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Brucella detection in blood: comparison of the BacT/Alert standard aerobic bottle, BacT/Alert FAN aerobic bottle and BacT/Alert enhanced FAN aerobic bottle in simulated blood culture

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The objective of this study was to compare the performances of the standard aerobic bottle (StAe), FAN aerobic (FANAe) and enhanced FAN aerobic (E-FANAe) (the charcoal component of the FANAe was revised recently to improve the feasibility of Gram smear interpretation) blood culture bottles for BacT/Alert system for the detection of *Brucella melitensis* in simulated blood culture. Triplicate strains of eight clinical isolates of *B. melitensis* were studied. Each bottle was inoculated with 5 mL of freshly collected human blood at three different targeted bacterial inocula (10^1, 10^2 and 10^3 CFU/bottle). All bottles were monitored for up to 21 days or until they became positive. The results of time to detection (TTD) on the eight *B. melitensis* samples were as follows: at 10^1 CFU/bottle, the E-FANAe had a mean TTD significantly shorter than the StAe (48 h vs. 56.2 h, $P < 0.05$); and at 10^3 CFU/bottle, the FANAe and E-FANAe had a mean TTD significantly shorter than the StAe (41.2 h and 40 h vs. 45.6 h, $P < 0.05$). The reproducibilities (no.of positive signals/no.of all bottles) of three bottle systems were as follows: at 10^1 CFU/bottle, the reproducibilities of StAe, FANAe and E-FANAe were 96, 83 and 58%, respectively. At 10^3 CFU/bottle, the reproducibilities of StAe, FANAe and E-FANAe were 95, 95 and 91%, respectively. Positive results for the presence of bacteria in Gram smears were confirmed in 68% of StAe, 54% of FANAe and 90% of E-FANAe. In case of suspected brucellosis, the combination of one StAe bottle and one E-FANAe bottle seems to provide the highest and fastest recovery of the organism.

Keywords Brucella, BacT/Alert blood culture system

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INTRODUCTION

Brucellae are intracellular parasites that cause septicemic febrile illness or localised infection of bone, tissue, or organ systems in humans. Brucellosis in humans has a variable incubation period, an insidious or abrupt onset, and no pathognomonic symptoms or signs [1]. Diagnosis of brucellosis is based on bacteriological and serological tests, and blood cultures should be obtained when brucellosis is suspected. Primary isolation of *Brucella* spp. is difficult because it is a slow-growing bacterium and conventional diphasic blood culture bottles may require an incubation period of weeks [2]. Automated blood culture systems seem to shorten the time to detect these organisms from blood and other body fluids [1].

The BacT/Alert Microbial Detection System (Organon Teknika Corp., Durham, NC, USA) was introduced in 1990 as an automated colorimetric blood culture system consisting of standard aerobic (StAe) and anaerobic blood culture bottles and pediatric aerobic bottles, containing growth sensors, for detecting microbial growth [3]. The manufacturer also developed an aerobic medium (FANAe) with a brain–heart infusion base containing Ecosorb. Ecosorb is a proprietary substance that contains adsorbent charcoal, Fuller's earth, and other components. FAN media were developed to enhance the recovery of fastidious organisms from blood, as well as to improve the detection of bacteremia and fungemia in patients receiving antimicrobial agents. Recently, the charcoal component of the FAN media was revised to improve the feasibility of Gram smear interpretation. These revised media were termed enhanced FAN (E-FANAe).

Brucellosis caused by *B. melitensis* is endemic in our region. In our laboratory, approximately 50–60 *B. melitensis* strains are isolated from 8000 blood cultures per year.

The aim of this study was to evaluate the performance of the three different bottles (StAe, FANAe and E-FANAe) for the detection of *B. melitensis* in seeded blood cultures.

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MATERIALS AND METHODS

Study design

StAe, FANAe, and E-FANAe blood culture bottles for the BacT/Alert system were used. The bottles were supplied by the manufacturer (Organon Teknika Corp.). Eight recent blood isolates of *B. melitensis*, all of which were selected arbitrarily, were tested. These were isolated from patients with brucellosis and identified by standard laboratory methods in our laboratory.

Brucella strains were inoculated on sheep blood agar and incubated for 48 h at 35 °C in an aerobic atmosphere. Suspensions of these growths were made in 10 mL of trypticase soy (TS) broth (BBL) and adjusted to a McFarland 0.5 standard. These suspensions (containing approximately 10^8 colony-forming units (CFU)/mL) were subsequently diluted with TS broth to achieve an organism concentration range of between 10^1 and 10^3 CFU/mL. Colony counts were performed to verify the actual concentrations. Blood culture bottles were each inoculated with 5 mL of fresh blood collected from known healthy volunteers who had been checked for Brucella antibodies by the Wright agglutination test before the study. Triplicate bottles were inoculated with 1 mL of each the bacterial suspensions containing 10^1, 10^2 and 10^3 CFU/mL. All bottles were transiently vented, placed in the BacT/Alert instrument under continuous agitation and monitored for up to 21 days or until they became positive. When a positive bottle was flagged, a Gram stain of the broth was performed, and a portion of the fluid was subcultured on TS agar medium with 5% sheep blood. In addition, blind subcultures of all terminally negative bottles were performed using sheep blood agar. Gram stain results, time to detection (TTD) of positive results and blind subculture results were recorded. Contaminated bottles were excluded from the study.

Statistical analysis

Data were analyzed by ANOVA and the posthoc Scheffe procedure. *P*-values lower than 0.05 were accepted as statistically significant.

RESULTS

Time to detection analyses of *B. melitensis* strains from the three different bottles are shown in Table 1. The recovery of *B. melitensis* strains in the FANAe and E-FANAe bottles was faster than in the StAe bottles. The performances of the three bottle systems are shown in Table 2. False negative (detection negative and subculture positive) results were 3, 4.6, and 7.5% in StAe, FANAe, and E-FANAe, respectively. The reproducibilities (no. of positive signals/no. of all bottles) of the FANAe and E-FANAe bottles were found to be lower when compared with the StAe bottles (Figure 1). Positive results for the presence of

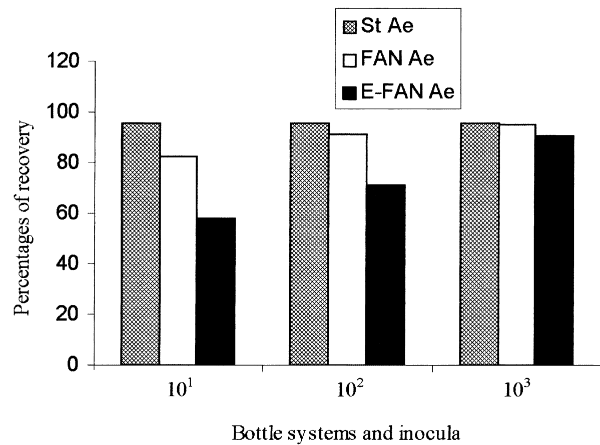

Figure 1 Percentages of recovery of *B. melitensis* by bottle systems and inocula.

Table 1 Time to detection analyses of *B. melitensis* strains from the three different bottles

Time to detection (h), mean ± SD							
CFU/bottle	***n***	**StAe**	***n***	**FANAe**	***n***	**E-FANAe**	***P***
10^1	23	56.2 ± 7.9	19	51.5 ± 5.8	14	48.0 ± 3.6[a]	<0.05
10^2	22	51.3 ± 7.6	21	46.7 ± 5.3[a]	15	44.2 ± 2.3[a]	<0.05
10^3	21	45.6 ± 5.3	19	41.2 ± 3.4[a]	20	40.0 ± 3.5[a]	<0.05
Total	66	51.2 ± 8.2	59	46.5 ± 6.4[a]	49	43.6 ± 4.6[a]	<0.05

[a]Significantly different from StAe.

Table 2 Overall performances of three bottle systems (%)

Bottle system/no.of bottles	Detection positive, subculture positive	Detection negative, subculture negative	Detection positive, subculture negative	Detection negative, subculture positive
StAe/69	95.7	1.3	0	3
FANAe/66	89.4	6	0	4.6
E-FANAe/67	73.1	19.4	0	7.5

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bacteria with Gram smears were confirmed in 68% of StAe, 54% of FANAe, and 90% of E-FANAe.

DISCUSSION

A definitive diagnosis of brucellosis is based on the culture of *Brucella* strains from different samples, mainly blood. Broth-based blood culture systems have gained universal acceptance for the isolation of Brucellae, but the slow growth of the organism has hampered this method. Blood cultures of patients with suspected brucellosis need a long incubation time (30 days) and periodic blind subcultures to avoid false negative results [4]. The time to isolation of the organism has been decreased by using biphasic Castañeda medium and a lysis-filtration method [2]. These techniques are time-consuming and labor-intensive and require extensive manipulation of specimens. They also pose a substantial risk for laboratory staff [5]. However, automated blood culture systems have reduced the detection time of these organisms [6–8]. The BacT/Alert is an automated blood culture system in which the growth of organisms produces increased amounts of CO_2, which diffuses through a semi-permeable membrane in the base of the culture bottle and reacts with water to generate hydrogen ions. This causes a decrease in pH, resulting in a color change of a built-in sensor. In this study we compared the performance of three bottle systems of BacT/Alert (StAe, FANAe, and E-FANAe) for the detection of *B. melitensis* in seeded blood cultures. The recovery of *B. melitensis* strains in FANAe and E-FANAe bottles was faster than in StAe bottles (Table 1). This may be due to the differences between the media types (brain–heart infusion broth vs. tripticase soy broth) as well as to the components found in the bottle systems. However, these data should not be significant in a clinical setting. Solomon et al. [9] demonstrated a mean detection time of 48 ± 1 h in eight of 10 replicates seeded with a stock containing 10^2 CFU/mL of *B. melitensis* by using BacT/Alert StAe bottles. Zimmermann et al. [10] performed seeded blood culture studies of a *B. abortus* isolate with fresh human blood and target inocula of both 5 and 500 CFU/mL with the BACTEC NR 730 system and indicated that the larger (500 CFU/mL) inoculum produced positive instrument detection within 2 days, whereas the smaller (5 CFU/mL) inoculum required 5.5–7.5 days for detection, depending on the medium used.

As expected, the number of seeded microorganisms was correlated inversely with the TTD of bacterial growth in all bottle systems. Yagupsky et al. [11] have shown that the magnitude of Brucella bacteremia correlated inversely with the TTD of the organism, using another automated system, BACTEC 9240.

The performances of the three bottle systems are shown in Table 2. No false positive (detection positive, subculture negative) result was detected in any bottle system. False negative (detection negative, subculture positive) results were found in 3% of StAe bottles, 4.6% of FANAe bottles, and 7.5% of E-FANAe bottles. StAe bottles showed a higher performance when compared with FANAe and E-FANAe (detection positive, subculture positive were 95.7, 89.4, and 73.1%, respectively), regardless of the magnitude of bacteria.

The reproduciblity of the FANAe and E-FANAe bottles was found to be lower when compared with StAe bottles (Figure 1). The amount of sodium polyanethol sulfonate (SPS) in StAe bottles was lower than in the FANAe and E-FANAe bottles. SPS, used as an anticoagulant in many blood cultures, exerts a harmful effect on the outer membrane of the bacteria, making it permeable to hydrophobic substances and thus hindering growth [12]. Gamazo et al. [13] found that growth values of *B. melitensis* in an automated blood culture system were lower in vials with SPS than in vials without this agent.

In our study, positive results for the presence of bacteria with Gram smears were confirmed in 68% of StAe, 54% of FANAe, and 90% of E-FANAe. The Gram stain results from the E-FANAe bottles were better than from the FANAe bottles since the charcoal component of the FAN media was revised to improve the feasibility of Gram smear interpretation. The poor counterstaining quality of brucellae might also preclude detection by Gram stain [14]. Therefore a negative Gram stain should not be used to rule out a signal positive result, especially in blood cultures of patients suspected of having brucellosis.

In the case of suspected brucellosis, the combination of one StAe bottle and one E-FANAe bottle seems to provide the highest and fastest recovery of the organism. The results of this study need to be supported by further clinical investigations.

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Serodiagnosis of tuberculosis by enzyme immunoassay using A60 antigen

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Tuberculosis continues to be a worldwide public-health problem, with an estimated 90 million new cases and 30 million deaths during the last decade of the 20th century [1]. It has been an ever-present health threat in developing countries. At the same time, it has been increasing in Europe and the USA following the emergence of AIDS in the world [2]. The prevalence of tuberculosis in Turkey is 0.35%, and 30 000–40 000 new cases are being reported every year [3]. An estimated 2 billion people are currently infected with *Mycobacterium tuberculosis* and *Mycobacterium intracellulare* complex throughout the world [4]. The rates of morbidity and mortality are also rising as a result of multidrug-resistant strains [5].

The diagnosis of mycobacterial diseases depends upon identifying the infecting organism in the secretion or tissues. However, there are several limitations of this method. One is that *Mycobacterium tuberculosis* is usually present in undetectable numbers (for smear 5×10^3 to 5×10^4 bacilli/mL, and for culture 10–100 bacilli/mL), so that it is recognized for the most part in advanced cases [6]. Second, most mycobacteria are slow-growing organisms and require long periods of time to culture, even if the most advanced techniques are used [6,7]. Third, negative smears are usually obtained until cavities form [8]. Thus, a fast, easy and reliable method was needed for the diagnosis of tuberculosis. Among several serologic techniques, it has been concluded that enzyme immunoassay (EIA) is a sensitive, reliable, simple and rapid method [6]. Several purified antigens, such as 38-kDa protein, 85A antigen, lipoarabinomannan, plasma membrane antigen, antigen 5 and antigen 60 (A60), have been used for the serodiagnosis of tuberculosis [8–12]. This study was undertaken to evaluate the usefulness of the EIA method using A60 antigen for the diagnosis of different forms of tuberculosis in Turkish patients.

Serum samples were collected from four groups of patients and the control group. Sera from active tuberculosis patients were collected before chemotherapy, and kept at $-70\,^{\circ}C$ until the EIA procedure.

Group 1 consisted of 112 patients (74 male, 38 female) with a mean age of 36.32 ± 12.25 years, who were diagnosed as having active lung tuberculosis by positive smear and/or culture and clinical and radiologic findings. They were all anti-HIV negative.

Group 2 consisted of 40 patients (29 male, 11 female) with a mean age of 59.47 ± 13.36 years, with the diagnosis of inactive lung tuberculosis by radiologic findings and patient history. Three of the patients had a history of tuberculosis during the previous 2 years, and 26 of them had a history of tuberculosis before that period. Eleven of 40 patients had no tuberculosis history but had findings related to a previous infection in their chest X-rays. None of their sputum smears and cultures were positive.

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