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Enhanced Near-Infrared Upconversion Emission Based Lateral Flow Immunoassay for Background-Free Detection of Avian Influenza Viruses

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Abstract : Avian influenza viruses (AIV) are the primary cause of highly contagious respiratory diseases caused by type A influenza viruses of the Orthomyxoviridae family. AIV are categorized on the basis of types of surface glycoproteins such as hemagglutinin and neuraminidase. Certain H5 and H7 subtypes of AIV have evolved to the high pathogenic avian influenza (HPAI) virus, which has caused considerable economic loss to the poultry industry and led to severe public health crisis. Several commercial kits have been developed for on-site detection of AIV. However, the sensitivity of these methods is too low to detect low virus concentrations in clinical samples and opaque stool samples. Here, we introduced a background-free near-infrared (NIR)-to-NIR upconversion nanoparticle-based lateral flow immunoassay (NNLFA) platform to yield a sensor that detects AIV within 20 minutes. Ca^{2+} ion in the shell was used to enhance the NIR-to-NIR upconversion photoluminescence (PL) emission as a heterogeneous dopant without inducing significant changes in the morphology and size of the UCNPs. In a mixture of opaque stool samples and gold nanoparticles (GNPs), which are components of commercial AIV LFA, the background signal of the stool samples mask the absorption peak of GNPs. However, UCNPs dispersed in the stool samples still show strong emission centered at 800 nm when excited at 980 nm, which enables the NNLFA platform to detect 10-times lower viral load than a commercial GNP-based AIV LFA. The detection limit of NNLFA for low pathogenic avian influenza (LPAI) H5N2 and HPAI H5N6 viruses was 10^2 EID_{50}/mL and $10^{3.5}$ EID_{50}/mL, respectively. Moreover, when opaque brown-colored samples were used as the target analytes, strong NIR emission signal from the test line in NNLFA confirmed the presence of AIV, whereas commercial AIV LFA detected AIV with difficulty. Therefore, we propose that this rapid and background-free NNLFA platform has the potential of detecting AIV in the field, which could effectively prevent the spread of these viruses at an early stage.

Keywords : avian influenza viruses, lateral flow immunoassay on-site detection, upconversion nanoparticles
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