

Use of sentinel blood culture system for analysis of specimens from potentially infected prosthetic joints

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Abstract

The Sentinel blood culture system was used for the analysis of 657 specimens from infected prosthetic joints and blood cultures (83 from prosthetic joints and 574 from standard blood cultures). The positivity rate was similar for specimens from prosthetic joints and blood cultures (18% compared with 14%). However, there was an unacceptable rate of false positive results with specimens from prosthetic joints (58% compared with 8%). This high false positivity rate was due to (i) prolonged incubation and (ii) the lack of blood in these specimens.

It is therefore recommended that the Sentinel system should only be used for the initial seven days of incubation of specimens taken from prosthetic joints. Further incubation should take place in a standard incubator and a terminal subculture performed after 21 days.

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The Sentinel system (Difco Laboratories Ltd, East Molesey, Surrey, England) is an automated blood culture system which permits the continuous monitoring of aerobic and anaerobic blood culture bottles. The blood culture bottles are contained in thermostatically controlled, self contained units (silos).

Each bottle contains 50 ml of growth medium. In the base of the bottles are two electrodes, one aluminium and the other gold plated, which enter the growth medium when the bottles are inserted into the silos. The bottle acts as an electric cell, generating a potential difference of 500 Mv. Bacterial growth requires electron acceptors (such as oxygen), making them unavailable for removal of electrons at the gold electrode, with a resulting decrease in voltage. The software measures the voltage at 15 minute intervals and determines both the absolute voltage and the rate of decrease in voltage. These two values are compared with pre-set thresholds to determine their relevance.

Since December 1991 the microbiology laboratory at Selly Oak Hospital has used the Sentinel system for processing specimens from presumed infected hip and knee arthroplasties. These specimens were from patients undergoing revision arthroplasty at the Royal Orthopaedic Hospital, Birmingham.

The Sentinel system underwent a Department of Health trial between 8 January

1991 and 15 July 1991 at three different sites. The system's performance was comparable with that of the Bactec system, but with the advantage of continuous monitoring. The Department of Health report on the Sentinel highlighted two problems relevant to the processing of fine needle aspirates (FNA) and tissues from prosthetic joint infections (PJI). Firstly, some Gram positive cocci will grow in the anaerobic bottle, but will not signal.¹ Secondly, there may be a problem in detecting bacteria of low metabolic activity, such as *Staphylococcus epidermidis* or *Propionibacterium* sp.¹

A problem with false negative results was thus anticipated when the Sentinel system was introduced for FNAs and biopsy specimens from patients with PJI. This prompted an investigation into the use of the Sentinel system for the processing of FNAs of articular fluid and biopsy specimens of hip and knee tissue.

Methods

FNAs were obtained from patients awaiting revision surgery. About 3 ml of aspirate were inoculated into each of a pair of Sentinel blood culture bottles, one aerobic and one anaerobic.

Tissues obtained during revision surgery were homogenised in 7 ml of sterile isotonic saline using a sealed unit homogeniser (Janke and Kunkel IKA Laborotechnik). Supernatant fluid (3.5 ml) was inoculated into each of a pair of Sentinel blood culture bottles.

Aspirates and homogenised tissue were incubated for 20 days on the Sentinel system. On day 21, both bottles were removed from the machine and subcultured on to two Columbia agar plates (Oxoid) with 5% blood and nicotinamide adenine dinucleotide (NAD). These were incubated in 5% CO₂ and in a WISE anaerobic cabinet (Don Whitley Ltd, Shipley, England) for 48 hours.²

Blood cultures obtained from patients on other wards were processed in parallel during the study period. The blood cultures were incubated for seven days, followed by subculture on day 8 on to Columbia blood agar plates which were incubated as above. Blood cultures from patients with suspected endocarditis were incubated for 21 days, followed by subculture and incubation also as above.

The results for blood culture specimens and FNAs and tissue specimens are shown in the table. False positive results were defined

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Positive and negative rates for blood cultures and specimens from prosthetic joint infections

	Blood cultures	Aspirates and tissues	Total
True positives	81 (14%)	15 (18%)	96 (15%)
False positives	45 (8%)	48 (58%)	93 (14%)
True negatives	428 (75%)	16 (19%)	444 (68%)
False negatives	20 (3.5%)	4 (5%)	24 (4%)
Total	574	83	657

as specimens which caused a positive signal, but were sterile on subculture. False negative results were defined as specimens which did not cause a positive signal on the Sentinel system but which yielded growth on terminal subculture.

Results

A total of 657 specimens were examined of which 574 were standard blood cultures. Eighty three samples were from prosthetic joints: of these, 36 were aspirates and 47 were tissues. Eighty one of the 574 (14%) blood cultures were positive compared with 15 of the 83 (18%) aspirates or tissues. There was no significant difference between the positivity rate for aspirates (six of 36, 16%) and for tissues (nine of 47, 19%).

The false negative rate was 3.5% for blood cultures and 5% for aspirates or tissues. The false positive rate was noticeably different in the two groups. Forty five of the 574 (8%) blood cultures gave a false positive signal in the Sentinel system; over half the aspirates or tissues gave a false positive result (48 of 83, 58%).

A further 19 blood cultures from patients with suspected infective endocarditis were examined. These specimens were incubated for 21 days and the false positive rate for these was three out of 19 (16%).

Overall, the Sentinel system failed to detect bacterial growth in 24 of 657 (4%) specimens. On terminal subculture *Staphylococcus epidermidis* was isolated in 12 of these 24 (50%) specimens. Diphtheroids and *Propionibacterium* sp were isolated in four of 24 (17%) of these specimens.

Discussion

This study has highlighted two problems with the use of the Sentinel system for prosthetic specimens. Firstly, some organisms fail to grow and flag as positive. Secondly, an unacceptable level of false positive results (58%) was generated by prosthetic specimens.

The two main causes of false positive results are: (a) an absence of blood within the sample (lack of blood affects the voltage within the bottle and will produce noisy and irregular algorithms; personal communication, Difco Laboratories Ltd); and (b) the long incubation period of prosthetic

specimens leads to a depletion of electron acceptors in the culture medium, thereby reducing the voltage over a period of time, producing a "tail off" effect.

To establish the relative importance of these two effects we measured the false positive rate of blood cultures from patients with bacterial endocarditis. These specimens were incubated for 21 days (as are specimens from PJI). The false positive rate was 16%, which is higher than for routine blood cultures but less than for specimens from PJI. This suggests that, although prolonged incubation itself resulted in a false positive reading, a lack of blood in the specimen was also an important factor.

False negative results were less of a problem. For most organisms, the Sentinel system provides a nutritious growth medium, but this investigation has highlighted a problem in detecting *Staphylococcus epidermidis*, diphtheroids, and *Propionibacterium* sp which are frequently isolated from prosthetic joint infections and which are clinically important in this setting.^{3,4}

False negative results are thought to arise from the lack of blood in the specimens, as fastidious or nutritionally dependent organisms, such as *Haemophilus* sp and some strains of streptococci and staphylococci require blood components such as NAD, haemin, pyridoxine or thymine for optimal growth.^{5,6} The methodology for these specimens was altered as follows. Prosthetic specimens were incubated for seven days in the Sentinel system. After day 7 both bottles were removed and subcultured on to two Columbia blood agar plates and incubated for 48 hours at 37°C, one in 5% carbon dioxide and the other anaerobically. The culture bottles were then incubated at 37°C in air and subcultured after 21 days. This method reduced the number of false positive readings from 58% to 7.4%.

We strongly recommend, therefore, that when the Sentinel system is used for specimens that do not contain blood and require more than seven days of incubation, further incubation should take place in a standard incubator and a terminal subculture performed after 21 days.

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