Basic Study of Angiotensin II Kit for Radioimmunoassay

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The determination of plasma angiotensin II, using a radioimmunoassay, had been reported by Valloton et al. and Catt et al. in 1967. Recently, as we had chance to use the radioimmunoassay Kit of angiotensin II which was based on the procedure described previously by Gocke, et al. and was supplied by CEA-CEN-SORIN, we report experience of its use.

(1) Adsorption ratio of ^{125}I-angiotension II for uncleaning glass tube was 1.5-4.0 times higher than they treated with silicone, chromic acid mixture, neutral cleaning material and polyethylene tube. In this fact, it is impossible to use uncleaning glass tube for this assay. The best result was gotten by siliconized tube, but in other three cases, it is possible to use. The calibration curves were almost same when used each of them.

(2) Calibration curves, using four kinds of angiotensin II-free pooled plasma which were prepared from stored blood plasma, heparinized plasma, plasma treated with EDTA and serum, were no significant difference, and if they were stored at –20°C for three months, it was possible to use for caribration curve.

(3) There was no significant difference of calibration curve between 0.2 ml of dextran-coated charcoal suspension and 0.5 ml, but in the decantation, 0.5 ml was better to decrease the counting error than 0.2 ml.

(4) It is not necessary to do pre-incubation for calibration curve, but in the case of patient samples, if pre-incubation is omitted, it is difficult to distinguish between normal and abnormal.

(5) It is necessary for assay to use the arterial blood, and to keep the temperature at 4°C during the measurement, and also necessary to clean the glass apparatus and operate the pipet exactly. As we have seen, it is possible to get the good result when users read very carefully the booklet of instruction for use with Kit.

Quantitative Measurement for Serum Autoantibody by Using Solid-State Competitive Binding Radioassay

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A new, rapid, sensitive and specific solid-state competitive binding radioassay methods for anti-thyroid antibodies and their clinical results were presented.

Cups on a disposable microtiter plate (Cooke Eng. Co.) were coated with human thyroid microsomal suspension. About 1% of radioiodinated Long-acting Thyroid Stimulator (LATS) IgG of very high biologic activity was found to be fixed onto the microsome coated cup by over-night incubation.

Addition to the incubate of unlabeled

LATS-IgG, LATS serum, anti-thyroglobulin antibody (Anti-TG) IgG from a patient with Hashimoto's thyroiditis resulted in a dose-response inhibition of the binding of the label. This binding system is proved highly specific or anfti-microsomal antibody (Anti-M), and permits a quantitative estimation of this antibody with a sensitivity of about 100 times that of immunofluorescent technique, and is able to detect the potency of antibody in only 0.05 μg standard LATS IgG.

Substituting purified thyroglobulin for microsomes and Anti-TG IgG for LATS-IgG, a quantitative binding assay for Anti-TG with comparable sensitivity was also devised. By using these two methods, serum antibody levels were studied in patients with various diseased conditions.

Of 55 untreated Graves' patients, all but one were found to have elevated serum Anti-M, and 89% had abnormal Anti-TG values.

Eighteen of 19 chronic thyroiditis were positive in Anti-M, and 86% were also abnormal in Anti-TG. All the untreated Graves' patients and patients with chronic thyroiditis were proved to have at least one of the two antibodies in their sera.

Primary hypothyroidism was also associated with high incidences of serum antibodies, however, all but one of simple or adenomatous goiter were negative in both antibodies. Anti-M were also measured in 330 hospitalized controls and their results were analysed respect to their clinical diagnoses. Thirty-one of them had moderately high serum Anti-M and 29 of them were found to be associated with either thyroid disorders, collagen or autoimmune diseases, widespread neoplasms, renal diseases, and endocrine-metabolic disorders. Elevated serum values were observed in 79 cases and 71% of them had above listed conditions, and were significantly different from the disease distribution in negative Anti-M group. Only 31% of negative group were associated with these diseases.

Demonstration of Antibody to "Alcalase" in the Laundry Detergents by Radioimmunoassay Technic

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The laundry detergents containing bacterial enzymes (Alcalase) derived from cultures of Bacillus subtilis have been marketed since several years ago. Respiratory symptoms such as asthmatic attacks, cough and chest pain were observed among workers adding powdery enzyme into the detergent. This study was undertaken to determine whether antibodies to enzymatic material could be detected in the plasma of exposed workers. Radioimmunoassay technic has proved successful in detectnig low concentrations of antibody because of its higher specificity and sensitivity than any other immune systems. Alcalase was dissolved and used for labeling and immunization of guinea pigs. Labeled Alcalase was purified on sephadex G 75 column and three peaks were obtained. These three components were incubated with 4 groups of plasma samples (exposed workers, unexposed control subjects, immunized guinea pigs and control guinea pigs) for adequate period. Incubation mixtures were applied on paper for electrophoresis, afterwards papers were scanned and contacted with film for radioautograph and finally stained with Amido Schwarz. Among three components only void volume component showed the difference as follows. In controls radioactivity migrated as a single peak in the region of