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## Development of 6E3 antibody-mediated SERS immunoassay for drug-resistant influenza virus

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### Abstract

Influenza viruses are responsible for several pandemics and seasonal epidemics and pose a major public health threat. Even after a major outbreak, the emergence of drug-resistant influenza viruses can pose disease control problems. Here we report a novel 6E3 monoclonal antibody capable of recognizing and binding to the H275Y neuraminidase (NA) mutation, which has been associated with reduced susceptibility of influenza viruses to NA inhibitors. The 6E3 antibody had a  $K_D$  of 72.74  $\mu\text{M}$  for wild-type NA and 32.76  $\mu\text{M}$  for H275Y NA, suggesting that it can identify drug-resistant pandemic H1N1 (pH1N1) influenza virus. Molecular modeling studies also suggest the high-affinity binding of this antibody to pH1N1 H275Y NA. This antibody was also subject to dot-blot, enzyme-linked immunosorbent assay, bare-eye detection, and lateral flow assay to demonstrate its specificity to drug-resistant pH1N1. Furthermore, it was immobilized on Au nanoplate and nanoparticles, enabling surface-enhanced Raman scattering (SERS)-based detection of the H275Y mutant pH1N1. Using 6E3 antibody-mediated SERS immunoassay, the drug-resistant influenza virus can be detected at a low concentration of  $10^2$  plaque-forming units/mL. We also detected pH1N1 in human nasopharyngeal aspirate samples, suggesting that the 6E3-mediated SERS assay has the potential for diagnostic application. We anticipate that this newly developed antibody and SERS-based immunoassay will contribute to the diagnosis of drug-resistant influenza viruses and improve treatment strategies for influenza patients.

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