

Ultrasensitive immunoassay for E.coli O157 using DNA-gold nanoparticles assisted enzyme amplification method

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E.coli (*Escherichia coli*) O157 can cause serious food poisoning in human. Immunoassay based on the enzyme amplification for the detection of protein or pathogenic bacteria typically involves two types of particles, a magnetic microparticle (MMP) functionalized with an monoclonal antibody that has an affinity for a target of interest and an Au nanoparticle (AuNP) functionalized with a polyclonal antibody that has an affinity for the same target of interest. The DIG-labeled Oligonucleotides with thiol terminal group was covalently attached on the AuNP. And then HRP labeled anti-DIG was bound to DIG labeled DNA on AuNP. By this process, the number of HRP on AuNP could be enhanced and effectively act as reporter groups for the target of interest. The immobilized enzyme on AuNP probes is reacted with tetramethylbenzidine (TMB) that is a substrate of HRP, followed by stopping the reaction with 2M H₂SO₄. The resulting end products were analyzed by UV-vis spectroscopy.