

RESEARCH COMMUNICATION

A RAPID MICROTITRATION SERUM AGGLUTINATION TEST FOR THE DETECTION OF CONTAGIOUS EQUINE METRITIS ANTIBODIES

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ABSTRACT

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A microtitration serum agglutination test, based on that used for brucellosis, has been developed to detect antibodies in the sera of horses exposed to the contagious equine metritis (CEM) organism. Two known positive sera were tested 100 times in 15 separate tests. The results were reproducible to within a twofold range. The test is capable of being carried out within 100 min.

INTRODUCTION

As a result of the possibility of *Taylorella equigenitalis* (Sugimoto, Isayama, Sakazaki & Kuramochi, 1983) being introduced into South Africa, bacterial isolation techniques and later the complement fixation test (CFT) were introduced to screen imported horses. Although the CFT is reliable at detecting chronically infected animals, it does not detect all cases of contagious equine metritis (CEM) (Brewer, 1983). It was therefore decided to introduce a backup serological test to supplement and cross-check the results of the CFT and to minimize the incidence of false negative results. The serum agglutination test (SAT) was decided upon as it has been found to be one of the most useful tests for diagnosing acute cases of CEM (Brewer, 1983). The method decided upon was an adaptation of the microtitration test developed by Herr, Te Brugge & Guiney (1982) for the detection of *Brucella abortus* antibodies.

MATERIALS AND METHODS

Serological procedures

The SAT was carried out with known positive CEM "high titre" and "medium titre" sera obtained from the National Veterinary Services Laboratory, Ames, Iowa, USA. The antigen used was produced at the Veterinary Research Institute, Onderstepoort (Gummow, Herr & Brett, 1986). Twofold serial dilutions of test sera (0,05 ml) were made in round-bottomed, microtitration plates with phenol-saline using an 8-channel micro diluter¹ to give serum dilutions of 1/2 to 1/256. To each serum dilution 0,05 ml of Ag was added, making a final volume of 0,1 ml per well. The microtitration plate was then placed on a shaker for 90 min at 37 °C. The plate was then removed and centrifuged for 30 s at 10 g. The plates were read with a lighting apparatus designed in our laboratory for easy reading of all SAT results done in microtitration plates. The "high titre" and "medium titre" sera were each tested 50 times in 7 and 8 batches, respectively, on different days. For each batch of positive sera tested at least 2 negative sera (received for routine dourine testing and negative for both dourine and CEM in the CFT) were also included.

Interpretation of serological end-points

A positive reaction was taken to be one which exhibited agglutination in the bottom of the well and consequently resulted in a clear supernate. The degree of clearing of the supernate was further graded from a 1+ to a 4+ with 4+ = 100 % agglutination; 3+ = 75 %; 2+ = 50 % and 1+ = 25 %. A positive reaction was read to the final dilution showing such a reaction. A negative reaction was taken to be one showing poor or no

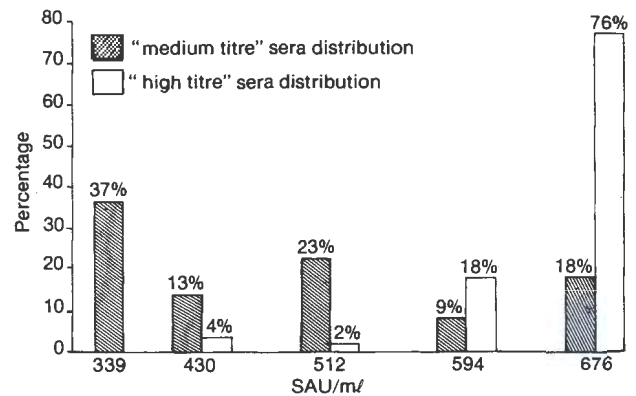


FIG. 1 The distribution of SAT end-point titres of repeated tests done on "medium titre" sera and "high titre" sera

agglutination and a turbid supernate. For easier recording of results, the South African units per ml (SAU/ml) system was used, whereby serum dilutions are converted to SAU/ml (Gummow *et al.*, 1986). By definition, 1 000 SAU/ml = 50 % agglutination at a final dilution of 1/500, where a final dilution is the dilution factor after all the reagents are added. This is not the same as the serum dilution which is the dilution factor of serum with phenol-saline only added.

RESULTS

Two sets of results were obtained, those for the "medium titre" serum and those for the "high titre" serum. For the medium titre serum tested, it was found that all the results fell within a twofold range of 339-676 SAU/ml. The median of this distribution was at 512 SAU/ml. Twenty-three per cent of the end-point titres for this serum were recorded at 512 SAU/ml. The mode (37 %) of the end-point titres were recorded at 339 SAU/ml (Fig. 1). The results of the "high titre" serum tested fell within less than a twofold range 430-676 SAU/ml. The commonest end-point titre for this serum was 676 SAU/ml, at which dilution 76% of the results were recorded (Fig. 1). There were no false negative or false positive reactions in the 130 tests carried out.

DISCUSSION

The advantages of this microtitration method over other methods (Benson, Dawson, Durrant, Edwards & Powell, 1978; Sahu, Rommel, Fales, Hamdy, Swerczek, Youngquist & Bryans, 1983) is described in detail by Herr, Te Brugge & Guiney (1982). Briefly they are; a

¹ Micro Compu-Pet, General Diagnostic Div. Warner. Lambert. Co., Morris Plains, New Jersey 07950 USA

saving of antigen, a reduction in apparatus and completion of the test with a 90-min incubation period as opposed to overnight incubation.

Initial test indicated that the antigen had a tendency to settle out far more rapidly than the *B. abortus* antigen. By systematically lowering the centrifugal force and time of centrifugation, an optimum time of 30 s at a centrifugal force of 10 g was eventually obtained. When this force and duration were used the larger Ag/Ab complexes pelleted out as desired, and the smaller Ag particles, remaining in suspension, enabled one to distinguish clearly between a positive and negative result. If the microtitration plates were left to stand at room temperature for any length of time (i.e. > 1 h), the antigen in the negative wells began to settle out, making the distinction between positive and negative reactions more difficult. This could be remedied if the plate was placed back on the shaker for 1 min and the correct centrifugation necessary for reading the plates applied.

From the results it is evident that the modified method is both reliable for detecting CEM antibodies and reproducible, as there were no false positive or false negative reactions and all the end points fell within a twofold range. This is certainly acceptable when compared to SAT's carried out for *B. abortus* (Herr, Roux & Pieter-son, 1982).

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