

THE RELATIONSHIP BETWEEN THE MICROTITRATION SERUM AGGLUTINATION AND COMPLEMENT FIXATION TESTS IN BOVINE BRUCELLOSIS SEROLOGY

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ABSTRACT

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The relationship between antibody titres in the microtitration serum agglutination test and the complement fixation test in bovine brucellosis is described. For low and high MSAT values there is good agreement between the 2 tests. This is not the case for MSAT values between 54 and 338 IU/ml. For practical reasons, results falling into this category cannot all be repeated. Repetitions are so structured that less than 4 % of the tests need to be repeated. If the level of repetitions should show an increase above 4 %, it is assumed that technical or human error has occurred.

INTRODUCTION

The serum agglutination test (SAT) has been reported to be of value in checking results of the complement fixation test (CFT) in bovine brucellosis (Morgan, 1977). When the microtitration SAT (MSAT) was developed, the relationship between antibody titres in this test and that found in the CFT was reported on (Herr, Te Brugge & Guiney, 1982). The report covered 4 dilutions, representing antibody levels of 0-212 International Units per millilitre (IU/ml) in the MSAT. Subsequently the range of dilutions in the MSAT was extended to include antibody levels of 0-1352 IU/ml. The results reported here examine the relationship between the MSAT and the CFT titres in this expanded range.

TABLE 1 The conversion of CFT end-point reactions to IU/ml on a scale where 50 % haemolysis in a 1/220 serum dilution was equivalent to 1 000 IU/ml^c

Serum dilution ^a	Final dilution ^b	End-point reading		IU/ml ^c
		% haemolysis	Reaction	
1/4	1/20	75	1-+	15
		50	2-++	18
		25	3-+++	21
		0	4-++++	24
1/8	1/40	75	1-+	30
		50	2-++	36
		25	3-+++	43
		0	4-++++	49
1/16	1/80	75	1-+	60
		50	2-++	72
		25	3-+++	86
		0	4-++++	98
1/32	1/160	75	1-+	120
		50	2-++	145
		25	3-+++	172
		0	4-++++	196
1/64	1/320	75	1-+	240
		50	2-++	290
		25	3-+++	344
		0	4-++++	392
1/128	1/640	75	1-+	480
		50	2-++	581
		25	3-+++	688
		0	4-++++	784
1/220	1/1100	50	2-++	1000

^a Serum dilution = dilution factor with veronal buffer only

^b Final dilution = final dilution factor after all reagents were added

^c IU/ml = International Units per millilitre

MATERIALS AND METHODS

Sera

The sera used were received from the field for routine brucellosis serology at the Veterinary Research Institute, Onderstepoort. The vaccination history of the animals from which sera were taken varied. In some the vaccination history was unknown, whereas others had never been vaccinated. Some had been vaccinated as heifers (3-10 months of age) and were tested for the first time as adults (more than 18 months of age). These heifers would have been vaccinated with *Brucella abortus* strain 19 vaccine containing 4-12 × 10¹⁰ viable organisms per

TABLE 2 The dilutions, reactions and International Units per ml (IU/ml) conversion used in the microtitration SAT

Serum dilution ^a	Final dilution ^b	End-point reading Agglutination reaction		IU/ml ^c
		%	Gradation	
1/4	1/8	0	0 or —	0
		25	1 or +	13
		50	2 or ++	16
		75	3 or +++	19
		100	4 or ++++	21
1/16	1/32	25	1 or +	54
		50	2 or ++	65
		75	3 or +++	74
		100	4 or ++++	85
1/64	1/128	25	1 or +	215
		50	2 or ++	256
		75	3 or +++	298
		100	4 or ++++	339
1/256	1/512	25	1 or +	860
		50	2 or ++	1024
		75	3 or +++	1188
		100	4 or ++++	1352
1/250	1/500	50	2 or ++	1000

^a Serum dilution = dilution factor with phenol saline only

^b Final dilution = final dilution factor after addition of antigen

^c IU/ml = International Units per millilitre

TABLE 3 The relationship between SAT and CFT, indicating the advisability of repeating both tests

If the SAT antibody titre is: IU/ml	and the CFT titre is: IU/ml
(a) 0-21	30 or more
(b) 54-85	120 or more
(c) 215-338	0
(d) 860-1352	98 or less

Both tests should then be repeated before such a result is accepted

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TABLE 4 The relationship between the frequencies for the SAT and CFT titres

MSAT in IU/ml	CFT in IU/ml						
	0	15-24	30-49	60-98	120-196	240-392	480-784
0-21	2205 (80,18%)	495 (18,0%)	45 (1,64%)	4 (0,14%)	1 (0,04%)	0	0
54-85	2928 (48,74%)	1664 (27,70%)	1006 (16,75%)	335 (5,6%)	61 (1,0%)	7 (0,11%)	6 (0,1%)
215-338	62 (6,2%)	99 (10,0%)	110 (11,1%)	144 (14,5%)	170 (17,2%)	188 (19,0%)	218 (22,0%)
860-1352	1 (0,15%)	1 (0,15%)	2 (0,3%)	3 (0,45%)	16 (2,4%)	58 (8,75%)	582 (87,8%)

dose. Some animals had been vaccinated when over 12 months of age with a reduced dose *Brucella abortus* strain 19 vaccine containing between 4×10^8 and 1×10^9 viable organisms per dose. These animals were tested at 2-7 months post-vaccination.

Serological methods

The CFT was done as described by Herr, Roux & Pieterse, 1982. Serum dilutions between 1/4 and 1/128 were used for the test proper, while the 1/2 serum dilution was used as an anti-complementary control. The end-point titres were expressed in IU/ml as set out in Table 1. CFT titres were therefore recorded between 0-784 IU/ml.

The microtitration SAT was done as described by Herr, Te Brugge & Guiney, 1982 except that fourfold dilutions were used instead of twofold. This resulted in final dilutions (after the addition of an antigen) of 1/8-1/512, representing titres of 0-1352 IU/ml (Table 2).

RESULTS

Of the 2 750 sera with an antibody titre of 0-21 IU/ml in the SAT, 98,18 % had CFT titres of between 0-24 IU/ml in the CFT. Less than 2 % showed CFT titres of 30 IU/ml or more.

When the 6 007 sera with a SAT titre of 54-85 IU/ml are considered, 1,21 % had CFT antibody titres of 120 IU/ml or higher.

Of the 991 sera with SAT titres of 215-338 IU/ml, 6,2 % were negative (less than 15 IU/ml) in the CFT.

Of the 663 sera with end-point readings of 860-1 352 IU/ml in the SAT, 1,05 % recorded titres of 98 IU/ml or less in the CFT.

DISCUSSION

There was a good correspondence between the 2 tests for the low and high MSAT-values. This was not true of MSAT-values between 54 and 338 IU and retesting was necessary in this area to verify the results. For practical reasons, not more than 4 % of tests could be repeated. A table has been constructed allowing for a practical amount of retesting (Table 3).

The value of carrying out both tests was that false negative CFT reactions could be identified by high antibody titres in the MSAT. Morgan (1977) described the use of the SAT to identify sera where false negative CFT reactions were due either to the prozone effect (Plackett & Alton, 1975) or to human error. Where the MSAT

titre lay between 0 and 21 IU/ml, a CFT titre of 30 IU/ml or higher was obtained in fewer than 2 % of cases. If such result was obtained on CFT, the result was accepted only when both the MSAT and CFT had been repeated.

After examination of the relationships between the MSAT and CFT antibody titres reported on in Table 4, a similar rule of thumb check on CFT titres could be formulated (Table 3). With sera with MSAT titres of 54-84 IU/ml, less than 2 % CFT titres of 120 IU/ml were seen. Sera which give such a result should be subjected to both tests again (Table 3). In sera with MSAT titres of 215-338 IU/ml, only 6,2 % showed a 0 IU/ml reaction in the CFT and had to be retested (Table 3). Where the MSAT was 860-1352 IU/ml, less than 2 % of the sera had a CFT titre of 98 IU/ml or lower and required retesting.

When this was applied in the routine testing of sera in the laboratory, 192 of the 10 411 (1,8 %) CFTs reported on in this series needed repeating. This is an acceptable level for the laboratory personnel to handle without affecting efficiency. In addition, if this level of repetitions should show an increase to above 4 % in certain batches of sera or on certain days, it was assumed that technical or human error had occurred and warranted investigation.

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