A modified test for *Brucella* agglutinins

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SYNOPSIS 0.1% Protamine sulphate in normal (0.85% w/v) saline has been used as a diluent in *Brucella* serology instead of saline as such. The use of protamine sulphate in this concentration has obviated the need for performing the modified Coombs test to detect non-agglutinating *Brucella* antibodies.

This method has been used in the Microbiological Diagnostic Unit of the University of Melbourne in the routine titration of 721 sera and has proved to give reliable and reproducible results.

The diagnosis of brucellosis often presents a difficult clinical and bacteriological problem. Harris (1946) has noted how readily brucellosis and psychoneurosis can be confused. Wilson (1957) estimated that the causative organism can only be isolated from 10% of those cases in which infection causes symptoms so it is not surprising that Dalrymple-Champneys (1960) suggested that only one case of brucellosis in 10 is diagnosed and notified.

The Joint FAO/WHO Expert Committee on Brucellosis (De Mello, 1963) after reviewing a number of diagnostic techniques, namely, blood culture, animal inoculation, skin tests, surface fixation, complement-fixation, and tube agglutination tests, considered that the last of these was the only reliable procedure for the diagnosis of individual cases of brucellosis. The direct tube agglutination test was described in 1930 (Fitch, Donham, Bishop, and Boyd, 1930) and has remained virtually unaltered since then. It is satisfactory in the large majority of cases but an appreciable proportion of patients, particularly those in the chronic phase of the illness, may possess non-agglutinating antibodies (Jones and Wilson, 1951).

Wilson and Merrifield (1951) made a significant contribution to the problem created by these antibodies by introducing the Coombs modification in which these non-agglutinating antibodies are demonstrated by the use of antihuman globulin. This greatly improved the test for antibodies to *brucella* but doubled the time taken for its performance.

Protamine sulphate in isotonic saline was used by Vaughan and Walter (1954) and by Roth and Frumin (1954) to detect antibodies in haemolytic anaemia which could otherwise only be demonstrated by the Coombs modification. It, therefore, seemed appro-

priate to try protamine sulphate in the test for *Brucella* antibodies and this paper records the success achieved.

MATERIALS AND METHODS

Brucella abortus strains, both carbon dioxide sensitive and independent, were kept in the lyophilised state. The dried organisms were resuscitated by suspending in *Brucella* (Albimi) broth and incubating in air or in 10% CO₂-air for 16 to 18 hours at 37°C. The broth cultures were then plated out on Albimi agar plates. Single colonies were confirmed as *Brucella abortus* serologically.

BRUCELLA AGGLUTINATING SUSPENSION Using the above strains, an agglutinating suspension was prepared and standardized as recommended by the Standards Laboratory, Central Public Health Laboratory, Colindale.

Protamine sulphate This was obtained from Koch-Light Laboratories, Bucks, England. A 1% stock solution was made by dissolving 1 g of protamine sulphate in 100 ml of normal (0.85% w/v) saline. A 0.1% solution was prepared by mixing 10 ml of stock solution in 90 ml of normal saline.

Coombs reagent (batch K7459) This was obtained from the Wellcome Research Laboratories, Beckenham, England, and was stored at 4°C. It was generally used at a dilution of 1/30 (prepared in normal saline). The diluted serum was kept at -20°C. As repeated freezing and thawing reduces the titre of this reagent, only small volumes of diluted serum were prepared at any one time.

Preparation of glassware Pyroneg (Diversey, A/asia), 1/3 oz, was dissolved in 1 gallon of water at 48° to 52°C. Dreyer tubes, bottles, etc, previously rinsed in tap water, were soaked in this detergent solution overnight. Next day the tubes were rinsed in two to three changes of tap water and then dried.

Sources of sera Most of the sera used in this project came from rural districts, from dairy farmers and allied workers who were suspected of suffering from brucellosis.

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TABLE I
EFFICIENCY OF 0.1% PROTAMINE SULPHATE AS A DILUENT TO DETECT NON-AGGLUTINATING BRUCELLA ANTIBODIES
IN HEATED AND UNHEATED SERA

Case No.	Conventional Test	Modified Coombs Test	Sera in 0.1% Protamine Sulphate	
			Unheated	Heated
1	< 20	80	40	80
2	< 20	640	80	320
3	< 20	80	40	40
4	< 20	40	20	40
5	< 20	80	40	80
6	< 20	320	40	160
7	< 20	640	80	320
8	< 20	20	< 20	< 20
9	< 20	80	40	40
10	< 20	20	< 20	< 20
11	< 20	80	20	40
12	< 20	80	40	80
13	< 20	160	40	80
14	< 20	160	40	40
15	80	160	80	160
16	160	640	80	320
17	640	640	640	640
18	640	1280	640	640
19	< 20	< 20	< 20	< 20
20	< 20	< 20	< 20	< 20

A much smaller number of sera came from suspected cases in the Melbourne metropolitan area.

PERFORMANCE OF TESTS FOR BRUCELLA ANTIBODY The conventional agglutination test was performed by adding equal volumes of the standardized *Brucella* suspension to serial dilutions, from 1/10 to 1/2,560, of sera in normal saline, in Dreyer tubes.

The tubes were examined for agglutination after being incubated at 37°C for 16 hours.

With protamine sulphate In this the conventional technique was employed but the sera were diluted in 0.1% protamine sulphate in normal saline.

Modified Coombs test This procedure was carried out as recommended by Wilson and Merrifield (1951).

RESULTS

Twenty sera which had showed a wide range of *Brucella* antibody titres by the conventional agglutination and antiglobulin tests were re-tested in parallel with the agglutination test employing the protamine sulphate diluent.

Preliminary experiments (Singh, 1968) had indicated that when protamine sulphate was used it was necessary to heat the sera at 56°C for 30 minutes, before testing, to get optimal results. Therefore, in the parallel tests the protamine sulphate diluent was used with both heated and unheated sera.

The results of the parallel tests are shown in Table I.

DISCUSSION

The failure of the conventional agglutination test in

chronic brucellosis and the success of the complement-fixation test and of the Coombs modification are consistent with the appearance of non-agglutinating 7S antibody.

Amerault, Manthei, Good, and Lambert (1961) heated sera to remove non-specific reactions when testing for bovine brucellosis but the use of protamine sulphate in tests for brucellosis has not been described before. The exact mechanism of its action is not fully understood. Various explanations have included suggestions that reaction sites on the antibody molecule are 'opened up' and that electrostatic forces repelling molecules of antigen and antibody are reduced as by the addition of bovine serum albumin (Pollack, 1965).

The modified agglutination test using heated sera and a protamine-sulphate-containing diluent has had its value confirmed by parallel trials with conventional methods by other members of the staff of this School (Rubbo, 1969). Since May 1968 it has been used as a routine technique in the diagnostic unit for the examination of 721 sera and has proved to give reliable and reproducible results effectively correlated with clinical findings, but more extensive studies over prolonged periods are planned before recommending it for general acceptance.

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