

## AN ECONOMICAL METHOD FOR THE ESTIMATION OF C<sub>3</sub> and C<sub>4</sub>

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### Summary

An economical, reliable method is described for the estimation of C<sub>3</sub> and C<sub>4</sub> levels in serum. On comparison with commercial kits, a correlation coefficient (*r*) of 0.91 was obtained for C<sub>3</sub> and 0.93 for C<sub>4</sub> 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)<sup>1,2</sup> 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<sup>4</sup> for measuring the levels of complement components C<sub>3</sub> and C<sub>4</sub>.

Currently available commercial plates for measuring C<sub>3</sub> and C<sub>4</sub> 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<sub>3</sub> and C<sub>4</sub> 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<sub>3</sub> (Behringwerke Anti-C<sub>3</sub> Globulin Serum OTEA<sub>0.5</sub> Batch No. 5110A) or anti-C<sub>4</sub> antisera (Behringwerke Anti-C<sub>4</sub> Globulin Serum OTNC<sub>0.5</sub> Batch No. 2898G) in buffer containing 1% bovine albumin. The final dilutions for the antisera were 1 in 15 for anti-C<sub>3</sub> and 1 in 40 for anti-C<sub>4</sub>. 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<sub>3</sub> AND C<sub>4</sub>

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<sub>3</sub> (Behringwerke Protein-Standard - Serum B (human), OTFG<sub>0.7</sub>, Batch No. 10036) and C<sub>4</sub> (Behringwerke Standard Human Serum ((Stabilised) ORDT<sub>0.3</sub> Batch No. 1001W) preparations were determined simultaneously. At the same time C<sub>3</sub> and C<sub>4</sub> levels were also determined on commercial kits (M-Partigen Immuno-diffusion 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<sub>3</sub> and C<sub>4</sub> 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<sub>3</sub>) and Fig. 2 (for C<sub>4</sub>). 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<sub>3</sub> (Fig. 1) and 0.93 for C<sub>4</sub> (Fig. 2).

DISCUSSION

The prohibitive cost of commercially available kits for estimating C<sub>3</sub> and C<sub>4</sub> 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<sub>3</sub> and C<sub>4</sub> 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<sup>3</sup>. 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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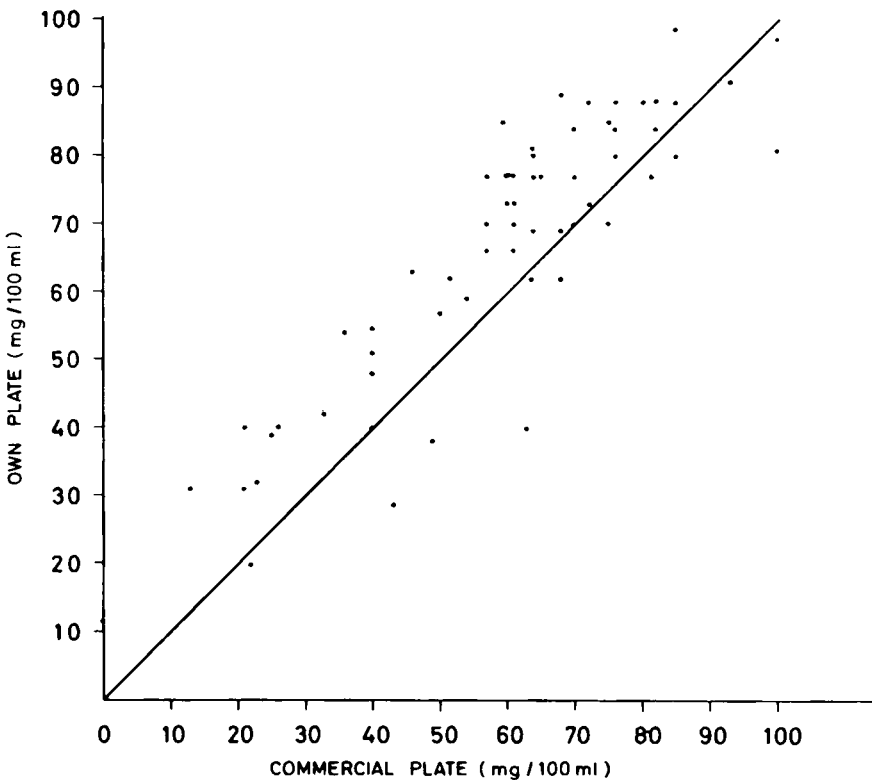


FIGURE 1: Correlation in values of C<sub>3</sub> between own plates and commercial kits.

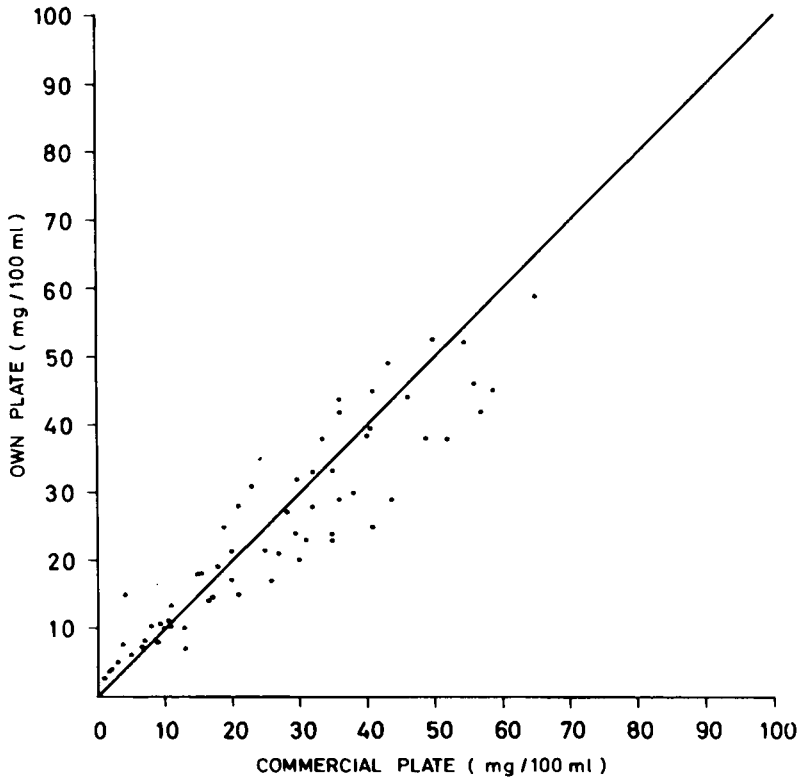


FIGURE 2: *Correlation in values of  $C_4$  between own plates and commercial kits.*

REFERENCES

- 1 Ruddy S, Gigli I and Austen KF: The complement system of man. *N Engl J. Med*, 287: 489-495, 545-549, 592-596, 642-646, 1972.
- 2 Osler AG: *Complement - Mechanisms and Functions*. Prentice-Hall, New Jersey, 1976.
- 3 Gewurz H. and Suyehira LA: Complement. *In Manual of Clinical Immunology*. Am Soc Microbiol edited by Noel R. Rose and Herman Friedman, pp. 36-47, 1976.
- 4 Mancini G, Carbonara AO and Heremans JF: Immunochemical quantitation of antigens by single radial immunodiffusion. *Int J. Immunochem* 2:235-254, 1965.