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Performance of two blood culture systems to detect anaerobic bacteria. Is there any difference?☆

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ABSTRACT

We studied the performance characteristics of two blood culture (BC) bottles/systems, (i) BacT/ALERT-FN Plus/3D (bioMérieux, Marcy l'Étoile, France) and (ii) BACTEC-Lytic/9000 (Becton Dickinson, Sparks, USA) for detection of growth and time-to-positivity (TTP) against a balanced and diverse collection of anaerobic bacterial strains (n = 48) that included reference strains (n = 19) and clinical isolates (n = 29) of 32 species (15 Gram-negative and 17 Gram-positive). Standard suspension of bacteria was inoculated to each bottle in duplicates and incubated in the corresponding system. Overall, 62.5% (n = 30) of strains were detected by both BC bottle types. Comparing the two, 70.8% (n = 34) and 79.2% (n = 38) of strains were detected by BacT/ALERT-FN Plus and BACTEC-Lytic bottles, respectively (p = 0.38). Among Gram-negative anaerobes (n = 25) the detection rate was 76.0% (n = 19) vs. 92.0% (n = 23) (p = 0.22), respectively. Among Gram-positive anaerobes (n = 23) the detection rate was 65.2% (n = 15) in both bottles (p = 1). The average TTP per bottle was calculated only for the strains detected by both systems (n = 30) and was 40.85 h and 28.08 h for BacT/ALERT-FN Plus and BACTEC-Lytic, respectively (p < 0.001). The mean difference was 12.76 h (95% CI: 6.21-19-31 h). Six anaerobic strains were not detected by any system, including Gram-negative *Porphyromonas gingivalis*, and five Gram-positive strains: *Finegoldia magna*, *Peptostreptococcus anaerobius*, *Propionibacterium acnes*, *Clostridium novyi* and *Clostridium clostridioforme*. Furthermore, *Eggerthella lenta* and *Prevotella bivia* were detected only by BacT/ALERT-FN Plus, while *Prevotella disiens* and *Prevotella intermedia* were detected only by BACTEC-Lytic bottles. There were no major differences in detection rate among clinical and reference strains. Anaerobic bacteria represent a minority of BC isolates, however, far from ideal detection rate was observed in this study for both tested bottle/system combinations. Nevertheless, in those cases where both gave positive signal, BACTEC-Lytic was superior to BacT/ALERT FN Plus with 12.76 h shorter mean TTP. Improvements of media in blood culture bottles available for detection of anaerobes are warranted.

1. Introduction

Anaerobic bacteria are important human pathogens and can cause infections of virtually any anatomical site including bloodstream infections [1,2]. They account for up to 10% of all positive blood culture (BC) isolates and it has been estimated that anaerobic bacteremia has an average mortality of 14–30%, depending on clinical setting [1–4]. Early recognition and initiation of treatment, both an effective antimicrobial therapy and adequate source control of infection, have been proved to have a protective effect on mortality of patients with anaerobic bacteremia [3,5,6]. The majority of anaerobic bacteremia are due to Gram-negative anaerobic bacilli, mostly *Bacteroides fragilis* group, which account for about half of all anaerobic isolates from blood. Other anaerobes that are commonly isolated from BCs are Gram-positive anaerobic cocci, *Clostridium* spp. and *Fusobacterium* spp. which account for about one fourth of all isolates. Furthermore, *Propionibacterium acnes* is frequently isolated from blood, but is mostly regarded as a contaminant from skin [1,2].

Detection of anaerobes from blood represents a well-known challenge in the clinical microbiology laboratory. This is partially

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because of the fastidious nature of anaerobic bacteria with special growth requirements and partially because of their slow growth. Development of automated BC systems with dedicated anaerobic BC bottles has improved the detection of anaerobes from blood of patients with signs of systemic infection. The two most commonly used automated BC systems worldwide are (i) BacT/ALERT (bioMérieux, Marcy l'Étoile, France) and (ii) BACTEC (Becton Dickinson, Sparks, USA). The two most commonly used anaerobic BC bottles from these two systems are (a) BacT/ALERT-FN Plus, which contains a complex anaerobic medium with adsorbent polymeric beads and is an evolution from an older medium, designed BacT/ALERT-FN, which contains charcoal as an adsorbent for antimicrobial agents in blood, and (b) BACTEC-Lytic/10 Anaerobic/F (BACTEC-Lytic in further text), which is a complex anaerobic medium that contains detergent saponin for lysis of leucocytes and recovery of extracellular as well as intracellular bacteria and does not contain any adsorbent agent. Additionally, both anaerobic bottles contain anticoagulant sodium polyanethol sulphonate (SPS) that is also a well-known growth inhibitor of several anaerobic species. Very few studies with conflicting results have compared the ability to grow and time-to-positivity (TTP) of anaerobic bacteria from these two anaerobic bottles/systems [7–9].

The aim of the present study was to comprehensively evaluate two commercially available BC bottles, BacT/ALERT-FN Plus and BACTEC-Lytic for growth detection and TTP against a balanced and diverse collection of anaerobic bacterial strains, comprising a selection of reference strains and most common clinical BC isolates.

2. Methods

2.1. Study design and bacterial strains

The study was performed at the Institute of Microbiology and Immunology, Medical Faculty, University of Ljubljana, Slovenia. This is the largest Slovenian microbiology laboratory that receives BC samples from two major tertiary-care hospitals in the area, (i) the University Medical Centre Ljubljana and (ii) the Institute of Oncology Ljubljana, comprising a total of 2400 beds.

A balanced and diverse collection of anaerobic bacterial strains (n = 48) that included reference strains (n = 19) and clinical isolates (n = 29) of 32 species (15 Gram-negative and 17 Gram-positive) was tested. Clinical isolates were selected based on the rank of all anaerobic bacteria isolated from BCs at the institute in the 5-years period between 2010 and 2014. We use both tested BC systems in routine diagnostics as well, so the rank was based on cumulative data from the two. Altogether, 25 most common clinical anaerobic bacterial isolates were included in the collection, the most prevalent ones with two distinct randomly selected strains. The tested collection included *Bacteroides* spp. (n = 10), *Prevotella* spp. (n = 8), Gram-positive anaerobic cocci (GPAC) (n = 8), *Clostridium* spp. (n = 8), *Fusobacterium* spp. (n = 5), *Eggerthella* spp. (n = 2), *Actinomyces* spp. (n = 2), *Propionibacterium* spp. (n = 2), *Porphyromonas* spp. (n = 1), *Veillonella* spp. (n = 1) and *Lactobacillus* spp. (n = 1). Bacterial strains used in the study are shown in Table 1.

Table 1
Anaerobic strains used in this study.

Strain	Origin of the strain (clin/ref)[a]
Gram-negative (n = 25)	
Bacteroides fragilis 1	CLIN
Bacteroides fragilis 2	CLIN
Bacteroides fragilis ATCC 23745	REF
Bacteroides ovatus	CLIN
Bacteroides ovatus BAA 1296	REF
Bacteroides thetaiotaomicron 1	CLIN
Bacteroides thetaiotaomicron 2	CLIN
Bacteroides thetaiotaomicron ATCC 29741	REF
Bacteroides uniformis	CLIN
Bacteroides vulgatus	CLIN
Fusobacterium necrophorum 1	CLIN
Fusobacterium necrophorum 2	CLIN
Fusobacterium necrophorum ATCC 25286	REF
Fusobacterium nucleatum	CLIN
Fusobacterium nucleatum ATCC 25586	REF
Porphyromonas gingivalis ATCC 33277	REF
Prevotella bivia ATCC 29303	REF
Prevotella buccae	CLIN
Prevotella disiens	CLIN
Prevotella intermedia	CLIN
Prevotella intermedia ATCC 25611	REF
Prevotella melaninogenica	CLIN
Prevotella melaninogenica ATCC 25845	REF
Prevotella nigrescens	CLIN
Veillonella parvula	CLIN
Gram-positive (n = 23)	
Actinomyces odontolyticus	CLIN
Actinomyces viscosus ATCC 15987	REF
Clostridium clostridioforme	CLIN
Clostridium innocuum	CLIN
Clostridium novyi ATCC 19402	REF
Clostridium perfringens	CLIN
Clostridium perfringens ATCC 13124	REF
Clostridium septicum ATCC 12464	REF
Clostridium sordelii ATCC 9714	REF
Clostridium sporogenes ATCC 19404	REF
Eggerthella (Eubacterium) lenta	CLIN
Eggerthella (Eubacterium) lenta ATCC 43055	REF
Finegoldia magna 1	CLIN
Finegoldia magna 2	CLIN
Finegoldia magna ATCC 29328	REF
Lactobacillus rhamnosus	CLIN
Parvimonas (Micromonas) micra	CLIN
Peptoniphilus asaccharolyticus	CLIN
Peptoniphilus harei	CLIN
Peptostreptococcus anaerobius	CLIN
Peptostreptococcus anaerobius ATCC 27337	REF
Propionibacterium acnes	CLIN
Propionibacterium acnes CO 62553	REF
Total (n = 48)	**CLIN** (n = 29), **REF** (n = 19)

[a] CLIN clinical isolate, REF reference strain.

2.2. Blood culture inoculation

Fresh bacterial cultures (i.e. second subculture from strains stored at −70 °C) grown for 48 h on Schaedler agar at 35 °C in an anaerobic atmosphere generated with Anoxomat system (Advanced Instruments, Norwood, USA), were suspended in the brain heart infusion broth to reach 0.5 McFarland standard density (corresponding to $\approx 10^8$ CFU/mL). After two serial dilutions of 1:100, an inoculating suspension of $\approx 10^4$ CFU/mL density was prepared and 0.1 mL (≈1000 CFU) inoculated to (i) BacT/ALERT FN Plus and (ii) BACTEC-Lytic BC bottles in duplicates. Simultaneously, 0.1 mL of inoculating suspension was spread on Schaedler agar for control of viability, inoculum concentration and confirmation of identification by MALDI-TOF MS. Additionally, 5 mL of defibrinated sterile horse blood (Becton Dickinson, Sparks, USA) was added to each inoculated bottle to simulate real BC specimens condition following the protocol from one of the manufacturers [10]. Since both BC bottle types contain 40 mL of the complex medium, the final starting concentration of bacteria in each bottle was ≈20–30 CFU/mL, which also corresponds well with the estimated real-time concentration of bacteria in blood of adult patients with sepsis [11]. Blood culture bottles were loaded into BacT/ALERT 3D and BACTEC 9000 BC systems respectively and incubated for 5-days or until the machines signaled positivity. The TTP was recorded. If no growth was detected in 5-days, terminal subcultivation on Schaedler agar was performed and bacterial quantity evaluated after 48 h anaerobic incubation.