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AN ECONOMICAL METHOD FOR THE ESTIMATION OF C_3 and C_4

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Summary

An economical, reliable method is described for the estimation of C_3 and C_4 levels in serum. On comparison with commercial kits, a correlation coefficient (r) of 0.91 was obtained for C_3 and 0.93 for C_4 estimations.

Serum complement levels are regularly depressed in a variety of disease states including active systemic lupus erythematosus (SLE), acute post-streptococcal glomerulonephritis (AGN) and membranoproliferative glomerulonephritis (MPGN)[1,2] and thus serve as valuable diagnostic aids and therapeutic guides. In recent years, immunochemical techniques utilizing monospecific antisera have become the main assays for serum complement in humans, especially the single radial immunodiffusion technique[4] for measuring the levels of complement components C_3 and C_4.

Currently available commercial plates for measuring C_3 and C_4 levels, although reliable, have proven to be prohibitive in terms of cost. We, therefore, set out to prepare our own plates in the laboratory. As this report demonstrates, the plates were found to be satisfactory and provide a reliable and economic method to measure C_3 and C_4 levels.

MATERIALS AND METHODS

Patients Sera

Sera were obtained from patients in the Renal Clinic and wards of the University Hospital, Kuala Lumpur. Sera were processed fresh in most cases or stored at -20°C for not more than 48 hours.

PREPARATION OF PLATES

A 3% agar solution (Purified Agar, Oxoid Ltd., London, England) was prepared in Veronal buffer (52.5 gm sodium diethyl-barbiturate, 8.3 gm diethylbarbituric acid, 7.7 gm calcium lactate in 5 litres deionized water, pH 8.6) and heated until boiling. This was then mixed with an equal volume of anti-C_3 (Behringwerke Anti-C_3 Globulin Serum $OTEA_{05}$ Batch No. 5110A) or anti-C_4 antisera (Behringwerke Anti-C_4 Globulin Serum $OTNC_{05}$ Batch No. 2898G) in buffer containing 1% bovine albumin. The final dilutions for the antisera were 1 in 15 for anti-C_3 and 1 in 40 for anti-C_4. The agar and antisera mixture was then brought to 56°C and 1 ml pipetted and spread evenly over a plastic template (40 x 95 mm, 1½ mm deep) and allowed to set. Plates can be stored for up to 2 weeks at 0–4°C (sealed in foil to minimize evaporation).

DETERMINATION OF C_3 AND C_4

2 mm diameter wells were punched on the agar using a well cutter. 5 μl of patient's serum (diluted 1 in 2 with normal saline) was then added to the wells, placed in a moist chamber and incubated at 37°C for 48 hours. The diameter of the precipitin rings was then measured to the nearest 0.1 mm using a precision viewer (Hyland diagnostics, Costa Mesa, Calif. U.S.A.). Standard C_3 (Behringwerke Protein-Standard – Serum B (human), $OTFG_{07}$, Batch No. 10036) and C_4 (Behringwerke Standard Human Serum ((Stabilised) $ORDT_{03}$ Batch No. 1001W) preparations were determined simultaneously. At the same time C_3 and C_4 levels were also determined on commercial kits (M-Partigen Immunodiffusion Plates, Behringwerke) for comparison purposes.

STATISTICAL METHODS

Standard curves were constructed by linear regression analysis and correlation coefficients (r) calculated using standard formulae.

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RESULTS

A total of 65 sera were evaluated for C_3 and C_4 levels; values were obtained from a standard curve prepared from 3 dilutions of known standards. The degree of correlation observed between values obtained with commercial plates and our own plates is depicted in Fig. 1 (for C_3) and Fig. 2 (for C_4). The results show a high degree of correlation between the values obtained by the two methods; a correlation coefficient (r) of 0.91 was obtained for the determination of C_3 (Fig. 1) and 0.93 for C_4 (Fig. 2).

DISCUSSION

The prohibitive cost of commercially available kits for estimating C_3 and C_4 levels has hampered the routine use of this test as an aid in diagnosing a variety of immune disorders and related syndromes. The present communication has shown that an economical method, at approximately 1/10th of the cost of commercial kits per test, can be used to give reliable estimates of C_3 and C_4 levels.

It should be pointed out, however, that there are great variations in the complement component patterns during the course of a disease as well as in healthy individuals[3]. A reflection of this variation can be seen in Fig. 1 and Fig. 2. Finally, it is also important to realise that complement levels in diseases must be interpreted as aids to suggesting and confirming given diagnoses, and not definitive of themselves.

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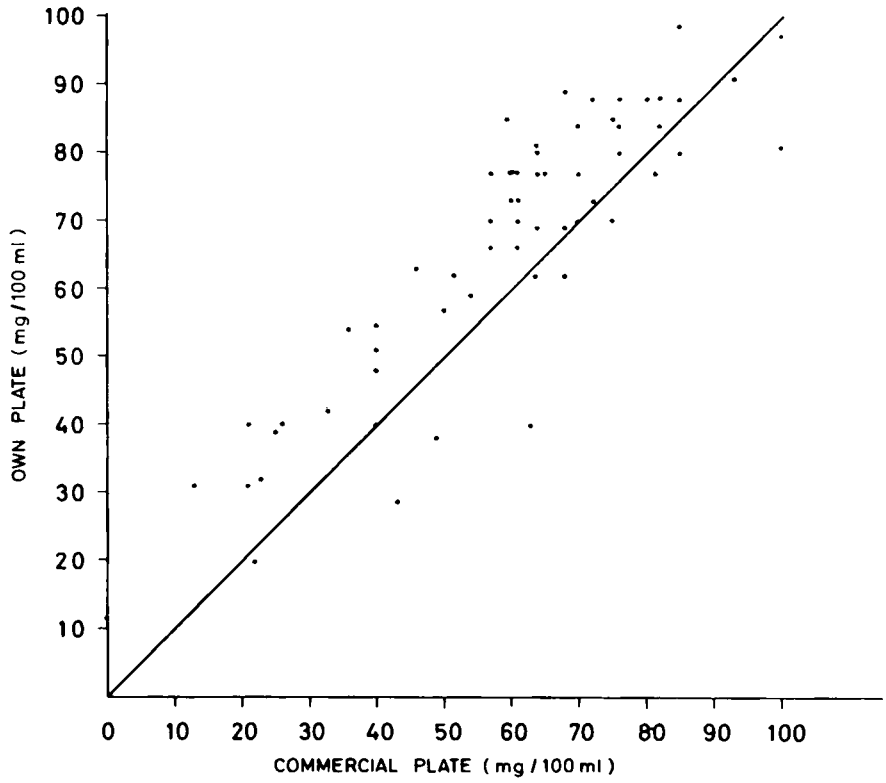

FIGURE 1: *Correlation in values of C_3 between own plates and commercial kits.*

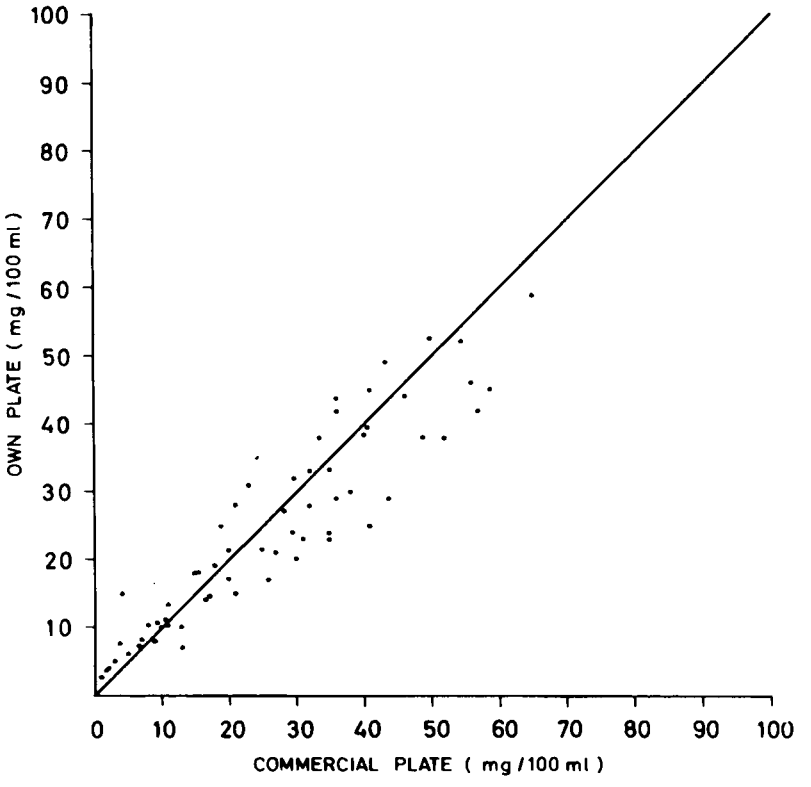

FIGURE 2: *Correlation in values of C_4 between own plates and commercial kits.*

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