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DEVELOPMENT OF ANGIOTENSIN I AND II ASSAY. Taeko Morimoto, Masaaki Aoyama. Special Reference Laboratory Inc.

For determining Angiotensin I(AI) and Angiotensin II(AII) rapidly and sensitively, we tried to produce specific antibodies and developed a method based on florisil adsorption.

We used synthetic AI, AII to produce the antibodies, and obtained the labeled antigen from NEN.

We used activated florisil adsorption capability to extract AI and AII from plasma, and eluted with ethanol ammonia solution, and assayed by RIA after evaporative dryness.

We determined AI by competitive assay, AII by delayed assay and used polyethylene-glycol for B/F separation.

Final dilution rate of our antibodies we had were 120 thousand times for AI, 600 thousand times for AII and the cross reactivity was negligible except 34% of cross reactivity of AII with AIII.

Extraction Rates using unlabeled Antigens were 82.5% for AI, 91.2% for AII respectively.

Minimum detectable sensitivities of the methodology were 4 pg for AI, 0.75 pg for AII.

We also had sufficient results in other fundamental studies such as reproducibility, recovery test, and dilution test.

We report here that the determination of AI, AII in our sufficient method is very useful in clinical diagnosis.

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STUDIES ON HUMAN C-PEPTIDE RADIOIMMUNOASSAY KIT. T. Hori, T. Iwanami, T. Tazaki and N. Yoshitani. Shionogi Clinical Laboratory and Shionogi Kaiseki Center. Settu and Osaka

C-peptide immunoreactivity (CPR) levels were measured in 33 normal adult male subjects during 50 g OGTT using a newly established CPR radioimmunoassay kit. The fasting level of serum CPR was 1.6 ± 0.4 ng/ml (Mean \pm SD) and reached to peak between 30 to 60 min after loading, the level of which was 5.4 ng/ml. Then values declined to the fasting level within 180 min.

Values obtained by our kit (Y) were correlated to those obtained by a commercial kit "C-Peptide kit Daiichi" with equation of $Y = 0.885X - 0.25$ ($r = 0.955$). In our kit, underestimation was noted in lower dosage range.

It was confirmed that our kit provided acceptable precision in the range from 0.2 to 50 ng/ml and it had several practical advantages as compared with the ordinary conventional double-antibody methods.

These results indicate that this kit is useful clinically for measurement of pancreas β cell function.

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EVALUATION OF NORMAL RANGES BY HOFFMANN'S METHOD. K.Imamura,M.Fujii,K.Hoshi,Y.Sasaki, K.Someya,F.Asaba and T.Sakaki. St. Marianna University School of Medicine and St. Marianna University Hospital. Kawasaki.

We applied Hoffmann's method to evaluate normal ranges of RIAs on the basis of daily patient measurements. On a frequency paper, modal Gaussian distribution is extracted as a largest component. Normal range is evaluated from the modal Gaussian distribution.

We analyzed the RIAs of T₄, T₃, TSH, AFP and CEA. Number of tests analyzed was 500 to 900. Four components were observed for T₄, T₃ RIAs and three for TSH, AFP and CEA RIAs. Normal ranges by this method were somewhat lower than the current normal range in our laboratory.

Number of tests largely effects the features of a cumulative frequency curve and the normal range evaluated.

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EVALUATION OF SPLINE FUNCTION, LOGISTIC, QUADRATIC AND CUBIC LOG-LOGIT AS REGRESSION MODELS IN RADIOIMMUNOASSAY.

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Selection of regression models is extremely important for automated data analysis in RIA, because a considerable bias could be shown at potency estimation when a model is erroneously used. Smoothing spline function, logistic, quadratic and cubic log-logit were evaluated for goodness-of-fit using several indicators in various RIA such as IRI, T-4, T-3, TSH, Digoxin, TBG, AFP, Cortisol and C-peptide. Coefficient of multiple determination (CMD%), MSL/Se² and a comparative fitting factor (CFF) were used as indicators for evaluating goodness-of-fit.

Several RIAs such as T-4, T-3, TBG, Digoxin and AFP showed constant results for goodness-of-fit. In these assays spline function gave excellent result. Cubic log-logit was also very flexible, especially when a sigmoid curve remained even after log-logit transformation in such an assay as solid phase cortisol. However no constant result was obtained as for goodness-of-fit in assays such as IRI, TSH and IRC. In these assays most suitable model was different from assay to assay. In an extreme case different equations showed some 20-30 % difference at potency estimation. These difference was far beyond predicted precision of the assay system calculated by Rodbard method and could make a considerable bias in assay results.