

| Segment | Position ^a | AIV residues | H3N2 CIV residues | H3N8 CIV residues | Function ^c |
|---------|-----------------------|--------------|-------------------------|-------------------|--|
| PB2 | 76 | T(96.5) | M(93.6)/I(17.4) | T(100) | Within PB1 and NP-binding domain (1) |
| | 147 | I(78) | T(100) | V(100) | Within NP-binding domain (1) |
| | 365 | M(97) | I(100) | M(100) | Within cap binding domain (2) |
| | 570 | M(94) | V(100) | M(100) | Within PB1 and NP-binding domain (1) |
| PB1 | 108 | L(96) | I(100) | L(100) | Within RNA binding domain (3) |
| | 361 | S(94) | N(100) | S(100) | Virulence marker (4) |
| | 377 | D(97) | N(78.3)/D(21.7) | D(100) | Within RNA binding domain (3) |
| | 517 | I(96) | V(95.64)/I(4.34) | I(100) | Host range (5) |
| | 723 | R(97) | Q(93.6)/R(17.4) | R(100) | N/A |
| | 744 | M(94) | V(100) | M(100) | N/A |
| PA | 65 | S(93) | Y(100) | S(100) | Host adaptation (6) |
| | 208 | T(93) | A(100) | T(100) | Within translocation domain(7) |
| | 234 | D(98) | N(83.3)/D(16.7) | D(100)d | Within translocation domain(7) |
| | 347 | D(99) | N(29.2)/D(70.8) | D(100) | N/A |
| | 369 | A(96) | V(100) | A(100) | N/A |
| | 441 | M(93) | K(87.5)/R(12.5) | M(95.1)/I(4.9) | N/A |
| | 615 | K(94) | R(100) | K(100) | Polymerase activity and adaptation (8) |
| HA | 10 | T(95) | A(100) | T(100) | N/A |
| | 81 | D(74) | N(100) | Y(98.75)/H(1.25) | Glycosylation sequon (9) |
| | 111 | L(96) | I(83.3)/V(16.7)* | I(100) | N/A |
| | 172 | D(95) | N(100) | E(8.75)/K(91.25) | N/A |
| | 222 | W(90) | L(100)* | L(100) | Receptor binding (10, 11) |
| | 435 | H(96) | N(91.7)/H(4.15)/K(4.15) | H(100) | N/A |
| | 489 | D(92) | N(100) | Y(100) | N/A |
| NP | 52 | Y(58) | H(95.8)/Y(4.2)* | H(69.8)/Y(30.2) | N/A |
| | 109 | I(97) | I(62.5)/V(37.5) | I(100) | Host range (12) |
| | 125 | N(95) | G(91.7)/N(8.4) | N(100) | N/A |
| | 159 | M(95) | L(91.7)/M(8.4) | M(100) | N/A |
| | 373 | T(50) | K(95.8)/T(4.2) | T(100) | Putative NLS (13) |
| | 428 | A(94) | T(95.8)/A(4.2) | A(100) | N/A |
| | 452 | R(79) | K(95.8)/R(4.2)* | K(100) | N/A |
| | 473 | N(93) | K(95.8)/N(4.2) | N(100) | N/A |
| NA | 24 | M(95) | L(100) | N/A | N/A |
| | 48 | N(80) | S(91.7)/N(8.3) | N/A | N/A |
| | 54 | E(89) | K(100) | N/A | N/A |
| | 81 | P(74) | S(100) | N/A | N/A |
| | 143 | D(62) | N(100) | N/A | N/A |
| | 156 | P(96) | S(100) | N/A | N/A |
| | 372 | S(95) | L(100) | N/A | Sialidase activity (14) |
| | 432 | R(96) | G(100) | N/A | N/A |
| NS1 | 60 | A(87) | I(100) | A(99.46)/T(0.54) | Within RNA-binding domain (15) |
| | 67 | R(91) | W(100) | Q(100) | Within RNA-binding domain (15) |
| | 75 | E(85) | K(100) | E(100) | Within effector domain (15) |
| | 152 | E(67) | N(100) | E(99.46)/D(0.54) | Within effector domain (15) |
| | 172 | E(95) | K(100) | E(100) | CPSF30 binding (15) |

Table 1. Amino acid changes that differentiate H3N2 CIV from AIVs. Fixed mutations are shown in bold. Asterisks indicate convergent mutations between H3N2 and H3N8 CIVs. ^a Codon position. H3 numbering is used for codon positions in HA. ^b Amino acid present at the consensus level. The prevalence of each amino acid is shown in brackets. ^c Putative function of the amino acid residue or domain in which is located.

1. **Poole E, Elton D, Medcalf L, Digard P.** 2004. Functional domains of the influenza A virus PB2 protein: identification of NP- and PB1-binding sites. *Virology* **321**:120-133.
2. **Guilligay D, Tarendeau F, Resa-Infante P, Coloma R, Crepin T, Sehr P, Lewis J, Ruigrok RW, Ortin J, Hart DJ, Cusack S.** 2008. The structural basis for cap binding by influenza virus polymerase subunit PB2. *Nature structural & molecular biology* **15**:500-506.
3. **Gonzalez S, Ortin J.** 1999. Distinct regions of influenza virus PB1 polymerase subunit recognize vRNA and cRNA templates. *The EMBO journal* **18**:3767-3775.
4. **Allen JE, Gardner SN, Vitalis EA, Slezak TR.** 2009. Conserved amino acid markers from past influenza pandemic strains. *BMC microbiology* **9**:77.
5. **Tamuri AU, Dos Reis M, Hay AJ, Goldstein RA.** 2009. Identifying changes in selective constraints: host shifts in influenza. *PLoS computational biology* **5**:e1000564.
6. **Miotto O, Heiny AT, Albrecht R, Garcia-Sastre A, Tan TW, August JT, Brusich V.** 2010. Complete-proteome mapping of human influenza A adaptive mutations: implications for human transmissibility of zoonotic strains. *PloS one* **5**:e9025.
7. **Nieto A, de la Luna S, Barcena J, Portela A, Ortin J.** 1994. Complex structure of the nuclear translocation signal of influenza virus polymerase PA subunit. *The Journal of general virology* **75 (Pt 1)**:29-36.
8. **Gabriel G, Dauber B, Wolff T, Planz O, Klenk HD, Stech J.** 2005. The viral polymerase mediates adaptation of an avian influenza virus to a mammalian host. *Proceedings of the National Academy of Sciences of the United States of America* **102**:18590-18595.
9. **Zhang M, Gaschen B, Blay W, Foley B, Haigwood N, Kuiken C, Korber B.** 2004. Tracking global patterns of N-linked glycosylation site variation in highly variable viral glycoproteins: HIV, SIV, and HCV envelopes and influenza hemagglutinin. *Glycobiology* **14**:1229-1246.
10. **Collins PJ, Vachieri SG, Haire LF, Ogrodowicz RW, Martin SR, Walker PA, Xiong X, Gamblin SJ, Skehel JJ.** 2014. Recent evolution of equine influenza and the origin of canine influenza. *Proceedings of the National Academy of Sciences of the United States of America* **111**:11175-11180.
11. **Yang G, Li S, Blackmon S, Ye J, Bradley KC, Cooley J, Smith D, Hanson L, Cardona C, Steinhauer DA, Webby R, Liao M, Wan XF.** 2013. Mutation tryptophan to leucine at position 222 of haemagglutinin could facilitate H3N2 influenza A virus infection in dogs. *The Journal of general virology* **94**:2599-2608.
12. **Chen GW, Chang SC, Mok CK, Lo YL, Kung YN, Huang JH, Shih YH, Wang JY, Chiang C, Chen CJ, Shih SR.** 2006. Genomic

- signatures of human versus avian influenza A viruses. *Emerging infectious diseases* **12**:1353-1360.
13. **Hutchinson EC, Fodor E.** 2012. Nuclear import of the influenza A virus transcriptional machinery. *Vaccine* **30**:7353-7358.
 14. **Kobasa D, Kodihalli S, Luo M, Castrucci MR, Donatelli I, Suzuki Y, Suzuki T, Kawaoka Y.** 1999. Amino acid residues contributing to the substrate specificity of the influenza A virus neuraminidase. *Journal of virology* **73**:6743-6751.
 15. **Hale BG, Randall RE, Ortin J, Jackson D.** 2008. The multifunctional NS1 protein of influenza A viruses. *The Journal of general virology* **89**:2359-2376.