

Loss of hemagglutination ability by H3N2 influenza A virus, subclade K

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Seasonal human H3N2 influenza viruses, subclade K (J.2.4.1), have been the predominant influenza A viruses in the Northern hemisphere influenza season of 2025/2026. Since 2024, the vaccine virus A/Darwin/6/21 has emerged into different antigenic variants. To assess antigenicity, the hemagglutination inhibition (HI) assay is the most widely used, and a loss of binding to turkey erythrocytes could significantly hamper this process. Antigenic changes are frequently caused by amino acid substitutions near the hemagglutinin (HA) receptor-binding pocket, which can also affect receptor binding properties, such as hemagglutination. In this study, we explored how substitutions in or around the HA receptor-binding site affect binding to glycans at the molecular level. We employed ELISA, glycan array, flow cytometry, hemagglutination assays, and tissue staining. Substitutions at positions 140, 192, and 223 establish a new HA background of the viruses that emerged in 2024. Computational analysis of HA in complex with elongated glycan reveals that mutation F192 forms a CH-Pi to stabilize the binding. Based on this background, substitutions in antigenic sites A and B within subclade K viruses exhibit a binding preference to elongated glycans, which are not displayed on turkey erythrocytes. Conversely, our previously established glyco-remodeled erythrocytes are efficiently bound by these subclade K H3N2 viruses and could support influenza surveillance and vaccine development.

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Yes, I am a junior scientist.

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