Onderstepoort J. vet. Res., 49, 23–28 (1982)

THE VALUE OF THE MICROTITRE SERUM AGGLUTINATION TEST AS A SECOND SCREENING TEST IN BOVINE BRUCELLOSIS

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ABSTRACT

HERR, S., TE BRUGGE, LESLEY A. & GUINEY, M. C. M., 1982. The value of the microtitre serum agglutination test as a second screening test in bovine brucellosis. *Onderstepoort Journal of Veterinary Research*, 49, 23–28 (1982).

The use of the serum agglutination test (SAT) as a 2nd screening test led to a reduction in false negative screening reactions especially in sera delayed in transit and provided an easy check on human error in the rose bengal test (RBT) and on each individual complement fixation test titre. The SAT in microtitration plates was more sensitive than when done in tubes and effected a saving of time, labour and materials.

INTRODUCTION

In the serology applied to bovine brucellosis, the serum agglutination test (SAT) has been discarded by many workers because of inherent drawbacks such as (a) its inability to differentiate vaccinal titres from true infection where *Brucella abortus* strain 19 (S 19) vaccine is used, (b) the fact that it tends to become positive later than other test following infection, (c) it may be delayed longer than other tests following abortion and (d) titres tend to wane in chronic infection (Alton, Maw, Rogerson & McPherson, 1975). Moreover 10–13% of culture positive animals test in the negative or inconclusive range, with the end-result that the SAT often cannot be relied on by itself to establish a diagnosis (Amerault, Manthei, Goode & Lambert, 1961; Morgan, 1970; Morgan & Richards, 1974; Alton *et al.*, 1975; Cunningham, 1977a; Alton, 1977; Morgan, Mackinnon, Gill, Gower & Norris, 1978). Nevertheless, it is still being used as a back-up in some countries, while others retain it as their definitive test, especially where rough *Brucella abortus* strain 45/20 killed vaccine (45/20) is used. Eradication of the disease, using the SAT only, has been effected in Norway and Tasmania (Alton, 1977; Cunningham, 1977b; McKeown, 1977; Plommet, 1977; Morgan *et al.*, 1978).

Although the SAT has been automated in tubes (Morgan, 1977), problems were experienced when the test was carried out in microtitration plates (Mittal & Tizard, 1980) in that titres were too low for its use to be considered in routine diagnosis. Our investigation was aimed at the development of a reliable test in the microsystem and at the same time at an attempt to reduce the incubation time. An aspect that has not been reported on is the correlation between titres in the SAT and those seen in the complement fixation test (CFT). In this study this correlation is examined and evaluated.

A factor often overlooked in laboratory work is that of human error (Morgan, 1977; Anon., 1980). As the SAT has the advantage of technical simplicity and international standardisation (Morgan, 1977), the possibility of its being used as a 2nd screening test was investigated, firstly, in an attempt to reduce the incidence of false negativity in the screening process and, secondly, to minimize the human error factor. At the same time the titre at which this test should be used to yield the greatest benefit with the least overload of laboratory capacity was examined.

MATERIALS AND METHODS

Experimental sera

Correlation of SAT and CFT titres. A total of 14 053 sera received for routine testing by the Veterinary Research Institute, Onderstepoort, during the period May to August 1980 were used to establish a correlation between SAT and CFT titres. These sera were all subjected to both the rose bengal (RBT) and SAT tests and all reactors in the RBT and those that had a titre of 34 IU/mℓ or higher in the SAT were subjected to the CFT. In addition 3 807 randomly selected sera were subjected to all 3 tests. The vaccination history of the animals concerned in all these cases was for the most part unknown, but only *Brucella abortus* strain 19 vaccine[1] could have been used. All 3 tests were also performed on 1 745 sera from cattle that had never been inoculated but were in the midst of a spreading infection. In all 3 of these series both the SAT and CFT were repeated where a large discrepancy was seen and the titres altered, if the results of the retest warranted it.

SAT as a 2nd screening test. The 2 groups of sera described above (the 3 807 and 1 745 series) were used to evaluate the efficacy of the SAT as a 2nd screening test. A further 992 sera, derived from animals with an unknown vaccination history, were included as a 3rd group. The 1 745 and 992 sera were tested within 2 days of collection, while the 3 807 sera were delayed in transit and only tested after at least 2 days had elapsed.

SAT in microtitration plates. A total of 2 510 sera submitted for routine diagnostic purposes were subjected to both the tube and microtitration plate SAT tests for the sake of comparison.

Serological methods

SAT in tubes. The methods of Morgan *et al.* (1978), as modified by Herr, Bishop, Bolton & Van der Merwe (1979), were followed, the essential difference being that the test was carried out in 2 mℓ volumes and that an automatic syringe with a 16 gge, 75 mm cannula was used for the serial dilutions. The reading of the test was based on the International Unitage (IU/mℓ) table with the 1 000 IU/mℓ end-point defined as 50% agglutination at a dilution of 1/500 (Herr *et al.*, 1979). A four-tube test was used giving final dilutions of 1/10, 1/20, 1/40 and 1/80 and a maximum reading of 212 IU/mℓ.

SAT in microtitration plates. Preliminary tests were carried out to determine whether the best results would be obtained in U- or V-bottomed plates. A preliminary observation that the agglutinate tended to stick to the bottom of the wells after 16–20 hours incubation led to the use of a shaker and centrifugation of the test at half-hourly intervals. In this way the optimum time for agglutination to occur was determined and the sticking of the agglutinate to the bottom of the wells prevented. It was reasoned that even if agglutination had occurred it would not immediately be seen, as the large molecules would still have to settle out. For this reason the plates were centrifuged at 300 g for 1 min at these same half-hourly intervals. From these results the following method evolved.

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Received 2 November 1981—Editor

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Sera were dispensed (0,1 mℓ) in 96 well, U-bottomed, microtitration plates. Using the micro Compu-Pet multidilutor[2], primed with phenolsaline set to pick up 0,02 mℓ and at a dilution of 1/5, 8 sera at a time were dispensed into rows 1, 5 and 9 of a clean microtitration plate giving 0,1 mℓ of a 1/5 dilution of sera in these rows. The Compu-Pet was then reset to pick up 0,05 mℓ and at a dilution of 1/2. A serial dilution was then done in 4 wells, picking up 0,05 mℓ, transferring 0,1 mℓ and discarding 0,05 mℓ from the 4th well, giving serum dilutions of 1/5, 1/10, 1/20 and 1/40. Antigen[3] was used to reprime the Compu-Pet and the machine was reset at a 1/1 dilution. All the wells received 0,05 mℓ antigen, giving final dilutions of 1/10, 1/20, 1/40 and 1/80. The plates were then placed on a shaker in an incubator at 37 °C for 90 min. After incubation the plates were centrifuged at 300 *g* for 1 min and read over a black background with a diffuse light source. This *g* force for this short a time is sufficient to pellet the larger molecules of the agglutinate but insufficient to spin down the *Brucella* organisms used as antigens.

RESULTS

Correlation between SAT and CFT titres

Patterns of distribution of CFT titres relative to SAT results in uninoculated animals were similar to those animals whose vaccination history was unknown. Out of 1 804 sera with SAT negative titres 1 716 (95,1%) were also negative to the CFT, 58 (3,2%) had titres of 15–24 IU/mℓ, 17 (0,9%) lay between 30 and 49 IU/mℓ, 9 (0,5%) read 60–98 IU/mℓ and 3 (0,2%) exceeded 120 IU/mℓ (Fig. 1a & b). The 360 sera with SAT titres of 17–27 IU/mℓ were distributed as follows: 200 (55,5%) were CFT negative, 77 (21,4%) had CFT titres of 15–24 IU/mℓ, 58 (16,1%) lay between 30 and 49 IU/mℓ, 6 (1,7%) were 60–98 IU/mℓ and 3 (0,8%) exceeded 120 IU/mℓ (Fig. 1c & d). Where the SAT results lay between 67 and 106 IU/mℓ, there was a fairly even distribution of CFT titres ranging from 0–392 IU/mℓ with only 3 specimens exceeding 480 IU/mℓ in the group whose vaccination history was unknown (Fig. 1 h), whereas in the uninoculated group no CFT titres below 60 IU/mℓ were seen (Fig. 1 g). Where SAT titres of 134–212 IU/mℓ were found, most CFT titres exceeded 60 IU/mℓ (Fig. 1 i & j). Of the cases where a SAT and CFT retest was deemed necessary, less than 1% showed substantial changes in the titres.

SAT as a second screening test

As the CFT is used as the definitive test, the efficacy of the SAT as a 2nd screening procedure will depend on the level at which the CFT is taken as indicating a positive result. When a titre of 34 IU/mℓ or higher in the SAT was taken as the criterion for applying the CFT to RBT negative sera, the incidence of false negative tests was reduced from 2,2% to 0,97%, 1,2–0,6% and 0,7–0,24% in the 3 807 series at CFT levels of 18, 30 and 60 IU/mℓ or higher respectively. In the 1 745 series no such reduction occurred (Table 2). With the 992 sera this reduction was of the order of 0,1% (Table 5). When the SAT was used at the 17–27 IU/mℓ level, further reductions in false negatives of 0,47%, 0,2% and 0,11% at the 3 CFT levels were seen in the 3 807 series and of 0,25%, 0,05% and 0,05% respectively for the 1 745 sera (Table 2). With the 992 sera a reduction in false negative levels of 0,1% for the 60 IU/mℓ CFT occurred (Table 4).

The work load of extra CFT's resulting from the use of the SAT at the 17–27 IU/mℓ level was 13,2%, 3,4% and 45,6% for the 3 807, 1 745 and 992 series respectively (Table 4), while the work load was 6,5%, 0% and 1,7% respectively where the 34 IU/mℓ or higher SAT was used. It was impossible to determine how many of these reductions were due to human error in recording results or processing wrong specimens, as no errors were specifically detected, but in routine testing this phenomenon was easily seen.

SAT in microtitration plates

In the series of 2 510 sera 246 were positive to RBT and therefore the same number of CFT's was done. The macro- and microtitres were in agreement within the twofold limit of repeatability in 2 415 (96,2%) of cases (Table 1). In 66 (2,6%) cases the microtitre was higher than the twofold limit but less than fourfold. In 5 (0,2%) cases the microtitre proved less sensitive in the two- to fourfold range. The discrepancy exceeded fourfold in 24 (1%) cases and these occurred exclusively where the macrotitre was either negative or at most 20 IU/mℓ. *In toto* titres of 34 IU/mℓ or higher were recorded in the microtitre system in 83 cases where the SAT in macrovolumes was either negative or between 17–27 IU/mℓ, and in 13 of these cases the microtitre exceeded 67 IU/mℓ.

DISCUSSION

Very broadly, the titres in the SAT so closely parallel those of the CFT (Fig. 1 a–j) that the SAT titre may be used to check each individual CFT titre. Herein lies the advantage of using the SAT in this manner, as there is no other easy way of achieving a control on individual CFT results. Furthermore, the laboratory is able to report on CFT results with much greater confidence. The decision on retests must take practicalities into consideration as a laboratory could very easily become bogged down in this type of work at the expense of efficiency. Nevertheless, keeping the number of retests at a practical, realistic level and yet rechecking all titres that fall outside an arbitrary norm serves a useful purpose in that it keeps a check on human errors which can so easily lead to incorrect results. For this purpose, and working from the figures quoted, a retest of sera in both the SAT and CFT is deemed necessary under the following conditions where distinct discrepancies are encountered:

SAT IU/mℓ	*CFT* IU/mℓ
— to 27	⩾ 60
34 to 53	⩾ 120
67 to 106	⩾ 480
134 to 212	⩽ 49

This policy results in a repeat of 4–6% of all CFT's done and is a load that a laboratory can easily handle. Invariably, where such retests substantially change the result, human error proves to be the cause of the original discrepancy.

International standards accept the incidence of 0,5–0,9% false negative reactions in the RBT screening test, an acceptable level because 100% detection of infected animals can never be achieved with any currently available test, mainly because of the long incubation period. The best tests available can only hope to minimize the potential for spread of infection on a property, but, for the above reason, will be unable to eliminate them except by repeated testing (Morgan, 1977). Thus, although extra tests may prove beneficial in reducing the incidence of false negative reactions and human error, a decision must be made as to what level one hopes to achieve and whether the results warrant the extra workload.

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[2] General Diagnostics, Eastleigh, Hants, SO5 3ZQ, England

[3] *Brucella abortus* SAT antigen, Veterinary Research Institute, Onderstepoort 0110

TABLE 1 Correlation between SAT titres in macro- and microsystems

Microtitre results	SAT macrotitres IU/mℓ					Total	%
	—	17–27	34–53	67–106	134–212		
Within twofold	2 196	75	37	25	82	2 415	96,2
Two- to fourfold microtitre more sensitive	50	10	4	2	—	66	2,6
Two- to fourfold microtitre less sensitive	—	—	—	3	2	5	0,2
More than fourfold microtitre more sensitive	23	1	—	—	—	24	1,0
						2 510	100

TABLE 2 The effect of the SAT as 2nd screening test on the level of false negative screening tests

Total number of sera	False negative RBT		CFT IU/mℓ	False negative screening test using SAT ⩾ 34 IU/mℓ as 2nd screening test		False negative screening tests using SAT ⩾ 17 IU/mℓ as 2nd screening test	
	No.	%		No.	%	No.	%
3 807[1]	85	2,2	⩾ 18	37	0,97	19	0,5
1 745[2]	10	0,5	⩾ 18	10	0,5	5	0,25
3 807	46	1,2	⩾ 30	23	0,6	15	0,4
1 745	2	0,1	⩾ 30	2	0,1	1	0,05
3 807	28	0,7	⩾ 60	9	0,24	5	0,13
1 745	1	0,05	⩾ 60	1	0,05	1	0,05

[1] Field sera of unknown vaccination history with delays of 2–5 days or longer between collection and testing

[2] Sera from unvaccinated animals tested within 2 days of collection

TABLE 3 The effect of the titre (IU/mℓ) in the SAT at which CFT's are carried out on the increase in CFT's done

Total sera tested	No. of CFT's done on RBT positive results	Extra CFT's done at SAT level ⩾ 17 IU/mℓ		Extra CFT's done at SAT level ⩾ 34 IU/mℓ	
		No.	%	No.	%
3 807	735	145	19,7	48	6,5
1 745	147	15	10,2	10	6,8

TABLE 4 Distribution of CFT titres when RBT negative sera are rescreened at SAT levels of 17–27 IU/mℓ

Total number of sera tested	CFT's done on RBT +ve[1]	Extra CFT's done		CFT IU/mℓ							
				—		18–24		30–49		⩾ 60	
		No.	%CFT[2]	No.	%CFT	No.	%FN[3]	No.	%FN	No.	%FN
3 807	735	97	13,2	79	10,7	10	0,2	4	0,1	4	0,1
1 745	147	5	3,4	4	2,72	1	0,05	—	—	—	—
992	57	26	45,6	24	42,1	1	0,1	—	—	1	0,1

[1] RBT +ve = RBT positive

[2] %CFT = percentage of CFT done normally on RBT +ve cases

[3] %FN = percentage false negative results on total number of sera tested

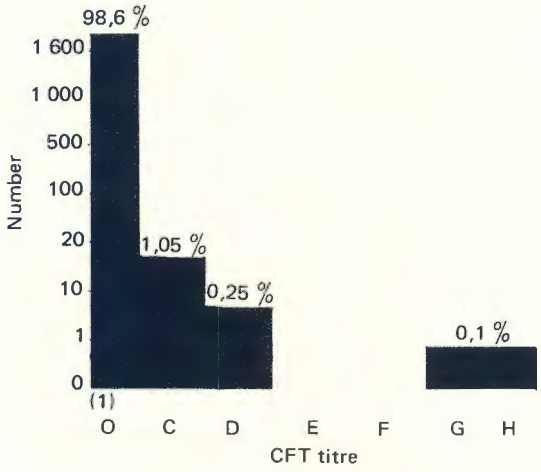
TABLE 5 Distribution of CFT titres when RBT negative sera are rescreened at SAT levels of 34 IU/mℓ or higher

Total number of sera tested	CFT's done on RBT +ve[1]	Extra CFT's done		CFT IU/mℓ							
				—		18–24		30–49		⩾ 60	
		No.	%CFT[2]	No.	%CFT	No.	%FN[3]	No.	%FN	No.	%FN
3 807	735	48	6,5	16	2,2	9	0,2	4	0,1	19	0,5
1 745	147	—	—	—	—	—	—	—	—	—	—
992	57	1	1,7	—	—	—	—	—	—	1	0,1

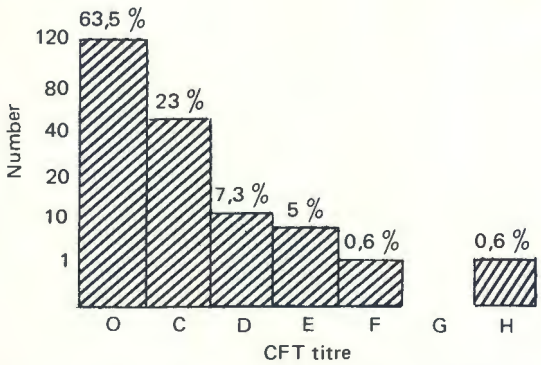
[1] RBT +ve = RBT positive

[2] %CFT = percentage of CFT done normally on RBT +ve cases

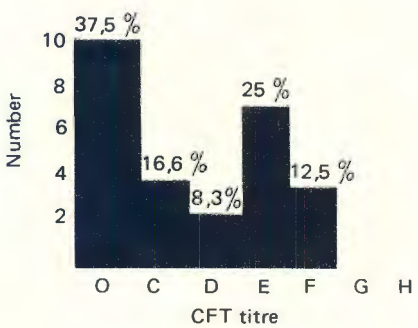
[3] %FN = percentage false negative results on total number of sera tested



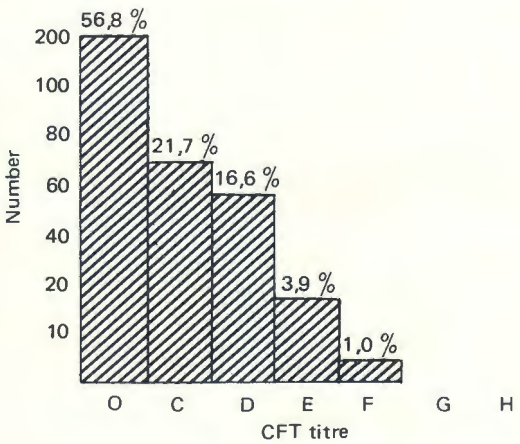
a. SAT 0 IU/mℓ
(unvaccinated)



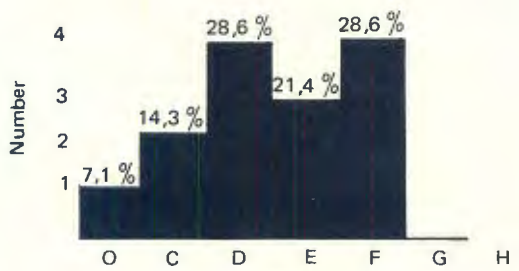
b. SAT 0 IU/mℓ
(vaccination history unknown)



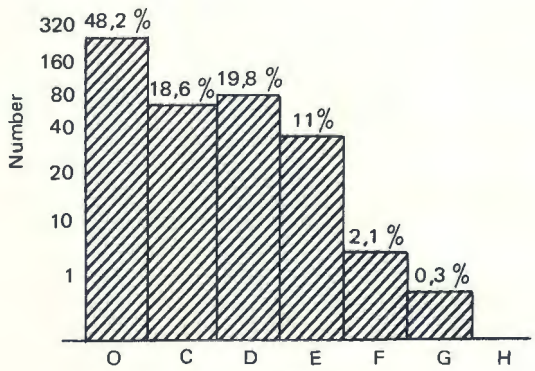
c. SAT 17-27 IU/mℓ
(unvaccinated)



d. SAT 17-27 IU/mℓ
(vaccination history unknown)



e. SAT 34-53 IU/mℓ
(unvaccinated)



f. SAT 34-53 IU/mℓ
(vaccination history unknown)

FIG. 1 (continued)

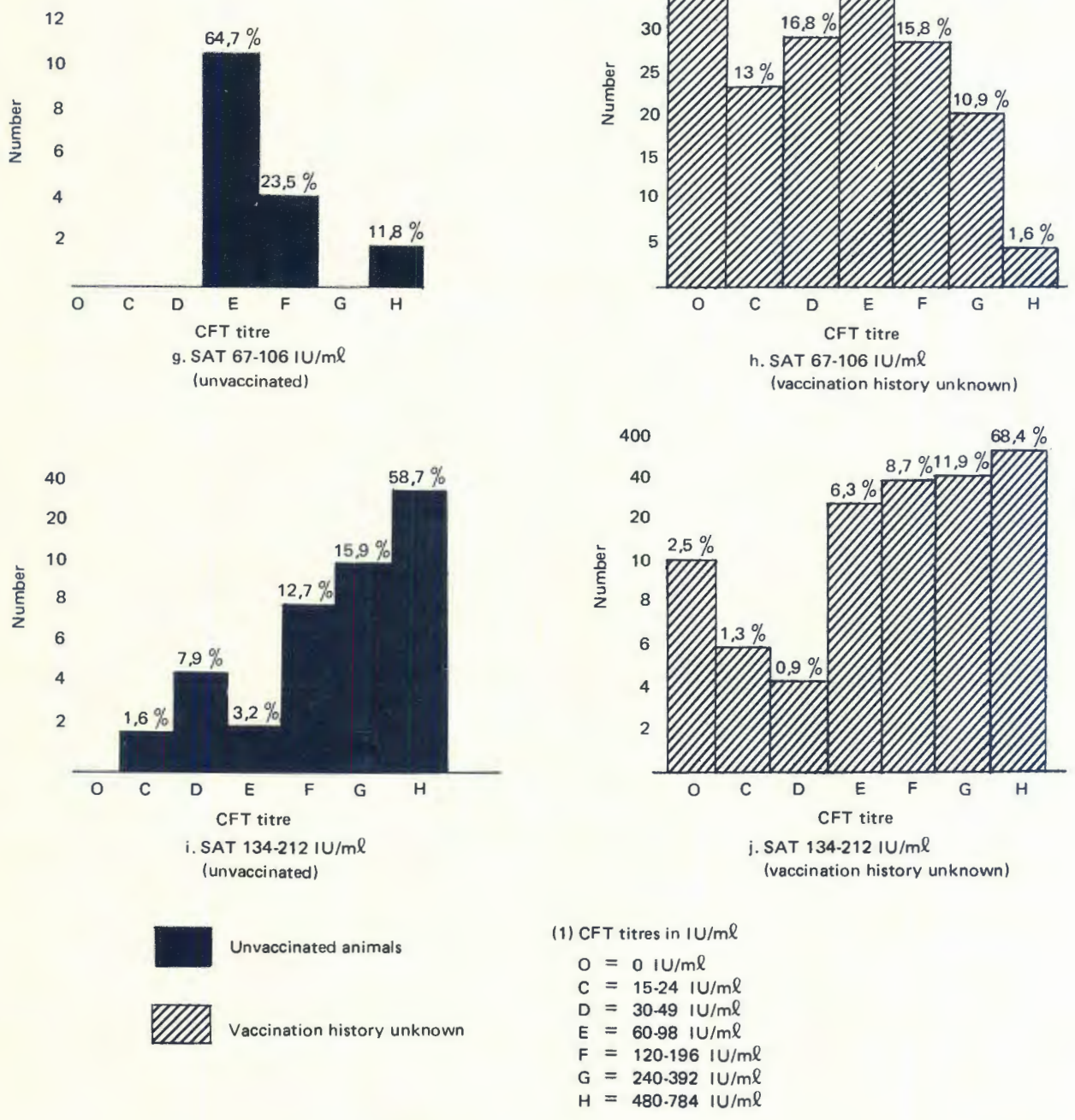


FIG. 1 Correlation between SAT and CFT titres of RBT positive sera

At the same time the significance of CFT titres must be taken into account. National policy in South Africa regards a CFT titre of less than 30 IU/mℓ as suspicious, warranting a retest, and only titres higher than this as indicative of infection in all but adult inoculated cattle (Bosman, P. P., Department of Agricultural Technical Services letter 12/1/8/6/8 July 1979). Thus, in summarizing the figures in Tables 4 and 5, 128/939 (13,6%) extra CFT's would have to be done to reduce the false negative screening tests at the 30 IU/mℓ or higher CFT level by a factor of 9/6544 (0,14%), when using the SAT level of 17–27 IU/mℓ. Where the 34 IU/mℓ or higher SAT titre is used 49/939 (5,2%) extra CFT's give a 24/6544 (0,36%) reduction in false negative screening reactions. Thus it is a decision of expediency to use the latter level in the SAT as a 2nd screening procedure.

Whereas some authorities recommend extra personnel for checking on human error in recording RBT results (Anon., 1980), errors can still occur in the CFT. The inclusion of the SAT saves the double manning of the RBT and at the same time provides an equally efficient check on the CFT.

The effect of using the SAT as a 2nd screening test in reducing the number of false negative screening results (Tables 4 & 5) is markedly greater in the 3 807 sera delayed in transit than in the fresh sera (1 745 and 992 series). This seems to indicate that the RBT is more easily affected by transport conditions than the SAT and delays result in false negative reactions.

In general, the microtitre system proved more sensitive than the tube test (Table 1) as there were only 5 cases where the former was lower than the latter outside the twofold limit of repeatability. A problem that was experienced with this oversensitivity occurred in the 83 cases of negative RBT, negative tube test SAT with microtitres of 34 IU/mℓ or higher. If this limit is used to determine the level at which a CFT is warranted, it would mean an extra 83 CFT's where 246 would suffice. This would be an unacceptable level, as it would mean an increase of 33% in CFT's. However, by selecting a level of 67 IU/mℓ or higher, only 13 (5,2%) extra CFT's become necessary, which is the same figure as applies to the 34 IU/mℓ level used for this pupose in the tube SAT. Using the suggested criteria, the micro SAT then gives sufficiently close results to those obtained with the macrotest to warrant its recommendation and use as a 2nd screening test.

The advantages of the micromethod are, firstly, the saving in antigen, since only 1/20th of the volume is used, secondly, the speed with which the test can be done allowing one person to do upwards of 1 000 tests per day with ease, thirdly, the reduction in apparatus needing to be washed. (For 1 000 tests this would be 4 000 tubes, whereas in the microsystem it is 42 microtitration plates). Fourthly, the fact that the test can be completed with a 90-min incubation period as opposed to 20 h in the macrosystem means that a 5-day-week can effectively be employed instead of the current 4 days used to avoid a Saturday laboratory service. Finally, the turnaround time per serum sample is reduced to a single day as opposed to 2 days when the tube SAT is used.

ACKNOWLEDGEMENTS

The authors wish to thank Mrs E. van der Merwe and Miss J. C. Peens for their technical assistance.

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Printed by and obtainable from the Government Printer, Bosman Street, Private Bag X85, Pretoria, 0001