Buffered Plate Antigen Test as a Screening Test for Diagnosis of Human Brucellosis

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Brucellosis in Argentina is currently investigated in bank donor blood by the standard plate agglutination test (PAT). This study evaluated the buffered plate antigen test (BPA), now used to screen for bovine brucellosis, as a screen for human disease. Of 57 sera from patients with culture-confirmed brucellosis, 100% were detected with the BPA. Of 142 sera positive by rose bengal (RB) and complement fixation (CF), from patients with clinical evidence of brucellosis, the BPA detected 100%. Of 307 sera from a nonsymptomatic population that were RB and CF negative, the BPA detected 99.67% of the negative sera. The data indicate that the BPA is satisfactory compared to the other agglutination tests employed. It is an inexpensive and practical screening test and reduces the nonspecific reactions detected by the PAT.

In Latin American countries with high animal densities and high infection rates in cattle, swine, and goats, brucellosis is a health hazard difficult to control (8). Close contact between human and animal populations in rural areas, packing houses, and slaughterhouses increases the transmission from animals to humans. However, its impact on public health is probably underestimated due to lack of reporting and inadequate diagnostic services (5).

Human brucellosis has been found to be afebrile and asymptomatic in some cases, so the need for efficient presumptive tests is great, especially in areas where it is endemic. Since blood for transfusions and organ donors is examined for brucellosis in some countries such as Argentina, the screening test must be improved (9). In many countries, the standard plate agglutination test (PAT), which may give false-negative results (11), is the routine test and is sometimes the only one used (5).

The buffered plate antigen test (BPA) described by Angus and Barton (2) is the officially accepted screening test for the diagnosis of bovine brucellosis in Argentina. The purpose of this study was to assess its use for the diagnosis of human brucellosis.

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Serological tests. The cold complement fixation test (CF) was run as described previously (3). The BPA, the rose bengal test (RB), the standard PAT, and the standard tube agglutination test (TAT) were performed as described by Alton et al. (1). Each test included a control standard serum whose titer was known.

All of the antigens used were prepared in our laboratory from a concentrated cell suspension of smooth *Brucella abortus* 1119-3 by using antigens supplied by the National Veterinary Services Laboratories, U.S. Department of Agriculture, as a reference. The BPA antigen is an 11% suspension of *B. abortus* 1119-3 stained with crystal violet and brilliant green and buffered to pH 3.65 ± 0.02 (1–2). Serum (80 μl) and antigen (30 μl) were mixed with a spreader on a glass plate divided into 4-cm squares and then incubated for 8 min at room temperature. The plate was hand rotated three times, at 4 and 8 min after mixing and just before reading. Any sign of agglutination was considered positive (1).

RB antigen is an 8% *Brucella* suspension stained with rose bengal, buffered to pH 3.65 ± 0.05. Serum (30 μl) was mixed at room temperature with 30 μl of antigen on a flat glass divided into 15-mm squares. The plate was then rotated six times clockwise and six times counterclockwise, placed on a rotary agglutinator for 4 min, and read. Results were considered positive or negative with the presence or absence, respectively, of agglutination.

The PAT antigen is an 11% *Brucella* suspension stained with crystal violet and brilliant green and diluted in physiological saline plus 0.5% phenol (PS). The pH of the antigen ranged from 6.4 to 7.0. First, 80, 40, 20, and 10 μl of the serum sample were placed in a row on 4-cm squares marked on a glass plate. Then, 30 μl of antigen was dropped onto each square and mixed with a spreader in circles, starting with 10 μl of serum and spreading it over an area 2 cm in diameter. The same procedure was used for the other serum dilutions, except that the diameter of the spread was increased up to 3 cm for the 80-μl serum sample. The plate was rotated to ensure proper mixing and allowed to stand for 8 min in a testing box, with one gentle rotation 4 min after mixing. The testing box has a light source that throws oblique light onto the serum-antigen mixture. It is painted black, and its cover prevents too rapid evaporation. After 8 min, the plate was tilted to allow the mixture to flow aside for the reading. The dilutions correspond to the 1:25, 1:50, 1:100, and 1:200 dilutions of the TAT (1). A titer of up to 1:100 was classified as positive, and 1:50 was classified as suspicious.

The TAT antigen is a 4.5% *Brucella* suspension in PS with a pH ranging from 6.4 to 7.0 that is diluted 1:100 in PS before use. The test was done in tubes (13 by 100 mm) arranged in rows of four. Decreasing quantities of 80, 40, 20, and 10 μl of serum were placed into the tubes, and 2 ml of appropriately diluted antigen was added to obtain dilutions of 1:25, 1:50, 1:100, and 1:200. The tubes were shaken and placed in an incubator at 37°C for 48 h. The results were read by observing the tubes against a black background with a light source behind. A titer of up to 1:100 was considered positive, and 1:50 was considered suspicious.

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TABLE 1. Results of three serological tests run on 506 sera from different populations

Serum source (no.)	BPA		PAT				TAT			
	Negative	Positive	Negative	Positive at endpoint titer of:			Negative	Positive at endpoint titer of:		
				1:25	1:50	≥1:100		1:25	1:50	≥1:100
Culture-positive patients (57)[a]	0	57	0	1	6	50	0	4	9	44
Suspected-brucellosis patients (142)[b]	0	142	0	1	20	121	0	4	34	104
Asymptomatic population (307)[c]	306	1	292	14	1	0	293	13	0	1

[a] Of the total of 57 positive isolates, 29 were *B. suis*, 15 were *B. abortus*, 6 were *B. melitensis*, and seven *Brucella* strains were not typed to the species level.
[b] Patients with clinical evidence of brucellosis and positive by RB and CF.
[c] Asymptomatic population negative by RB and CF.

Bacteriological studies. *Brucella* organisms were isolated from three blood cultures incubated in 10% CO_2 as previously described (6). Cultures were kept for 45 days before being considered negative. A suspected *Brucella* culture was subcultured on solid medium for identification. *Brucella* organisms were typed basically as recommended by the International Committee on Bacterial Nomenclature, Subcommittee on Taxonomy of the Genus *Brucella* (4), at the Instituto Nacional de Microbiología "Dr. C. G. Malbrán."

Brucella-infected patients. Fifty-seven patients with symptomatic brucellosis characterized by a wide variety of clinical manifestations, such as moderate or high fever, sweating, headache, anorexia, fatigue, etc., which corresponded to the epidemiological information were selected after the isolation of *Brucella* spp.

Asymptomatic population. Three hundred seven human sera from hospitals in Buenos Aires, Argentina (195 from blood donors, 89 from pre-employment tests, and 23 from laboratory technicians), were selected for this study. The sera belonged to healthy, asymptomatic people (78 women and 229 men) ranging in age from 18 to 65 years.

Suspected-brucellosis patients. One hundred forty-two RB- and CF-positive sera from suspected-brucellosis patients based on epidemiological and clinical information were included in the study. Forty of these patients had negative bacteriological results.

Table 1 summarizes the agglutination test results for the 506 sera divided into three groups. The first group presents the tests' capacity to detect 57 sera from patients with *Brucella* sp.-positive cultures. Isolated were 29 *B. suis*, 15 *B. abortus*, and 6 *B. melitensis* strains, but 7 *Brucella* sp. strains were not typed to the species level. BPA, TAT at 1:25, and PAT at 1:25 identified 100% of the positive sera, while PAT at 1:50 detected 56 cases (98.24%) and TAT at 1:50 identified only 53 cases (92.98%). PAT and TAT at ≥1:100 detected 50 (87.71%) and 44 (77.19%) cases, respectively.

The second group shows the ability of the tests to detect brucellosis infection in patients with suspected clinical and epidemiological evidence of disease. Of 142 sera positive by RB and CF, all were detected by BPA, TAT at 1:25, and PAT at 1:25. PAT and TAT at 1:50 detected 141 (99.29%) and 138 (97.18%) cases, while PAT and TAT at ≥1:100 detected 121 (85.21%) and 104 (73.23%) cases, respectively.

Of the 307 sera that were negative by RB and CF from the asymptomatic urban population, only 1 was positive by BPA, with a titer by TAT of 1:100 and by PAT of 1:50. It is interesting that 17 sera presented suspicious or nonspecific reactions to PAT and TAT at 1:25 (10 were positive by PAT at 1:25 and TAT at 1:25, 3 were positive by TAT at 1:25, and 4 were positive by PAT at 1:25).

Although the definitive test is bacteriological isolation of the organism, *Brucella* cultures are not always positive (28.5% in afebrile patients) (10), so that serological methods must be used as indirect evidence for diagnosis. Attempts to assess the usefulness of a screening test for brucellosis are complicated by the difficulties in bacteriological isolation and the lack of consensus among investigators as to which serological titer reflects a positive case. Differences occur because of the stage of the infection, since brucellosis presents a wide range of incubation periods.

Of the newest serological tests, enzyme-linked immunosorbent assay appears to be the most sensitive. However, it poses several interpretation problems and more experience is needed before it can replace the agglutination tests for human brucellosis (13).

The TAT has become the standard method, is the test recommended for collection of quantitative information on immune responses, and is the most frequently used confirmatory serological test. A TAT response is detected in the early stage of the disease, when immunoglobulin M antibodies are elicited, but individuals having antibodies caused by cross-reacting bacteria may exhibit a similar pattern of serological reactivity. Some authors have suggested that a 1:80 titer could have diagnostic value in urban areas or areas where the disease is not endemic, whereas in rural areas, higher diagnostic titers (up to 1:320) should be used (7, 12); however, the endpoint agglutination titer has not been satisfactorily established. In our study, positive titers of up to 1:100 and 1:50 were considered suspicious for brucellosis.

The serological survey was run on 506 sera. With a cutoff point of ≥1:100, PAT detected 87.71% of the patients with positive cultures from the first group, 85.21% of the suspected patients in the second group, and 100% of the negative cases from the third group. PAT at 1:25 detected 100% of culture-positive sera and 100% of the sera of suspected-brucellosis patients but only 95.43% of negative sera in the asymptomatic population that were negative by CF, RB, and BPA (four sera were negative by TAT at 1:25).

The occurrence of nonspecific reactions and a low rate of false-positive results could be due to healthy individuals who had been exposed to smooth *Brucella* or other species of gram-negative bacteria and therefore showed cross-reactivity. One area of research focused on nonspecific reactions reduced by a lowered pH. The BPA does not identify as many positive reactions to PAT that are of questionable significance.

In our study, BPA detected 100% of the definitely infected cases, 100% of the RB- and CF-positive cases in the group of presumptively infected patients, and 99.67% of the RB- and CF-negative sera in the group of healthy people. It is an inexpensive and practical screening test that effectively reduces the nonspecific reactions detected by PAT. Another advantage is

that, as a simple procedure, it is useful for field laboratories and hospitals lacking skilled personnel. Supplementary tests such as the TAT and the CF must be run on all BPA-positive samples to ensure diagnostic specificity. Evaluation of the quality of the antigen is essential for consistent and replicable results; an unsatisfactory antigen preparation could induce differences. Both the antigen and the test can be standardized (1).

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