0612 ENZYME AMPLIFIED TIME-RESOLVED FLUOROIMMUNOASSAYS.

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The advantages of enzyme immunoassay and Eu^{3+}-chelate based time-resolved fluorometry, TRF are combined for the first time, to give improved heterogeneous immunoassays. Horseradish peroxidase (HRP) is used to label the immunoreactants. The hydrogen donor is a biotin-tyramine conjugate (BT). In the presence of H_2O_2, peroxidase catalyzes the covalent attachment of many BT molecules to the proteins which are coated on the solid phase. The mechanism of BT attachment may involve free radical formation and reaction with electron rich moieties on the solid phase (e.g. tyrosines of protein). Therefore, the tyramine moiety of BT acts as an anchor to the bottom while biotin is exposed for binding to labelled streptavidin. The biotins are quantitated on the solid phase by adding a Eu^{3+} containing highly fluorescent streptavidin reagent of the form $SA[TG_3(BCPDA)_{480}]$ where TG: thyroglobulin and BCPDA: 4,7-bis (chlorosulfophenyl)-1,10-phenanthroline-2,9 dicarboxylic acid, a Eu^{3+} chelator. A "two-site" assay for AFP is used to test the performance of the proposed configuration. The assay protocol is: Pipet 50 µL of sample (range 0.02 to 5 ng/mL) and 50 µL of biotinylated anti-AFP into antibody coated polystyrene microtiter wells. Incubate 2h. Add 100 µL of HRP labelled streptavidin. Incubate 15 min. Add 100 µL of H_2O_2/BT solution. Incubate 30 min. Add 100 µL of a 0.3 mg/L $SA[TG_3(BCPDA)_{480}]$ solution. Incubate 30 min. Measure the Eu^{3+}-fluorescence on the washed and dried solid phase with CFI-615 Immunoanalyzer. The optimized assay was compared with the conventional TRF assay where $SA[TG_3(BCPDA)_{480}]$ is added immediately after the sandwich is formed. A 20-fold improvement in the signal and a 6 to 8 fold improvement in the signal to background (S/B) ratio was achieved. The precision was 8% and the highest measurable AFP level was 5 ng/mL. Furthermore, we conjugated HRP with the AFP antibody and performed the same assay but eliminating the SA-HRP addition step. The results were similar as in the first protocol. In conclusion, the extension of the capabilities of TRF-immunoassays was achieved by using enzymatic generation of immobilized biotins.