

Thesis Title Bioluminescence assay for Totale Bile Acids and
Primary Bile Acids in serum using coimmobilized Enzymes.

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ABSTRACT

A simple rapid, and sensitive bioluminescence method for measuring total bile acids and primary bile acids has been developed and validated. The method is based on enzymatic dehydrogenation of 3α -hydroxybile acid and 7α -hydroxybile acid using 3α - and 7α -hydroxysteroid dehydrogenase, those are coimmobilized on Sepharose 4B beads with diaphorase, and bacterial luciferase. The assay is specific for 3α -hydroxybile acids and 7α -hydroxybile acid. The detection limit of total bile acids assay is $3 \mu\text{Mol/l}$, and $2 \mu\text{Mol/l}$ for primary bile acids assay. The detection limit is depend on activity of enzymes that coimmobilized on the Sepharose bead. In both assay shows good precision (5-10 % within run, 8-10 % between run in total bile acids, and 7-10 % within run, 9-10 % between run in primary bile acids). The recovery of added standards in both assay were satisfactory. The values obtained with the bioluminescence assay of total bile acids showed good agreement with those obtained by endpoint enzymatic spectrophotometric assay revealed an excellent correlation ($r = 0.91$, $Y = 3.67 + 0.26X$, $n = 50$). Comparison of the values by total bile acids and primary bile acids assay in the patients with liver disease, shows that the secondary bile acids higher than primary bile acids, and it shows correlation of total bile acids and primary bile acids ($r = 0.828$, $Y = 0.226X - 1.269$, $n = 46$). Both methods were no interference from the effect of other dehydrogenase activity in serum.