Rapid Complement Fixation Technique for Estimating Complement-Fixing Antigen Elution Profiles of Viruses from Gel Filtration Columns

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The complement fixation elution profiles of dengue-2 virus-infected suckling mouse brain supernatant fluids from Sephadex G-200 columns were compared by the antigen end-point titration procedure and by a complement dilution technique. The latter technique was found to be a quick method for approximating the complement-fixing antigen in eluates.

When dengue virus-infected suckling mouse brain supernatant fluids are fractionated by Sephadex gel filtration, two complement-fixing (CF) antigens are eluted (3). The larger antigen is associated with hemagglutinating activity and is eluted in the void volume, whereas the smaller soluble complement-fixing antigen is not associated with hemagglutinating activity and is eluted in a volume in which a globular protein of 39,000 daltons would be expected. When the CF antigen profiles were determined, the antigen end-point titration procedure was utilized which involved testing serially diluted fractions against an excess of CF antibody and 2 units of complement (C'). In this report a procedure is described for determining the approximate CF elution profiles of antigens by a rapid C' dilution procedure. This is essentially a microtest based on principles described by Mayer et al. (8). Each fraction is tested against excess CF antibody utilizing various dilutions of C'. The results are graded from 0 to +4, and the 50% hemolytic end point is estimated by eye instead of spectrophotometrically determined.

In preparing the antigens, suckling mice were injected intracerebrally and sacrificed when moribund, and their brains were harvested and homogenized in a Lourdes blender at a 20% (w/v) concentration in pH 9.0 boratesaline (1). After clarification by centrifugation at 13,200 × g for 30 min, 5.0 ml of the supernatant liquid was placed on a standardized (5) Sephadex G-200 column (2.5 by 45 cm) equilibrated at 4 C. Fractions of 6 ml were collected, and the CF antigen elution profile was determined simultaneously by both the antigen end-point titration technique and the C' dilution procedure described below. The antibody was hyperimmune mouse ascitic fluid (AF) prepared by the method of Brandt et al. (2) against crude infected mouse brain suspensions.

Each lot of antigen or antiserum, or both, was initially titrated to determine its potency by a standard cross-block titration (4, 6). Maximum antibody titers in the presence of a crude 20% infected suckling mouse brain antigen always occurred with the highest concentration of antigen tested. Inhibition from antigen excess was never observed when such antigens were tested against a 1:4 dilution of homologous immune AF. Russel et al. (9) also observed this pattern with similar reagents. Therefore, no dilution of the fractions had to be made to assure an excess of CF antibody since fractionation by gel filtration effected even greater dilution of the antigen.

The test consists of adding one drop of a fraction to be tested into each of 12 wells in a V Microtiter plate (Cooke Engineering Co., Alexandria, Va.). Wells 1 through 6 serve as the test for CF antigen activity, and 7 through

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12 serve as anticomplementary antigen controls (Table 1). All fractions to be tested are added to their respective rows prior to addition of the other reagents. Then in wells 1 through 6 one drop of chilled, inactivated immune AF, which is in antibody excess, is added; to wells 7 through 12 one drop of Veronal buffer diluent (7) is added. Then one drop of the appropriate dilution of C' is added as indicated in Table 1. The dilutions of C' used depend upon the potency of the C' preparation. With fresh guinea pig C', the usual dilutions of C' were 1:40, 1:80, 1:100, 1:120, 1:160, and 1:200 (prior titrations indicated that a 1:100 dilution represented 2 exact units). With one lot of a commercially lyophilized C', 1:10, 1:20, 1:40, 1:60, 1:80, and 1:100 dilutions were used. If a particular reaction pattern is obtained regularly with a given lot of reagents (antigen, AF, C'), adjustments in the serial dilutions of C' can be made to obtain a more precise titration end point. In addition, if previous results indicated that the fractions were not anticomplementary, they can be tested against nine dilutions of C' while the anticomplementary controls need consist of only the three higher dilutions. Antibody and C' controls are performed concomitantly with the test. Antibody controls consist of one drop of the diluted, inactivated AF, one drop of the Veronal buffer diluent, and one drop of the appropriately diluted C'. The C' control consists of two drops of the Veronal buffer diluent and one drop of the appropriately diluted C'.

The test plate is covered and allowed to incubate for 1 hr at 37 C after which two drops of 0.4% sensitized sheep cells (4) equilibrated at 37 C are added to each well. Following an additional incubation at 37 C for 30 min, the cells are allowed to settle and the results are recorded. One C'H50 unit of C' is taken to be that dilution of C' which lysed approximately 50% (+2) of the cells in the C' control; two C'H50 units is twice that concentration of C', etc., providing that all controls are satisfactory. If the antibody or antigen fraction is slightly anticomplementary in the higher dilutions of C', the built-in controls permit readjustment of the C'H50 unit to a higher concentration of C'. If a fraction is found to give a +4 reaction in a particular dilution of C' but is negative in the next higher concentration, it is assumed that the 50% hemolytic end point would have occurred at a concentration between the two dilutions and the results are plotted as such. In any fraction fixing C' in which a +2 end point is not obtained but both greater and lesser fixation does occur, the intermediate dilution is estimated.

The variation obtained from duplicate tests utilizing the complement dilution technique with the above interpretations has on occasions been as much as +1 C'H50 unit; however, the elution patterns have always been similar on retests of the same series of fractions.

When the complement dilution technique was compared with the CF antigen end-point titration method, the CF elution patterns were similar, but the tests could not be quantitatively compared (Fig. 1). No advantage could be seen in utilizing the time-consuming antigen end-point titration test over the complement dilution test for approximating quickly the CF antigen elution profiles of dengue virus antigens from gel filtration columns. This technique was also found applicable to tests on rate zonal centrifugation fractions containing dengue virus CF antigens, gel filtration frac-

| Table 1. A representative plate used for the complement dilution technique for estimating dengue virus complement-fixing antigens in eluates |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Fraction no.    | 1  | 2  | 3  | 4  | 5  | 6  | 7  | 8  | 9  | 10 | 11 | 12 |
|                 | 1* | 20 | 40 | 60 | 80 | 100| 120|
|                 | 2  | 40 | 60 | 80 | 100| 120|
|                 | 3  | 40 | 60 | 80 | 100| 120|
|                 | 4  | 40 | 60 | 80 | 100| 120|
|                 | etc.|    |    |    |    |    |    |    |    |    |    |    |

Test | Antibody control

| 1  | 2  | 3  | 4  | To each well: 1 drop of appropriate fraction, antibody, and indicated dilution of complement |
| 2  | 4  |    |    | To each well: 1 drop of appropriate fraction, buffer, and indicated dilution of complement |
| 3  |    |    |    | |
| 4  |    |    |    | |
| etc.|    |    |    | |

Complement control | Two drops of buffer and 1 drop of appropriately diluted complement

Antibody control | One drop of antibody, buffer, and appropriately diluted complement

* Well number.
* Reciprocal of complement dilution.
**Fig. 1.** CF elution profiles of 5.0 ml of 20% dengue-2 virus-infected suckling mouse brain supernatant fluid from a Sephadex G-200 column (2.5 by 45 cm) as simultaneously determined by the complement dilution technique and the antigen titration end-point technique. (These fractions have been described previously by Brandt et al. [3].) Vo, void volume; “SCF,” soluble complement-fixing antigen; C'H5O units is defined in text.

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