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Title: The nanoparticle based optical and electrochemical immunosensor for detection of hepatitis B surface antigen

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Abstract: The high affinity of antigen-antibody interaction is the basis for precise detection and diagnosis of antigens by means of immuno-sensor. In the present study, immunoassay was incorporated with two strong physical methods: chemiluminescence and electrochemistry, to develop the immuno-sensor for detection of hepatitis-B virus at low concentration. In the first method, hepatitis B surface antigen (HBs-Ag) was detected by a chemiluminescence immunosensor. In a sandwich type immunoassay method, the primary antibody (anti-hepatitis B surface antigen, anti-HBs-Ag) was immobilized in polystyrene wells and the secondary antibody was conjugated to luminol coated gold nanoparticles (GNPs) as label. Then, HBs-Ag was conjugated between primary and secondary antibodies. The immunosensor responded toward HBs-Ag in a wide linear range of 0.125 to 30 ng/ml. The proposed method has successfully applied to determine the HBs-Ag in patient sera with a detection limit of 14 pg/ml (signal/noise=3). In the electrochemical method, the biotinylated hepatitis B surface antibody was immobilized on streptavidin-magnetic nanoparticles and used for targeting the HBsAg. In the presence of horseradish peroxidase conjugated secondary antibody (HRP-HBsAb), a sandwich-type immunoassay format was formed. O-aminophenol and hydrogen peroxide as substrates for HRP were used to produce 3-aminophenoxazone (3-APZ). The electroactive enzymatic production (3-APZ) was transferred into an electrochemical cell and monitored by voltammetry. Under optimal conditions, the cathodic current response of 3-APZ which was proportional to HBsAg concentration was measured by a glassy carbon electrode. The immunosensor response was linear towards HBsAg in the concentration range from 0.001 to 0.015 ng/ml with a detection limit of 0.9 pg/ml (signal/noise=3). Both proposed methods were successfully applied to determine the HBs-Ag in patient sera.

Hepatitis B surface antigen, Luminol, Gold nanoparticles, Chemiluminescence, Immunosensor; Magnetic nanoparticles; Aminophenole; Aminophenoxazone

Presentation: Oral