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DUSIT ROONPHO : OPTIMISATION AND APPLICATION OF
SERUM TRIODOTHYRONINE (T₃) BY SOLID - PHASE ANTIBODY.
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Separation of the bound antigen - antibody complex from the free fraction is one of the major sources of error in an RIA test. Solid - phase RIA by magnetic particle method has been shown to be simple and rapid, requiring a few minutes to complete separation. Therefore, the object of this study is to devise, in - house develop and optimise a solid - phase RIA for serum T₃. Twenty to forty microns of magnetic cellulose particles which were activated with 1,1'-carbonyldiimidazole (CDI). Neat T₃ - antiserum was then coupled to the CDI - activated magnetic cellulose in the ratio of 1 : 10 (v/w) at room temperature (20 - 25 °C) for 48 hrs with continuous mixing, producing the immunoabsorbent or immobilisation of T₃ - antibody on the surface of magnetic cellulose. The activation and coupling procedure was found to be easy to prepare, reproducible and less hazardous than cyanogen bromide method. Stability of the immunoabsorbent in phosphate buffer pH 7.4 at 4 °C was examined at various time intervals up to 15 months.

Selections of reagents, correct incubation time and optimisation of the assay protocol are important factors in achieving satisfactory assay performance. Under optimised conditions, concentrations of 0.15 mg immuno-adsorbent / tube, 2.0 mg ANS/ml, 0.2 % BSA (w/v) and 0.2 % Triton X -100 (v/v) were used throughout this study. Magnetic separation was carried out after 4 hrs incubation at ambient temperature to accomplish the best fitting curve.

In order to monitor validation of assay techniques, QC profiles in details such as sensitivity, precision, accuracy, specificity, precision profile and drift effect were evaluated and satisfactory results were obtained. Correlation coefficient (r) between the solid - phase RIA and the charcoal separation as a reference method was excellent (r = 0.993). Thus inexpensive, simple, rapid, precise, sensitive and specific RIA for total serum T₃ was established and adopted. This solid - phase RIA was applied to get the normal range of 75 - 173 ng/dl with the mean (± SD) of 123.8 ± 24.5 ng/dl from 286 adult euthyroid subjects, yielding very good discrimination (p < 0.0005) between euthyroids and patients with hypothyroid, hyperthyroid and T₃ - toxicosis. Moreover, the mean values of serum T₃ in aging euthyroids from Bangkok (112.6 ± 25.5 ng/dl) and Ubonrajchathani (113 ± 24.6 ng/dl) provinces were significantly lower (p < 0.0005) than in adult euthyroids.

The magnetic particle technique can be universally applied in many RIAs (limited reagent immunoassays) and is also ideally suited to immunometric assays (excess reagent immunoassays), when may lead to improvement in the diagnosis of endocrinopathies. Therefore, measurements of serum T₃ by solid - phase should be a useful adjunct to both clinical diagnosis and physiological evaluation of thyroid functions, especially suspected T₃ - toxicosis.