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Rapid Diagnosis of *Brucella melitensis* in Blood: Some Operational Characteristics of the BACT/ALERT

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A clinical isolate of *Brucella melitensis* was detected in a blood culture with the BACT/ALERT after an incubation period of 2.8 days. Dilution studies revealed an inverse linear relationship between the log of the initial concentration of the organism and the time to detection of a positive result. Reproducibility studies demonstrated a mean detection time of 48 ± 1 h in 8 of 10 replicates seeded with a stock containing 10^2 CFU/ml.

Primary isolation of *Brucella* spp. in the clinical laboratory is difficult, since all species are slow growing and fastidious (3, 10). Culture in a biphasic medium (Castaneda technique) and lysis concentration have been recommended to improve the recovery of *Brucella* spp. from clinical specimens (4). However, even under these conditions development of colonies may take as long as 35 days (1). Recently, a new automated system for detection of microbial growth in blood cultures (BACT/ALERT) has been introduced, and it is claimed to have a shorter time to detection in cases of bacteremia and fungemia than other currently available blood culture systems (7). In a patient with a febrile illness, subsequently identified as having been caused by *Brucella melitensis*, we established the presence of bacteremia after an incubation period of only 2.8 days by using the BACT/ALERT with the standard aerobic culture bottles.

A previously healthy 37-year-old male noted the onset of an intermittent low-grade fever, arthralgia, and malaise in January 1991. This occurred 1 month following his return from Saudi Arabia, where he had worked during the preceding 2 years as a nurse at a hospital in Riyadh. During that time, he was not exposed to domesticated animals nor did he knowingly ingest unpasteurized dairy products. In March 1991, he developed fever (oral temperature, 38.5°C) and swelling and tenderness of the left testicle and epididymis. His hemoglobin was 12.7 g/dl, and both leukocyte and differential counts were normal. Blood cultures were positive for *B. melitensis*. An initial brucella agglutinin titer of 1:160 was noted, and a follow-up titer 5 weeks later was 1:320. He was treated with doxycycline 100 mg twice a day for 5 days and then 100 mg daily for 25 days. His recovery was uneventful.

Three blood cultures (10 ml) were collected from the patient approximately 20 min apart and transferred to aerobic and anaerobic BACT/ALERT bottles (5 ml each). The aerobic bottle of each set was transiently vented. Both bottles were then incubated in the BACT/ALERT at 36°C with continuous agitation and monitoring. When positive, the contents of the bottles were Gram stained and subcultured to sheep blood agar and chocolate agar. Preliminary identification of the organism as a *Brucella* species was confirmed by the Bacteriology Laboratory of the Georgia Department of Human Resources and the Clinical Microbiology Section, Special Bacteriology Unit, Centers for Disease Control, Atlanta, Ga.

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The *Brucella* isolate was inoculated to chocolate agar and incubated for 48 h at 36°C in an atmosphere of 5% CO_2. A suspension of that growth was made in 5 ml of tryptic soy broth (TSB) and adjusted to approximately a McFarland 1 standard. This suspension (10^8 CFU/ml) was then diluted with TSB to 10^6 CFU/ml. Subsequent 10-fold dilutions were made in TSB to include the range between 10^0 and 10^4 CFU/ml. Ten BACT/ALERT aerobic bottles were each inoculated with 5 ml of fresh blood collected from healthy volunteers. Duplicate bottles were then inoculated with 0.4 ml of each of the dilutions described above. All bottles were transiently vented, inserted into the instrument, and monitored for 5 days or until they became positive.

For the replication study, 50 ml of fresh blood was collected from healthy volunteers and 5 ml was added to each of 10 aerobic bottles. A suspension of the *Brucella* isolate was made and diluted with TSB to a final concentration of 10^2 CFU/ml. A 0.4-ml aliquot of this dilution was inoculated into each bottle. The bottles were then transiently vented and placed in the BACT/ALERT, where they were continuously agitated and monitored for 5 days or until they became positive.

CO_2 production by *B. melitensis* from our patient's blood is shown in Fig. 1. In contrast to the early and sustained increase in CO_2 production observed in our laboratory with clinical isolates of *Escherichia coli* and the later, less pronounced increase noted with *Staphylococcus aureus*, the increase with *B. melitensis* occurred more slowly and was the lowest in magnitude and the briefest in duration. Nevertheless, this pattern was recognized by the BACT/ALERT as positive. Despite the subsequent return to the baseline rate of CO_2 production, *B. melitensis* remained viable, as demonstrated by terminal subculture.

When different concentrations of the organism were incubated in the BACT/ALERT, the general appearance of the curves of reflectance units versus time were similar in size and shape but differed in the times at which they were recognized as positive by the instrument. The effect of the initial concentration of *B. melitensis* in the inoculum on the time required to produce a positive result is shown in Fig. 2. An inverse linear relationship was demonstrated between the log of the initial concentration of the organism and the time to detection of a positive result.

The reproducibility of the system was tested by determining the time to detection of positive results in 10 replicate culture bottles initially inoculated with 0.4 ml of TSB containing 10^2 CFU/ml. In eight of these bottles sufficient rates of CO_2 production occurred to justify interpretation as

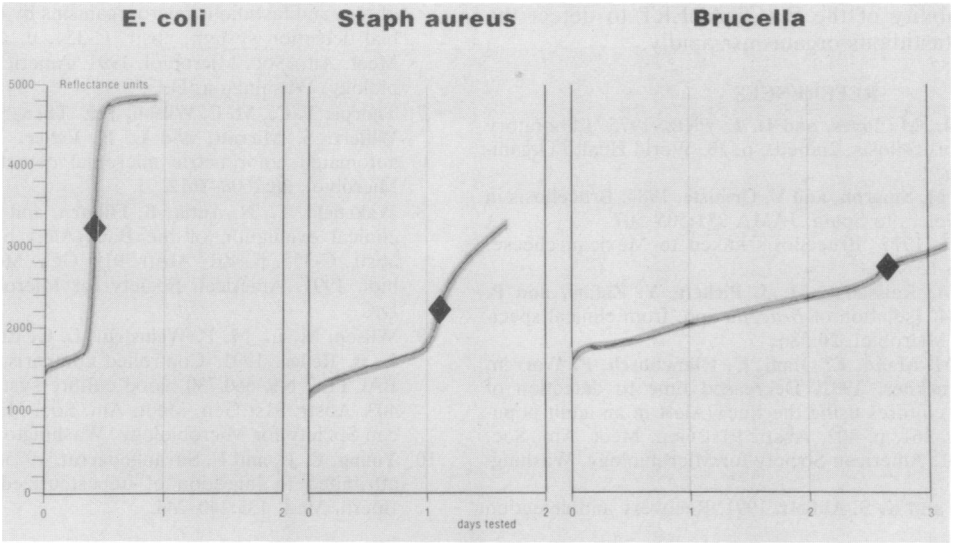

FIG. 1. CO_2 production by microorganisms present in blood from bacteremic patients as measured by the BACT/ALERT.

positive by the BACT/ALERT. Mean time to detection was 48 h, with a standard deviation of ± 1.0 h. The two remaining bottles were not detected as positive, even after 120 h of incubation.

In the BACT/ALERT, growth of organisms produces increased amounts of CO_2, which diffuses through a semipermeable membrane in the base of the culture bottle and reacts with water to generate hydrogen ions. This causes a decrease in pH, which results in a change in the color of a built-in sensor. Reflectance values from the sensor of each culture bottle are monitored. A complex algorithm allows the system to differentiate microbial CO_2 production from background CO_2 produced by other components in the blood (7). Positive results are characterized by an acceleration in the rate of production of CO_2. Results for patients are presented as individual plots of the reflectance units (production of CO_2) as a function of time of incubation.

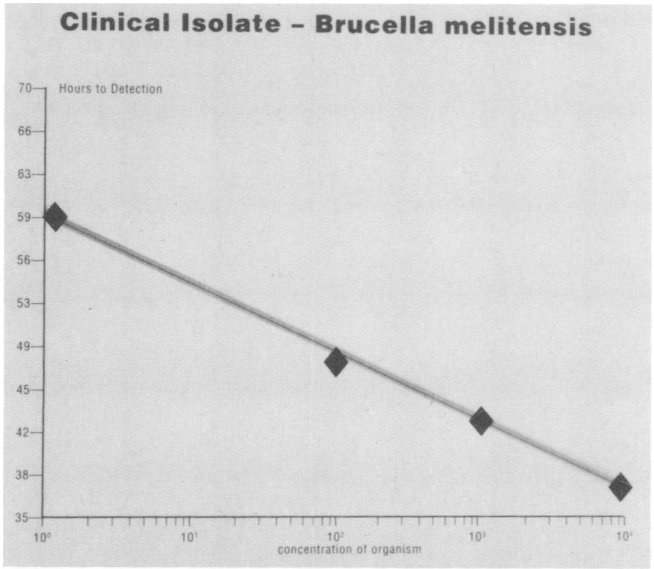

FIG. 2. Relationship between the initial concentration of *B. melitensis* in blood and time to detection of positive results in the BACT/ALERT.

The initial study documenting the reliability and accuracy of a prototype BACT/ALERT in detecting many organisms which are frequently encountered in clinical isolates has since been confirmed by several reports of studies in which commercially available instruments were utilized (5, 7, 8, 9). Recently, the effectiveness of the BACT/ALERT in detecting growth in blood cultures seeded with various fastidious microorganisms has been reported (6).

The low recovery rates for *Brucella* spp. from blood culture specimens improved with the introduction of the BACTEC, which detected $^{14}CO_2$ produced by metabolism of ^{14}C-labeled substrates. In one study involving 6 patients infected with *B. melitensis*, at least one specimen from each individual and 15 (79%) of 19 blood cultures were positive in the radiometric system within 4 to 8 days of inoculation (2). All three blood cultures from our patient were positive and were detected by the BACT/ALERT after a mean incubation period of only 2.8 days (range, 2.7 to 2.9 days). This decreased time to detection has also been observed with a number of other pathogenic organisms (5, 8, 9).

Our study of the effect of the concentration of an organism on the time to detection of a positive result suggests that a critical number of bacteria must be reached to generate CO_2 at a rate which is recognized by the BACT/ALERT as positive. The longer times required for the lower initial concentrations of the organism to yield positive results are presumably a function of the doubling time of the organism and reflect the time necessary for the critical number to be attained in each culture bottle. Over the range of concentrations studied, the doubling time of the organism is independent of the number present. Hence, the relationship between the log number of organisms and the time to detection is linear.

The results of our replication study, in which 8 of 10 culture bottles seeded with a *B. melitensis* isolate were detected, suggests that the BACT/ALERT should reliably detect this organism when it is present in blood samples from patients. Further studies with additional isolates of various *Brucella* species from patients are necessary to characterize

more fully the ability of the BACT/ALERT to detect the growth of these fastidious organisms rapidly.

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