Thesis Title Estimation of Serum Thyroxine by Magnetic Cellulose Solid-phase

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ABSTRACT

Misclassification error can arise from incomplete separation of bound and free antigen, so the separation technique is an essential step in RIA, which is the most commonly achieved by centrifugation. Many of the existing methods are complex and time-consuming. An attempt was made to develop and optimise the solid-phase RIA for serum thyroxine utilizing magnetic cellulose as a solid matrix. Activation of magnetic cellulose with 1,1'- carbonyldiimidazole (CDI) was performed and found to be simple, rapid and reproducible. Sheep anti-thyroxine antiserum was coupled to CDI-activated magnetic cellulose under optimised condition producing covalent linkage between magnetic cellulose and the antibody. Concentrations of assay reagents such as ANS, BSA, Triton X-100 and coupled magnetic cellulose were optimised and assessed to obtain the best fitting curve. Optimal incubation time to reach equilibrium was only 2 h at ambient temperature with continual mixing. The separation procedure was performed on a magnetic separator that enables washing steps to be carried out rapidly and efficiently with reduction of non-specific

binding effect.

Complete quality control (QC) profiles in details for the magnetic cellulose RIA, such as reproducibility, accuracy, specificity, sensitivity, precision profile, parallelism and drift tests were investigated. Thus simple, rapid, inexpensive, sensitive, precise and specific RIA for total serum thyroxine was established and adopted.

The correlation coefficient (r) of the conventional magnetic cellulose and the PEG assisting double (second) antibody separation was excellent ($r = 0.987$). This permits discrimination between euthyroid subjects from patients with thyroid disorders.

In addition, the magnetic cellulose RIA was applied to get the normal range which would discriminate euthyroid neonates from the thyroid diseases. Therefore, serum thyroxine concentrations in 167 newborns during the first 7 days of life were measured and found that the mean value ($\pm$ SD) was 11.73 ± 3.36 ug/dl.

The solid-phase RIA using magnetic cellulose particles is suitable for routine use and applicable in any laboratory that does not have a centrifuge particularly those in provincial hospitals.

Magnetic cellulose particles offer an excellent solution to a number of problems associated with the separation step in RIA and related techniques. They are universally applicable and convenient for use.