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RESEARCH COMMUNICATION

THE RELATIONSHIP BETWEEN THE MINIMAL AND THE 50% HAEMOLYTIC DOSE IN COMPLEMENT TITRATIONS

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ABSTRACT

HERR, S., PIETERSON, P. M. & BOSHOFF, J. A., 1981. The relationship between the minimal and the 50% haemolytic dose in complement titrations. *Onderstepoort Journal of Veterinary Research*, 48, 259–260 (1981).

The 50% spectrophotometric complement titration end-point ($C'H_{50}$) was found to be more reproducible than the 100% end-point. The relationship of the 100% end-point (MHD) to the ($C'H_{50}$) was 1:2,5. The use of thrombin tubes induced clotting within 2 min and enabled the process of complement preparation to be completed well within 1 hour.

Résumé

LA RELATION ENTRE LA DOSE MINIMALE ET LA DOSE HAEMOLYTIQUE A 50% DANS DES TITRATIONS DE COMPLÉMENT

Le point terminal 50% ($C'H_{50}$) de la titration de complément spectrophotométrique fut trouvé être plus reproductible que le point terminal 100% (MHD). La relation entre le MHD et le ($C'H_{50}$) fut 1: 2,5. L'emploi de tubes thrombines induisit le coagulation endéans 2 minutes et permit d'accomplir le procédé de préparation de complément bien endéans 1 heure.

INTRODUCTION

In the complement titration for use in the complement fixation test (CFT) the spectrophotometrically determined 50% end-point ($C'H_{50}$) was found to be more sensitive and displayed greater reproducibility than the 100% end-point (minimum haemolytic dose—MHD) (Alton, 1977). Alton (1977) used 5 $C'H_{50}$'s and worked with a single volume of 3% red blood cells (RBC). Where Hill's method is employed in the CFT (Morgan, Mackinnon, Gill, Gower & Norris, 1978), $1\frac{1}{4}$ MHD's of complement are used, and Hill's method uses a double volume of 3% RBC. In adapting the 50% spectrophotometric titration for use in the latter method it becomes necessary to establish the relationship between 1 MHD and 1 $C'H_{50}$. This relationship was reported to be 1: 2,5 (Alton & Mackinnon, personal communications, 1980). The purpose of this study was to confirm this relationship experimentally.

Complement was produced from donor male guinea pigs fed on a commercial pelleted ration supplemented with vitamin C in the water and a daily supply of oranges, carrots and/or guavas. They were starved for 12 hours before collection. Blood was collected in 10 mℓ vacuum tubes(3) by heart puncture from at least 5 guinea-pigs and, using serum separators(4), centrifuged at 1000/*g* for 10 min with a minimum of 2 min delay after collection. The serum was pooled, Richardson's preservative was added (Alton, 1977) and the preserved complement stored in screw-capped bottles at 4 °C. The entire process from collection to storage never exceeded 1 hour.

The method of Herr, Bishop, Bolton & Van der Merwe (1979), modified by using dilutions of complement up to 1/120 and by keeping all reagents in a water-bath at 4 °C before and between the incubation periods, was used for the 100% end-point complement titration. The complement titration using the 50% end-point was done by the method of Herr *et al.* (1979), modified in that a 1/250 dilution of complement was used exclusively and all reagents were kept in a water-bath at 4 °C before and between the incubation periods. A Spectronic 21(1) spectrophotometer was used to determine the percentage heamolysis present. In both titrations the 3% RBC and haemolytic system were prepared and titrated as described by Herr *et al.* (1979), except that in the latter case sensitization was done at 37 °C for 30 min in a water-bath.

The results from 3 different batches of complement (Tables 1 A, B & C) showed that the relationship of 1 MHD: 1 $C'H_{50}$ was 1: 2,4–2,6. The repeatability of the titration using the 50% end-point was such that all results fell within 10% of the mean (Table 1), while with the 100% end-point results varied by as much as 30–40% from the mean.

TABLE 1 Results of complement titrations using the 100% and 50% end-points

A. Complement 81.02.24

Date	50% end-point 1 $C'H_{50}$	100% end-point 1 MHD
24/2.....	169*	80*
24/2.....	161	70
24/2.....	158	70
25/2.....	151	60
25/2.....	166	70
26/2.....	156	50
26/2.....	151	40
27/2.....	166	50
Average.....	160	61
Range.....	151–169	40–80

* Reciprocal of complement dilution containing 1 $C'H_{50}$ or 1 MHD respectively

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(3) Vacutainer 6525, Becton-Dickinson, Rutherford, New Jersey 07070, USA
(4) Sure-Sep II, General Diagnostics, Morris Plains, New Jersey 07950, USA

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(1) Bausch & Lomb, Rochester, New York 14625, USA

B. Complement 81.01.28

Date	50% end-point 1 $C'H_{50}$	100% end-point 1 MHD
29/1	168	40
2/2	170	50
3/2	170	70
3/2	170	80
4/2	170	80
10/2	153	70
13/2	182	80
16/2	170	70
17/2	155	70
17/2	172	70
18/2	154	70
Average	167	68
Range	153–182	40–80

C. Complement 81.02.19

Date	50% end-point 1 $C'H_{50}$	100% end-point 1 MHD
19/2	150	70
20/2	155	60
23/2	150	60
Average	152	63
Range	150–155	60–70

The use of the thrombin tubes for blood collection ensured clotting of the blood within 2 min and allowed the whole process of complement preparation to be completed well within 1 hour. In our hands the repeatability of the complement titration was very much better when the spectrophotometric 50% rathere than 100% end-point was used. Nevertheless, the rang of results (Table 1) leaves room for improvement.

The relationship between MHD and $C'H_{50}$ was found to be close enough to the reported 2,5 (Alton & Mackinnon, personal communications, 1980) to accept this figure as the correct one to use in conversion.

References

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