Evaluation of Conventional Castaneda and Lysis Centrifugation Blood Culture Techniques for Diagnosis of Human Brucellosis

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We investigated the role of the lysis centrifugation blood culture technique over the conventional Castaneda technique for the diagnosis of human brucellosis. The lysis centrifugation technique has been found to be more sensitive in both acute (20% higher sensitivity; \( P < 0.00001 \)) and chronic (40% higher sensitivity; \( P = 0.087 \)) forms of brucellosis. The major advantage of lysis centrifugation was in the mean detection time, which was only 2.4 days in acute and 2.7 days in chronic cases, with 103 out of 110 (93.6%) and 17 out of 20 (85%) cultures from acute and chronic brucellosis, respectively, detected before the conventional culture was positive. Our results confirmed the potential usefulness of the lysis technique in diagnosis and institution of appropriate antibiotic therapy.

The spectrum of human brucellosis, a zoonosis, ranges from subclinical infection to acute (less than 2 months), subacute (2 to 12 months), and chronic illness, often manifested by recurrent symptoms over many years (1, 10). A definitive diagnosis of this infection is based on culture from different samples, mainly blood. With acute forms produced by Brucella melitensis, the number of positive results from blood cultures by the conventional (Castaneda) technique is usually 70 to 80% (2). This figure is notably reduced for patients with long illness and focal complications; in these cases the percentage of positives rarely exceeds 30 to 50% (3, 6). Although a prior study (4) of the lysis centrifugation technique has demonstrated the possibility of detecting brucellae early along with an increased isolation rate, information concerning the use of this technique in the diagnosis of human brucellosis is scarce, especially for chronic illness. This study compares lysis centrifugation to the conventional blood culture technique for the diagnosis of acute and chronic brucellosis.

The study group comprised 121 acute and 27 chronic brucellosis patients. A case of brucellosis was identified if the titters were \( \geq 1:160 \) (9) by standard tube agglutination testing (Brucella abortus plain antigen; Indian Veterinary Research Institute, Iztanagar, India).

Five milliliters of venous blood was inoculated aseptically into the broth phase of Castaneda’s biphasic medium consisting of brain heart infusion agar and broth (High Media, Mumbai, India), in duplicate. The media were incubated at 37°C with and without a CO₂ atmosphere for 30 days, and the broth-blood mixtures were tilted over the solid phase every day.

A modification of the method described by Etemadi et al. (4) was employed for lysis centrifugation. A 5-ml aliquot of blood drawn simultaneously along with that used for the Castaneda culture was added to a 50-ml screw-cap sterile centrifuge tube containing 20 ml of sterile distilled water and 1.5 ml of 4% sodium citrate. The contents were gently mixed, and the tube was centrifuged (model no. R8C; Remi, Mumbai, India) at \( 2,000 \times g \) for 30 min. The supernatant was discarded, and the sediment was inoculated onto brain heart infusion agar plates in duplicate. The plates were incubated at 37°C with and without carbon dioxide for 7 days.

The bottles and plates were observed daily. The date of the appearance of the first colony was recorded for comparison of growth rates.

Brucella isolates were identified with the help of Gram staining, Kinyoun staining, urease testing, and monitoring of H₂S production (4 days) and sensitivity to dyes, such as basic fuchsin (1:50,000 and 1:100,000) and thionin (1:25,000; 1:50,000; and 1:100,000). Slide agglutination testing was performed using B. abortus and B. melitensis monospecific antisera (Murex Biotech Ltd., Dartford, England). The results were confirmed at the Indian Veterinary Research Institute.

Of 121 blood specimens from acute cases, 87 (71.8%) showed growth of B. melitensis by the conventional technique, whereas lysis centrifugation yielded 110 (90.9%; \( P < 0.00001 \)) isolates (Table 1). The mean time to positive detection by the conventional technique was 6.7 days (standard deviation [SD], \( \pm 2.2 \) days), whereas with the lysis centrifugation technique it was only 2.4 days (SD, \( \pm 0.9 \) days; \( P < 0.00001 \)). The earliest detection of growth by the conventional technique was at 4 days, compared to 2 days with lysis centrifugation. A total of 103 (93.6%) cultures were positive by the lysis centrifugation technique before the first conventional-culture positive was detected.

Of 27 blood specimens from chronic cases, only 9 (33.3%) grew B. melitensis as determined by the conventional technique, compared to 20 (74.1%; \( P = 0.087 \)) as determined by lysis centrifugation (Table 1). The mean time to positive detection by the conventional culture system was 7.2 days (SD, \( \pm 2.6 \) days), and it was only 2.7 days (SD, \( \pm 1.4 \) days; \( P < 0.00001 \)) with the lysis centrifugation technique. The earliest detection of positive culture was 4 days with the conventional technique, compared to 2 days with lysis centrifugation. Sev-
enteen (85%) cultures were positive by the lysis centrifugation technique before the first conventional-culture positive was detected.

All the isolates were identified as *B. melitensis* biotype 1. There was no correlation to titers of culture positives and negatives by both techniques.

The conventional culture system was found to be contaminated for only 4 (2.7%) blood specimens, whereas lysis centrifugation showed contaminants in cultures of 13 (8.7%) blood specimens. Most of the contaminants in the lysis centrifugation group were obtained in the initial phase of the study, and with increased efficiency, the contamination rate came down.

While techniques introduced by Ruiz-Castaneda improved the chance of culturing *Brucella* spp., the rate of isolation from patients with chronic or subacute forms of the disease remains low. Although a presumptive diagnosis of brucellosis can be made by demonstrating high or rising titers of antibodies to *Brucella* antigens, isolation of the organism from blood, bone marrow, or tissue is the only irrefutable proof of the disease (8, 11). Overall, blood cultures are positive in 53.4 to 90% of cases. With 110 (90.9%) of 121 cases being detected by the lysis centrifugation technique with a mean detection time of 2.4 days, compared to only 87 (71.8%) cases detected by the conventional technique. Here also, 103 (93.6%) out of 110 cultures were detected before the conventional culture was positive. Etemadi et al. (4) have also reported the rapid recovery of *brucellae* within 48 h in their study of the lysis centrifugation procedure. The lysis concentration technique has not only detected the pathogen earlier (>90%; *P* < 0.00001) but also picked up a larger number of cases (20% more acute and 40% more chronic cases; *P* < 0.00001). We would have missed 34 cases if conventional culture alone had been performed—a point worth noting.

The superiority of the lysis technique is in the rapid confirmation of clinical diagnosis and also its sensitivity in confirmation of a larger number of cases, especially of chronic illness. This method would be the ideal one since it is technologically simple and uses equipment and reagents which are already available in most clinical laboratories. The rapid confirmation of the etiological agent would permit the institution of appropriate therapy, thereby decreasing morbidity.

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### REFERENCES


### TABLE 1. *Brucella* recovery rates of blood culture systems*^a^*

<table>
<thead>
<tr>
<th>Brucellosis group</th>
<th>No. of patients</th>
<th>No. of specimens (%) positive in:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Conventional system</td>
</tr>
<tr>
<td>Acute</td>
<td>121</td>
<td>87 (71.8)</td>
</tr>
<tr>
<td>Chronic</td>
<td>27</td>
<td>9 (33.3)</td>
</tr>
</tbody>
</table>

* The chi-square (Yates-corrected) test was used for statistical analysis. For the acute (chi square, 20.00), chronic (chi square, 2.92), and combined (chi square, 27.82) groups, the *P* values were as follows: <0.000001 (significant), 0.087 (not significant), and <0.000001 (significant), respectively.