

monoclonal capture antibody and a polyclonal detection antibody labelled with Alkaline Phosphatase (ALP). In this two-step assay, 25 μL of the standards or samples and 50 μL of the assay buffer are pipetted into the coated wells and incubated for 1 h at room temperature. After washing the wells, detection antibody labelled with ALP is added and incubated for another 30 min. After washing, the amount of bound antibody which is proportional to the AFP concentration in serum, is determined by measuring the activity of the ALP. The enzymatic activity is quantified with a new detection technique as follows: Diflunisal Phosphate (DFP) is used as the enzyme substrate for 10 min; the released Diflunisal (DF) but not the DFP, 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 0.03 $\mu\text{g/L}$; recovery $94 \pm 7.6\%$; within-run imprecision 2-3%; between run imprecision 4-5%. Interference testing, including albumin, bilirubin, lipids and hemoglobin shows no significant effects on the assay. Comparison of this AFP assay with an established enzymatic assay gave the following regression equation: $y = 1.82 + 0.93x$, $r = 0.995$ ($n = 45$). The above method is fast, very sensitive and suitable for routine use.

“ A highly sensitive alpha-fetoprotein (AFP) immunoassay based on enzymatically amplified time-resolved fluorometry

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We describe an immunoassay for the determination of AFP in human serum. The assay is performed in white opaque microtitration wells and uses a solid-phase