for 10 min; the released 5-fluorosalicylate (FSA), but not FSAP, forms highly fluorescent complexes with Tb-EDTA at pH 13, which are long lived and thus quantified by time-resolved fluorometry. The assay has the following characteristics: detection limit 10 nmol/L; recovery 98-108%; within run imprecision 2-4%; between run imprecision 3-5%. Comparison with a radioimmunoassay (RIA) method (x) gave the correlation (n=80) y = -1.03 +1.03 x; r=0.98. The above method is very fast, reliable and suitable for routine use.

53 Total thyroxine immunoassay based on enzymatically amplified time-resolved fluorometry

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We have developed a competitive-type immunoassay for total thyroxine (T4) in serum using a new assay concept, as follows:

Thyroxine is labelled with the enzyme Alkaline Phosphatase (ALP). T4-ALP competes with T4 in the sample (released from serum binding proteins by anilinonaphthalene sulfonic acid and salicylate) for binding to a monoclonal anti-thyroxine antibody. The competition occurs in a microtiter well coated with goat anti-mouse immunoglobulin (GAM Ig). After incubation for 30 min, the microtiter well is washed. The following immunocomplex is formed on the solid phase: GAM Ig-mouse anti-T4-T4-ALP. The activity of ALP, which is inversely related to the amount of T4 in the sample, is quantified with a new detection technique as follows: 5-fluorosalicyl-phosphate (FSAP) is used as the enzyme substrate.