

**Title :** Development of Competitive Electrochemical Immunosensor based on Gold Nanoparticle-Sandwiched Structure for SARS-CoV-2 Detection.

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## ABSTRACT

The COVID-19 pandemic, caused by the SARS-CoV-2 virus, has significantly impacted global public health and economies, highlighting the urgency for fast, sensitive, and reliable diagnostic tools. Rapid and precise detection of viral antigens is key to controlling the spread of infections and enabling swift treatment. In this study, we successfully developed a competitive electrochemical immunosensor for detecting the SARS-CoV-2 antigen, utilizing a gold nanoparticle-sandwiched structure composed of polyethyleneimine-coated gold nanoparticles (PEI-AuNPs) and nanotags. A 40-fold dilution of PEI-AuNPs is optimal for modifying a screen-printed carbon electrode (SPCE), offering a high specific surface area, rapid electron transfer, excellent electrical conductivity, and good biocompatibility for antigen loading. Subsequently, the spike protein S1+S2 is immobilized onto the modified electrode, resulting in the formation of spike protein S1+S2/PEI-AuNPs/SPCE for specific recognition of antibody. In parallel, gold nanotags were prepared by conjugating PEI-AuNPs with an anti-spike protein S1+S2 antibody, forming PEI-AuNPs/anti-spike protein S1+S2. In the presence of the target, immunocomplexes form through antigen-antibody interactions in the gold nanotag solution. Furthermore, the interaction between spike protein S1+S2/PEI-AuNPs/SPCE and gold/antibody nanotags forms competitive immunocomplexes, facilitating the sensitive detection of the SARS-CoV-2 antigen. Under optimal conditions, the sensor achieved sensitive detection of SARS-CoV-2 antigen within a range of 0.10 to 100 ng/mL with a limit of detection (LOD) of 0.09 ng/mL. The electrochemical immunosensor exhibits high selectivity, reliable reproducibility, and exceptional stability. In addition, the proposed competitive immunosensor can detect SARS-CoV-2 in human serum samples with a good recovery. The outstanding performance offers an important tool for the early screening and detection of the SARS-CoV-2 antigen, especially in combating the ongoing COVID-19 pandemic.

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