Use of BACTEC 9240 Blood Culture System for Detection of *Brucella melitensis* in Synovial Fluid

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Synovial fluid specimens aspirated from patients with arthritis were inoculated into an aerobic Peds Plus blood culture bottle and monitored by the BACTEC 9240 instrument for 4 weeks. A total of 1,072 synovial fluid cultures were processed, and 15 (0.14%) were positive for *Brucella melitensis*. A single culture, harboring 1.3 CFU of viable organisms per ml, was missed by the instrument. All 14 positive BACTEC cultures were detected within 3 to 7 days.

Isolation of brucellae from blood and other normally sterile body fluids or tissues remains the only irrefutable evidence for the disease, but detection of the organism in clinical specimens is frequently hampered by its slow growth. On the basis of experience gained with traditional methods, incubation of cultures for 30 days has been advocated to maximize the recovery of these fastidious organisms (1, 8).

During the last decade, the use of blood culture systems for the culture of normally sterile body fluids other than blood is gaining increasing acceptance (3–7, 10–12). These systems have been shown to improve the overall recovery of bacteria from synovial fluid cultures of specimens from patients with septic arthritis and that of fastidious organisms such as *Kingella kingae* (3–7, 10–12). No information on the performance of this approach for the diagnosis of brucellar arthritis has been published, and the optimal incubation period for these cultures has not been established yet. *Brucella melitensis*, which is endemic in countries of the Middle East, is more virulent for humans than other biovars, and infections with this organism are frequently associated with arthritis (15). A prospective study was conducted to examine the performance of the BACTEC 9240 system (Becton Dickinson Diagnostic Instrument Systems, Towson, Md.) for the detection of *B. melitensis* in synovial fluid specimens.

Specimens of joint fluid aspirated from patients with clinical arthritis were sent to the Microbiology Laboratory of the Soroka Medical Center (which is in southern Israel) in a closed sterile syringe. Once in the laboratory, the amount of fluid was measured and recorded. When less than 0.5 ml of synovial fluid was available, the entire specimen was inoculated into an aerobic BACTEC 9240 Peds Plus bottle (referred to as a BACTEC culture). When the synovial fluid volume ranged between 0.6 and 1.5 ml, the specimen was divided into two aliquots. One-half of the fluid was inoculated into an aerobic bottle, as described above, and the second aliquot was inoculated into an Isolator 1.5 Microbial Tube (Wampole Labora-

tories, Cranbury, N.J.) (referred to as an Isolator culture). When the synovial fluid specimen exceeded 1.5 ml, a third aliquot was plated onto chocolate plates and Trypticase soy agar plates supplemented with 5% sheep blood (referred to as a conventional culture).

Bottles were incubated at 35°C in BACTEC 9240 cabinets and monitored on a continuous basis for 4 weeks. Detection of positive bottles by the instrument was followed by performance of a Gram stain and subculture of the broth in sheep blood agar, chocolate agar, and MacConkey agar plates. The Isolator tube was processed by the methodology recommended by the manufacturer for blood cultures, and the synovial fluid lysate was plated onto solid media similar to those described above for conventional cultures. Plates seeded with the Isolator culture lysate and conventional culture media were incubated at 35°C in a CO₂-enriched atmosphere and were examined for bacterial growth once a day for 10 days. Identification of brucellae was performed by conventional microbiological procedures (1, 8).

The fraction of blood cultures in which brucellae were detected by the instrument within a 1-week incubation period out of the total number of cultures positive for the organism detected during the 4-week period was determined.

During the 4.5-year period from 1 January 1997 to 30 June 2000, a total of 1,072 synovial fluid cultures were processed. *B. melitensis* was isolated from 15 (0.14%) cultures of samples obtained from 13 pediatric patients aged 11 months to 15 years and 10 months (median, 12 years) and from two adults aged 23 and 71 years, respectively. In addition, *Staphylococcus aureus* was isolated from 71 patients, *Streptococcus pyogenes* was isolated from 16 patients, other streptococcal species were isolated from 10 patients, *Kingella kingae* was isolated from 5 patients, members of the family *Enterobacteriaceae* were isolated from 4 patients, and other gram-negative organisms and anaerobes were isolated from 3 patients each.

Brucellar arthritis involved the knee in six patients, the shoulder or the elbow in three patients each, the hip in two patients, and the wrist in one patient. *B. melitensis* grew in 14 of 15 (93.3%) BACTEC cultures, 6 of 8 (75.0%) Isolator cultures, and 4 of 7 (57.1%) conventional cultures. The BACTEC instrument detected 3 of the 14 positive cultures (21.4%) by

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day 3, 7 of the 14 positive cultures (50.0%) by day 4, 12 of the 14 positive cultures (85.7%) by day 5, and all 14 positive cultures (100.0%) by day 7. The single positive culture missed by the BACTEC instrument was obtained from a child with arthritis of the hip. A 0.8-ml volume of synovial fluid was aspirated from this patient and was processed by the three culture methods. A single *Brucella* colony was recovered in the Isolator culture and conventional culture plates, suggesting that the low concentration of circulating organisms (1.3 CFU/ml) may have been the reason for the false-negative result.

Brucellosis is an important cause of human morbidity in areas of endemicity. Involvement of the skeletal system and especially septic arthritis occur in up to 40% of patients (15). The diagnosis of the disease, however, is frequently difficult because brucellosis may mimic other clinical conditions such as rheumatic disorders, and thus, it should be confirmed by laboratory methods.

In recent years, an automated generation of blood culture systems has been introduced into clinical practice, resulting in reduced detection times for a wide range of blood pathogens. Experience accumulated with the BACTEC 9240 instrument has demonstrated that the system enables detection of *B. melitensis* from blood cultures within the routine 7-day incubation period, obviating the need for prolonged incubation of blood culture bottles, and is superior to the Isolator 1.5 Microbial Tube in terms of sensitivity and time to detection of circulating brucellae (2, 9, 13, 14).

The results of the present study also suggest that the aerobic Peds Plus BACTEC blood culture bottles may be a convenient tool for the culture of brucellae from synovial fluid of patients with arthritis. All cultures positive with the BACTEC instrument were detected within a week, indicating that prolonged incubation of negative bottles may not be necessary. This results in a more convenient laboratory work flow and considerable savings in labor, time, and incubation space. From the clinical point of view, the rapid detection of brucellae with the BACTEC 9240 system may lead to an earlier diagnosis of *Brucella* arthritis and improve case management. In addition, monitoring of bacterial growth is performed by a noninvasive

technology that avoids creation of dangerous aerosols, an important laboratory safety consideration for dangerous and transmissible organisms such as brucellae.

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