

Comparison of BACTEC 9240 Peds Plus Medium and Isolator 1.5 Microbial Tube for Detection of *Brucella melitensis* from Blood Cultures

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The sensitivity and time to detection of *Brucella melitensis* by the BACTEC 9240 and the Isolator blood culture systems were compared in a prospective volume-controlled study. Blood sample aliquots, obtained from children with suspected brucellosis, were inoculated into a BACTEC 9240 Peds Plus bottle and into an Isolator 1.5 Microbial Tube. Overall, 122 pairs of blood samples for culture were obtained, and 28 (23%) were positive by at least one method. The BACTEC 9240 system detected all 28 positive cultures (sensitivity, 100%), and the Isolator system detected 22 positive cultures (sensitivity, 79%) ($P = 0.023$). Among those 22 cultures positive by both methods, 21 (95%) and 15 (68%) were found to be positive within 3 days by the BACTEC and the Isolator systems, respectively; 8 (36%) were found to be positive at least 1 day earlier by the BACTEC instrument, and the remaining 14 were found to be positive by the two systems on the same day ($P = 0.045$). The BACTEC 9240 blood culture system is more sensitive than the Isolator system for the detection of *B. melitensis* and is superior in terms of time to detection of the organism.

Infections caused by *Brucella* spp. continue to affect human populations living in countries where the disease is endemic (1, 12, 13). The diagnosis of brucellosis is established with certainty by the isolation of the organism from blood and other body fluids or tissues (2, 4, 7, 13). Because brucellae tend to grow slowly, current recommendations advise prolonged incubation of blood culture media to maximize the recovery of this fastidious bacterium (2, 7).

The BACTEC 9240 system (Becton Dickinson Diagnostic Instrument Systems, Towson, Md.) is one of the new automated continuously monitoring blood culture systems. Detection of positive cultures by the BACTEC instrument is based on continuous monitoring of CO₂ production by growing organisms with a fluorescence sensor (9). Its main advantages over previous generations of blood culture instruments include full automation once the bottles are loaded, shortened time for detection of blood pathogens, considerable labor savings, and improved laboratory work flow (9). No data on the performance of this system for the detection of *Brucella* spp. have been published. On the other hand, the Isolator blood culture system (Wampole Laboratories, Cranbury, N.J.), a nonautomated lysis-based method, is also gaining acceptability because it has been shown to increase the recovery of intracellular pathogens such as mycobacteria and fungi (8, 10). Limited experience with this technique suggests that the Isolator system may shorten the time to detection of circulating brucellae to 2 to 5 days (8).

A prospective volume-controlled study was conducted to compare the performance of the BACTEC 9240 and the Isolator blood culture systems for the recovery of *Brucella melitensis* in southern Israel.

MATERIALS AND METHODS

Background. In the southern region of Israel *B. melitensis* is endemic among the seminomadic Bedouin population of the area, who maintain herds of unvaccinated sheep and goats and who consume unpasteurized dairy products. An incidence rate of the disease as high as 52 per 100,000 inhabitants was reported in the region in 1988 (1).

Blood culture methods. At the physician's discretion, blood for culture was drawn from febrile Bedouin children referred to the emergency room of the Soroka Medical Center, the only medical facility in the region. Whenever brucellosis was suspected on the basis of epidemiological or clinical grounds, positive *Brucella* serology, or isolation of the organism from a culture of normally sterile body fluid, the following protocol was followed. By a strict sterile technique, 3 ml of blood was obtained by venipuncture. The blood specimen was divided into two 1.5-ml aliquots, and one aliquot was inoculated into a BACTEC 9240 Peds Plus blood culture bottle and one was inoculated into an Isolator 1.5 Microbial Tube. The bottles and tubes were sent to the Clinical Microbiology Laboratories without delay and were processed within half an hour.

Inoculated Peds Plus medium bottles were incubated at 35°C and were monitored on a continuous basis for 4 consecutive weeks by the BACTEC 9240 instrument. No venting of the inoculated bottles was performed, and the growth index thresholds and change in growth index values used to identify positive cultures were those recommended by the manufacturer. When a positive bottle was detected, a Gram stain of the broth was performed, and a portion of the fluid was subcultured onto Trypticase soy agar medium with 5% sheep blood, a chocolate agar plate, and a MacConkey agar plate. In addition, blind subcultures of negative bottles were performed on chocolate agar medium once a week, disregarding the growth index readings.

Inoculated Isolator tubes were processed in a type II biological safety cabinet following the manufacturer's recommendations. The blood lysate was dispersed on the surface of Trypticase soy agar medium with 5% sheep blood and on chocolate agar plates.

All plates seeded with either Peds Plus medium or Isolator blood lysate were incubated at 35°C in a 5% CO₂-enriched atmosphere and were examined for bacterial growth once a day for 10 days.

Presumptive identification of *Brucella* species was performed on the basis of a typical microscopic picture showing small gram-negative coccobacilli; positive oxidase, catalase, and urease tests; and negative fermentation of sugars and was confirmed by a positive agglutination with specific antiserum (7).

The overall sensitivity for the recovery of brucellae, time to detection of bacterial growth, and cumulative positivity rates of the BACTEC 9240 and Isolator methods were established and compared. The Fisher exact test was used to assess the statistical significance of the observed differences between the two blood culture methods (5).

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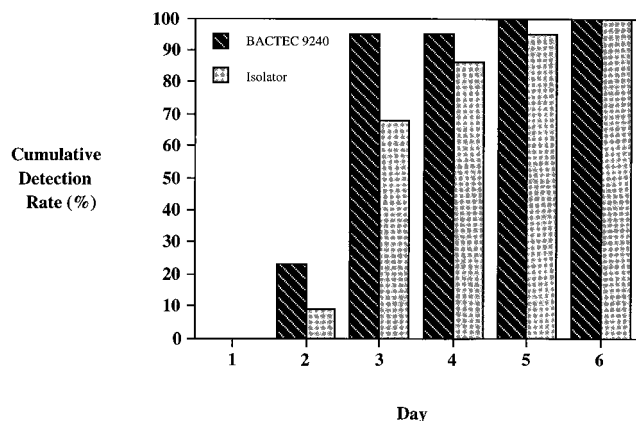


FIG. 1. Cumulative detection rates of bacterial growth by the BACTEC 9240 and the Isolator blood culture systems.

RESULTS

A total of 122 pairs of blood samples obtained from 66 pediatric patients in whom the diagnosis of brucellosis was entertained and inoculated into Peds Plus medium bottles and Isolator tubes were available for comparison, and 28 (23%) of the cultures of blood, drawn from 11 patients were positive by at least one method. The BACTEC instrument detected all 28 positive cultures (sensitivity, 100%), and the Isolator system detected 22 positive cultures (sensitivity, 79%) ($P = 0.023$). Three of the six positive blood samples missed by the Isolator system were obtained from children with known brucellosis who were being treated with doxycycline or rifampin and gentamicin, and two others were drawn from children in whom the disease relapsed after completing a course of antimicrobial therapy.

The cumulative detection rates of the 22 blood samples in which brucellae were detected by both culture systems are presented in Fig. 1. By day 3, 21 positive cultures (95%) were already detected by the BACTEC system, but only 15 (68%) were positive by the Isolator system ($P = 0.045$). Growth was detected earlier by the BACTEC instrument for 8 paired cultures, and for the remaining 14 paired cultures, growth was detected on the same day by both systems.

The magnitude of brucella bacteremia, as determined by the quantitative results for the 22 positive Isolator cultures, ranged between 1.3 and 1,000 CFU/ml (median, 88 CFU/ml) and correlated inversely to the time to detection of the organism ($r = -0.22$ and $P = 0.32$ and $r = -0.26$ and $P = 0.24$ for the BACTEC 9240 and Isolator systems, respectively).

DISCUSSION

Although broth-based blood culture systems have gained universal acceptance because of automation and labor savings, the isolation of brucellae by this method is frequently hampered by the slow growth of the organism. To avoid false-negative results, incubation of cultures of blood from patients with suspected brucellosis for 30 days and performance of periodic blind subcultures have traditionally been recommended (2, 7).

Over the years, alternative strategies have been attempted to decrease the time to isolation of the organism, including the use of the biphasic Castañeda medium and a lysis-filtration method (2-4, 6, 7). These methods have generally been successful in detecting brucellae within 1 week of incubation, but

their use has been limited by several drawbacks. These techniques are not commercially available, are time-consuming and labor-intensive, and require extensive manipulation of specimens potentially contaminated with virulent *Brucella* organisms, posing a substantial risk for laboratory personnel (2-4, 6, 7).

In 1993, Navas et al. (8) reported their experience with the use of the 10-ml Isolator tube, a commercial lysis-based method, and compared its performance to that of the BACTEC 660NR system for the detection of *B. melitensis*. All seven cultures positive by at least one of the two methods were detected by the BACTEC system, whereas the Isolator tube detected six positive cultures. The average time to detection, however, was 20.6 days for the BACTEC 660NR system compared with only 3.1 days for the Isolator tube. It should be pointed out, however, that although an equal blood volume was used to inoculate each system, half of each blood sample in the BACTEC culture was divided between aerobic and anaerobic media. Because anaerobic bottles cannot adequately sustain the growth of obligatory aerobic brucellae, for practical purposes, the effective blood volume inoculated into the BACTEC system was only half of that seeded into the Isolator tubes.

Our own experience with the BACTEC 660NR system for the detection of *B. melitensis* has been more favorable. In a prospective study, this blood culture system enabled the detection of 79% of cultures positive for the organism within 7 days and without requiring terminal blind subculturing, although incubation of blood culture bottles for up to 3 weeks was needed to maximize isolation of the organism (12).

In the present study, the performances of the Isolator and the BACTEC 9240 blood culture systems were compared for the first time. The Isolator system does not require expensive equipment, and since colonies are already developed by the time that detection of bacterial growth occurs, the identification of the organism can be made without delay. The system, however, is not suitable for automation, is time-consuming, is more prone to contamination, and poses a substantial threat to the laboratory technicians while they are working with transmissible pathogens (2, 7, 8, 10). In addition, because the Isolator system is not used by most clinical laboratories for routine blood cultures, inoculation of an Isolator tube to detect brucellosis will only be done when the diagnosis of the disease has already been entertained.

Use of the BACTEC 9240 system for the isolation of brucellae offers some clear advantages. Because the system is fully automated, it allows for the simultaneous processing of a large number of bottles. Monitoring of growth is performed by non-invasive sampling, and therefore, the risks of contamination of media or dispersion of aerosols are minimized, an important consideration for highly transmissible and dangerous safety level 3 organisms such as *Brucella* species (7, 9).

The quantitative information provided by the Isolator system shows that the usual concentration of circulating brucellae in children with acute disease ranges on the order of a few dozens to a few hundred CFU per milliliter. It is possible, however, that the relatively high bacterial counts found in the present study are the result of the fact that the population studied consisted of children in whom, generally, higher bacterial counts are encountered compared to those found in adult bacteremic patients (11). In addition, the vast majority of our patients had been sick for a few days only, and therefore, the high bacterial concentration found may characterize the initial phase of the disease before partial immunity has developed. Whether the present observations are also representative of the disease among adults or in patients with a more

prolonged or subacute clinical course remain to be determined in future studies.

As expected, the number of circulating microorganisms correlated inversely with the time to detection of bacterial growth. Even when the magnitude of bacteremia was low, the BACTEC system was able to detect brucellae within the routine 1-week incubation period instituted by most clinical microbiology laboratories. The practical implication of this observation is that isolation of the organism will not generally be missed when negative bottles are routinely discarded after 1 week of incubation and blind subcultures are not performed. Although the BACTEC 9240 blood culture system frequently detected bacterial growth 1 to 3 days earlier than the Isolator 1.5 Microbial Tube did, it should be pointed out that this apparent advantage was compensated for by the need to subculture the contents of positive bottles for 24 to 36 h before identification of the organism could be performed.

The reasons for the improved performance of the BACTEC 9240 system compared to the performances of previous generations of blood culture instruments are not clear. Continuous rather than sporadic monitoring of CO₂ production may result in only a modest shortening of the time to detection of positive bottles. It is possible that growth factors included in the enriched Peds Plus medium accelerate the growth of fastidious brucella organisms. Whether other broth media, and especially those in use for culturing the blood of adult patients, will also support the rapid growth of *B. melitensis* deserves further investigation.

The present results also indicate that the overall sensitivity of the BACTEC 9240 blood culture system was superior to that of the Isolator 1.5 Microbial Tube, especially for antibiotic-treated patients, suggesting that the resins contained in the Peds Plus medium had a salutary effect under these circumstances. In the present study, detection of viable organisms during or after completion of antimicrobial regimens allowed for the recognition of therapeutic failures and bacteriological relapses that were missed by serological tests, making an important contribution to the management of our patients.

The overall sensitivity, the short time to detection, and the full automation and safety considerations make the BACTEC 9240 Peds Plus system more convenient than the Isolator 1.5 Microbial Tube system for processing cultures of blood from children with suspected brucellosis.

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