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Tumor markers defined as biochemical substances that are produced by tumors or produced by the host in response to a tumor. They can be detected in the serum or other body secretions.

Early detection of cancer offers the best chance for cure. The goal is to early diagnose cancer when a tumor is still small enough to be completely removed surgically. Alphafetoprotein (AFP), one of the widely used oncofetal antigens. It is an important diagnostic marker for primary liver cancer and teratocarcinomass. Due to increased consumption of AFP kits in Egypt, it has been found necessary to start the local preparation and evaluation of primary reagents for radioimmunoassay of AFP.

The preparation of the primary reagents of AFP assay is important to formulate RIA kit. The primary reagents include the radioiodinated tracer, anti-AFP antibody, standards and quality control (QCs). This work concentrated on preparation of the radioiodinated tracer with different techniques. In these techniques, different oxidizing agents like chloramine-T, Iodogen, N-bromosuccinimide and lactoperoxidase were used.

The different conditions for radioiodination were tested in order to optimize the best radioiodination conditions. The different parameters studied were the

following reaction time, reaction volume, oxidizing agent concentration and reaction pH. The influence of the storage condition on the stability of the iodinated product was also studied in order to minimize the degradation of the iodinated product (tracer) during storage. The factors studied were storage temperature and specific activity.

From this work, chloramine-T was found to be the suitable oxidizing agent which gave high yield and fast iodination. Iodogen was come in the second order after chloramine-T. This is a solid phase oxidizing agent that can be removed after complete iodination from reaction mixture.

N-bromosuccinimide came after the iodogen where it is a mild oxidizing agent. At the end lactoperoxidase was used in presence of hydrogen peroxide. This is enzymatic-oxidizing agent so the potential damage of protein is very low.

The second primary reagent in RIA kit is standards. In this work, standards were prepared using cord blood sample. The cord blood sample contains high level of AFP concentration. Firstly, the cord blood serum was estimated using NETRIA - IRMA kit and the cord blood serum sample was contained about 40 KIU/ml. After that, the cord blood serum was purified by using ammonium sulphate precipitation method. Then, U.V spectrophotometer was used to estimate the accurate concentration of AFP in this sample. The cord blood serum was diluted with IRMA assay buffer to give set of standards from 5 to

500 IU/ ml. IRP standards and NETRIA-IRMA kit were used to correct the value of AFP set of standards.

The third primary reagent in RIA kit is a polyclonal anti-AFP antibody. The study comprised 4 New-Zealand rabbits, weighing 2000 - 30000 gm. They were kept under the same hygienic conditions, well balanced diet and water was supplied. Immunizing the four rabbits with highly purified AFP-antigen (0.1 mg AFP per rabbit) was carried out for the production of the polyclonal anti-AFP antibody. This production was carried out through, primary immunization and 4 boosters.

Immunogen was prepared by mixing and emulsifying 0.5 mg of highly purified AFP antigen in 0.4 ml sterile distilled water and 1.6 ml of Freund's complete adjuvant. The mixing between the aqueous phase (AFP) and oil phase (adjuvant) was performed 300 times until stable water in oil emulsion was produced.

The first booster was given after one month from the primary immunization. The second one was given after three weeks interval from the first one. In booster dose the complete Freund's adjuvant was replaced by incomplete adjuvant. Blood samples were collected individually just at the day before the subsequent booster immunization. The collected antisera were assessed in terms of titre using double antibody RIA technique.

Formulation of AFP RIA kit was carried out after the preparation of all primary reagents were completed. The RIA reagents prepared were the tracer, standards, quality control sera and anti-AFP-antibody in addition to separating reagents. The separating reagents are nonimmunorabbit serum, Donkey antirabbit serum in addition to polyethylene glycol (8000). These reagents come through TC-project (No. 9914/$R_0$) with the International Atomic Energy Agency (IAEA).

The RIA-AFP assay is carried out as follows: in duplicate tubes, 100 µl anti-AFP-antibody (1:50,000), 100 µl tracer were added to 100µl sample or standard and the tubes were incubated at 37°C for about 3hs. Then, 100 µl non-immunorabbit serum, 100µl Donkey antirabbit serum and 500 µl PEG (4%) were added. The tubes were incubated for 30 minutes at 25 °C, after that, the tubes were centrifuged at 4000 rpm for about 20 minutes at 4°C. The tubes were decanted carefully and radioactive bound fraction was counted (cpm). The optimization of the assay conditions like incubation time, sample volume and radioactivity of tracer were tested.

Due to the advantage of RIA solid phase technique compared to RIA double antibody precipitation technique the solid phase coated tubes were prepared using two techniques namely phosphate technique and borate technique.

The optimization of solid phase RIA assay was carried. The performance characteristics for the two techniques [Liquid phase and solid phase (coated tube)] were studied.

The results and observations of the present study were used to prepare a precise and sensitive assay to detect AFP concentration in human serum.