JOURNAL OF CLINICAL MICROBIOLOGY, Oct. 2002, p. 3878 Vol. 40, No. 10
0095-1137/02/$04.00+0 DOI: 10.1128/JCM.40.10.3878.2002

Use of the Isolator 1.5 Microbial Tube for Detection of *Brucella melitensis* in Synovial Fluid

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 the low sensitivity of routine culture methods. Because brucellae have the ability to survive and multiply within phagocytic cells, we speculated that phagocytosis of the organism by leukocytes accumulated in the joint space may contribute to the failure to recover bacteria noted in some patients with brucellar arthritis.

In recent years, the Isolator blood culture system (Wampole Laboratories, Cranbury, N.J.) has been introduced into clinical practice. The system is based on lysis of blood leukocytes and release of already phagocytosed but still viable organisms, which are then suitable for plating and incubation. The Isolator microbial tube increases the recovery of intracellular pathogens such as mycobacteria and fungi from blood cultures and shortens the time to detection of circulating brucellae to 2 to 6 days (1).

In a previous study, we inoculated synovial fluid specimens of patients with septic arthritis into Isolator 1.5 microbial tubes. Use of this approach resulted in improved bacterial recovery compared to conventional cultures, but no persons with brucellosis were included in the patient's population (2). A prospective study was conducted to compare the performance of Isolator and conventional cultures for the detection of *Brucella melitensis* in synovial fluid.

Samples of joint fluid, aspirated from patients with clinical arthritis between 1 January 1997 and 31 October 2001, were sent to the Clinical Microbiology Laboratory of the Soroka University Medical Center, Beer-Sheva, Israel, in a closed sterile syringe. The amount of fluid was measured and recorded, one half of the volume was plated onto chocolate plates and Trypticase soy agar plates supplemented with 5% sheep blood ("conventional culture"), and the second half was inoculated into an Isolator 1.5 microbial tube ("Isolator culture").

The Isolator tube was processed according to the method recommended for blood cultures, and the synovial fluid lysate was plated onto solid media similar to those described for conventional cultures. All plates were incubated at 35°C in a CO_2-enriched atmosphere and examined for bacterial growth once a day for 10 days.

During the study period, 10 synovial fluid specimens, obtained from three adult and seven pediatric patients, grew *B. melitensis* in conventional and/or Isolator cultures. Culture results including time to detection and concentration of organisms in the synovial fluid sample (expressed as CFU per milliliter) are summarized in Table 1.

TABLE 1. Culture results, time to detection, and concentration of brucellae in Isolator and conventional cultures of synovial fluid

Patient	Isolator culture			Conventional culture		
	Result[a]	Day	CFU/ml	Result[a]	Day	CFU/ml
1	Pos.	6	2	Neg.	–	–
2	Pos.	7	6	Neg.	–	–
3	Pos.	5	>500	Pos.	5	100
4	Pos	7	1.3	Pos.	7	1.3
5	Pos.	5	22	Pos.	5	16
6	Pos.	6	46	Pos.	6	30
7	Pos.	7	5	Neg.	–	–
8	Pos.	4	270	Pos.	4	33
9	Pos.	7	5	Neg.	–	–
10	Pos.	3	300	Pos.	3	33

[a] Pos., positive; Neg., negative.

Although the figures herein presented are not large enough to reach statistical significance, it seems that adoption of this practice enhanced bacterial isolation in cases that would have been missed by conventional cultures. Examination of the quantitative data shows that, in those specimens positive by both methods, plates seeded with the Isolator lysate usually yielded the largest number of colonies. This observation explains the superior sensitivity of the Isolator technique, because the concentration of bacteria in the synovial fluid of patients with brucellar arthritis is usually of low magnitude.

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