JOURNAL OF CLINICAL MICROBIOLOGY, Oct. 1982, p. 737–738
0095-1137/82/100737-02$02.00/0

Vol. 16, No. 4

Microtiter-Adapted Method That Facilitates the Coombs Test for Brucellosis

JOAQUÍN R. OTERO,* ANTONIO FUERTES, ELIA PALENQUE, AND ANTONIO R. NORIEGA
Servicio de Microbiología, Hospital Primero de Octubre, Madrid 26, Spain

Received 19 February 1982/Accepted 2 July 1982

A simple method for carrying out the Coombs test with U-shaped microtiter plates instead of tubes is described. This technique provides reliable and more rapid testing of large numbers of sera in suspected cases of human brucellosis.

Both the complement fixation test and the anti-human globulin (Coombs) test (CT) are generally considered adequate techniques for detecting anti-*Brucella* non-agglutinating antibodies, being especially useful in the diagnosis of chronic brucellosis (2, 3). Our own experiments, which we describe below, confirm the efficacy of CT in cases of brucellosis in which low or undetectable titers have been obtained by the tube agglutination test (TAT). However, although CT is technically easier to perform than the complement fixation test and is therefore more suitable for diagnostic laboratories, even well-equipped laboratories have difficulties in carrying it out on a daily basis if current techniques are used (1, 4). The many tubes handled and the necessity of centrifuging several times can prove tedious and time-consuming. For this reason, we have developed a microtiter-adapted Coombs technique (CM).

A conventional agglutination reaction was reproduced with U-shaped microtiter plates instead of tubes. Twofold serum dilutions were made between 1:20 and 1:40,960 with saline as the diluent (50 μl per well). These dilutions were then doubled by adding 50 μl of standardized *Brucella abortus* 99 antigen (Bio-Mérieux) diluted in normal saline. The plates were briefly vibrated in a microshaker (Dynatech Corp.) and incubated, suitably covered to prevent evaporation, at 37°C for 2 h and then at 4°C until the next day, when the plates were washed three times with saline. Each washing consisted of a 15-min centrifugation at 3,500 rpm in a Hettich Universal 2S centrifuge until a compact button of antigen formed at the bottom of the well. This was followed by an energetic inversion of the plates to eliminate the supernatant. Finally, 0.2 ml of saline was placed into each well, and the plates were vibrated once more so that the antigen button was totally suspended. After the third washing, saline was not added, but the plates were vibrated again to suspend the antigen in the small quantity of saline which inevitably remained in each well. By this time, the day after the serum was received in the laboratory, we knew the preliminary TAT result, and it was possible to carry out an initial screening of CM. If TAT was negative, (titer, <10), washed antigen was taken with a micropipette from the first well of each row (serum dilution, 1:40). If TAT was positive (titer, ≥10), antigen was taken from the well corresponding to two dilutions beyond the preliminary TAT titer. In either case, the entire contents of the well (ca. 15 μl) were mixed with 20 μl of a Coombs reagent (Pronadisa) on a glass slide, using the tip of a toothpick to facilitate the mixing. The glass slide was suitably marked so that simultaneous screening of many sera could be carried out. After incubation in a humid chamber for 30 min at 37°C, the results were read against an appropriate luminous background. Those sera showed by this initial screening to be positive could be titrated by the same technique, with the exception that washed antigen suspension was taken from all the wells corresponding to the serum, from the highest to

TABLE 1. CM results for the first serum sample[a] from 16 bacteriologically confirmed cases of brucellosis with TAT titers of <160.

No. of sera tested by TAT	TAT titer[b]	No. of sera tested by CM	CM titer[b]
5	<10	2	<40
		1	80
		2	1,280
6	40	3	160
		2	320
		1	640
5	80	3	160
		1	320
		1	640

[a] The serum sample was taken simultaneously with blood for culture.
[b] Reported as the reciprocal of dilution.

TABLE 2. Comparison of titers obtained with CT and CM in 52 sera

CT titer[a]	No. of specimens with indicated CM titer[a]									
	40	80	160	320	640	1,280	2,560	5,120	10,240	20,480
40										
80		**1**	1							
160			**4**							
320			2	**2**	2					
640				2	**4**	2				
1,280				1	2	**5**	2			
2,560						2	**7**	1		
5,120							3	**6**		
10,240								1	**1**	
20,480										**1**

[a] Reported as the reciprocal of dilution. Numbers in boldface type represent the numbers of sera for which results coincided.

the lowest dilution. For convenience, all sera with a positive initial screening were gathered for titration on specially made glass plates (Comercial Anger) on which six sera with eight dilutions per serum can be read simultaneously.

To evaluate the technique described, we reviewed the TAT results in the first serum sample from 73 random cases in which brucellosis was confirmed by blood culture and carried out CM on the 16 sera (22%) showing TAT titers of <160, generally accepted as a borderline dilution below which diagnosis is not conclusive (5). The results are shown in Table 1. In 14 of the 16 sera, CM titers exceeded TAT titers in the range of one to seven dilutions. This was particularly significant in three of the five sera with undetectable agglutinating antibodies. In addition, we compared the results obtained by CM with those obtained by the conventional CT. For this, 52 previously recorded sera in which CT titers exceeded TAT titers by a minimum of three dilutions were used. These sera come from cases either bacteriologically confirmed or diagnosed clinically and serologically. The results are shown in Table 2. It is unusual to find differences of more than one dilution between the two Coombs techniques, and the results are satisfactorily reproducible.

In conclusion, bearing in mind the relative frequency with which low TAT titers are observed in cases of active brucellosis, it would seem reasonable to systematically carry out a test complementary to agglutination and capable of detecting non-agglutinating antibodies. CM provides results very similar to those obtained with the conventional CT, but it can be completed much more rapidly and easily. Its use is especially recommended for diagnostic laboratories receiving a great number of serum samples from suspected cases of brucellosis.

We thank Ramón Díaz and Avelino Gutiérrez for critical reading of the manuscript.

LITERATURE CITED

1. **Alton, G. C., L. M. Jones, and D. E. Pietz.** 1975. Laboratory techniques in brucellosis. World Health Organization, Geneva.
2. **Foz, A., and S. Garriga.** 1954. Relation entre la fixation du complément et les "anticorps incomplets" (test de Coombs) dans la brucellose humaine. Rev. Immunol. **18**:288–298.
3. **Kerr, W. R., J. D. Coghlan, D. J. H. Payne, and L. Robertson.** 1966. The laboratory diagnosis of chronic brucellosis. Lancet **ii**:1181–1183.
4. **Kerr, W. R., W. J. McCaughey, J. D. Coghlan, D. J. H. Payne, R. A. Quaife, L. Robertson, and I. D. Farrell.** 1968. Techniques and interpretations in the serological diagnosis of brucellosis in man. J. Med. Microbiol. **1**:181–193.
5. **Renner, E. D.** 1979. Brucellosis. Clin. Microbiol. Newsletter **1**:1–4.