaccine is designed to stimulate a protective immune response against the predominant and expected influenza virus strains (Park et al., 2004).

There are three general types of influenza viruses, Type A, Type B and Type C, which are defined by the absence of serological crossreactivity between their internal proteins. Influenza Type A viruses are further classified into subtypes based on antigenic and genetic differences of their glycoproteins, the HA and NA proteins. All the known HA and NA subtypes (H1 to H15 and N1 to N9) have been isolated from aquatic birds, which are thought to act as a natural reservoir for influenza. H7N7 and H3N8 Type A viruses are the most common causes of equine influenza, and those subtypes are generally incorporated into equine influenza vaccines.

Thus, there is a continuing need to isolate new influenza virus isolates, e.g., for vaccine production.

SUMMARY OF THE INVENTION

The invention provides isolated H3 equine derived influenza type A virus that was isolated from a foal that succumbed to a fatal pneumonia, which virus has characteristic substitutions at residues 78 and 159 of HA (numbering of positions is that in the mature protein which lacks a 15 amino acid signal peptide), i.e., the residue at position 78 of HA is not valine and the residue at position 159 is not asparagine. In one embodiment, the isolated H3 influenza A virus of the invention has a conservative substitution at residue 78, e.g., a valine to an alanine substitution, and a nonconservative substitution at residue 159, e.g., an asparagine to a serine substitution. In one embodiment, the isolated H3 influenza A virus of the invention has a residue other than methionine at position 29, e.g., a

other than serine at position 83, e.g., a nonconservative substitution, a residue other than asparagine at position 92, e.g., a nonconservative substitution, a residue other than leucine at position 222, e.g., a nonconservative substitution, a residue other than alanine at position 272, e.g., a conservative substitution, and/or a residue other than threonine at position 328, e.g., a conservative substitution. Conservative amino acid substitutions refer to the interchangeability of residues having similar side chains. For example, a group of amino acids having aliphatic side chains is glycine, alanine, valine, leucine, and isoleucine; a group of amino acids having aliphatic-hydroxyl side chains is serine and threonine; a group of amino acids having amide-containing side chains is asparagine and glutamine; a group of amino acids having aromatic side chains is phenylalanine, tyrosine and tryptophan; a group of amino acids having basic side chains is lysine, arginine and histidine; and a group of amino acids having sulfur-containing side chain is cysteine and methionine. In one embodiment, conservative amino acid substitution groups are: threonine-valine-leucine-isoleucine-alanine; phenylalanine-tyrosine; lysine-arginine; alanine-valine; glutamic-aspartic; and asparagine-glutamine.

In one embodiment, the influenza virus of the invention includes one or more viral proteins (polypeptides) having substantially the same amino acid sequence as one of SEQ ID NOs:1-8, 17 and/or 18, so long as the HA has the characteristic substitutions at residues 78 and 159. An amino acid sequence which is substantially the same as a reference sequence has at least 95%, e.g., 96%, 97%, 98% or 99%, amino acid sequence identity to that reference sequence, and may include sequences with deletions, e.g., those that result in a deleted viral protein having substantially the same activity or capable of being expressed at substantially the same level as the corresponding full-length, mature viral protein, insertions, e.g., those that result in a modified viral protein having substantially the same activity or capable of being expressed at substantially the same level as the corresponding full-length, mature viral protein, and/or substitutions, e.g., those that result in a viral protein having substantially the same activity or capable of being expressed at substantially the same level as the reference protein. In one embodiment, the one or more residues which are not identical to those in the reference sequence may be conservative or nonconservative substitutions which one or more substitutions do not substantially alter the expressed level or activity of the protein with the substitution(s), and/or the level of virus obtained from a cell infected with a virus having that protein. As used herein, "substantially the same expressed level or activity" includes a detectable protein level that is about 80%, 90% or more, the protein level, or a measurable activity that is about 30%, 50%, 90%, e.g., up to 100% or more, the activity, of a full-length mature polypeptide corresponding to one of SEQ ID NOs:1-8, 17 or 18. In one embodiment, the virus comprises a polypeptide with one or more, for instance, 2, 5, 10, 15, 20 or more, amino acid substitutions, e.g., conservative substitutions of up to 5% of the residues of the full-length, mature form of a polypeptide having SEQ ID NOs:1-8, 17 or 18. The isolated virus of the invention may be employed alone or with one or more other virus isolates, e.g., other influenza virus isolates, in a vaccine, to raise virus-specific antisera, in gene therapy, and/or in diagnostics. Accordingly, the invention provides host cells infected with the virus of the invention, and isolated antibody specific for the virus.

The invention also provides an isolated nucleic acid molecule (polynucleotide) comprising a nucleic acid segment corresponding to at least one of the proteins of the virus of the invention, a portion of the nucleic acid segment for a viral

protein having substantially the same level or activity as a corresponding polypeptide encoded by one of SEQ ID NOs: 1-8, 17 or 18, or the complement of the nucleic acid molecule. In one embodiment, the isolated nucleic acid molecule encodes a polypeptide which has substantially the same amino acid sequence, e.g., has at least 95%, e.g., 96%, 97%, 98% or 99%, contiguous amino acid sequence identity to a polypeptide having one of SEQ ID NOs: 1-8, 17 or 18. In one embodiment, the isolated nucleic acid molecule comprises a nucleotide sequence which is substantially the same as, e.g., has at least 50%, e.g., 60%, 70%, 80% or 90% or more, contiguous nucleic acid sequence identity to, one of SEQ ID NOs: 9-16, or the complement thereof, and encodes a polypeptide having at least 95%, e.g., 96%, 97%, 98% or 99%, contiguous amino acid sequence identity to a polypeptide having one of SEQ ID NOs: 1-8, 17 or 18.

The isolated nucleic acid molecule of the invention may be employed in a vector to express influenza proteins, e.g., for recombinant protein vaccine production or to raise antisera, as a nucleic acid vaccine, for use in diagnostics or, for vRNA production, to prepare chimeric genes, e.g., with other viral genes including other influenza virus genes, and/or to prepare recombinant virus, e.g., see Neumann et al. (1999) which is incorporated by reference herein. Thus, the invention also provides isolated viral polypeptides, recombinant virus, and host cells contacted with the nucleic acid molecule(s) and/or recombinant virus of the invention, as well as isolated virus-specific antibodies, for instance, obtained from mammals infected with the virus or immunized with an isolated viral polypeptide or polynucleotide encoding one or more viral polypeptides.

The invention further provides at least one of the following isolated vectors, for instance, one or more isolated influenza virus vectors, or a composition comprising the one or more vectors: a vector comprising a promoter operably linked to an influenza virus PA DNA for a PA having substantially the same amino acid sequence as SEQ ID NO: 5 linked to a transcription termination sequence, a vector comprising a promoter operably linked to an influenza virus PB 1 DNA for a PB1 having substantially the same amino acid sequence as SEQ ID NO: 3 linked to a transcription termination sequence, a vector comprising a promoter operably linked to an influenza virus PB2 DNA for a PB2 having substantially the same amino acid sequence as SEQ ID NO: 4 linked to a transcription termination sequence, a vector comprising a promoter operably linked to an influenza virus HA DNA for a HA having substantially the same amino acid sequence as SEQ ID NO: 1 linked to a transcription termination sequence, a vector comprising a promoter operably linked to an influenza virus NP DNA for a NP having substantially the same amino acid sequence as SEQ ID NO: 6 linked to a transcription termination sequence, a vector comprising a promoter operably linked to an influenza virus NA DNA for a NA having substantially the same amino acid sequence as SEQ ID NO: 2 linked to a transcription termination sequence, a vector comprising a promoter operably linked to an influenza virus M DNA for a M having substantially the same amino acid sequence as SEQ ID NO: 7 (M1) and/or SEQ ID NO: 17 (M2), linked to a transcription termination sequence, and/or a vector comprising a promoter operably linked to an influenza virus NS DNA for a NS having substantially the same amino acid sequence as SEQ ID NO: 8 (NS1) and/or SEQ ID NO: 18 (NS2), linked to a transcription termination sequence. Optionally, two vectors may be employed in place of the vector comprising a promoter operably linked to an influenza virus M DNA linked to a transcription termination sequence, e.g., a vector comprising a promoter operably linked to an

influenza virus M1 DNA linked to a transcription termination sequence and a vector comprising a promoter operably linked to an influenza virus M2 DNA linked to a transcription termination sequence. Optionally, two vectors may be employed in place of the vector comprising a promoter operably linked to an influenza virus NS DNA linked to a transcription termination sequence, e.g., a vector comprising a promoter operably linked to an influenza virus NS1 DNA linked to a transcription termination sequence and a vector comprising a promoter operably linked to an influenza virus NS2 DNA linked to a transcription termination sequence. An influenza virus vector is one which includes at least 5' and 3' noncoding influenza virus sequences.

Hence, the invention provides vectors, e.g., plasmids, which encode influenza virus proteins, and/or encode influenza vRNA, both native and recombinant vRNA. Thus, a vector of the invention may encode an influenza virus protein (sense) or vRNA (antisense). Any suitable promoter or transcription termination sequence may be employed to express a protein or peptide, e.g., a viral protein or peptide, a protein or peptide of a nonviral pathogen, or a therapeutic protein or peptide. In one embodiment, to express vRNA, the promoter is a RNA polymerase I promoter, a RNA polymerase II promoter, a RNA polymerase III promoter, a T3 promoter or a T7 promoter. Optionally the vector comprises a transcription termination sequence such as a RNA polymerase I transcription termination sequence, a RNA polymerase II transcription termination sequence, a RNA polymerase III transcription termination sequence, or a ribozyme.

A composition of the invention may also comprise a gene or open reading frame of interest, e.g., a foreign gene encoding an immunogenic peptide or protein useful as a vaccine. Thus, another embodiment of the invention comprises a composition of the invention as described above in which one of the influenza virus genes in the vectors is replaced with a foreign gene, or the composition further comprises, in addition to all the influenza virus genes, a vector comprising a promoter linked to 5' influenza virus sequences linked to a desired nucleic acid sequence, e.g., a cDNA of interest, linked to 3' influenza virus sequences linked to a transcription termination sequence, which, when contacted with a host cell permissive for influenza virus replication optionally results in recombinant virus. In one embodiment, the DNA of interest is in an antisense orientation. The DNA of interest, whether in a vector for vRNA or protein production, may encode an immunogenic epitope, such as an epitope useful in a cancer therapy or vaccine, or a peptide or polypeptide useful in gene therapy.

A plurality of the vectors of the invention may be physically linked or each vector may be present on an individual plasmid or other, e.g., linear, nucleic acid delivery vehicle.

The invention also provides a method to prepare influenza virus. The method comprises contacting a cell, e.g., an avian or a mammalian cell, with the isolated virus of the invention or a plurality of the vectors of the invention, e.g., sequentially or simultaneously, for example, employing a composition comprising a plurality of the vectors, in an amount effective to yield infectious influenza virus. The invention also includes isolating virus from a cell infected with the virus or contacted with the vectors and/or composition. The invention further provides a host cell infected with the virus of the invention or contacted with the composition or vectors of the invention. In one embodiment, a host cell is infected with an attenuated (e.g., cold adapted) donor virus and a virus of the invention to prepare a cold-adapted reassortant virus useful as a cold-adapted live virus vaccine.

The invention also provides a method to induce an immune response in a mammal, e.g., to immunize a mammal, against

one more pathogens, e.g., against a virus of the invention and optionally a bacteria, a different virus, or a parasite or other antigen. An immunological response to a composition or vaccine is the development in the host organism of a cellular and/or antibody-mediated immune response to a viral polypeptide, e.g., an administered viral preparation, polypeptide or one encoded by an administered nucleic acid molecule, which can prevent or inhibit infection to that virus or a closely (structurally) related virus. Usually, such a response consists of the subject producing antibodies, B cell, helper T cells, suppressor T cells, and/or cytotoxic T cells directed specifically to an antigen or antigens included in the composition or vaccine of interest. The method includes administering to the host organism, e.g., a mammal, an effective amount of the influenza virus of the invention, e.g., an attenuated, live virus, optionally in combination with an adjuvant and/or a carrier, e.g., in an amount effective to prevent or ameliorate infection of an animal such as a mammal by that virus or an antigenically closely related virus. In one embodiment, the virus is administered intramuscularly while in another embodiment, the virus is administered intranasally. In some dosing protocols, all doses may be administered intramuscularly or intranasally, while in others a combination of intramuscular and intranasal administration is employed. The vaccine may further contain other isolates of influenza virus including recombinant influenza virus, other pathogen(s), additional biological agents or microbial components, e.g., to form a multivalent vaccine. In one embodiment, intranasal vaccination with inactivated equine influenza virus and a mucosal adjuvant, e.g., the non-toxic B chain of cholera toxin, may induce virus-specific IgA and neutralizing antibody in the nasopharynx as well as serum IgG.

The equine influenza vaccine may employed with other anti-virals, e.g., amantadine, rimantadine, and/or neuraminidase inhibitors, e.g., may be administered separately in conjunction with those anti-virals, for instance, administered before, during and/or after.

Further provided is a diagnostic method which employs a virus of the invention, an isolated viral protein encoded thereby, or antisera specific for the virus or protein, to detect viral specific antibodies or viral specific proteins.

BRIEF DESCRIPTION OF THE FIGURES

FIG. 1A-R. Sequences of A/Equine/Wisconsin/1/03. SEQ ID NOs:1-8, 17 and 18 represent the deduced amino acid sequence for HA, NA, PB1, PB2, PA, NP, M1, NS1, M2, and NS2, respectively, of A/Equine/Wisconsin/1/03. SEQ ID NOs:9-16 represent the mRNA sense nucleotide sequence for HA, NA, PB1, PB2, PA, NP, M (M1 and M2) and NS (NS1 and NS2), respectively, of A/Equine/Wisconsin/1/03.

FIG. 2. Sequence alignment of HA-1 of A/Equine/NewYork/99 (SEQ ID NO:19) and A/Equine/Wisconsin/1/03 (SEQ ID NO:20).

DETAILED DESCRIPTION OF THE INVENTION

Definitions

As used herein, the term “isolated” refers to in vitro preparation and/or isolation of a nucleic acid molecule, e.g., vector or plasmid, peptide or polypeptide (protein), or virus of the invention, so that it is not associated with in vivo substances, or is substantially purified from in vitro substances. An isolated virus preparation is generally obtained by in vitro culture and propagation, and is substantially free from other infectious agents.

As used herein, “substantially purified” means the object species is the predominant species, e.g., on a molar basis it is more abundant than any other individual species in a composition, and preferably is at least about 80% of the species present, and optionally 90% or greater, e.g., 95%, 98%, 99% or more, of the species present in the composition.

As used herein, “substantially free” means below the level of detection for a particular infectious agent using standard detection methods for that agent.

A “recombinant” virus is one which has been manipulated in vitro, e.g., using recombinant DNA techniques, to introduce changes to the viral genome.

As used herein, the term “recombinant nucleic acid” or “recombinant DNA sequence or segment” refers to a nucleic acid, e.g., to DNA, that has been derived or isolated from a source, that may be subsequently chemically altered in vitro, so that its sequence is not naturally occurring, or corresponds to naturally occurring sequences that are not positioned as they would be positioned in the native genome. An example of DNA “derived” from a source, would be a DNA sequence that is identified as a useful fragment, and which is then chemically synthesized in essentially pure form. An example of such DNA “isolated” from a source would be a useful DNA sequence that is excised or removed from said source by chemical means, e.g., by the use of restriction endonucleases, so that it can be further manipulated, e.g., amplified, for use in the invention, by the methodology of genetic engineering.

Influenza Virus Type A Structure and Propagation

Influenza A viruses possess a genome of eight single-stranded negative-sense viral RNAs (vRNAs) that encode at least ten proteins. The influenza virus life cycle begins with binding of the hemagglutinin (HA) to sialic acid-containing receptors on the surface of the host cell, followed by receptor-mediated endocytosis. The low pH in late endosomes triggers a conformational shift in the HA, thereby exposing the N-terminus of the HA2 subunit (the so-called fusion peptide). The fusion peptide initiates the fusion of the viral and endosomal membrane, and the matrix protein (M1) and RNP complexes are released into the cytoplasm. RNP complexes consist of the nucleoprotein (NP), which encapsidates vRNA, and the viral polymerase complex, which is formed by the PA, PB1, and PB2 proteins. RNP complexes are transported into the nucleus, where transcription and replication take place. The RNA polymerase complex catalyzes three different reactions: synthesis of an mRNA with a 5' cap and 3' polyA structure, of a full-length complementary RNA (cRNA), and of genomic vRNA using the cRNA as a template. Newly synthesized vRNAs, NP, and polymerase proteins are then assembled into RNPs, exported from the nucleus, and transported to the plasma membrane, where budding of progeny virus particles occurs. The neuraminidase (NA) protein plays a crucial role late in infection by removing sialic acid from sialyloligosaccharides, thus releasing newly assembled virions from the cell surface and preventing the self aggregation of virus particles. Although virus assembly involves protein-protein and protein-vRNA interactions, the nature of these interactions is largely unknown.

Any cell, e.g., any avian or mammalian cell, such as a human, canine, bovine, equine, feline, swine, ovine, mink, e.g., MvLu1 cells, or non-human primate cell, including mutant cells, which supports efficient replication of influenza virus can be employed to isolate and/or propagate influenza viruses. Isolated viruses can be used to prepare a reassortant virus, e.g., an attenuated virus. In one embodiment, host cells for vaccine production are those found in avian eggs. In another embodiment, host cells for vaccine production are

continuous mammalian or avian cell lines or cell strains. It is preferred to establish a complete characterization of the cells to be used, so that appropriate tests for purity of the final product can be included. Data that can be used for the characterization of a cell includes (a) information on its origin, derivation, and passage history; (b) information on its growth and morphological characteristics; (c) results of tests of adventitious agents; (d) distinguishing features, such as biochemical, immunological, and cytogenetic patterns which allow the cells to be clearly recognized among other cell lines; and (e) results of tests for tumorigenicity. Preferably, the passage level, or population doubling, of the host cell used is as low as possible.

It is preferred that the virus produced by the host cell is highly purified prior to vaccine or gene therapy formulation. Generally, the purification procedures result in the extensive removal of cellular DNA, other cellular components, and adventitious agents. Procedures that extensively degrade or denature DNA can also be used.

Equine Influenza Virus Detection

Disease causing equine influenza viruses are generally Type A influenza viruses of the H7N7 (equi-1) and H3N8 (equi-2) subtypes. These generally differ from the subtypes that cause infection in man (H1N1, H2N2 and H3N2). Equine influenza is contracted by either inhalation or contact with secretions (e.g., physiological fluid) containing live virus. The virus infects the epithelial cells of the upper and lower airways and can cause deciliation of large areas of the respiratory tract within 4 to 6 days. As a result, the mucociliary clearance mechanism is compromised and tracheal clearance rates may be reduced for up to 32 days following infection. Bronchitis and bronchiolitis develop followed by interstitial pneumonia accompanied by congestion, edema and leukocyte infiltration. In general, H3N8 viruses cause more severe disease than H7N7 viruses; viruses of the H3N8 subtype are more pneumotropic and have also been associated with myocarditis.

Clinical signs in previously influenza-naïve animals are easily recognizable. Influenza is characterized by its sudden onset with an incubation period of 1 to 3 days. The first sign is an elevation of body temperature (up to 41° C.), which is usually biphasic. This is followed by a deep dry cough that releases large quantities of virus into the atmosphere often accompanied by a serous nasal discharge, which may become mucopurulent due to secondary bacterial infection. The other most commonly observed clinical signs are myalgia, inappetence, and enlarged submandibular lymph nodes. Edema of the legs and scrotum is observed very rarely. The severity of the disease varies with the dose and strain of virus and the immune status of the horse.

Previously healthy, immunocompetent adult horses usually recover from uncomplicated influenza within 10 days, although coughing may persist for longer. If secondary bacterial infection occurs, it can prolong the recovery period. However, relatively high mortality rates have been recorded in foals, animals in poor condition and donkeys. If maternal antibody is absent at the time of exposure, young foals may develop a viral pneumonia leading to death. Deaths among adult animals are usually a consequence of secondary bacterial infection leading to pleuritis, suppurative pneumonia or rarely, purpura haemorrhagica. Sequelae of equine influenza can include chronic pharyngitis, chronic bronchiolitis, myocarditis, and alveolar emphysema, which can contribute to heaves, and secondary sinus and guttural pouch infections.

Clinical signs in animals partially immune as a result of vaccination or previous infection are more difficult to recog-

nize as there may be little or no coughing or pyrexia. Whereas spread of infection throughout a group of naïve animals is always rapid, there have been outbreaks in which the infection circulated subclinically in vaccinated horses for 18 days before inducing recognizable clinical signs.

Outbreaks of infectious respiratory disease may be caused by various agents, including equine herpes viruses, rhinoviruses, adenoviruses, and arteritis viruses, *Streptococcus equi*, or *S. zooepidemicus*. A presumptive diagnosis of influenza based on clinical signs should be confirmed by virus isolation or detection, or by serological testing. Laboratory confirmation of a clinical diagnosis may be by traditional isolation of virus from nasopharyngeal swabs or serology to demonstrate seroconversion, or by rapid diagnostic tests which detect the presence of viral antigens, viral nucleic acid, or virally infected cells in respiratory secretions. Rapid diagnostic tests, despite their convenience and ease of use, provide little or no information about genetic or antigenic characteristics of the infecting strain of virus and do not allow isolation of the virus.

Nasopharyngeal swabs for virus isolation or detection should be taken as promptly as possible. Results of experimental challenge studies suggest that peak viral titers are obtained during the initial 24 to 48 hours of fever, on the second or third day after infection, and the duration of viral shedding is usually not more than 4 or 5 days. Nasal swab samples are taken by passing a swab as far as possible into the horse's nasopharynx via the ventral meatus to absorb respiratory secretions. Swabs should be transferred immediately to a container with virus transport medium and transported on ice to maintain viability of the virus. Virus is unlikely to survive if dry swabs are taken and there is an increased chance of contamination if bacterial transport medium is used. Nasal swab samples may be inoculated into the allantoic (or amniotic) cavity of 9- to 11-day-old embryonated hens' eggs. After incubation at 33-35° C. for 3 days, the allantoic fluid is harvested and tested for haemagglutinating activity. Alternatively, cell culture may be used to isolate viruses. Influenza infection can also be diagnosed by comparison of the results of serological testing of an acute serum sample taken as soon as possible after the onset of clinical signs and a convalescent serum sample taken 2 to 4 weeks later.

The haemagglutination inhibition (HI) test measures the capacity of influenza-specific antibody present in serum samples to inhibit the agglutination of red blood cells by virus. Sera are heat-inactivated and pre-treated to reduce non-specific reactions and serially diluted prior to incubation with a standard dose of virus in a U-bottomed microtiter plate. A suspension of red blood cells is added and, after a further incubation period, examined for agglutination. A four-fold rise in virus-specific antibodies indicates infection. Whole virus antigen may be used for H7N7 viruses, but Tween 80-ether disrupted antigen is usually required to enhance the sensitivity of the assay for H3N8 viruses. In repeatedly vaccinated horses, infection may fail to stimulate a 4-fold increase in HI titer.

The single-radial haemolysis (SRH) test, although less strain-specific, is more reproducible and less error prone than the HI test and, as it is a linear test, is more sensitive, enabling detection of smaller increases in antibody induced by infection in heavily vaccinated horses. The SRH test is based on the ability of influenza-specific antibodies to lyse virus-coated red blood cells in the presence of complement. Test sera are added to wells punched in agarose containing coated red blood cells and complement and allowed to diffuse through the agarose for 20 hours. The areas of clear zones of haemolysis around the wells are proportional to the level of influenza antibody present in the serum samples.

If horses are vaccinated in the face of infection, it may not be possible, using the HI and SRH assays, to determine whether any increase in antibody levels is due to vaccination or infection.

Influenza Vaccines

A vaccine of the invention includes an isolated influenza virus of the invention, and optionally one or more other isolated viruses including other isolated influenza viruses, West Nile virus, equine herpes virus, equine arteritis virus, equine infectious anemia lentivirus, rabies virus, Eastern and/or Western and/or Venezuelan equine encephalitis virus, one or more immunogenic proteins or glycoproteins of one or more isolated influenza viruses or one or more other pathogens, e.g., an immunogenic protein from one or more bacteria, non-influenza viruses, yeast or fungi, or isolated nucleic acid encoding one or more viral proteins (e.g., DNA vaccines) including one or more immunogenic proteins of the isolated influenza virus of the invention. In one embodiment, the influenza viruses of the invention may be vaccine vectors for influenza virus or other pathogens.

A complete virion vaccine may be concentrated by ultrafiltration and then purified by zonal centrifugation or by chromatography. It is inactivated before or after purification using formalin or beta-propiolactone, for instance.

A subunit vaccine comprises purified glycoproteins. Such a vaccine may be prepared as follows: using viral suspensions fragmented by treatment with detergent, the surface antigens are purified, by ultracentrifugation for example. The subunit vaccines thus contain mainly HA protein, and also NA. The detergent used may be cationic detergent for example, such as hexadecyl trimethyl ammonium bromide (Bachmeyer, 1975), an anionic detergent such as ammonium deoxycholate (Layer & Webster, 1976); or a nonionic detergent such as that commercialized under the name TRITON X100. The hemagglutinin may also be isolated after treatment of the virions with a protease such as bromelin, then purified by a method such as that described by Grand and Skehel (1972).

A split vaccine comprises virions which have been subjected to treatment with agents that dissolve lipids. A split vaccine can be prepared as follows: an aqueous suspension of the purified virus obtained as above, inactivated or not, is treated, under stirring, by lipid solvents such as ethyl ether or chloroform, associated with detergents. The dissolution of the viral envelope lipids results in fragmentation of the viral particles. The aqueous phase is recuperated containing the split vaccine, constituted mainly of hemagglutinin and neuraminidase with their original lipid environment removed, and the core or its degradation products. Then the residual infectious particles are inactivated if this has not already been done.

Inactivated Vaccines. Inactivated influenza virus vaccines are provided by inactivating replicated virus using known methods, such as, but not limited to, formalin or β -propiolactone treatment. Inactivated vaccine types that can be used in the invention can include whole-virus (WV) vaccines or subvirion (SV) (split) vaccines. The WV vaccine contains intact, inactivated virus, while the SV vaccine contains purified virus disrupted with detergents that solubilize the lipid-containing viral envelope, followed by chemical inactivation of residual virus.

In addition, vaccines that can be used include those containing the isolated HA and NA surface proteins, which are referred to as surface antigen or subunit vaccines.

Live Attenuated Virus Vaccines. Live, attenuated influenza virus vaccines can be used for preventing or treating influenza virus infection. Attenuation may be achieved in a single step

by transfer of attenuated genes from an attenuated donor virus to a replicated isolate or reassorted virus according to known methods (see, e.g., Murphy, 1993). Since resistance to influenza A virus is mediated primarily by the development of an immune response to the HA and/or NA glycoproteins, the genes coding for these surface antigens must come from the reassorted viruses or clinical isolates. The attenuated genes are derived from the attenuated parent. In this approach, genes that confer attenuation preferably do not code for the HA and NA glycoproteins.

Viruses (donor influenza viruses) are available that are capable of reproducibly attenuating influenza viruses, e.g., a cold adapted (ca) donor virus can be used for attenuated vaccine production. Live, attenuated reassortant virus vaccines can be generated by mating the ca donor virus with a virulent replicated virus. Reassortant progeny are then selected at 25° C., (restrictive for replication of virulent virus), in the presence of an appropriate antiserum, which inhibits replication of the viruses bearing the surface antigens of the attenuated ca donor virus. Useful reassortants are: (a) infectious, (b) attenuated for seronegative non-adult mammals and immunologically primed adult mammals, (c) immunogenic and (d) genetically stable. The immunogenicity of the ca reassortants parallels their level of replication. Thus, the acquisition of the six transferable genes of the ca donor virus by new wild-type viruses has reproducibly attenuated these viruses for use in vaccinating susceptible mammals both adults and non-adult.

Other attenuating mutations can be introduced into influenza virus genes by site-directed mutagenesis to rescue infectious viruses bearing these mutant genes. Attenuating mutations can be introduced into non-coding regions of the genome, as well as into coding regions. Such attenuating mutations can also be introduced into genes other than the HA or NA, e.g., the PB2 polymerase gene (Subbarao et al., 1993). Thus, new donor viruses can also be generated bearing attenuating mutations introduced by site-directed mutagenesis, and such new donor viruses can be used in the production of live attenuated reassortants vaccine candidates in a manner analogous to that described above for the ca donor virus. Similarly, other known and suitable attenuated donor strains can be reassorted with influenza virus to obtain attenuated vaccines suitable for use in the vaccination of mammals (Enami et al., 1990; Muster et al., 1991; Subbarao et al., 1993).

It is preferred that such attenuated viruses maintain the genes from the virus that encode antigenic determinants substantially similar to those of the original clinical isolates. This is because the purpose of the attenuated vaccine is to provide substantially the same antigenicity as the original clinical isolate of the virus, while at the same time lacking pathogenicity to the degree that the vaccine causes minimal chance of inducing a serious disease condition in the vaccinated mammal.

The virus can thus be attenuated or inactivated, formulated and administered, according to known methods, as a vaccine to induce an immune response in an animal, e.g., a mammal. Methods are well-known in the art for determining whether such attenuated or inactivated vaccines have maintained similar antigenicity to that of the clinical isolate or high growth strain derived therefrom. Such known methods include the use of antisera or antibodies to eliminate viruses expressing antigenic determinants of the donor virus; chemical selection (e.g., amantadine or rimantidine); HA and NA activity and inhibition; and nucleic acid screening (such as probe hybridization or PCR) to confirm that donor genes encoding the antigenic determinants (e.g., HA or NA genes) are not present

in the attenuated viruses. See, e.g., Robertson et al., 1988; Kilbourne, 1969; Aymard-Henry et al., 1985; Robertson et al., 1992.

Pharmaceutical Compositions

Pharmaceutical compositions of the present invention, suitable for inoculation, e.g., nasal, parenteral or oral administration, comprise one or more influenza virus isolates, e.g., one or more attenuated or inactivated influenza viruses, a subunit thereof, isolated protein(s) thereof, and/or isolated nucleic acid encoding one or more proteins thereof, optionally further comprising sterile aqueous or non-aqueous solutions, suspensions, and emulsions. The compositions can further comprise auxiliary agents or excipients, as known in the art. See, e.g., Berkow et al., 1987; *Avery's Drug Treatment*, 1987; Osol, 1980. The composition of the invention is generally presented in the form of individual doses (unit doses).

Conventional vaccines generally contain about 0.1 to 200 µg, e.g., 30 to 100 µg, of HA from each of the strains entering into their composition. The vaccine forming the main constituent of the vaccine composition of the invention may comprise a single influenza virus, or a combination of influenza viruses, for example, at least two or three influenza viruses, including one or more reassortant(s).

Preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and/or emulsions, which may contain auxiliary agents or excipients known in the art. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Carriers or occlusive dressings can be used to increase skin permeability and enhance antigen absorpion. Liquid dosage forms for oral administration may generally comprise a liposome solution containing the liquid dosage form. Suitable forms for suspending liposomes include emulsions, suspensions, solutions, syrups, and elixirs containing inert diluents commonly used in the art, such as purified water. Besides the inert diluents, such compositions can also include adjuvants, wetting agents, emulsifying and suspending agents, or sweetening, flavoring, or perfuming agents. See, e.g., Berkow et al., 1992; *Avery's*, 1987; and Osol, 1980.

When a composition of the present invention is used for administration to an individual, it can further comprise salts, buffers, adjuvants, or other substances which are desirable for improving the efficacy of the composition. For vaccines, adjuvants, substances which can augment a specific immune response, can be used. Normally, the adjuvant and the composition are mixed prior to presentation to the immune system, or presented separately, but into the same site of the organism being immunized. Examples of materials suitable for use in vaccine compositions are provided in Osol (1980).

Heterogeneity in a vaccine may be provided by mixing replicated influenza viruses for at least two influenza virus strains, such as 2-20 strains or any range or value therein. Influenza A virus strains having a modern antigenic composition are preferred. Vaccines can be provided for variations in a single strain of an influenza virus, using techniques known in the art.

A pharmaceutical composition according to the present invention may further or additionally comprise at least one chemotherapeutic compound, for example, for gene therapy, immunosuppressants, anti-inflammatory agents or immune enhancers, and for vaccines, chemotherapeutics including, but not limited to, gamma globulin, amantadine, guanidine, hydroxybenzimidazole, interferon-α, interferon-β, interferon-γ, tumor necrosis factor-alpha, thiosemicarbazones, methisazone, rifampin, ribavirin, a pyrimidine analog, a

purine analog, foscarnet, phosphonoacetic acid, acyclovir, dideoxynucleosides, a protease inhibitor, or ganciclovir.

The composition can also contain variable but small quantities of endotoxin-free formaldehyde, and preservatives, which have been found safe and not contributing to undesirable effects in the organism to which the composition is administered.

Pharmaceutical Purposes

The administration of the composition (or the antisera that it elicits) may be for either a "prophylactic" or "therapeutic" purpose. When provided prophylactically, the compositions of the invention which are vaccines are provided before any symptom or clinical sign of a pathogen infection becomes manifest. The prophylactic administration of the composition serves to prevent or attenuate any subsequent infection. When provided prophylactically, the gene therapy compositions of the invention, are provided before any symptom or clinical sign of a disease becomes manifest. The prophylactic administration of the composition serves to prevent or attenuate one or more symptoms or clinical signs associated with the disease.

When provided therapeutically, an attenuated or inactivated viral vaccine is provided upon the detection of a symptom or clinical sign of actual infection. The therapeutic administration of the compound(s) serves to attenuate any actual infection. See, e.g., Berkow et al., 1992; and *Avery*, 1987. When provided therapeutically, a gene therapy composition is provided upon the detection of a symptom or clinical sign of the disease. The therapeutic administration of the compound(s) serves to attenuate a symptom or clinical sign of that disease.

Thus, an attenuated or inactivated vaccine composition of the present invention may be provided either before the onset of infection (so as to prevent or attenuate an anticipated infection) or after the initiation of an actual infection. Similarly, for gene therapy, the composition may be provided before any symptom or clinical sign of a disorder or disease is manifested or after one or more symptoms are detected.

A composition is said to be "pharmacologically acceptable" if its administration can be tolerated by a recipient mammal. Such an agent is said to be administered in a "therapeutically effective amount" if the amount administered is physiologically significant. A composition of the present invention is physiologically significant if its presence results in a detectable change in the physiology of a recipient patient, e.g., enhances at least one primary or secondary humoral or cellular immune response against at least one strain of an infectious influenza virus.

The "protection" provided need not be absolute, i.e., the influenza infection need not be totally prevented or eradicated, if there is a statistically significant improvement compared with a control population or set of mammals. Protection may be limited to mitigating the severity or rapidity of onset of symptoms or clinical signs of the influenza virus infection.

Pharmaceutical Administration

A composition of the present invention may confer resistance to one or more pathogens, e.g., one or more influenza virus strains, by either passive immunization or active immunization. In active immunization, an inactivated or attenuated live vaccine composition is administered prophylactically to a host (e.g., a mammal), and the host's immune response to the administration protects against infection and/or disease. For passive immunization, the elicited antisera can be recovered and administered to a recipient suspected of having an infection caused by at least one influenza virus strain. A gene

therapy composition of the present invention may yield prophylactic or therapeutic levels of the desired gene product by active immunization.

In one embodiment, the vaccine is provided to a mammalian female (at or prior to pregnancy or parturition), under conditions of time and amount sufficient to cause the production of an immune response which serves to protect both the female and the fetus or newborn (via passive incorporation of the antibodies across the placenta or in the mother's milk).

The present invention thus includes methods for preventing or attenuating a disorder or disease, e.g., an infection by at least one strain of pathogen. As used herein, a vaccine is said to prevent or attenuate a disease if its administration results either in the total or partial attenuation (i.e., suppression) of a clinical sign or condition of the disease, or in the total or partial immunity of the individual to the disease. As used herein, a gene therapy composition is said to prevent or attenuate a disease if its administration results either in the total or partial attenuation (i.e., suppression) of a clinical sign or condition of the disease, or in the total or partial immunity of the individual to the disease.

At least one influenza virus isolate of the present invention, including one which is inactivated or attenuated, one or more isolated viral proteins thereof, one or more isolated nucleic acid molecules encoding one or more viral proteins thereof, or a combination thereof, may be administered by any means that achieve the intended purposes.

For example, administration of such a composition may be by various parenteral routes such as subcutaneous, intravenous, intradermal, intramuscular, intraperitoneal, intranasal, oral or transdermal routes. Parenteral administration can be accomplished by bolus injection or by gradual perfusion over time.

A typical regimen for preventing, suppressing, or treating an influenza virus related pathology, comprises administration of an effective amount of a vaccine composition as described herein, administered as a single treatment, or repeated as enhancing or booster dosages, over a period up to and including between one week and about 24 months, or any range or value therein.

According to the present invention, an "effective amount" of a composition is one that is sufficient to achieve a desired effect. It is understood that the effective dosage may be dependent upon the species, age, sex, health, and weight of the recipient, kind of concurrent treatment, if any, frequency of treatment, and the nature of the effect wanted. The ranges of effective doses provided below are not intended to limit the invention and represent dose ranges.

The dosage of a live, attenuated or killed virus vaccine for an animal such as a mammalian adult organism can be from about 10²-10¹⁵, e.g., 10³-10¹², plaque forming units (PFU)/kg, or any range or value therein. The dose of inactivated vaccine can range from about 0.1 to 1000, e.g., 30 to 100 µg, of HA protein. However, the dosage should be a safe and effective amount as determined by conventional methods, using existing vaccines as a starting point.

The dosage of immunoreactive HA in each dose of replicated virus vaccine can be standardized to contain a suitable

amount, e.g., 30 to 100 µg or any range or value therein, or the amount recommended by government agencies or recognized professional organizations. The quantity of NA can also be standardized, however, this glycoprotein may be labile during purification and storage.

Compositions and Dosing for Equine Influenza Vaccines

Equine influenza vaccines generally include representative strains of H7N7 and H3N8 subtypes either as inactivated whole virus or their subunits. They provide protection against influenza by inducing antibody to the surface glycoproteins, in particular to HA, which is essential for viral attachment and entry into cells, and/or potentially important cell-mediated immune responses to other viral proteins. Vaccination is helpful in preventing influenza but the protection is short-lived (3-4 months using conventional inactivated virus vaccines), so the frequency of vaccination varies according to how often the horse will likely come in contact with the virus (see Table 1). The usual procedure for the primary course is vaccination with a single dose followed 3 to 6 weeks later with a second dose. Vaccine manufacturers recommend that booster vaccinations be given at 6- to 12-month intervals thereafter. Alternatively, a horse is administered one 1 to 2 ml dose, e.g., via intramuscular (IM) injection, a second 1 to 2 ml dose 3 to 4 weeks later at a different injection site, e.g., via IM injection, and optionally a third 1 to 2 ml dose, e.g., IM or intranasal (IN) administration. Each 1 to 2 ml dose of vaccine may contain approximately 1-500 billion virus particles, and preferably 100 billion particles. Horses in contact with a large number of horses, for example, at a boarding stable, training centers, racetracks, shows, and other such events, are often vaccinated every 2-3 months. A three-dose primary series has been shown to induce a higher and more persistent immunity than the recommended two-dose series regardless of the age.

Using conventional vaccines, it is advisable to vaccinate young horses, particularly racehorses and other competition horses, at 4 to 6 month intervals for several years after their primary course of vaccinations. It has been demonstrated that inclusion of an additional booster vaccination between the second and third vaccination recommended by the vaccine manufacturers is of benefit to young horses. An annual booster will usually suffice for older horses such as show jumpers and brood mares that have been vaccinated regularly since they were foals. Vaccination in the face of an ongoing outbreak is sometimes practiced, but is not likely to be effective without an interval of at least 7 to 10 days before the freshly vaccinated horses are exposed to infection. Equine influenza outbreaks are not seasonal as in man but are frequently associated with sales or race meets where horses from different regions congregate and mix. It may therefore be advantageous to time additional booster vaccinations to be given prior to such events.

Brood mares should be vaccinated in the later stages of pregnancy, but not later than 2 weeks prior to foaling, to ensure a good supply of colostral antibodies for the foal. Foal vaccinations should begin at 3-6 months of age, with a booster at 4-7 months, again at 5-8 months, and repeated every three months if the foal is at high risk of exposure.

TABLE 1

	Foals & Weanlings from Vaccinated Mares	Foal & Weanlings from non-Vaccinated Mares	Yearlings	Performance Horse	Pleasure Horses	Brood-mares
Influenza inactivated injectable	1st Dose: 9 months 2nd Dose: 10 months 3rd Dose: 11-12 months	1st Dose: 6 months 2nd Dose: 7 months 3rd Dose: 8 months	Every 3-4 months	Every 3-5 months	Annual with Boosters prior to	At least semi-annual, with 1 Booster

TABLE 1-continued

	Foals & Weanlings from Vaccinated Mares	Foal & Weanlings from non-Vaccinated Mares	Yearlings	Performance Horse	Pleasure Horses	Brood- mares
Influenza intranasal cold-adapted live virus	Then at 3 month intervals 1st Dose: 12 months; has been safely administered to foals less than 11 months	Then at 3 month intervals 1st Dose: 12 months; has been safely administered to foals less than 11 months	Every 4-6 months	Every 4-6 months	likely exposure Every 4-6 months	4-6 weeks prepartum Annual before breeding

Influenza vaccines may be combined with tetanus or herpesvirus antigens as well as other pathogens, e.g., equine pathogens. The immune response elicited by tetanus toxoid is much more durable than that induced by influenza antigen. In an intensive influenza vaccination program, vaccines containing influenza only are thus preferred.

Levels of antibody (measured by the SRH assay) required for protection of horses have been identified through vaccination and challenge studies and from field data. Because the vaccine-induced antibody response to HA in horses is remarkably short-lived, adjuvants such as aluminum hydroxide or carboxylate are normally included to enhance the amplitude and duration of the immune response to whole virus vaccines. Subunit equine influenza vaccines containing immune stimulating complexes (ISCOMs) are also immunogenic.

Historically, antigenic content in inactivated vaccines has been expressed in terms of chick cell agglutinating (CCA) units of HA and potency in terms of HI antibody responses induced in guinea pigs and horses, neither of which yields reproducible results. The single radial diffusion (SRD) assay is an improved in vitro potency test that measures the concentration of immunologically active HA (expressed in terms of micrograms of HA) and can be used for in-process testing before the addition of adjuvant.

The invention will be further described by the following non-limiting example.

EXAMPLE

An approximately 36-hour-old Morgan/Friesian colt was referred to the large animal hospital at the University of Wisconsin for an evaluation of altered mentation (mental status), first noticed shortly after birth. Parturition had been unobserved, but the foal had been found separated from the mare by a fence at a few hours of age. The foal was ambulatory and able to nurse when first discovered but showed progressive disorientation, apparent blindness, and aimless wandering during the following 36-hour period. A SNAP immunoglobulin G (IgG) assay (Idexx Laboratories, Westbrook, Me.) at 24 hours of age had shown an IgG concentration >800 mg/dL, and a CBC performed at that time was normal. The foal was treated twice with dimethyl sulfoxide 1 g/kg IV, diluted in 5% dextrose before referral.

At presentation, the colt wandered aimlessly, bumped into objects, and appeared blind with sluggish but intact pupillary light responses. When positioned under the mare, the foal nursed successfully. Physical examination was unremarkable. A CBC and serum biochemistry were normal, including a serum IgG concentration of 937 mg/dL measured by radioimmunodiffusion.

Initial treatment for presumptive hypoxemic, ischemic encephalopathy included a 250 mL loading dose of 20% magnesium sulfate for 1 hour, followed by a constant rate

infusion at 42 mL/h and thiamine hydrochloride 2.2 mg/kg IV q24h. Antimicrobial therapy consisted of amikacin 20 mg/kg IV q24h and procaine penicillin G 22,000 U/kg IM q12h. Omeprazole 1 mg/kg PO q24h also was administered to the foal to help prevent the development of gastric ulcers.

The foal's mental status remained static during the next 24 hours, and additional treatment with mannitol 1 g/kg IV q24h and dexamethasone sodium phosphate 0.1 mg/kg IV q24h on days 2 and 3 of hospitalization was not associated with improvement. On day 3, the foal underwent general anesthesia for a computerized tomographic scan of the skull and proximal spine, which was normal. A cerebrospinal fluid sample was obtained from the lumbosacral space and was normal on cytologic evaluation and had a normal protein concentration.

On day 4 of hospitalization, the foal developed a right-sided head tilt but otherwise remained static through day 5 of hospitalization. Magnesium sulfate therapy was discontinued on day 5, but the remainder of the therapeutic regimen was unchanged. On day 6, the foal had 2 brief, generalized seizures that were controlled with midazolam 0.05 mg/kg IV. Between seizures, the foal was still bright, afebrile, and nursing.

On day 7 of hospitalization, the foal became febrile (40° C.) and developed a mucopurulent nasal discharge and progressive tachypnea with diffuse adventitious crackles and wheezes on auscultation. Fever, mucopurulent nasal discharge, and coughing had been noted in several other mares and foals in the neonatal care unit during the previous 7 days. Antimicrobial therapy was changed to ticarcillin/clavulanic acid 50 mg/kg IV q8h had gentamicin 6.6 mg/kg IV q24h, and the foal was treated with polyionic fluids, although it was still nursing. During days 8-10, the foal's neurologic status continued to improve, with a resolution of the head tilt and a return to normal mentation, but the tachypnea, dyspnea, and adventitious lung sounds worsened. Thoracic radiography at this time showed a severe, diffuse bronchointerstitial pattern. Aminophylline 0.5 mg/kg IV q12h by slow infusion and nasal insufflation of oxygen were instituted on days 9 and 10 of hospitalization. Serial arterial blood gas analysis identified severe hypoxemia (PaO₂, 52 mm Hg), hypercapnia (PaCO₂, 68.4 mm Hg), and reduced oxygen saturation (76%) by the end of day 10. Consequently, the foal was placed on a mechanical ventilator. Ventilatory support and total parenteral nutrition were continued for 48 hours, during which time arterial blood gas values normalized on 100% oxygen. Antimicrobial therapy was continued as before. When challenged on day 13 by the removal of ventilatory support, the foal developed severe dyspnea and cyanosis and was euthanized at the owner's request. An aerobic culture of a transtracheal aspirate obtained on day 13 grew *Klebsiella pneumoniae* and *Escherichia coli* resistant to ticarcillin/clavulanic acid and gentamicin.

A complete gross and histopathologic postmortem examination was performed, as well as a real-time quantitative polymerase chain reaction (PCR) evaluation for the presence of equine herpes virus (EHV)-1 and EHV-4 in samples of nasal secretions; serologic tests to determine if there was exposure to equine viral arteritis virus; and a Directigen Flu A assay (Bectin Dickinson and Co., Franklin, N.J.) and virus isolation from samples of nasal secretions to test for the presence of influenza virus. Samples of nasal secretions were collected with Dacron swabs that were subsequently placed in 2 mL of viral transport media containing phosphate-buffered saline, 0.5% bovine serum albumin, and penicillin G, streptomycin, nystatin, and gentamicin. The nasal swab samples were collected on day 8 of hospitalization. Follow-up evaluations for the influenza virus included immunohistochemistry on snap-frozen and formalin-fixed lung, abdominal viscera, and central nervous system tissues for the presence of influenza nucleoprotein (NP) expression, virus isolation from frozen lung tissue, and viral sequence analyses. Gross post-mortem examination identified severe diffuse interstitial pneumonia and subdural hemorrhage on the caudal ventral surface of the brain around the pituitary gland but no evidence of sepsis or pathology in other organs. Histopathologic examination of the lung identified necrotizing bronchitis and bronchiolitis, diffuse squamous metaplasia, and multifocal interstitial pneumonia. A mild mononuclear infiltrate lined the lower airways and, occasionally, areas of alveolar collapse associated with congestion and exudate. Evaluation of the brain tissue revealed a mild dilatation of the ventricular system with diffuse white matter vacuolation, particularly in the cerebellum. Cresyl violet staining for the presence of myelin was performed on multiple sections and showed diminished but present myelin throughout the brain and spinal cord when compared to tissues from an age-matched control stained in parallel. Additional histopathologic abnormalities in the central nervous system included an apparent absence of the molecular layer within the cerebellum. Serologic tests for equine viral arteritis and a real-time PCR assay for EHV-1 and EHV-4 DNA were negative.

The presence of influenza virus in nasal secretions initially was confirmed by a positive Directigen assay. Previous studies have documented the sensitivity and specificity of this assay when applied to equine nasal secretion samples (Morely et al., 1995 and Chambers et al., 1994). Samples of the nasal swab transport media also were inoculated into the allantoic cavity of embryonated chicken eggs and onto Madin-Darby canine kidney (MDCK) cells growing in 24-well cell culture plates. Cytopathologic effects consistent with influenza virus growth were observed in the inoculated MDCK cells, and an agent that caused the hemagglutination of chicken red blood cells was isolated from the inoculated eggs (Palmar et al., 1975). The presence of influenza virus in the MDCK cell cultures was confirmed by the immunocytochemical staining (Landolt et al., 2003) of the inoculated cells with an anti-NP monoclonal antibody (Mab) 68D2 (kindly provided by Dr. Yoshihiro Kawaoka, University of Wisconsin-Madison School of Veterinary Medicine) with positive (swine influenza virus inoculated) and negative (mock inoculated) control cells included on the same plate. The identity of the virus as an H3-subtype equine influenza virus was confirmed by reverse transcription-PCR amplification of the hemagglutinin (HA) gene from the isolate, with primers described in Olsen et al. (1997), followed by cycle sequencing of the full-length protein coding region of the HA gene and pairwise comparisons to viral sequences available in GenBank (DNASTAR software, version 4.0 for Win32, Bestfit, Madison, Wis.). The virus was shown to be derived from

the North American lineage of H3 equine influenza viruses by a phylogenetic analysis that used a maximum parsimony bootstrap analysis (PAUP software, version 4.0b6; David Swofford, Smithsonian Institution, Washington, D.C.) of the HA sequence compared to reference virus strains with a fast-heuristic search of 1,000 bootstrap replicates. Similar analyses of portions of the nucleotide sequences of the nonstructural protein gene (544 nucleotides sequenced) and the NP gene (885 nucleotides sequenced) further confirmed the identity of the virus as a North American-lineage equine influenza virus. This virus is now defined as A/Equine/Wisconsin/1/03. FIG. 1 provides sequences for the coding region of each gene of that virus.

The presence of influenza virus also was assessed in the lungs and other tissues of the foal. Specifically, immunohistochemistry with Mab 68D2 showed scattered, widely dispersed areas of influenza virus NP expression (predominantly localized around airways) in the frozen as well as the formalin-fixed lung tissue samples. NP expression was not shown in the other viscera or in the central nervous system. In addition, influenza virus was isolated in MDCK cells (and confirmed by immunocytochemistry and HA gene sequencing) from a sample of the frozen lung tissue.

Acute respiratory distress syndrome (ARDS) in neonatal foals has been documented as a consequence of bacterial sepsis (Wilkins, 2003; Hoffman et al., 1993), perinatal EHV-1 (Frymus et al., 1986; Gilkerson et al., 1999) and EHV-4 (Gilkerson et al., 1999), and equine viral arteritis infection (Del Piero et al., 1997). Less severe lower airway disease occasionally is documented with adenovirus and EHV-2 infections, particularly in the immunocompromised patient (Webb et al., 1981; Murray et al., 1996). Bronchointerstitial pneumonia and ARDS are high-mortality respiratory diseases of older foals with several potential causes, including bacterial and viral infections (Lakritz et al., 1993). Whether it occurs in neonates experiencing septic shock or in older foals with diffuse bronchointerstitial pneumonia, ARDS is characterized by acute-onset, rapidly progressive, severe tachypnea. The increased respiratory effort, worsening cyanosis, hypoxemia, and hypercapnia that accompany ARDS frequently are poorly responsive to aggressive therapy (Wilkins, 2003; Lakritz et al., 1993). It is a category of respiratory disease with several potential etiologies and a mortality rate that frequently exceeds 30% despite intensive treatment with antimicrobials, oxygen, anti-inflammatory agents, bronchodilators, and thermoregulatory control. Equine influenza is a well-documented cause of upper respiratory disease in horses worldwide (Wilkins, 2003; Van Maanen et al., 2002; Wilson, 1993), but very little information exists in the literature about the manifestations of this disease in neonates. A single report describes bronchointerstitial pneumonia in a 7-day-old foal from which equine influenza A was isolated (Britton et al., 2002); this foal resembles the foal described herein.

The foal detailed in this study was one of several hospitalized horses that developed fever, mucopurulent nasal discharge, and coughing during a 2- or 3-week period. Clinical signs in the other affected horses, including high-risk neonates, generally were confined to the upper respiratory tract, except for mild systemic signs of fever and inappetance. The reason for the severity of the pulmonary failure in this foal is unclear. Treatment did include the potentially immunosuppressive drug dexamethasone and general anesthesia for a diagnostic procedure, both of which may have predisposed the foal to the development of pneumonia. The impact of the foal's neurologic disease on the development and progression of respiratory disease also is unclear. The histologic findings of diffuse vacuolization, decreased myelin throughout the

central nervous system, and absent molecular layer within the cerebellum do not fit any specific clinical or histopathologic diagnosis. The foal could have had impaired central control of respiration, because the areas of the brain involved in the control of respiration (the pons and medulla oblongata) showed diffuse vacuolization and diminished myelin staining. Any subsequent impairment of ventilation would likely have been a terminal event given the normalcy of ventilatory function until several days after hospitalization. However, the abnormal mentation from birth, the vacuolization, the decreased myelinization in the central nervous system, and the cerebellar abnormalities are suggestive of a concurrent, congenital neurologic abnormality, which may have compromised the foal's ability to respond to worsening respiratory function. The focal hemorrhage observed on the caudal ventral aspect of the brain was mild and was possibly a consequence of trauma during one of the seizures the foal experienced.

The mare had been vaccinated semiannually against influenza for the past 2 years with a killed product and was given a booster vaccination in late pregnancy. Considering the evidence of adequate passive transfer in this foal, these antibodies apparently did not confer adequate protection for the foal. Furthermore, phylogenetic analysis of the isolate obtained from the foal characterized it as an H3N8 subtype, and the commercial product used to vaccinate the mare in late pregnancy contained an influenza virus strain of the same subtype, suggesting that passive transfer cannot be guaranteed to protect against natural infection under certain circumstances. This lack of vaccine efficacy is consistent with a recent study by Mumford et al. (2003) that describes the failure of commercially available H7N7 and H3N8 equine influenza virus vaccines to protect adults against clinical respiratory disease that results from a natural infection with certain H3N8 virus strains. The transtracheal recovery of 2 bacterial species that were resistant to the antimicrobial regimen in place at the time of death confounds the conclusion that influenza was the sole cause of death. However, postmortem examination identified no gross or histopathologic evidence of sepsis, and synergism occurs between the influenza virus and some bacterial pathogens, combining to cause pneumonia with increased mortality (McCullers et al., 2003; Simonsen, 1999). Furthermore, the isolation of the infectious virus and the immunohistochemical demonstration of viral antigen from the lung tissue obtained postmortem, 6 days after the virus initially was recovered by a nasopharyngeal swab, provide strong evidence of a pathologic contribution from influenza virus in this foal's respiratory failure.

To compare the growth characteristics of avian, equine, human, and porcine lineage viruses in primary canine respiratory epithelial cells and to investigate the species influence on their growth characteristics, cultured cells were infected at an MOI of 3 with viruses including A/Equine/Wisconsin/1/03 and incubated for up to 10 hours. The other viruses included six human and swine influenza A virus isolates (A/Philippines/08/98, A/Panama/2002/99, A/Costa Rica/07/99; A/Swine/NorthCarolina/44173/00, A/Swine/Minnesota/593/99, A/Swine/Ontario/00130/97, and two equine influenza viruses (A/Equine/Kentucky/81 and A/Equine/Kentucky/91). At the end of the experiment, the cells were formalin fixed for immunocytochemistry and flow cytometry analyses.

The six human and swine influenza virus isolates mentioned above readily infected substantially all (80-90%) of the canine respiratory epithelial cells and grew to high titers ($10^{5.3}$ - 10^7 TCID₅₀/ml) in those cells. A/Equine/Kentucky/81 and A/Equine/Kentucky/91 were highly restricted in their

infectivity (<10% of the cells infected) with little ($10^{1.7}$ TCID₅₀/ml for A/Equine/Kentucky/81) or no (for A/Equine/Kentucky/91) detectable viral growth. In contrast, A/Equine/Wisconsin/1/03 infected a larger percentage (about 30%) of the primary canine respiratory epithelial cells and grew to substantially higher titers (about $10^{4.8}$ TCID₅₀/ml) in those cells. The results demonstrated that all influenza A viruses tested were able to infect canine primary respiratory epithelial cells. However, the infectivity and replication characteristics of the viruses were strongly lineage-dependent.

Dubovi et al. (2004) noted recurrent outbreaks of severe respiratory disease characterized by coughing and fever in greyhound dogs at racing kennels in Florida. Most affected dogs recovered, but some succumbed to a fatal hemorrhagic pneumonia. Lung tissues from 5 of the dogs that died from the hemorrhagic pneumonia syndrome were subjected to virus isolation studies in African green monkey kidney epithelial cells (Vero), Madin-Darby canine kidney epithelial cells (MDCK), primary canine kidney epithelial cells, primary canine lung epithelial cells, primary bovine testicular epithelial cells, canine tumor fibroblasts (A-72), and human colorectal adenocarcinoma epithelial cells (HRT-18) (Dubovi et al., 2004). Cytopathology in the MDCK cells was noted on the first passage of lung homogenate from one of the dogs, and the loss of cytopathology upon subsequent passage to cells cultured without trypsin coupled with the presence of hemagglutinating activity in culture supernatants suggested the presence of an influenza virus (Dubovi et al., 2004). The virus was initially identified as influenza virus by PCR using primers specific for the matrix gene. The canine influenza virus has been designated as the A/Canine/Florida/43/04 strain. Based on virus isolation from the lungs, the presence of viral antigens in lung tissues by immunohistochemistry, and seroconversion data, Dubovi et al. (2004) concluded that the isolated influenza virus was most likely the etiological agent responsible for the fatal hemorrhagic pneumonia in racing greyhounds during the Jacksonville 2004 outbreak, and that this was the first report of an equine influenza virus associated with respiratory disease in dogs (Dubovi et al., 2004). The HA protein of the canine isolate differs from the A/Equine/Wisconsin/1/03 strain by only 6 amino acids.

REFERENCES

- Avery's Drug Treatment: Principles and Practice of Clinical Pharmacology and Therapeutics, 3rd edition, ADIS Press, Ltd., Williams and Wilkins, Baltimore, Md. (1987).
- Aymard-Henry et al., *Virology: A Practical Approach*, Oxford IRL Press, Oxford, 119-150 (1985).
- Bachmeyer, *Intervirology*, 5:260 (1975).
- Berkow et al., eds., *The Merck Manual*, 16th edition, Merck & Co., Rahway, N.J. (1992).
- Britton et al., *Can. Vet. J.*, 43:55 (2002).
- Chambers et al., *Vet. Rec.*, 135:275 (1994).
- Daly and Mumford, In: *Equine Respiratory Diseases* Lekeux (ed.) International Veterinary Information Science, Ithaca, N.Y. (2001).
- Del Piero et al., *Equine Vet. J.*, 29:178 (1997).
- Dubovi et al., *Proceedings of the American Association of Veterinary Laboratory Diagnostics*, p. 158 (2004).
- Enami et al., *Proc. Natl. Acad. Sci. U.S.A.*, 87:3802 (1990).
- Frymus et al., *Pol. Arch. Med. Wewn.*, 26:7 (1993).
- Gilkerson et al., *Vet. Microbiol.*, 68:27 (1999).
- Grand and Skehel, *Nature, New Biology*, 238:145 (1972).
- Hoffman et al., *Am. J. Vet. Res.*, 54:1615 (1993).
- Kilbourne, *Bull. M2 World Health Org.*, 41: 653 (1969).
- Lakritz et al., *J. Vet. Intern. Med.*, 7:277 (1984-1989).

Landolt et al., *J. Clin. Microbiol.*, 41:1936 (2001).
 Layer & Webster, *Virology*, 69:511 (1976).
 Marriott et al., *Adv. Virus Res.*, 53:321 (1999).
 McCullers et al., *J. Infect. Dis.*, 187:1000 (2003).
 Morley et al., *Equine Vet. J.*, 27:131 (1995).
 Mumford et al., *Equine Vet. J.*, 35:72 (2003).
 Murphy, *Infect. Dis. Clin. Pract.*, 2: 174 (1993).
 Murray et al., *Equine Vet. J.*, 28:432 (1996).
 Muster et al., *Proc. Natl. Acad. Sci. USA*, 88: 5177 (1991).
 Neumann et al., *Proc. Natl. Acad. Sci. U.S.A.*, 96:9345 (1999).
 Ogra et al., *J. Infect. Dis.*, 134: 499 (1977).
 Olsen et al., *Vaccine*, 15:1149 (1997).
 Osol (ed.), *Remington's Pharmaceutical Sciences*, Mack Publishing Co., Easton, Pa. 1324-1341 (1980).
 Palmar et al., Madison Wis.: University of Wisconsin Department of Health, Education and Welfare Immunology Series (1975).
 Park et al., *Proc. R. Soc. London B.*, 271:1547 (2004).

Robertson et al., *Biologicals*, 20:213 (1992).
 Robertson et al., *Giornale di Igiene e Medicina Preventiva*, 29:4 (1988).
 Simonsen, *Vaccine*, 17:S3 (1999).
 Subbarao et al., *J. Virol.*, 67:7223 (1993).
 Van Maanen et al., *Vet. Q.*, 24:79 (2002).
 Webb et al., *Aust. Vet. J.*, 57:142 (1981).
 Wilkins, *Vet. Clin. North Am. Equine Pract.*, 19:19 (2003).

All publications, patents and patent applications are incorporated herein by reference. While in the foregoing specification this invention has been described in relation to certain preferred embodiments thereof, and many details have been set forth for purposes of illustration, it will be apparent to those skilled in the art that the invention is susceptible to additional embodiments and that certain of the details described herein may be varied considerably without departing from the basic principles of the invention.

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 20

<210> SEQ ID NO 1

<211> LENGTH: 565

<212> TYPE: PRT

<213> ORGANISM: Influenza A Virus

<400> SEQUENCE: 1

```

Met Lys Thr Thr Ile Ile Leu Ile Leu Leu Thr His Trp Ala Tyr Ser
 1                               5                               10          15

Gln Asn Pro Ile Ser Gly Asn Asn Thr Ala Thr Leu Cys Leu Gly His
 20                               25                               30

His Ala Val Ala Asn Gly Thr Leu Val Lys Thr Ile Ser Asp Asp Gln
 35                               40                               45

Ile Glu Val Thr Asn Ala Thr Glu Leu Val Gln Ser Ile Ser Met Gly
 50                               55                               60

Lys Ile Cys Asn Asn Ser Tyr Arg Ile Leu Asp Gly Arg Asn Cys Thr
 65                               70                               75          80

Leu Ile Asp Ala Met Leu Gly Asp Pro His Cys Asp Ala Phe Gln Tyr
 85                               90                               95

Glu Asn Trp Asp Leu Phe Ile Glu Arg Ser Ser Ala Phe Ser Asn Cys
 100                              105                              110

Tyr Pro Tyr Asp Ile Pro Asp Tyr Ala Ser Leu Arg Ser Ile Val Ala
 115                              120                              125

Ser Ser Gly Thr Leu Glu Phe Thr Ala Glu Gly Phe Thr Trp Thr Gly
 130                              135                              140

Val Thr Gln Asn Gly Arg Ser Gly Ala Cys Lys Arg Gly Ser Ala Asp
 145                              150                              155          160

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

Pro Thr Leu Asn Val Thr Met Pro Asn Asn Lys Asn Phe Asp Lys Leu
 180                              185                              190

Tyr Ile Trp Gly Ile His His Pro Ser Ser Asn Gln Glu Gln Thr Lys
 195                              200                              205

Leu Tyr Ile Gln Glu Ser Gly Arg Val Thr Val Ser Thr Lys Arg Ser
 210                              215                              220

Gln Gln Thr Ile Ile Pro Asn Ile Gly Ser Arg Pro Trp Val Arg Gly

```


-continued

Val	Leu	Val	Leu	Asn	Asn	Asn	Arg	Thr	Asp	Leu	Asn	Cys	Lys	Gly	Thr
	35						40					45			
Ile	Ile	Arg	Glu	Tyr	Asn	Glu	Thr	Val	Arg	Val	Glu	Lys	Ile	Thr	Gln
	50				55						60				
Trp	Tyr	Asn	Thr	Ser	Thr	Ile	Lys	Tyr	Ile	Glu	Arg	Pro	Ser	Asn	Glu
65					70					75					80
Tyr	Tyr	Met	Asn	Asn	Thr	Glu	Pro	Leu	Cys	Glu	Ala	Gln	Gly	Phe	Ala
			85						90					95	
Pro	Phe	Ser	Lys	Asp	Asn	Gly	Ile	Arg	Ile	Gly	Ser	Arg	Gly	His	Val
			100					105					110		
Phe	Val	Ile	Arg	Glu	Pro	Phe	Val	Ser	Cys	Ser	Pro	Ser	Glu	Cys	Arg
			115				120					125			
Thr	Phe	Phe	Leu	Thr	Gln	Gly	Ser	Leu	Leu	Asn	Asp	Lys	His	Ser	Asn
	130					135						140			
Gly	Thr	Val	Lys	Asp	Arg	Ser	Pro	Tyr	Arg	Thr	Leu	Met	Ser	Val	Lys
145					150						155				160
Ile	Gly	Gln	Ser	Pro	Asn	Val	Tyr	Gln	Ala	Arg	Phe	Glu	Ser	Val	Ala
				165					170						175
Trp	Ser	Ala	Thr	Ala	Cys	His	Asp	Gly	Lys	Lys	Trp	Met	Thr	Val	Gly
			180					185					190		
Val	Thr	Gly	Pro	Asp	Asn	Gln	Ala	Ile	Ala	Val	Val	Asn	Tyr	Gly	Gly
		195					200						205		
Val	Pro	Val	Asp	Ile	Ile	Asn	Ser	Trp	Ala	Gly	Asp	Ile	Leu	Arg	Thr
	210					215					220				
Gln	Glu	Ser	Ser	Cys	Thr	Cys	Ile	Lys	Gly	Asp	Cys	Tyr	Trp	Val	Met
225					230					235					240
Thr	Asp	Gly	Pro	Ala	Asn	Arg	Gln	Ala	Lys	Tyr	Arg	Ile	Phe	Lys	Ala
				245					250					255	
Lys	Asp	Gly	Arg	Val	Ile	Gly	Gln	Thr	Asp	Ile	Ser	Phe	Asn	Gly	Gly
			260						265				270		
His	Ile	Glu	Glu	Cys	Ser	Cys	Tyr	Pro	Asn	Glu	Gly	Lys	Val	Glu	Cys
		275					280						285		
Ile	Cys	Arg	Asp	Asn	Trp	Thr	Gly	Thr	Asn	Arg	Pro	Ile	Leu	Val	Ile
	290					295					300				
Ser	Ser	Asp	Leu	Ser	Tyr	Thr	Val	Gly	Tyr	Leu	Cys	Ala	Gly	Ile	Pro
305					310					315					320
Thr	Asp	Thr	Pro	Arg	Gly	Glu	Asp	Ser	Gln	Phe	Thr	Gly	Ser	Cys	Thr
				325					330					335	
Ser	Pro	Leu	Gly	Asn	Lys	Gly	Tyr	Gly	Val	Lys	Gly	Phe	Gly	Phe	Arg
		340						345					350		
Gln	Gly	Thr	Asp	Val	Trp	Ala	Gly	Arg	Thr	Ile	Ser	Arg	Thr	Ser	Arg
		355					360						365		
Ser	Gly	Phe	Glu	Ile	Ile	Lys	Ile	Arg	Asn	Gly	Trp	Thr	Gln	Asn	Ser
	370					375						380			
Lys	Asp	Gln	Ile	Arg	Arg	Gln	Val	Ile	Ile	Asp	Asp	Pro	Asn	Trp	Ser
385					390					395					400
Gly	Tyr	Ser	Gly	Ser	Phe	Thr	Leu	Pro	Val	Glu	Leu	Thr	Lys	Lys	Gly
				405					410					415	
Cys	Leu	Val	Pro	Cys	Phe	Trp	Val	Glu	Met	Ile	Arg	Gly	Lys	Pro	Glu
		420						425					430		
Glu	Thr	Thr	Ile	Trp	Thr	Ser	Ser	Ser	Ser	Ile	Val	Met	Cys	Gly	Val
		435					440						445		
Asp	His	Lys	Ile	Ala	Ser	Trp	Ser	Trp	His	Asp	Gly	Ala	Ile	Leu	Pro

-continued

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

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

Thr Lys Lys Lys Ile Glu Lys Ile Arg Pro Leu Leu Val 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 Arg 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 Thr 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 Lys 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 Ser 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 Asp Glu Phe Ala Glu Ile Met Lys Ile Cys Ser Thr Ile Glu Glu
 740 745 750

Leu Arg Arg Gln Lys
 755

-continued

```

<210> SEQ ID NO 4
<211> LENGTH: 759
<212> TYPE: PRT
<213> ORGANISM: Influenza A Virus

<400> SEQUENCE: 4

Met Glu Arg Ile Lys Glu Leu Arg Asp Leu Met Leu 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 Ala Leu Arg Met Lys
 35           40           45
Trp Met Met Ala Met Lys Tyr Pro Ile Thr Ala Asp Lys Arg Ile Met
 50           55           60
Glu Met Ile Pro Glu Arg Asn Glu Gln Gly Gln Thr Leu Trp Ser Lys
 65           70           75           80
Thr Asn 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 Thr Thr Ser Thr Ile His Tyr Pro
100          105          110
Lys Val 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 Val 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 Asp Cys Lys Ile Ala 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 Ile Asp
245          250          255
Gln Ser Leu Ile Ile Ala Ala Arg Asn Ile Val Arg Arg Ala Thr 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 Ile Arg Met Val Asp Ile Leu Lys 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 Met 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 Arg
355          360          365
Arg Ala Thr Ala Ile Leu Arg Lys Ala Thr Arg Arg Leu Ile Gln Leu
370          375          380

```

-continued

```

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 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 Leu Arg Gly Val 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 Ile Leu
                500                505                510

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

Ile Ile 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 Ile Val
545                550                555                560

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

Phe Glu Pro Phe Gln Ser Leu Val Pro Arg Ala Thr 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 Ala Gln Ile Ile Lys Leu Leu Pro Phe Ala Ala Ala
                610                615                620

Pro Pro Glu 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 Ala Thr Lys Arg Leu Thr Val Leu Gly Lys Asp Ala
                660                665                670

Gly Ala 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 Asn Lys Arg Tyr
                690                695                700

Gly Pro Ala Leu Ser Ile Asn Glu Leu Ser Lys 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> SEQ ID NO 5

<211> LENGTH: 716

<212> TYPE: PRT

<213> ORGANISM: Influenza A Virus

<400> SEQUENCE: 5

-continued

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 Asn Glu Leu Ser Glu Ser Val Val Ile 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 Thr Met Ala Trp Thr Val Val Asn Ser Ile Cys Asn
85 90 95

Thr Thr Arg Ala Glu Lys Pro Lys Phe Leu Pro Asp Leu Tyr Asp Tyr
100 105 110

Lys Glu Asn Arg Phe Val 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 Asn Tyr Ser Leu Pro Pro Asn Phe Ser
210 215 220

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

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

Ile Glu Pro Phe Ser Lys Thr Thr Pro Arg Pro Leu Lys Met Pro Gly
260 265 270

Gly Pro Pro 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

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

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

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

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

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

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

-continued

Leu Thr Asp Ser Ser Trp Ile Glu Leu Asp Glu Ile Gly Glu Asp Val
 420 425 430
 Ala Pro Ile Glu Tyr 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 Glu 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 Val 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 Phe Glu Pro His Lys Trp Glu Lys Tyr Cys Val Leu Glu
 530 535 540
 Ile Gly Asp Met Leu Leu Arg Thr Ala Val 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 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 Ile Gly Gly Leu Tyr Glu
 675 680 685
 Ser 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> SEQ ID NO 6

<211> LENGTH: 498

<212> TYPE: PRT

<213> ORGANISM: Influenza A Virus

<400> SEQUENCE: 6

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

-continued

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

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

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

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

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

Gln Val Arg Glu Gly 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 Leu Ala Val Thr Ser Gly
 275 280 285

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

Lys Leu Leu Gln Asn Ser Gln Ile Phe Ser Leu Ile Arg Pro Lys 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 Val Leu Asn Phe Ile Arg Gly Thr Lys Val
 340 345 350

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

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

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

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

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

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

Glu Asn Ala Lys Ser Glu Asp Val Ser Phe Gln 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 Phe
 485 490 495

-continued

Asp Ser

<210> SEQ ID NO 7
 <211> LENGTH: 252
 <212> TYPE: PRT
 <213> ORGANISM: Influenza A Virus

<400> SEQUENCE: 7

```

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 Ser 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 Val Ala Leu Ser Tyr Ser Thr Gly Ala Leu Ala Ser Cys Met
115          120          125
Gly Leu Ile Tyr Asn Arg Met Gly Thr 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 Arg
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 Asp 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> SEQ ID NO 8
 <211> LENGTH: 230
 <212> TYPE: PRT
 <213> ORGANISM: Influenza A Virus

<400> SEQUENCE: 8

```

Met Asp Ser Asn Thr Val Ser Ser Phe Gln Val Asp Cys Phe Leu Trp
 1           5           10           15
His Val Arg Lys Arg Phe Ala Asp Gln Glu Leu Gly Asp Ala Pro Phe
 20           25           30
Leu Asp Arg Leu Arg Arg Asp Gln Lys Ser Leu Arg Gly Arg Gly Ser
 35           40           45
Thr Leu Gly Leu Asp Ile Glu Thr Ala Thr His Ala Gly Lys Gln Ile
 50           55           60
Val Glu Gln Ile Leu Glu Lys Glu Ser Asp Glu Ala Leu Lys Met Thr

```

-continued

65		70		75		80									
Ile	Ala	Ser	Val	Pro	Thr	Ser	Arg	Tyr	Leu	Thr	Asp	Met	Thr	Leu	Asp
			85						90					95	
Glu	Met	Ser	Arg	Asp	Trp	Phe	Met	Leu	Met	Pro	Lys	Gln	Lys	Val	Thr
			100					105					110		
Gly	Ser	Leu	Cys	Ile	Arg	Met	Asp	Gln	Ala	Ile	Met	Asp	Lys	Asn	Ile
		115					120					125			
Ile	Leu	Lys	Ala	Asn	Phe	Ser	Val	Ile	Phe	Glu	Arg	Leu	Glu	Thr	Leu
	130					135					140				
Ile	Leu	Leu	Arg	Ala	Phe	Thr	Glu	Glu	Gly	Ala	Val	Val	Gly	Glu	Ile
145					150					155					160
Ser	Pro	Leu	Pro	Ser	Leu	Pro	Gly	His	Thr	Asn	Glu	Asp	Val	Lys	Asn
				165					170					175	
Ala	Ile	Gly	Val	Leu	Ile	Gly	Gly	Leu	Lys	Trp	Asn	Asp	Asn	Thr	Val
			180					185					190		
Arg	Ile	Ser	Glu	Thr	Leu	Gln	Arg	Phe	Ala	Trp	Arg	Ser	Ser	His	Glu
		195					200					205			
Asn	Gly	Arg	Pro	Ser	Phe	Pro	Ser	Lys	Gln	Lys	Arg	Lys	Met	Glu	Arg
	210					215					220				
Thr	Ile	Lys	Pro	Lys	Ile										
225					230										

<210> SEQ ID NO 9
 <211> LENGTH: 1701
 <212> TYPE: DNA
 <213> ORGANISM: Influenza A Virus

<400> SEQUENCE: 9

tcatgaagac aaccattatt ttgatactac tgaccattg ggcttacagt caaaacccaa	60
tcagtggcaa caacacagcc acattgtgtc tgggacacca tgcagtagca aatggaacat	120
tggtaaaaac aataagtgat gatcaaattg aggtgacaaa tgctacagaa ttagttcaaa	180
gcatttcaat ggggaaaata tgcaacaact catatagaat tctagatgga agaaattgca	240
cattaataga tgcaatgcta ggagaccccc actgtgacgc ctttcagtat gagaattggg	300
acctctttat agaaagaagc agcgctttca gcaattgcta cccatagac atccctgact	360
atgcatcgct cccatccatt gtagcatcct caggaacatt ggaattcaca gcagagggat	420
tcacatggac aggtgtcact caaaacggaa gaagtggagc ctgcaaaagg ggatcagccg	480
atagtttctt tagccgactg aattggctaa caaaatctgg aagctcttac cccacattga	540
atgtgacaat gcttaacaat aaaaatttcg acaagctata catctggggg attcacacc	600
cgagctcaaa tcaagagcag acaaaattgt acatccaaga atcaggacga gtaacagtct	660
caacaaaaag aagtcaacaa acaataatcc ctaacatcgg atctagaccg tgggtcagag	720
gtcaatcagg taggataagc atatactgga ccattgtaaa acctggagat atccaatga	780
taaacagtaa tggcaactta gttgcaccgc ggggatattt taaattgaaa acagggaaaa	840
gctctgtaat gagatcagat gtaccatag acatttgtgt gtctgaatgt attacccaa	900
atggaagcat ctccaacgac aagccattcc aaaatgtgaa caaagttaca tatggaaaat	960
gccccagta tatcaggcaa aacactttaa agctggccac tgggatgagg aatgtaccag	1020
aaaagcaaat cagaggaatc tttggagcaa tagcgggatt catcgaaaaa ggctgggaag	1080
gaatggttga tgggtggtat gggttccgat atcaaaaactc tgaaggaaca gggcaagctg	1140
cagatctaaa gagcactcaa gcagccatcg accagattaa tggaagtta aacagagtga	1200

-continued

```

ttgaaagaac caatgagaaa ttccatcaaa tagagaagga attctcagaa gtagaaagaa 1260
gaattcagga cttggagaaa tatgtagaag acacccaaat agacctatgg tcctacaatg 1320
cagaattgct ggtggctcta gaaaatcaac atacaattga cttaacagat gcagaaatga 1380
ataaattatt tgagaagact agacgccagt taagagaaaa cgcagaagac atgggagggtg 1440
gatgtttcaa gatttaccac aatgtgata atgcatgcat tggatcaata agaaatggga 1500
catatgacca ttacatatac agagatgaag cattaacaa ccgatttcag atcaaagggtg 1560
tagagttgaa atcaggctac aaagattgga tactgtggat ttcatcgcgc atcatcatgct 1620
tcttaatttg cgtgtttcta ttgggtttca ttatgtgggc ttgccaaaaa ggcaacatca 1680
gatgcaacat ttgcatttga g 1701

```

```

<210> SEQ ID NO 10
<211> LENGTH: 1413
<212> TYPE: DNA
<213> ORGANISM: Influenza A Virus

```

```

<400> SEQUENCE: 10

```

```

atgaatccaa atcaaaagat aatagcaatt ggatttgcatt cattggggat attaatacatt 60
aatgtcattc tccatgtagt cagcattata gtaacagtac tggtcctcaa taacaataga 120
acagatctga actgcaaagg gacgatcata agagagtaca atgaaacagt aagagtagaa 180
aaaattactc aatggtataa taccagtaca attaagtaca tagagagacc ttcaaatgaa 240
tactacatga acaacactga accactttgt gaggcccaag gctttgcacc attttccaaa 300
gataatggaa tacgaattgg gtcgagaggc catgtttttg tgataagaga accttttgta 360
tcatgttcgc cctcagaatg tagaaccttt ttcctcacac agggctcatt actcaatgac 420
aaacattcta acggcacagt aaaggaccga agtccgtata ggactttgat gagtgtcaaa 480
atagggcaat cacctaattg atatcaagct aggtttgaat cgggtggcatg gtcagcaaca 540
gcatgccatg atgaaaaaaa atggatgaca gttggagtca cagggcccga caatcaagca 600
attgcagtag tgaactatgg aggtgttccg gttgatatta ttaattcatg ggaggggat 660
atnttaagaa cccaagaatc atcatgcacc tgcattaaag gagactgta ttgggtaatg 720
actgatggac cggcaaatag gcaagctaaa tataggatat tcaaagcaaa agatggaaga 780
gtaattggac agactgatat aagtttcaat gggggacaca tagaggagtg ttcttgttac 840
cccaatgaag ggaaggtgga atgcatatgc agggacaatt ggactggaac aaatagacca 900
attctggtaa tatcttctga tctatcgtac acagttggat atttgtgtgc tggcattccc 960
actgacactc ctaggggaga ggatagtoaa ttcacaggct catgtacaag tcctttggga 1020
aataaaggat acggtgtaaa aggtttcggg tttcgacaag gaactgacgt atgggccgga 1080
aggacaatta gtaggacttc aagatcagga ttcgaaataa taaaaatcag gaatggttgg 1140
acacagaaca gtaaagacca aatcaggagg caagtgatta tcgatgaccc aaattggtca 1200
ggatatagcg gttctttcac attgccggtt gaactaacia aaaagggatg tttggtcccc 1260
tgtttctggg ttgaaatgat tagaggtaaa cctgaagaaa caacaatatg gacctctagc 1320
agctccattg tgatgtgtgg agtagatcat aaaattgcca gttggtcatg gcacgatgga 1380
getattcttc cctttgacat cgataagatg taa 1413

```

```

<210> SEQ ID NO 11
<211> LENGTH: 2277
<212> TYPE: DNA

```

-continued

<213> ORGANISM: Influenza A Virus

<400> SEQUENCE: 11

```

atggatgtca atccgactct acttttctta aagggtgccag cgcaaaatgc tataagcaca    60
acatttcctt atactggaga tcctccctac agtcatggaa cagggacagg atacaccatg    120
gatactgtca acagaacaca ccaatattca gaaaaaggga aatggacaac aaacactgag    180
attggagcac cacaaactta tccaatcgat ggaccacttc ctgaagacaa tgaaccaagt    240
gggtacgccc aaacagattg tgtattggaa gcaatggctt tccttgaaga atcccatccc    300
ggaatctttg aaaattcgtg tcttgaacg atggaggtga ttcagcagac aagagtggac    360
aaactaacac aaggccgaca aacttatgat tggaccttga ataggaatca acctgcccga    420
acagcacttg ctaatacgat tgaagtattc agatcaaatg gtctgacttc caatgaatcg    480
gggagattga tggacttcct caaagatgtc atggagtcca tgaacaagga agaaatggaa    540
ataacaacac acttccaacg gaagagaaga gtaagagaca acatgacaaa gagaatggta    600
acacagagaa ccatagggaa gaaaaaaca cgattaaaca gaaagagcta tctaatacaga    660
acattaaccc taaacacaat gaccaaggac gctgagagag ggaaattgaa acgacgagca    720
atcgctaccc cagggatgca gataagaggg tttgtatatt ttggtgaaac actagcccga    780
agaatatgtg aaaagcttga acaatcagga ttgccagttg gcggtaatga gaaaaaggcc    840
aaactggcta atgtcgtcag aaaaatgatg actaattccc aagacactga actctccttc    900
accatcactg gggacaatac caaatggaat gaaaatcaga acccacgcat attcctggca    960
atgatcacat acataactag aaaccagcca gaatggttca gaaatggtct aagcattgca   1020
ccgattatgt tctcaataa aatggcaaga ctggggaaaag gatatatgtt tgaaagcaaa   1080
agtatgaaat tgagaactca aataccagca ggaatgcttg caagcattga cctgaaatat   1140
ttcaatgatc caacaaaaa gaaaattgaa aagatacgac cacttctggt tgacgggact   1200
gcttcactga gtcctggcat gatgatggga atgttcaaca tgttgagcac tgtgctaggt   1260
gtatccatat taaacctggg ccagaggaaa tacacaaaga ccacatactg gtgggatggt   1320
ctgcaatcat ccgatgactt tgctttgata gtgaatgcgc ctaatcatga aggaatacaa   1380
gctggagtag acagattcta taggacttgc aaactggtcg ggatcaacat gagcaaaaag   1440
aagtctaca taaatagaac tggaaacttc gaattcaca gctttttcta ccggtatggt   1500
ttttagacca atttcagcat ggaactaccc agttttgggg tttccggaat aaatgaatct   1560
gcagacatga gcattggagt gacagtcac taaaacaaca tgataaataa tgatctcggg   1620
cctgccacgg cacaaatggc actccaactc ttcattaagg attatcggt cacataccgg   1680
tgccatagag gtgataccca gatacaaac agaagatctt ttgagttgaa gaaactgtgg   1740
gaacagactc gatcaaagac tggctactg gtatcagatg ggggtccaaa cctatataac   1800
atcagaaacc tacacatccc ggaagtctgt ttaaaatggg agctaatagga tgaagattat   1860
aaggggaggg tatgcaatcc attgaatcct ttcgtagtc acaaagaaat tgaatcagtc   1920
aacagtgcag tagtaatgcc tgcgcatggc cctgccaaaa gcatggagta tgatgctggt   1980
gcaacaacac attcttgat cccaagagg aaccggtcca tattgaacac aagccaaagg   2040
ggaatactcg aagatgagca gatgtatcag aaatgctgca acctgtttga aaaattcttc   2100
cccagcagct catacagaag accagtcggg atttctagta tggttgaggg catggtgtcc   2160
agggcccgca ttgatgcacg aattgacttc gaatctggac ggataaagaa ggatgagttc   2220
gctgagatca tgaagatctg ttccaccatt gaagagctca gacggcaaaa atagtga    2277

```

-continued

```

<210> SEQ ID NO 12
<211> LENGTH: 2281
<212> TYPE: DNA
<213> ORGANISM: Influenza A Virus

<400> SEQUENCE: 12
atggagagaa taaaagaact gagagatctg atgttacaat cccgcacccg cgagatacta    60
acaaaaacta ctgtggacca catggccata atcaagaaat acacatcagg aagacaagag    120
aagaaccctg cacttaggat gaaatggatg atggcaatga aatacccaat tacagcagat    180
aagaggataa tggagatgat tcctgagaga aatgaacagg gacaaaacct ttggagcaaa    240
acgaacgatg ctggctcaga ccgctaata gttacacctc tggcagtgcac atggtggaat    300
aggaatggac caacaacaag cacaattcat tatccaaaag tctacaaaac ttattttgaa    360
aaggttgaaa gattgaaaca cggaaccttt ggccccgttc attttaggaa tcaagtcaag    420
ataagacgaa gagttgatgt aaacctggtt cacgcggacc tcagtgccaa agaagcacia    480
gatgtgatca tggaaattgt tttcccaaat gaagtgggag ccagaattct aacatcggaa    540
tcacaactaa caataaccaa agagaaaaag gaagaacttc aggactgcaa aattgctccc    600
ttgatggtag catacatgct agaaagagag ttggtccgaa aaacaagggt cctcccagta    660
gcaggcggaa caagcagtgt atacattgaa gtgttgcata tgactcaggg aacatgctgg    720
gagcaaatgt acaccccagg aggagaagtt agaaacgatg atattgatca aagtttaatt    780
attgcagccc ggaacatagt gagaagagca acagtatcag cagatccact agcatcccta    840
ctggaaatgt gccacagtac acagattggt ggaataagga tggtagacat ccttaagcag    900
aatccaacag aggaacaagc tgtggatata tgcaaagcag caatgggatt gagaattagc    960
tcatcattca gctttggtgg attcaccttc aagagaacaa gtggatcata agtcaagaga   1020
gaagaagaaa tgcttacggg caaccttcaa acattgaaaa taagagtgca tgagggctat   1080
gaagaattca caatggctcg aagaagagca acagccattc tcagaaaggc aaccagaaga   1140
ttgattcaat tgatagtaag tgggagagat gaacagtcaa ttgctgaagc aataattgta   1200
gccatggtgt tttcgcaaga agattgcatg ataaaagcag ttcgaggcga tttgaacttt   1260
gttaatagag caaatcagcg cttgaacccc atgcatcaac tcttgaggca tttccaaaag   1320
gatgcaaaaag tgcttttcca aaattggggg attgaacca tcgacaatgt aatgggaatg   1380
attggaatat tgcctgacat gaccccgaag accgagatgt cattgagagg agtgagagtc   1440
agcaaatgg gagtggatga gtactccagc actgagagag tgggtggtgag cattgaccgt   1500
tttttaagag ttcgggatca aaggggaaac atactactgt cccctgaaga agtcagtgaa   1560
acacaaggaa cggaaaagct gacaataatt tattcgtcat caatgatgtg ggagattaat   1620
ggtcccgaat cagtgttggc caatacttat caatggatca tcaggaactg gaaattgta   1680
aaaattcagt ggtcacagga ccccaaatg ttatacaata agatagaatt tgagccattc   1740
caatccctgg tocctagggc taccagaagc caatacagcg gtttcgtaag aacctgttt   1800
cagcaaatgc gagatgtact tggaacatth gatactgctc aaataataaa actcctccct   1860
tttgccgctg ctcctccgga acagagtagg atgcagtctc cttctttgac tgtaaatgta   1920
agaggttcgg gaatgaggat acttgtaaga ggcaattccc cagtgttcaa ctacaataaa   1980
gccactaaaa ggctcacagt cctcgaaag gatgcagggt cgcttactga ggaccagat   2040
gaaggtacgg ctggagtaga atctgctggt ctaagagggt ttctcatttt aggtaaagaa   2100

```


-continued

```

aataagagat atggcccagc actaagcadc aatgaactaa gcaaacttgc aaaaggggag 2160
aaagccaatg tactaattgg gcaaggggac gtagtggttg taatgaaacg gaaacgtgac 2220
tctagcatac ttactgacag ccagacagcg accaaaagga ttcggatggc catcaattag 2280
t                                                                                   2281

```

```

<210> SEQ ID NO 13
<211> LENGTH: 2209
<212> TYPE: DNA
<213> ORGANISM: Influenza A Virus

```

```

<400> SEQUENCE: 13

```

```

atggaagact ttgtgcgaca atgcttcaat ccaatgatcg tcgagcttgc ggaaaaggca 60
atgaaagaat atggagagga cccgaaaatc gaaacaaaca aatttgacg aatatgcact 120
cacttggaag tctgcttcat gtactcggat ttccacttta ttaatgaact gagtgagtca 180
gtggtcatag agtctggtga cccaaatgct cttttgaaac acagatttga aatcattgag 240
gggagagatc gaacaatggc atggacagta gtaaacagca tctgcaacac cacaagagct 300
gaaaaaccta aatttcttcc agattttatac gactataagg agaacagatt tgttgaatt 360
ggtgtgacaa ggagagaagt tcacatatac tacctggaga aggccaaaca aataaagtct 420
gagaaaaacac atatccacat tttctcattt acaggagagg aaatggctac aaaagcggac 480
tatactcttg atgaagagag tagagccagg atcaagacca gactattcac tataagacaa 540
gaaatggcca gtagaggcct ctgggattcc tttcgtcagt ccgagagagg cgaagagaca 600
attgaagaaa gatttgaat cacagggacg atgcgcaagc ttgccaatta cagtctccca 660
ccgaacttct ccagccttga aaattttaga gtctatgtgg atggattcga accgaacggc 720
tgcattgaga gtaagcttcc tcaaatgtcc aaagaagtaa atgccagaat cgaaccattt 780
tcaaagacaa cccccgacc actcaaaatg ccaggtggtc caccctgcca tcagcgatct 840
aaattcctgc taatggatgc tctgaaactg agcattgagg acccaagtca cgagggagag 900
ggaataccac tatatgatgc catcaaatgc atgaaaactt tctttggatg gaaagagccc 960
agtattgtta aaccacatga aaaggttata aaccgcaact atctccaaac ttggaagcaa 1020
gtattagcag aattacaaga ccttgagaac gaagaaaagg accccaagac caagaatatg 1080
aaaaaaacaa gccaatgaa atgggcactt agtgaaaata tggcaccaga gaaagtggat 1140
tttgaggatt gtaaagacat cagtatttata aaacagtatg acagtgatga gccagaaaca 1200
aggtctcttg caagttggat tcaaagttag ttcaacaaag cttgtgaact gacagattca 1260
agctggatag agctcgatga aattggggag gatggtgccc caatagaata cattgagagc 1320
atgaggagaa attattttac tgctgaggtt tcccattgta gagcaacaga atatataatg 1380
aaggagatgt acatcaacac tgctctactc aatgcatcct gtgctgcatg ggatgaattc 1440
caattaatcc cgatgataag taaatgcagg accaaagaag ggagaaggaa gacaaattta 1500
tatggattca tagtaaaggg aaggtcccat ttaagaaatg atactgacgt ggtgaacttt 1560
gtaagtatgg aattttctct cactgatcca agatttgagc cacacaaatg ggaaaaatac 1620
tgcttcttag aaattggaga catgcttcta agaactgctg taggtcaagt gtcaagacct 1680
atgtttttgt atgtaaggac aaatggaacc tctaaaatta aaatgaaatg gggaatggaa 1740
atgaggcgct gcctccttca gtctctgcaa cagattgaaa gcatgatcga agctgagttc 1800
tcagtcaaag aaaaggacat gaccaaagaa ttttttgaga acaaatcaga gacatggcct 1860
ataggagagt ccccaaaagg agtggaagag ggctcaatcg ggaaggtttg caggacctta 1920

```

-continued

```

ttagcaaaa ctgtgtttaa cagtttgtat gcatctccac aactggaagg gttttcagct 1980
gaatctagga aattacttct cattgttcag gctcttaggg ataacctgga acctggaacc 2040
tttgatattg gggggttata tgaatcaatt gaggagtgcc tgattaatga tccttggggt 2100
ttgcttaatg catcttggtt caactccttc cttacacatg cactgaagta gttgtggcaa 2160
tgctactatt tgctatccat actgtccaaa aaagtacctt gtttctact 2209

```

```

<210> SEQ ID NO 14
<211> LENGTH: 1498
<212> TYPE: DNA
<213> ORGANISM: Influenza A Virus

```

```

<400> SEQUENCE: 14

```

```

atggcgtctc aaggcaccac acgatcctat gaacagatgg aaactgatgg ggaacgccag 60
aatgcaactg aatcagagc atctgtcggg aggatggtgg gaggaatcgg cgggttttat 120
gttcagatgt gtactgagct taaactaac gaccatgaag ggcggctgat tcagaacagc 180
ataacaatag aaaggatggt actttcggca ttcgacgaaa gaagaaacaa gtatctcgag 240
gagcatccca gtgctgggaa agaccctaag aaaacaggag gcccgatata cagaaggaaa 300
gatgggaaat ggatgaggga actcatctc catgataaag aagaaatcat gagaatctgg 360
cgtcaggcca acaatggtga agacgctact gctggtctta ctcatatgat gatctggcac 420
tccaatctca atgacaccac ataccaaga acaagggtc ttgttcggac tgggatggat 480
cccagaatgt gctctctgat gcaaggctca accctccac ggagatctgg agccgctggt 540
gctgcagtaa aaggtgttgg aacaatggta atggaactca tcagaatgat caaacgcgga 600
ataaatgatc ggaatttctg gagaggtgaa aatggtcgaa gaaccagaat tgcttatgaa 660
agaatgtgca atatcctcaa agggaaattt cagacagcag cacaacgggc tatgatggac 720
caggtgaggg aaggccgcaa tcctggaaac gctgagattg aggatctcat tttcttggca 780
cgatcagcac ttattttgag aggatcagta gcccataaat catgcctacc tgctgtgtt 840
tatggccttg cagtaaccag tgggtatgac tttgagaagg aaggatactc tctggttggg 900
attgatcctt tcaaaactact ccagaacagt caaattttca gtctaactag accaaaagaa 960
aaccagcac acaagagcca gttggtgtgg atggcatgcc attctgcagc atttgaggac 1020
ctgagagttt taaatttcat tagaggaacc aaagtaatcc caagaggaca gttaacaacc 1080
agaggagttc aaatagcttc aaatgaaaac atggagacaa tagattctag cacacttgaa 1140
ctgagaagca aatattgggc aataaggacc agaagcggag gaaacaccag tcaacagaga 1200
gcatctgcag gacagataag tgtgcaacct actttctcag tacagagaaa tcttccctt 1260
gagagagcaa ccattatggc tgcattcact ggttaacctg aaggaggac ttccgacatg 1320
agaacgaaa tcataaggat gatggaaaat gccaaatcag aagatgtgtc tttccagggg 1380
cggggagtct tcgagctctc ggacgaaaag gcaacgaacc cgatcgtgcc ttccttgac 1440
atgagcaatg aaggtctta tttcttggg gacaatgctg aggagtttga cagttaa 1498

```

```

<210> SEQ ID NO 15
<211> LENGTH: 982
<212> TYPE: DNA
<213> ORGANISM: Influenza A Virus

```

```

<400> SEQUENCE: 15

```

```

atgagtcttc taaccgaggt cgaaacgtac gttctctcta tcgtaccatc aggccctc 60

```

-continued

```

aaagccgaga tcgcgagag acttgaagat gtctttgcag ggaagaacac cgatcttgag 120
gactcatgg aatggctaaa gacaagacca atcctgtcac ctctgactaa agggatttta 180
ggatttgat tcacgctcac cgtgccagc gagcgaggac tgcagcgtag acgctttgtc 240
caaaatgccc ttagtggaag cggagatcca aacaacatgg acagagcagt aaaactgtac 300
aggaagctta aaagagaaat aacattccat ggggcaaaag aggtggcact cagctattcc 360
actggtgcac tagccagctg catgggactc atatacaaca gaatgggaac tgttacaacc 420
gaagtggcat ttggcctggt atgcgccaca tgtgaacaga ttgctgattc ccagcatcgg 480
tctcacaggc agatggtgac aacaaccaac ccattaatca gacatgaaaa cagaatggta 540
ttagccagta ccacggctaa agccatggaa cagatggcag gatcagatga gcaggcagca 600
gaggccatgg aggttgctag tagggctagg cagatggtac aggcaatgag aaccattggg 660
accacccta gctccagtgc cggtttgaaa gatgatctcc ttgaaaattt acaggcctac 720
cagaaacgga tgggagtgca aatgcagcga ttcaagtgat cctctcgta ttgcagcaag 780
tatcattggg atcttgact tgatattgtg gattcttgat cgtctttct tcaaattcat 840
ttatcgtcgc cttaaatcgc ggttgaaaag agggccttct acggaaggag tacctgagtc 900
tatgagggaa gaatacggc aggaacagca gaatgctgtg gatgttgacg atggtcattt 960
tgtcaacata gagctggagt aa 982

```

```

<210> SEQ ID NO 16
<211> LENGTH: 838
<212> TYPE: DNA
<213> ORGANISM: Influenza A Virus

```

```

<400> SEQUENCE: 16

```

```

atggattcca acactgtgtc aagctttcag gtagactgtt ttctttggca tgtccgcaaa 60
cgattcgag accaagaact gggatgatgc ccattccttg accggcttcg ccgagaccag 120
aagtcctcaa ggggaagagg tagcactcct ggtctggaca tcgaaacagc cactcatgca 180
ggaaagcaga tagtggagca gattctggaa aaggaatcag atgaggcact taaaatgacc 240
attgcctctg ttccacttc acgctactta actgacatga ctcttgatga gatgtcaaga 300
gactggttca tgctcatgcc caagcaaaa gtaacaggct ccctatgtat aagaatggac 360
caggcaatca tggataagaa catcactact aaagcaaaact ttagtgtgat tttcgaaagg 420
ctggaacac taatactact tagagccttc accgaagaag gagcagctgc tggcgaaatt 480
tcaccattac cttctcttc aggacatact aatgaggatg tcaaaaatgc aattggggtc 540
ctcatcggag gacttaaatg gaatgataat acggtttaga tctctgaaac tctacagaga 600
ttcgttgga gaagcagtca tgagaatggg agaccttcat tcccttcaaa gcagaaaacga 660
aaaatggaga gaacaattaa gccaaaaatt tgaagaaata agatggttga ttgaagaagt 720
gcgacataga ttgaaaaata cagaaaatag ttttgaacaa ataacattta tgcaagcctt 780
acaactattg cttgaagttag aacaagagat aagaacttct tcgtttcagc ttatttaa 838

```

```

<210> SEQ ID NO 17
<211> LENGTH: 97
<212> TYPE: PRT
<213> ORGANISM: Influenza A Virus

```

```

<400> SEQUENCE: 17

```

```

Met Ser Leu Leu Thr Glu Val Glu Thr Pro Thr Arg Asn Gly Trp Glu
  1           5           10           15

```

-continued

Cys Lys Cys Ser Asp Ser Ser Asp Pro Leu Val Ile Ala Ala Ser Ile
 20 25 30
 Ile Gly Ile Leu His Leu Ile Leu Trp Ile Leu Asp Arg Leu Phe Phe
 35 40 45
 Lys Phe Ile Tyr Arg Arg Leu Lys Tyr Gly Leu Lys Arg Gly Pro Ser
 50 55 60
 Thr Glu Gly Val Pro Glu Ser Met Arg Glu Glu Tyr Arg Gln Glu Gln
 65 70 75 80
 Gln Asn Ala Val Asp Val Asp Asp Gly His Phe Val Asn Ile Glu Leu
 85 90 95
 Glu

<210> SEQ ID NO 18
 <211> LENGTH: 119
 <212> TYPE: PRT
 <213> ORGANISM: Influenza A Virus

<400> SEQUENCE: 18

Met Asp Ser Asn Thr Val Ser Ser Phe Gln Leu Met Arg Met Ser Lys
 1 5 10 15
 Met Gln Leu Gly Ser Ser Ser Glu Asp Leu Asn Gly Met Ile Ile Arg
 20 25 30
 Leu Glu Ser Leu Lys Leu Tyr Arg Asp Ser Leu Gly Glu Ala Val Met
 35 40 45
 Arg Met Gly Asp Leu His Ser Leu Gln Ser Arg Asn Glu Lys Trp Arg
 50 55 60
 Glu Gln Leu Ser Gln Lys Phe Glu Glu Ile Arg Trp Leu Ile Glu Glu
 65 70 75 80
 Val Arg His Arg Leu Lys Asn Thr Glu Asn Ser Phe Glu Gln Ile Thr
 85 90 95
 Phe Met Gln Ala Leu Gln Leu Leu Leu Glu Val Glu Gln Glu Ile Arg
 100 105 110
 Thr Phe Ser Phe Gln Leu Ile
 115

<210> SEQ ID NO 19
 <211> LENGTH: 344
 <212> TYPE: PRT
 <213> ORGANISM: Influenza A Virus

<400> SEQUENCE: 19

Met Lys Thr Thr Ile Ile Leu Ile Leu Leu Thr His Trp Ala Tyr Ser
 1 5 10 15
 Gln Asn Pro Ile Ser Gly Asn Asn Thr Ala Thr Leu Cys Leu Gly His
 20 25 30
 His Ala Val Ala Asn Gly Thr Leu Val Lys Thr Ile Ser Asp Asp Gln
 35 40 45
 Ile Glu Val Thr Asn Ala Thr Glu Leu Val Gln Ser Ile Ser Met Gly
 50 55 60
 Lys Ile Cys Asn Asn Ser Tyr Arg Ile Leu Asp Gly Arg Asn Cys Thr
 65 70 75 80
 Leu Ile Asp Ala Met Leu Gly Asp Pro His Cys Asp Ala Phe Gln Tyr
 85 90 95
 Glu Asn Trp Asp Leu Phe Ile Glu Arg Ser Ser Ala Phe Ser Asn Cys
 100 105 110
 Tyr Pro Tyr Asp Ile Pro Asp Tyr Ala Ser Leu Arg Ser Ile Val Ala

-continued

Val	Thr	Gln	Asn	Gly	Arg	Ser	Gly	Ala	Cys	Lys	Arg	Gly	Ser	Ala	Asp
145					150					155					160
Ser	Phe	Phe	Ser	Arg	Leu	Asn	Trp	Leu	Thr	Lys	Ser	Gly	Asn	Ser	Tyr
				165				170						175	
Pro	Thr	Leu	Asn	Val	Thr	Met	Pro	Asn	Asn	Lys	Asn	Phe	Asp	Lys	Leu
			180					185					190		
Tyr	Ile	Trp	Gly	Ile	His	His	Pro	Ser	Ser	Asn	Gln	Glu	Gln	Thr	Lys
		195					200					205			
Leu	Tyr	Ile	Gln	Glu	Ser	Gly	Arg	Val	Thr	Val	Ser	Thr	Lys	Arg	Ser
	210					215					220				
Gln	Gln	Thr	Ile	Ile	Pro	Asn	Ile	Gly	Ser	Arg	Pro	Trp	Val	Arg	Gly
225					230					235					240
Gln	Ser	Gly	Arg	Ile	Ser	Ile	Tyr	Trp	Thr	Ile	Val	Lys	Pro	Gly	Asp
			245					250						255	
Ile	Leu	Met	Ile	Asn	Ser	Asn	Gly	Asn	Leu	Val	Ala	Pro	Arg	Gly	Tyr
			260				265						270		
Phe	Lys	Leu	Lys	Thr	Gly	Lys	Ser	Ser	Val	Met	Arg	Ser	Asp	Ala	Pro
		275					280					285			
Ile	Asp	Ile	Cys	Val	Ser	Glu	Cys	Ile	Thr	Pro	Asn	Gly	Ser	Ile	Ser
	290					295					300				
Asn	Asp	Lys	Pro	Phe	Gln	Asn	Val	Asn	Lys	Val	Thr	Tyr	Gly	Lys	Cys
305					310					315					320
Pro	Lys	Tyr	Ile	Arg	Gln	Asn	Thr	Leu	Lys	Leu	Ala	Thr	Gly	Met	Arg
				325				330						335	
Asn	Val	Pro	Glu	Lys	Gln	Ile	Arg								
			340												

35

What is claimed is:

1. An isolated H3 equine influenza virus comprising a gene segment with sequences for a HA having SEQ ID NO:1 or a HA having at least 98% amino acid sequence identity to SEQ ID NO:1 which HA does not have a valine at position 78 or an asparagine at position 159.

2. The isolated equine influenza virus of claim 1 which comprises at least one of the following gene segments: a gene segment with sequences for a NA having SEQ ID NO:2 or having at least 95% amino acid sequence identity to SEQ ID NO:2, a gene segment with sequences for a PB1 having SEQ ID NO:3 or having at least 95% amino acid sequence identity to SEQ ID NO:3, a gene segment with sequences for a PB2 having SEQ ID NO:4 or having at least 95% amino acid sequence identity to SEQ ID NO:4, a gene segment with sequences for a PA having SEQ ID NO:5 or having at least 95% amino acid sequence identity to SEQ ID NO:5, a gene segment with sequences for a NP having SEQ ID NO:6 or having at least 95% amino acid sequence identity to SEQ ID NO:6, a gene segment with sequences for a M1 having SEQ ID NO:7 or having at least 95% amino acid sequence identity to SEQ ID NO:7, a gene segment with sequences for a M2 having SEQ ID NO:17 or having at least 95% amino acid sequence identity to SEQ ID NO:17, a gene segment with sequences for a NS1 having SEQ ID NO:8 or having at least 95% amino acid sequence identity to SEQ ID NO:8, or a gene segment with sequences for a NS2 having SEQ ID NO:18 or having at least 95% amino acid sequence identity to SEQ ID NO:18.

3. The isolated equine influenza virus of claim 1 which comprises negative-strand nucleic acid corresponding to nucleic acid sequences encoding at least one of SEQ ID NOs:1-8, 17 or 18.

4. The isolated equine influenza virus of claim 1 which has HA having SEQ ID NO:1 and NA having SEQ ID NO:2.

5. A method to prepare influenza virus, comprising: contacting an avian or mammalian cell with the virus of claim 1.

6. The method of claim 5 further comprising isolating the virus.

7. The isolated virus virus obtained by the method of claim 6.

8. An isolated host cell infected with the virus of claim 1 or 7.

9. A method to immunize a mammal against influenza, comprising administering to the mammal an effective amount of the virus of claim 1 or 7.

10. The method of claim 9 wherein the mammal is a dog or a horse.

11. A vaccine comprising the virus of claim 1 or 7 in an amount effective to induce a prophylactic or therapeutic response against influenza infection.

12. The vaccine of claim 11 further comprising a different isolated influenza virus.

13. The vaccine of claim 11 wherein the isolated equine influenza virus is an attenuated virus.

14. The vaccine of claim 11 wherein the isolated equine influenza virus is a reassortant virus.

15. The vaccine of claim 11 wherein the equine influenza virus has been altered by chemical, physical or molecular means.

63

16. The vaccine of claim 11 further comprising an adjuvant.

17. The vaccine of claim 11 further comprising a pharmaceutically acceptable carrier.

18. The vaccine of claim 17 wherein the carrier is suitable for intranasal or intramuscular administration.

19. The vaccine of claim 11 which is in freeze-dried form.

20. An isolated H3 equine influenza virus comprising a gene segment with sequences for a HA having SEQ ID NO:1 or a HA having at least 95% amino acid sequence identity to SEQ ID NO:1 which HA has an alanine at position 78 and a serine at position 159.

21. The isolated equine influenza virus of claim 1 which has an HA with at least 99% amino acid sequence identity to SEQ ID NO:1 and does not have a valine at position 78 or an asparagine at position 159.

22. A vaccine comprising the virus of claim 20 in an amount effective to induce a prophylactic or therapeutic response against influenza infection.

23. The vaccine of claim 22 further comprising a different isolated influenza virus.

24. The vaccine of claim 22 wherein the isolated equine influenza virus is an attenuated virus.

64

25. The vaccine of claim 22 wherein the isolated equine influenza virus is a reassortant virus.

26. The vaccine of claim 22 wherein the equine influenza virus has been altered by chemical, physical or molecular means.

27. The vaccine of claim 22 further comprising an adjuvant.

28. The vaccine of claim 22 further comprising a pharmaceutically acceptable carrier.

29. The vaccine of claim 22 wherein the carrier is suitable for intranasal or intramuscular administration.

30. The vaccine of claim 20 which is in freeze-dried form.

31. A method to immunize a mammal against influenza, comprising administering to the mammal an effective amount of the virus of claim 20.

32. The method of claim 31 wherein the mammal is a dog or a horse.

33. A diagnostic method comprising contacting a physiological sample of an animal suspected of containing anti-influenza virus antibodies with the virus of claim 1, 7 or 20; and determining whether the sample comprises antibodies specific for the virus.

* * * * *