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Microtiter Plate Agglutination Test for *Brucella canis* Antibodies

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A micro-agglutination test for the antibodies to *Brucella canis* produced similar results to those obtained with the standard tube agglutination method in human and canine sera. The micromethod does provide an economical means of screening sera for the presence of antibodies.

Macro-tube agglutination (TA) methods have been utilized in the study of the agglutinins present in the sera of humans and animals infected with *B. canis*. A Microtiter plate agglutination (PA) test has been formulated which provides results comparable to the TA method, using a minimal amount of equipment, time, reagents, and serum. *B. canis* has zoonotic potential both in man and the canine; therefore, an economical screening test is desirable.

A comparison of the TA and PA tests was made on 106 sera obtained from dogs, humans, rabbits, and guinea pigs. A standardized, reliable TA method (1) was used in this laboratory throughout the study. The same reagents were used in both tests. *B. canis* antigen was prepared according to the method of Carmichael (1). Phosphate-buffered saline (0.15 M and pH 7.25) was used as a diluent and as a negative control. The TA test served as a positive control.

Dilutions in the TA test ranged from 1:10 to 1:4,000. Sera positive at 1:10 or 1:50 were then further diluted to yield this range. One milliliter of the antigen was added to each 13- by 100-mm glass test tube containing 0.1 ml of diluted sera. The tubes were shaken and incubated in a 50 C water bath for 48 h. They were then read, by using a slit illuminator. Tubes were read for clearing with a clumped mass at the bottom. Negative samples remained uniformly cloudy.

The PA test was run, utilizing equipment purchased from Cooke Engineering Company, Alexandria, Va. A drop of phosphate-buffered saline was added to each well of a round-bottomed microtest plate with a 25-μliter micropipette. Twenty-five microliters of undiluted sera was placed in the first well and diluted twofold serially with a microdiluter, and 50 μliters of antigen was added to each well. Negative controls consisted of 25 μliters of phosphate-buffered saline and 50 μliters of antigen. The plates were covered with a precut plate sealer and incubated in a moist chamber at 50 C for 24 h. The plates were read by using a Microtiter mirror and high intensity lamp. Agglutination appeared as a round or folded-over circle. Negative reactions appeared as partially agglutinated clumps of antigen, and negative controls were cloudy throughout the well. Partial agglutination reactions were read as negative. Titers were calculated based on final dilution of serum in the TA tubes and the PA systems.

The results of both the PA and TA tests have been summarized in Table 1. Similar results were obtained for the two tests. A number of PA tests were incubated and read at 48 h. This reading occasionally yielded higher titers.

Identical titers using the PA and TA meth-

TABLE 1. *Comparison of TA and PA tests on 106 sera tested for B. canis agglutinins*

Determination	No. positive on both TA and PA	No. negative on both TA and PA	No. positive on PA, negative on TA
No dilution difference	36	13	
One-dilution difference	35		1
Two-dilution difference	9		1
Three-dilution difference	4		1
No correlation	3		3
Total	87	13	6
Percentage	82%	12%	6%

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ods have been reported in the study of a number of antigen-antibody systems including *Brucella abortus* (3). The PA test described is a valid screening method for *B. canis* agglutinins. No false negatives were found and the PA test compared with the TA test identically, or with only one dilution difference in 80% of the samples tested. The results obtained in the present study compare favorably with the universal deviation of one dilution which exists when comparing TA and PA tests for agglutination and complement fixation (2).

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