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NEW ASSAY CONFIGURATIONS FOR COMPETITIVE-TYPE TIME-RESOLVED FLUOROIMMUNOASSAYS OF HAPTENS.

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We previously described a time-resolved fluorescence immunoassay system suitable for competitive and non-competitive type assays (Diamandis, E.P., Clin Biochem, 1988; 21:139-50). In this system, haptens were measured using the immobilized antigen approach. We now report new assay configurations based on the antibody immobilization approach. For a thyroxine (T4) or a triiodothyronine (T3) assay, specific antibodies are immobilized in white opaque microtitration wells. In the assay, T4 or T3 present in the sample, released from their binding proteins by thimerosal, compete with a T4-biotin or a T3-biotin conjugate for binding to the specific antibody. Alternatively, T4-protein-biotin and T3-protein-biotin conjugates can be used. After incubating for 1h at room temperature, all unbound species are washed out and the degree of binding of the biotinylated conjugate (which is inversely related to the concentration of T4 or T3 in the sample) is quantified by adding streptavidin labeled with the europium chelator EuroFluor S® [4,7-bis(chlorosulfophenyl)-1, 10-phenanthroline 2,9 dicarboxylic acid] in the presence of excess Eu^{3+}. The fluorescence of the final immunocomplex, e.g., antibody-T4-biotin-streptavidin-EuroFluor S®-Eu^{3+}, is measured on the dried solid-phase by time-resolved fluorometry. The assay characteristics were found to be similar to those of other immunological techniques for these analytes. These assay configurations have general applicability for the measurement of antigens in serum.