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Enhanced sensitivity of lateral flow immunoassay for double-antigen detection of influenza A using dual-layered gold nanoparticles

We designed and developed a highly sensitive lateral flow immunoassay (LFIA) for double-antigen detection of influenza A. The sensitivity enhancement of the assay was achieved by using two-layered gold nanoparticles in combination with double-target detection format. First, gold nanoparticles were conjugated to monoclonal antibodies (mAb) specific to the two most abundant influenza A proteins, nucleoprotein (NP) and matrix protein (M) and used as detector probes to detect two target antigens simultaneously. Then, the detection signal was enhanced via a signal amplification strategy of two-layered gold nanoparticles. Gold nanoparticles with the size of 15 nm and 40 nm were used to form a complex for signal amplification. Under optimum conditions, the system is capable to detect influenza A antigens in infected cells at levels as low as $47 \text{ TCID}_{50} \cdot \text{mL}^{-1}$, which was lower than the conventional LFIA based on single-target detection. This proof-of-principle of dual-layered and double-targeted gold nanoparticles based LFIA is promising for further development of single-step, rapid, and sensitive tests for screening and diagnosis of various diseases.

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