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**Brief Communication**

# Evaluation of the routine use of the anaerobic bottle when using the BACTEC blood culture system

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The established practice of sending blood cultures in an aerobic-anaerobic pair of bottles has been questioned in recent years, and this study was conducted to evaluate the routine use of an anaerobic bottle in the BACTEC blood culture set at the University of Malaya Medical Centre, Kuala Lumpur, from January to December 2004. A total of 11,663 paired blood culture sets were received, of which 3326 were from pediatric patients and 8337 were from adult patients. The overall positive isolation rate was 15%; the positive isolation rate on excluding the anaerobic bottles was 13%. Overall, there were significantly more organisms isolated from the aerobic bottle ($p<0.05$); however, the best yield was obtained on using the paired aerobic-anaerobic bottles. Among the positive blood culture sets, organisms were isolated from the anaerobic bottle alone in 15.2% of the pediatric sets and in 18.1% of the adult sets. Organisms that grew more frequently in the anaerobic bottle were anaerobes and some facultative anaerobes; however, the difference was not statistically significant except for anaerobes in the adult sets. We recommend that when culturing blood, an aerobic-anaerobic pair of bottles be used rather than an aerobic-aerobic pair, to optimize the recovery of a wider spectrum of organisms, including obligatory anaerobes.

**Key words:** Bacteremia; Bacteria, aerobic; Bacteria, anaerobic; Bacteriological techniques; Predictive value of tests

## Introduction

Blood culture systems and practices that maximize the recovery of pathogens are imperative in the investigation of septicemic patients to ensure the timely delivery of appropriate antimicrobials. Traditionally, it has been recommended that blood be cultured in aerobic and anaerobic pairs of bottles [1]; however, with reports of declining rates of anaerobic bloodstream infections [2,3], some authors have questioned the need for the routine inclusion of an anaerobic bottle in blood cultures [2,4]. It is clear, however, that the volume of blood cultured is one of the most important variables in optimizing overall recovery of isolates from adult patients [5].

Numerous studies have addressed the issue of whether paired blood culture bottles should routinely include an anaerobic bottle or a second aerobic bottle, and the findings have been varied [2,4,6-8]. Murray et al [4] suggested that individual hospitals should evaluate their own experience with regard to the advantages and disadvantages of using an anaerobic bottle, as findings may differ according to different patient populations and practices. With this in mind, we investigated the current practice of the routine inclusion of an anaerobic bottle when performing blood cultures using the BACTEC™ (BD Diagnostics, Becton Dickinson and Company, Sparks, MD, USA) blood culture system in both adult and pediatric patients in our hospital.

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## Methods

### Inclusion criteria

All positive blood cultures received in paired aerobic and anaerobic sets at the University of Malaya Medical Centre (UMMC), Kuala Lumpur, from January to December 2004 were reviewed. The UMMC is a 900-bed teaching hospital, which has medical, hematological, surgical, orthopedic, pediatric, and obstetric and

gynecology units as well as a bone marrow transplant unit, a general intensive care unit, and pediatric and neurology intensive care units.

### Blood culture collection, system, and identification of isolates

Blood culture samples from patients suspected of septicemia at the UMMC were equally divided into an aerobic BACTEC bottle and an anaerobic BACTEC bottle. The BD BACTEC Peds Plus/F bottle was used for the aerobic component of pediatric patients, whereas the BD BACTEC Plus Aerobic/F bottle was used for adults. The anaerobic bottle used was the BD BACTEC Plus Anaerobic/F. Bottles were run on a 5-day cycle or until flagged as positive, except for bottles from patients with suspected infective endocarditis, which were incubated for up to 3 weeks. Organisms isolated were identified by standard biochemical tests and the API system (bioMérieux, Marcy l'Etoile, France) when necessary.

### Data collection

Data recorded included whether growth from blood culture bottles was in the aerobic bottle alone, anaerobic bottle alone, or in both bottles. No attempt was made to determine the clinical significance of any isolate.

### Statistical analysis

Data was analyzed by the McNemar's chi-squared test, which was used to evaluate the extent to which organisms grew more favorably in the aerobic vs the anaerobic bottle. We compared the number of times an organism was isolated in the aerobic bottle to the number of times it was isolated in the anaerobic bottle among the paired blood culture sets.

## Results and Discussion

A total of 11,663 blood culture sets were received, of which 3326 and 8337 sets were from pediatric and adult patients, respectively. From this total, 1766 sets were positive either in the aerobic bottle, anaerobic bottle or both bottles, corresponding to an overall positive isolation rate of 15%. Omitting the anaerobic bottles would have given a rate of 13%.

Among the pediatric patients (Table 1), there were 389 positive blood cultures, giving a positive isolation rate of 11.7%, or 10.3% if the anaerobic bottles were excluded. Organisms were isolated on 217 occasions (50.6%) from both aerobic and anaerobic bottles, on 147 occasions (34.3%) from the aerobic bottle only, and on 65 occasions (15.2%) from the anaerobic bottle only. Overall, there were significantly more organisms isolated from the aerobic bottle and *Acinetobacter* spp., *Pseudomonas aeruginosa*, other pseudomonad organisms and *Burkholderia* spp., other Gram-negative organisms (*Serratia* spp., *Aeromonas* spp., *Campylobacter* spp. and others), Gram-positive bacilli (*Bacillus* spp., *Corynebacterium* spp. and *Brevibacterium* spp.) and all fungi (*Candida* spp., *Cryptococcus neoformans*, and others) grew significantly more often in the aerobic bottle. On the contrary, although the difference was not statistically significant, anaerobes and *Escherichia coli* grew more frequently in the anaerobic bottle. Coagulase-negative staphylococci were the commonest organisms isolated (38.0% of total isolates), followed by *Klebsiella* spp., (9.3%). Fungi comprised 4.4% and anaerobes contributed to only 0.7% of the total isolates.

Among the adult patients (Table 2), there were 1377 positive blood culture sets with a positive isolation rate of 16.5%, or 14.1% if the anaerobic bottles were omitted. Organisms were isolated on 825 occasions (50.8%) from both aerobic and anaerobic bottles, on 503 occasions (31.0%) from the aerobic bottle alone, and on 295 occasions (18.2%) from the anaerobic bottle only. Overall, there were significantly more organisms isolated from the aerobic bottle and *P. aeruginosa*, *Acinetobacter* spp., other pseudomonad organisms (*Pseudomonas* spp. and *Stenotrophomonas maltophilia*) and *Burkholderia* spp. (*Burkholderia pseudomallei* and *Burkholderia cepacia)*, other Gram-negative organisms (*Serratia* spp., *Aeromonas* spp., *Alcaligenes* spp., *Flavobacterium* spp., and others), fungi (*Candida* spp., *C. neoformans*, and *Penicillium marneffei*), other Gram-positive cocci (*Micrococcus* spp., *Aerococcus* spp., and others), and Gram-positive bacilli (*Bacillus* spp., *Corynebacterium* spp., and *Cellulomonas* spp.) grew significantly more frequently in the aerobic bottle, whereas anaerobes, which accounted for only 0.99% of the total isolates, were isolated significantly more frequently in the anaerobic bottle. Although facultative anaerobes such as *E. coli*, *Klebsiella* spp., *Proteus* spp. and *Morganella* spp., *Streptococcus* spp., and *Enterococcus* spp. grew more often in the anaerobic bottle, the difference was not statistically significant. Coagulase-negative staphylococci were the most common organisms isolated (32.5%), followed by *Staphylococcus aureus* (11.6%); fungi (the majority of which were *Candida* spp.) accounted for only 1.7% of the total organisms.

**Table 1.** Comparative yields of microorganisms isolated in the aerobic bottle alone, anaerobic bottle alone, and in both bottles among pediatric patients

| Microorganisms | No. of isolates detected | | | | *p* |
|---|---|---|---|---|---|
| | Aerobic bottle alone | Anaerobic bottle alone | Both aerobic and anaerobic bottles | Total organisms | |
| Gram-positive cocci | | | | | |
| *Staphylococcus aureus* | 5 | 5 | 22 | 32 | NS |
| Coagulase-negative staphylococci | 37 | 34 | 92 | 163 | NS |
| *Streptococcus* spp.[a] | 5 | 3 | 12 | 20 | NS |
| Other Gram-positive cocci[b] | 1 | 1 | 0 | 2 | NS |
| *Enterococcus* spp. | 3 | 1 | 3 | 7 | NS |
| Gram-positive bacilli[c] | 11 | 5 | 2 | 18 | <0.05 |
| Gram-negative organisms | | | | | |
| *Klebsiella*[d] | 5 | 5 | 30 | 40 | NS |
| *Escherichia coli* | 2 | 5 | 15 | 22 | NS |
| *Acinetobacter* spp. | 8 | 0 | 1 | 9 | <0.05 |
| *Pseudomonas aeruginosa* | 18 | 1 | 11 | 30 | <0.05 |
| *Citrobacter* spp. | 1 | 0 | 0 | 1 | NS |
| *Enterobacter* spp. | 3 | 1 | 6 | 10 | NS |
| *Salmonella* spp.[e] | 4 | 1 | 6 | 11 | NS |
| *Haemophilus* spp.[f] | 0 | 0 | 7 | 7 | NA |
| *Proteus* spp. | 1 | 0 | 3 | 4 | NS |
| Other *Pseudomonas* organisms and *Burkholderia* spp.[g] | 19 | 0 | 1 | 20 | <0.05 |
| Other Gram-negative organisms[h] | 7 | 0 | 4 | 11 | <0.05 |
| Anaerobes[i] | 0 | 3 | 0 | 3 | NS |
| All fungi[j] | 17 | 0 | 2 | 19 | <0.05 |
| All microorganisms | 147 | 65 | 217 | 429 | <0.05 |

Abbreviations: NS = not significant; NA = not analyzable

[a]Includes 2 *Streptococcus pyogenes*, 6 group B streptococcus, 8 viridans streptococci, and 4 *Streptococcus pneumoniae*.

[b]Includes 1 *Micrococcus* sp. and 1 *Abiotrophia defectiva*.

[c]Includes 13 *Bacillus* sp., 1 *Brevibacterium* sp., and 4 *Corynebacterium* spp.

[d]Includes 27 *Klebsiella pneumoniae* and 13 *Klebsiella* spp.

[e]Includes 10 *Salmonella* spp. and 1 *Salmonella enterica* serovar Paratyphi B.

[f]Includes 3 *Haemophilus influenzae* and 4 *Haemophilus aphrophilus*.

[g]Includes 2 *Stenotrophomonas maltophilia*, 5 *Burkholderia cepacia*, and 13 *Pseudomonas* spp.

[h]Includes 3 *Serratia marcescens*, 2 *Aeromonas* spp., 1 *Neisseria* sp., 2 *Brevundimonas vesicularis*, 1 *Agrobacter radiobacterium*, and 2 *Campylobacter* spp.

[i]Includes 1 *Peptostreptococcus* sp., 1 *Bacteroides* sp., and 1 *Bacteroides fragilis*.

[j]Includes 11 *Candida* spp., 4 *Cryptococcus neoformans*, 1 *Rhodotorula mucilaginosa*, 1 *Fusarium* sp., and 2 *Paecilomyces* spp.

Our study found the best isolation rates from paired sets of aerobic-anaerobic bottles. However, when evaluating yields from a single bottle, the aerobic bottle had significantly more organisms isolated for both adult and pediatric patients. Omitting the anaerobic bottle however, would have missed the isolation of some organisms, which were not limited to anaerobes. A previous study by Riley and Parasakthi [8] evaluating the use of the BACTEC anaerobic bottle in children over a 1-year study period from September 1994 to 1995 found an overall decrease in isolation rates from 11.0% to 9.9% if anaerobic bottles were excluded. There were no anaerobes isolated over this study period. In the present study, excluding the anaerobic bottle in pediatric blood cultures would have decreased the isolation rate from 11.7% to 10.3%. The anaerobes, although accounting for only a small fraction of isolates from both adult and pediatric blood cultures, were more frequently isolated in the anaerobic bottle than in the aerobic bottle, but the difference was only statistically significant among the adult patients. Therefore, we recommend that in cases where a single bottle is being used routinely for blood cultures, an anaerobic bottle be included if there are risk factors for anaerobic bacteremia, such as malignant

**Table 2.** Comparative yields of microorganisms isolated in the aerobic bottle alone, anaerobic bottle alone, and in both bottles among adult patients

| Microorganisms | No. of isolates detected | | | | *p* |
|---|---|---|---|---|---|
| | Aerobic bottle alone | Anaerobic bottle alone | Both aerobic and anaerobic bottles | Total organisms | |
| Gram-positive cocci | | | | | |
| *Staphylococcus aureus* | 21 | 19 | 148 | 188 | NS |
| Coagulase-negative staphylococci | 153 | 128 | 246 | 527 | NS |
| *Streptococcus* spp.[a] | 11 | 15 | 61 | 87 | NS |
| Other Gram-positive cocci[b] | 13 | 1 | 2 | 16 | <0.01 |
| *Enterococcus* spp. | 13 | 15 | 39 | 67 | NS |
| Gram-positive bacilli[c] | 25 | 9 | 8 | 42 | <0.01 |
| Gram-negative organisms | | | | | |
| *Klebsiella*[d] | 17 | 20 | 100 | 137 | NS |
| *Escherichia coli* | 30 | 40 | 91 | 161 | NS |
| *Pseudomonas aeruginosa* | 49 | 3 | 27 | 79 | <0.05 |
| *Acinetobacter* spp. | 66 | 2 | 14 | 82 | <0.05 |
| *Citrobacter* spp. | 0 | 2 | 8 | 10 | NS |
| *Enterobacter* spp. | 11 | 10 | 30 | 51 | NS |
| *Salmonella* spp.[e] | 2 | 0 | 17 | 19 | NS |
| *Haemophilus* spp.[f] | 6 | 2 | 0 | 8 | NS |
| *Proteus* spp. and *Morganella* spp. | 2 | 7 | 10 | 19 | NS |
| Other *Pseudomonas* organisms and *Burkholderia* spp.[g] | 47 | 1 | 6 | 54 | <0.05 |
| Other Gram-negative organisms[h] | 18 | 4 | 11 | 33 | <0.05 |
| Anaerobes[i] | 0 | 16 | 0 | 16 | <0.05 |
| All fungi[j] | 19 | 1 | 7 | 27 | <0.05 |
| All microorganisms | 503 | 295 | 825 | 1623 | <0.05 |

Abbreviation: NS = not significant

[a]Includes 7 *Streptococcus pyogenes*, 13 group B streptococcus, 6 group G streptococcus, 6 *Streptococcus* spp., 24 viridans streptococci, 12 *Streptococcus pneumoniae*, 3 *Streptococcus milleri*, and 6 *Streptococcus bovis*.

[b]Includes 10 *Micrococcus* spp., 3 *Aerococcus* spp., 2 *Leuconostoc* spp., and 1 *Rhodococcus* sp.

[c]Includes 23 *Bacillus* spp., 3 *Corynebacterium jeikeium*, 12 *Corynebacterium* spp., and 4 *Cellulomonas* spp.

[d]Includes 98 *Klebsiella pneumoniae* and 39 *Klebsiella* spp.

[e]Includes 18 *Salmonella* spp. and 1 *Salmonella typhi*.

[f]Includes 2 *Haemophilus influenzae*, 3 *Haemophilus parainfluenzae*, and 3 *Haemophilus* spp.

[g]Includes 24 *Stenotrophomonas maltophilia*, 10 *Burkholderia pseudomallei*, 6 *Burkholderia cepacia*, and 14 *Pseudomonas* spp.

[h]Includes 1 *Edwardsiella tarda*, 3 *Serratia marcescens*, 3 *Serratia* spp., 8 *Aeromonas* spp., 3 *Neisseria* spp., 2 *Agrobacterium radiobacter*, 1 *Sphingomonas paucimobilis*, 3 *Alcaligenes* spp., 1 *Moraxella* sp., 1 *Chryseomonas* sp., 2 *Flavobacterium* spp., 4 *Flavobacterium meningosepticum*, and 1 *Oerskovia* sp.

[i]Includes 2 *Bacteroides* spp., 4 *Bacteroides fragilis*, 1 *Eubacterium lentis*, 1 *Clostridium perfringens*, 2 *Fusobacterium* spp., 1 *Bifidobacterium* sp., 2 *Clostridium* spp., 2 *Propionibacterium* spp., and 1 *Porphyromonas* sp.

[j]Includes 2 *Cryptococcus neoformans*, 24 *Candida* spp., and 1 *Penicillium marneffei*.

neoplasms, hematological disorders, recent gastrointestinal or obstetric gynecologic surgery, intestinal obstruction, and diabetes mellitus [9-12]. It has been suggested that recognizing anaerobic bacteremia microbiologically was not crucial, as affected patients could be recognized clinically as probably having anaerobic bacteremia and treated empirically for it [13-15]. However, it is important to bear in mind that the anaerobic bottle not only supports the growth of anaerobes but also that of facultative anaerobes.

Murray et al [4] found that facultative anaerobes grew better in the Septichek aerobic bottle; however, in the present study, although some facultative anaerobes were detected more frequently in anaerobic bottles, the difference was not statistically significant. Because this was a retrospective study, we could not determine whether the blood sample was evenly distributed between the aerobic and anaerobic bottles. Uneven distribution of blood volume between bottles may affect yields in either bottle, as shown by a study that found

that a 1-mL increase in the volume of blood cultured resulted in an increase in yield of about 3% [5].

Other factors known to influence blood culture isolation rates include the number and timing of cultures [13]. The patient demographics and type of organisms in the particular hospital setting are also important [7]. High incidence of aerobic pathogens such as *Pseudomonas* and *Acinetobacter* would suggest they might be better isolated in 2 aerobic bottles rather than in an aerobic-anaerobic pair. Similarly, if fungemia is likely, blood should also be sent for fungal culture in fungal culture bottles.

Comparison of time to positivity in each bottle for isolates that grow in both aerobic and anaerobic bottles is also useful in determining the value of the routine inclusion of an anaerobic bottle in a blood culture set. Riley and Parasakthi [8] reported that when both aerobic and anaerobic bottles were positive, clinically significant isolates were isolated in 19.8% occasions from the aerobic bottle first and in 9.0% occasions from the anaerobic bottle first. However, it is not known whether facultative anaerobes predominated in the group where isolates were first detected in the anaerobic bottle. We did not evaluate time to positivity in our study.

In summary, this study showed significantly more organisms isolated in the aerobic bottle compared to the anaerobic bottle; however, we recommend that when culturing blood, an aerobic-anaerobic pair rather than an aerobic-aerobic pair of bottles be used to optimize the recovery of a wider spectrum of organisms, including the obligatory anaerobes.

Further studies comparing the time to positivity for facultative anaerobes between the aerobic and anaerobic bottle would be helpful in determining the ideal combination of bottles to be used.

## References


1. Balows A, Hausler WJ, Herrmann KL, Isenberg HD, Shadomy HJ, eds. Manual of clinical microbiology. 5th ed. Washington DC: American Society for Microbiology; 1991.
2. Sharp SE. Routine anaerobic blood cultures: still appropriate today? Clin Microbiol Newsl.1991;13:179-81.
3. Dorsher CW, Rosenblatt JE, Wilson WR, Ilstrup DM. Anaerobic bacteremia: decreasing rate over a 15-year period. Rev Infect Dis. 1991;13:633-6.
4. Murray PR, Traynor P, Hopson D. Critical assessment of blood culture techniques: analysis of recovery of obligate and facultative anaerobes, strict aerobic bacteria, and fungi in aerobic and anaerobic blood culture bottles. J Clin Microbiol. 1992;30:1462-8.
5. Mermel LA, Maki DG. Detection of bacteremia in adults: consequence of culturing an inadequate volume of blood. Ann Intern Med. 1993;119:270-2.
6. Dunne WM, Tillman J, Havens PL. Assessing the need for anaerobic medium for the recovery of clinically significant blood culture isolates in children. Pediatr Infect Dis J. 1994; 13:203-6.
7. Riley JA, Heiter BJ, Bourbeau PP. Comparison of recovery of blood culture isolates from two BacT/ALERT FAN aerobic blood culture bottles with recovery from one FAN aerobic bottle and one FAN anaerobic bottle. J Clin Microbiol. 2003; 41:213-7.
8. Riley PA, Parasakthi N. Evaluation of the use of Bactec anaerobic blood cultures in the detection of bacteremia and fungemia in children. Malays J Pathol. 1996;18:31-4.
9. Donaldson SS, Moore MR, Rosenberg SA, Vosti KL. Characterization of postsplenectomy bacteremia among patients with and without lymphoma. N Engl J Med. 1972;287:69-71.
10. Bodner SJ, Koenig MG, Goodman JS. Bacteremic *Bacteroides* infections. Ann Intern Med. 1970;73:537-44.
11. Alpern RJ, Dowell VR. *Clostridium septicum* infections and malignancy. JAMA. 1969;209:385-8.
12. Felner JM, Dowell VR. "*Bacteroides*" bacteremia. Am J Med. 1971;50:787-96.
13. Reimer LG, Wilson ML, Weinstein MP. Update on detection of bacteremia and fungemia. Clin Microbiol Rev. 1997;10: 444-65.
14. Morris AJ, Wilson ML, Mirrett S, Reller LB. Rationale for selective use of anaerobic blood cultures. J Clin Microbiol. 1993;31:2110-3.
15. Sharp SE, McLaughlin JC, Goodman JM, Moore J, Spanos SM, Keller DW, et al. Clinical assessment of anaerobic isolates from blood cultures. Diagn Microbiol Infect Dis. 1993;17: 19-22.


 