

42 Time-resolved enzyme immunoassay for the determination of thyrotropin in serum

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A highly sensitive immunoenzymometric assay for human thyrotropin (hTSH) has been developed. The assay is performed in white opaque microtitration wells and uses a solid-phase monoclonal capture antibody and a polyclonal detection antibody conjugated to alkaline phosphatase. In this one-step assay format, the standards or samples and the alkaline phosphatase conjugate are added into the wells and incubated at room temperature. After the incubation, the wells are washed and the enzymatic activity which is proportional to the concentration of hTSH in the sample, is quantified with a new detection technique as follows: the enzyme substrate, Diflunisal Phosphate (DFP) is added into the wells and incubated for 10 min; the released Diflunisal (DF) forms highly fluorescent complexes with Tb-EDTA at pH 13, which are long lived and thus quantified by time-resolved fluorometry (Cyberfluor™ 615 Immunoanalyzer). The assay has the following characteristics: detection limit 0.007 mIU/L; dynamic range up to 100 mIU/L; intra-assay imprecision 2 - 3%; inter-assay imprecision 3 - 5%; average recovery $97.9 \pm 4.9\%$; cross-reactivity with pituitary glycoprotein hormones was negligible.

Comparison with an established IRMA assay (x) gave the regression equation $y = 0.21 + 0.93x$, $r = 0.995$, $n = 40$.