INTRODUCTION

The Bactec 9240 system (Becton Dickinson Europe, Meylan, France) is a fully automated continuous blood culture monitoring system. The Bactec Plus Aerobic/F and Anaerobic/F vials contain two types of resins: a cationic strongly acidic resin that binds positively charged antibiotics (e.g., aminoglycosides) and an adsorbant polymeric resin that fixes hydrophobic portions of most antibiotics. The Bactec 9240 incubator also serves as an agitator that continually shakes each vial. Fluorescence is monitored every 10 min to detect CO₂ release indicating microbial growth. The Bactec 9240 system improves the isolation rate and reduces time to detection of pathogenic micro-organisms from blood as compared with other blood culture systems [1, 2], including the Bactec NR 660 system [3], as a result of continuous monitoring and the use of resin-containing media, especially in patients with previous antibiotic therapy.

The conventional methods used for culture of sterile body fluids by plating on to agar media lack sensitivity because the number of organisms per ml of fluid is often very low in these infections. Several authors have described the superiority of blood culture systems for culture of infected ascitic fluid [4, 5], peritoneal fluid in peritonitis secondary to continuous ambulatory peritoneal dialysis (CAPD) [6–8] and joint effusion in bacterial arthritis [9, 10]. Many of these studies found improved isolation of micro-organisms from sterile body fluids by using the Bactec system from generations of equipment previous to the 9240 [6–12].

In the present study, sensitivity and time to detection of micro-organisms from deep-seated infections were compared by culturing body fluids in three different culture systems: the Bactec Plus Aerobic/F broth, conventional culture in Schaedler enrichment broth and...
a set of three blood agar plates inoculated directly and incubated in different atmospheric conditions.

Materials and methods

Specimen collection and processing
All normally sterile body fluids (peritoneal, ascitic, CAPD, pericardial, pleural, amniotic, synovial, cerebrospinal) collected for bacterial culture by puncture from Feb. to Oct. 1997 were included in the study. Body fluids obtained from post-operative drains were excluded. A total of 336 specimens was analysed: 98 peritoneal fluids (29%), 74 ascitic fluids (22%), 70 CAPD fluids (21%), 43 pleural fluids (13%), 27 cerebrospinal fluids (CSF; 8%), 17 synovial fluids (5%), 4 pericardial fluids (1%) and 3 amniotic fluids (1%). Direct microscopy of a gram-stained preparation was performed on cytocentrifuged specimens of pericardial, pleural, amniotic, synovial and CSF fluids and CAPD fluids which appeared macroscopically turbid; other specimens were examined by preparing a smear of uncentrifuged fluid. Fluids showing leucocytes or micro-organisms, or both, on direct smear were included in the study, as well as all submitted peritoneal fluids irrespective of cytology. For direct culture on agar media, 100 μl of fluid were inoculated without prior concentration – except for CSF samples and grossly turbid CAPD fluids, which were concentrated by centrifugation at 1300 g for 10 min before plating out the deposit. The media used were Columbia agar with sheep blood 5%, chocolate blood agar enriched with polvivates and Schaedler blood agar (BBL, Becton Dickinson). They were incubated in aerobic, air with CO₂ 5% and anaerobic atmospheres, respectively. The anaerobic atmosphere was generated in jars filled with gas mixture (H₂ 5%, CO₂ 7%, N₂ 88%) obtained with the Anoxomat system (Mart Microbiology BV). Approximately 1 ml of specimen was also inoculated into a BBL Schaedler broth (Becton Dickinson) and 3–10 ml were injected into a Bactec Plus Aerobic/F™ vial. If the volume of specimen available was <3 ml, a Bactec Peds Plus/F™ was inoculated instead. No anaerobic blood culture bottle was used. All inoculated broths were incubated for 5 days and a Gram’s stain was done when broths gave positive indication of growth. Organisms were subcultured on Columbia blood agar incubated aerobically and Schaedler blood agar incubated anaerobically and additional appropriate media and atmospheres were used according to the micro-organisms seen.

Clinical assessment
When a positive culture was obtained, the investigators interviewed the patient’s attending physician and consulted the medical records to determine whether the micro-organism(s) isolated were considered to be clinically significant or were contaminants and to record any antimicrobial therapy. They also assessed whether the notification by the laboratory of isolation of a significant organism prompted new therapeutic decisions.

Statistical analysis
The statistical significance of differences in proportion of positive cultures by system was determined by McNemar χ² analysis with StatMost 2.01 software. The results of cultures on agar media and in Schaedler broth were each compared with those obtained with the Bactec system. No comparison was made between agar media and Schaedler broth culture results. A p value <0.05 was considered significant.

Results

Culture results
Micro-organisms were isolated from 81 specimens (24%). These samples were divided among two categories: 50 (15%) clinically significant specimens that yielded 71 isolates and 31 (9%) contaminated specimens that contained 34 micro-organisms. The 50 clinically significant specimens were distributed as follows: 25 were peritoneal (50%), 7 ascitic (14%), 3 CAPD (6%), 4 pleural (8%), 4 CSF (8%), 6 synovial (12%) and 1 amniotic fluid (2%). The distribution of the 31 contaminated specimens, most of which (65%) grew coagulase-negative staphylococci, was as follows: 22 were CAPD (71%), 3 peritoneal (10%), 2 ascitic (6%), 2 pleural (6%), 1 CSF (3%) and 1 amniotic (3%). Of these contaminated cultures, 20 (65%) were detected by the Bactec system alone.

As strictly anaerobic bacteria were not detectable by the Bactec medium used in the conditions of the study, only 43 specimens in which significant aerobic pathogens were isolated were included in the statistical analysis (Table 1). Aerobic micro-organisms considered clinically relevant were isolated significantly more frequently with the Bactec system from peritoneal and ascitic fluids as well as from other fluid types considered together (Table 1).

In comparison with Schaedler broth culture (Table 2) of the 43 relevant specimens from which aerobic pathogens were isolated, 15 (35%) were detected by the Bactec system only (p = 0.001). The difference in isolation of pathogens was, as expected, even more important when direct cultures on agar media were compared with those in the Bactec vial: 26 aerobic isolates from Bactec vials were not isolated with direct plating (44% of all significant isolates, with a predominance of streptococci and enterococci) (Table 2). However, 12 clinically significant anaerobes (1 Bacteroides capillosus, 2 B. fragilis, 2 B. vulgatus, 1 B. nodosus, 1 Fusobacterium necrophorum, 1 F. nucleatum, 1 F. varium, 1 Peptostreptococcus magnus,
1 Prevotella denticola and 1 Pr. oulorum) were isolated by conventional methods (Table 2).

There was no statistically significant difference in time to detection of micro-organisms isolated by Bactec and conventional culture systems, combining direct plating on agar media and Schaedler broth enrichment (data not shown).

**Clinical assessment**

In 27 infected patients (8% of all specimens and 54% of the significantly positive cultures) the isolation of a pathogen from the cultured fluid led to a modification of the initial empiric antibiotic therapy. In eight of these patients (30%) a pathogen was isolated in the Bactec system only. In 19 patients a diagnosis of infected fluid was made on the basis of specimens collected while they were already on antibiotic therapy. Among these patients, six had aerobic pathogens detected only with the Bactec system (p = 0.04); however, the empirical treatment was not modified in any of these patients.

**Discussion**

In this study, the use of resin-containing Bactec aerobic blood culture media analysed in the Bactec 9240 automated system provided a significant increase in the isolation rate of aerobic and facultative anaerobic pathogens from normally sterile body fluids as compared with conventional agar and broth cultures. However, this increased yield was probably related in large part to the increased volume of sample inoculated into the Bactec vials. Alternative methods such as sample concentration by centrifugation before inoculation of conventional agar media were not evaluated in this study, except in the case of CSF and CAPD fluid cultures which were performed on concentrated samples.

The result of a positive culture from a sterile body fluid from only one Bactec or Schaedler broth was frequently used to guide the patient management, contrary to other reports [7, 13]. In total, eight clinically relevant positive cultures detected only by the Bactec system led to therapeutic changes. This represents 2.4% of fluids cultured during the study and 30% of all positive cultures that were found to have an impact on therapy. No therapeutic change was made when pathogens were isolated only in the Bactec broth in the small number of patients who were already on antibiotic therapy at the time of sampling, in contrast with a previous report [14].

This study was designed to compare the isolation of aerobic pathogens by various culture methods. In seven cases (14% of all infections detected) clinically significant anaerobes were isolated, and would have been missed if Schaedler agar or broth media had not been inoculated and incubated anaerobically. As strict anaerobes comprised only a minority of pathogens sought in the study specimens, Bactec Plus Anaerobic/F* vials were not included in this evaluation, because this would have resulted in a marked increase in cost for a potentially marginal advantage. It would have been of interest to determine if the number of anaerobic isolates could have been increased as significantly by the use of the anaerobic Bactec Plus Anaerobic/F culture bottles as the Bactec Aerobic/F culture bottles did for aerobic isolates. Moreover, anaerobes are typically covered by empirical antibiotic therapy of infections such as peritonitis. Nevertheless, strict anaerobes were present in 14% of the significant specimens, because of the predominance of peritoneal samples in the present study. Therefore, these organisms must be taken into consideration in the selection of appropriate media for culture of these specimens [12, 14].

No fastidious organism, such as Kingella and Haemophillus species, was isolated in this study. This may be because this institution does not provide paediatric care, thereby introducing a bias in the selection of patients and the spectrum of micro-organisms encountered. The use of fastidious organism growth supplement was not evaluated because it was shown to be of little benefit in previous studies [12, 14]. The contamination rate (9%) in this series was greater than in a previous report [12]. Contaminated specimens were mostly found in CAPD fluids: 31% of inoculated samples and 88% of positive cultures were contaminated, probably due to the systematic mode of collection irrespective of symptoms. Based on these findings, specimen selection for microbiological culture

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**Table 1. Comparison of isolation rates of clinically significant aerobic micro-organisms from infected body fluids by fluid type and culture media**

<table>
<thead>
<tr>
<th>Fluid type (number positive)</th>
<th>Number of specimens detected positive by</th>
<th>p value (Bactec system vs Schaedler broth)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Schaedler broth and Bactec system</td>
<td>Schaedler broth only</td>
</tr>
<tr>
<td>Peritoneal ascitic (29)</td>
<td>19</td>
<td>1</td>
</tr>
<tr>
<td>Other (14)</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>Total (43)</td>
<td>27</td>
<td>1</td>
</tr>
</tbody>
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</tr>
<tr>
<td>Total (43)</td>
<td>27</td>
<td>1</td>
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</table>
Table 2. Comparative yield of clinically significant isolates by type of micro-organism and culture media

<table>
<thead>
<tr>
<th>Micro-organism category number of isolates</th>
<th>Bactec system and agar media</th>
<th>Bactec system only</th>
<th>Bactec system and agar media only</th>
<th>p value (Bactec system only vs Sabadell broth)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerobic (28)</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td>0.0001</td>
</tr>
<tr>
<td>Other gram-positive organisms (3)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>NS</td>
</tr>
<tr>
<td>Other anaerobic bacteria (3)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>NS</td>
</tr>
<tr>
<td>Enterococcus spp (10)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>NS</td>
</tr>
<tr>
<td>Anaerobic (24)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>NS</td>
</tr>
</tbody>
</table>

Some disadvantages of using Bactec blood culture vials for culture of sterile body fluids should be recognised. Time for identification and antibiotic susceptibility testing was shorter when bacterial pathogens were isolated from agar media than from either broth or Bactec media, because no subculture was then needed. Therefore, at least one agar medium incubated in a CO₂-enriched atmosphere should be used for culture of sterile body fluids. Furthermore, direct plating enables semi-quantitative estimation of the abundance of bacteria in infected fluids. This information may be useful in assessing the clinical significance of isolation or response to therapy.

An important concern is the cost of using the Bactec system, which was three-fold greater than that of the conventional method for aerobic culture for the purchase cost of the media alone. Working up a greater number of contaminants for identification and antibiotic susceptibility testing may also result in increased costs associated with use of Bactec vials. A second concern is the increased risk of needlestick injury for technical staff as a result of the need to use syringes to inoculate sterile body fluids into Bactec vials. These disadvantages should be balanced against the clinical advantage of better isolation rates of pathogens from infected body fluids.

In summary, the present study found that the Bactec Plus Aerobic/F® vial monitored by the Bactec system significantly increased the sensitivity of culture for diagnosis of infection of normally sterile body fluids as compared with the conventional method and contributed to 30% of all therapeutic changes made on the basis of a positive culture of normally sterile body fluids. In the case of previous antibiotic therapy, the clinical relevance was less obvious. However, the inoculation of the Bactec vial alone would have been inadequate in the case of infection by anaerobic or mixed aerobic-anaerobic bacteria. Based on these findings, culture of the sterile body fluids collected in this institution is now performed by inoculating specimens showing leucocytes on direct smear or cytological examination in Bactec Plus Aerobic/F® vial, chocolate agar medium incubated in a 5% CO₂-enriched atmosphere and one Schaedler blood agar plate incubated anaerobically, in accordance with the recommended procedure [16].

References


