EVALUATION OF PROLACTIN RIA KIT USING DOUBLE ANTIBODY METHOD


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Prolactin RIA kit (Dainabot) was used in two institutes (A and B) simultaneously for the measurement of plasma prolactin levels.

Patient's serum (0.1 ml) was incubated with 125I-PRL and anti-PRL rabbit serum for 72 hours at 4°C. The second antibody was then added. After 24 hours incubation at 4°C, the bound was separated by centrifugation. The radioactivity of the bound was measured in a well counter.

Dilution studies performed in the 2 institutes showed good linearity. There was some discrepancy in recovery test between the 2 institutes in that institute B revealed slightly higher recovery rate (106.5% in mean) than institute A (82.0%).

Precision of each assay was nearly identical with the mean slope of response error relationship (RER); 0.025±0.005 (M±S.D.) in institute A and 0.030±0.010. Precision profile showed similar pattern in 2 institutes.

Quality control samples of which PRL concentration were low, middle and high were measured in each assay. Their intra-assay variance ranged from 3.2 to 11.9% in coefficient of variation (C.V.). Inter-assay variance was 4.9 to 18.9% in C.V.. High variance was obtained in high PRL concentration over 95 ng/ml.

The same sample were measured independently in two institutes. In lower level of PRL concentration (<50 ng/ml) the obtained values agreed well. In higher level (>80 ng/ml) institute B showed higher values than institute A. Overall correlation was good (r = 0.99). When compared with another PRL RIA kit (Daiichi), present kit gave lower values, though the correlation was good (y=0.72x+5.0, r=0.98).

In TRH tests PRL measured by the present kit showed the same response as PRL Daiichi kit, though the former showed lower values. The measurement in two institutes gave identical data in TRH tests.

AS a antigen partially purified Somatomedin A (Som A) donated from AB KABI, Stockholm was used. Som A was conjugated with ovalbumin by glutaraldehyde method and immunized to 4 rabbits by Vaitukaitis method. For iodination purified Som A donated from KABI was used. Iodination was performed by chloramin T method. Incubation was performed with veronal buffer at 4°C for 1 day and separation of B and P was performed by polyethylene glycol method.

After the 5th immunization, antibody which binds 38% of total radioactivity at final concentration of 12,000 dilution was obtained. Using this antibody dose dependent displacement by Som A which has the biological activity of 78 U/ml was observed between the dosages of 4 to 100 ng/ml. Other Somatomedins, such as Som C, IGF-I, IGF-II, MSA and NSILP did not interfere the binding of 125I-Som A until 100 ng/ml. Other growth factors such as EGF, Som B and insulin did not interfere the binding of 125I-Som A until 25μg/ml. Som A activity in intermediate fraction during purification of Som A from Cohn Fraction IV was assayed using both our antibody and antibody produced in chicken by Hall et al. (1978). The 4th fraction from Sephadex G 50 interfered the binding of 125I-Som A with both antibodies. The 1st and 2nd fractions after electrophoresis at pH 5.0 of the above 4th fraction interfered the binding of 125I-Som A with our antibody, but only the 2nd fraction interfered the binding of 125I-Som A with Hall's antibody. These findings showed the difference of both antibodies.

After gel chromatography on Sephadex G 200, most of the Som A determined by our radioimmunoassay appears in large molecular form. Using whole serum, dose-response displacement was obtained between 0.25 and 5.0 μl/ml and this was paralleled to that obtained

The mean level of Som A in serum from acromegaly was higher than that obtained from pituitary deficiency. However the differences between these two values were not greater than those obtained by radioreceptor assay. The reasons for this discrepancy are now under investigation.