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Original Article

BACTEC Anaerobic Lytic/F bottles in the detection of obligate and facultative anaerobic bacteria in blood samples: laboratory evaluation and one-year experience of clinical use

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ABSTRACT

The detection of anaerobic bacteria from blood cultures may be challenging. BACTEC Anaerobic Lytic/F bottles (BALB) were developed to optimize the detection of obligate and facultative anaerobic organisms. This study compared the performance of BALB to resin-supplemented BACTEC Anaerobic/F bottles (BAB). The time-to-detection (TTD) and detection rate (DR) of BALB and BAB were compared in two study stages: first, a laboratory evaluation, including bottles spiked with human blood and 20 facultative and obligate anaerobic bacterial strains; and second, data from patients in the emergency room over two one-year periods: 2015-2016 with BAB and 2017-2018 with BALB. A total of 160 bottles (80 of each type) were included in the first part of the study. The DR of all species in BALB was higher than that in BAB (92.25% vs. 82.50%). The TTD was shorter in BALB than in BAB by 18.9 and 1.4 h for obligate and facultative anaerobic organisms, respectively. Data from patients in two one-year periods showed no significant differences in the numbers of positive anaerobic bottles growing any bacteria (9.29% and 9.52% with BAB and BALB, respectively, $p > 0.05$). However, the growth of obligate anaerobic bacteria was higher with BALB than with BAB (0.73% vs 0.46%, $p = 0.018$). The performance of BALB in terms of DR and TTD was significantly superior to BAB for obligate anaerobic species, suggesting that the use of these bottles can improve the detection of these bacteria from blood samples.

KEYWORDS: anaerobic bacteria, blood culture, BACTEC bottle, bacteremia, sepsis.

INTRODUCTION

Blood cultures (BCs) represent an invaluable diagnostic tool for the detection of potentially life-threatening infections [1-3]. The results of BCs can provide a definitive diagnosis that can guide the course of therapy and offer key prognostic information. Achieving this goal may be problematic, as the detection and identification of anaerobic bacteria in BCs is a well-recognized challenge in clinical microbiology, and some microorganisms are typically fastidious, slow growing, and difficult to culture [4]. Laboratories should try to overcome these difficulties, as early recognition and appropriate treatment of anaerobic bloodstream infections are of great clinical importance [5].

Automated continuous-monitoring BC systems together with anaerobic BC bottles have improved the detection of microorganisms [6, 7]. Furthermore, BC bottles able to reduce the time to positivity and increase the detection rate (DR) could potentially lead to a considerable advantage in decreasing morbidity and mortality rates [8]. According to the manufacturer, BD BACTEC™ Lytic/10 Anaerobic/FBC bottles (BALB) (Becton, Dickinson and Co, Sparks, MD, USA) contain

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0.26% saponin as a lysing agent and provide faster time-to-detection (TTD) for facultative and obligate anaerobic organisms as compared to BD BACTEC™ Anaerobic Plus/FBCs bottles (BAB) from the same manufacturer. The lytic medium optimizes the detection of obligate anaerobic and facultative organisms by lysing phagocytes. Phagocytized organisms are then released into the culture medium, thereby enabling more to be recovered. In addition, decreased metabolic activity from lysed blood cells increases the detection sensitivity and reduces false positives.

The aim of the present study is to compare the TTD and DR of BALB and BAB blood culture bottles for all bacteria in a laboratory evaluation with simulated positive bottles, and the DR for obligate anaerobic bacteria between two one-year periods, each with other type of bottles. In addition, the performance of both types of bottles in direct identification with the Sepsityper™ kit (Bruker Daltonics, Bremen, Germany) was evaluated.

MATERIALS AND METHODS

Study design

This study was performed at the Microbiology Laboratory of Emek Medical Center between January and November 2017 and compared the performance of BALB with that of BAB bottles for the detection of anaerobic bacteria. The study comprised two stages, a laboratory evaluation with spiked bottles simulating positive BCs and a comparison of DR in two one-year periods, one with BAB (2015/6) and the other with BALB (2017/8) using samples from patients presenting with suspected bacteremia in the emergency room.

Simulated blood cultures

In the first stage, in order to simulate positive BCs, 5 mL of sterile human blood and the tested organisms were injected to the BALB and BAB bottles. Following the detection of positivity, the TTD and DR were calculated for each set of both bottle types. The TTD was defined as the time elapsed from the placement of the bottles in the BACTEC™ FX unit until flagging of positivity by the instrument. The DR was defined as the percentage of positive bottles among all inoculated bottles with the same inoculum concentration for each species. Overall, nine obligate anaerobic bacterial species and 11 facultative anaerobic species were cultured on anaerobic CDC anaerobe blood agar (Hy Laboratories Ltd, Rehovot, Israel) and 5% defibrinated sheep blood-supplemented tryptic soy agar (TSA) (Novamed Ltd, Jerusalem, Israel), respectively. *Haemophilus influenzae* was cultured on supplemented chocolate agar from the same manufacturer.

The anaerobic agar medium was incubated under anaerobic conditions for 48 h and the TSA and chocolate agar plate were incubated under a 5% CO_2 atmosphere for 24 h. Colonies from agar plates were then suspended in saline to match a 1.0 McFarland (1×10^8 CFU/mL) standard, and diluted to final concentrations of 1×10^2 colony forming units (CFU)/mL and 1×10^1 CFU/mL. A volume of 100 µL from each suspension was inoculated into BC bottles in duplicate for each bottle type. Inoculum densities were verified by culturing 100 µL from the final suspensions in relevant agar plates and then incubating under anaerobic or aerobic conditions accordingly.

Following inoculation, all bottles were placed in a BACTEC™ FX continuous-monitoring system (Becton, Dickinson and Co, Sparks, MD, USA) for a total of six days or until positivity. The positive BCs were Gram stained, directly identified using the Sepsityper™ kit and a matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) Microflex system (both from Bruker Daltonics, Bremen, Germany) according to manufacturer recommendations, and sub-cultured on agar plates depending on the organism, as previously described.

In order to rule out any interference between lytic bottles and the Sepsityper™ kit, identification score categories were compared for both types of bottles. According to the Microflex manufacturer, the following categorical interpretation of scores was applied: score $\geq$ 2.0 (high confidence identification for genus and species level), score 1.7-1.99 (low confidence identification usually, only genus level reported), and score $\leq$ 1.699 (no organism identification possible). Final identification was confirmed from colonies after 24 h using the same technology.

The following 20 bacterial species were included in the study: *Bacteroides fragilis*, *Porphyromonas sp*., *Clostridium septicum*, *Fusobacterium necrophorum*, *Actinomyces odontolyticus*, *Clostridium perfringens*, *Veillonella atypica*, *Peptoniphilus harei*, *Prevotella bivia*, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 13883, *Proteus mirabilis*, *Citrobacter koseri*, *Enterobacter cloacae*, *H. influenzae*, *Staphylococcus aureus*, *Streptococcus pyogenes* ATCC 195615, *Enterococcus faecalis* ATCC 219212, *Streptococcus pneumoniae*, *and Staphylococcus epidermidis*.

Clinical evaluation

In the second stage of the study, instead of BAB, the BALB were introduced to routine use in the emergency room for a period of nine months, between March and November 2017. By the end of the trial, we were able to compare the performance of BALB to that of BAB in terms of the DR in blood samples drawn from adult patients admitted to the emergency room with suspected sepsis. Positive BCs were processed according to standard routine procedures: 3 mL of fluid extracted from the bottles were Gram stained and sub-cultured on four agar plates: 5% defibrinated sheep blood-supplemented TSA, supplemented chocolate agar, gentamicin-supplemented anaerobic blood agar (all from Novamed Ltd, Jerusalem, Israel), and MacConkey Agar (Hy Laboratories Ltd, Rehovot, Israel).

Following overnight incubation at 37 °C, identification of the bacterial colonies was performed using a MALDI-TOF Microflex system, and antimicrobial susceptibility testing was performed using routine techniques.

For the clinical stage of the study, the DR was defined as the percentage of positive BCs among all anaerobic bottles, BALB or BAB, during each period, respectively. The DR for the period between March and November 2017 with BALB was compared to the DR of BAB for the same period in the previous year (2016). The DR of obligatory anaerobic bacteria was also calculated and compared between both periods of time.

Statistical methods

The positivity rates for the four bottle types were compared using the Chi-squared test. Values of $p < 0.05$ were considered statistically significant.

RESULTS

The first stage of the study included a total of 160 anaerobic BCs, comprising two sets of different bottles (BALB and BAB) inoculated with the same suspension of 20 facultative and obligatory anaerobic bacterial species in two different concentrations. As shown in Table 1, compared with BAB, the DR was higher and the average TTD was shorter in BALB for both concentrations of facultative and obligate anaerobic bacteria. At the higher inoculum concentration, the TTD was shorter with BALB than with BAB by 18.9 h and 1.4 h for obligate and facultative anaerobic bacteria, respectively (Table 1). At the lower concentration, the average TTD with BALB for facultative anaerobic bacteria was shorter by only 0.7 h. The TTD for obligate anaerobic bacteria could not be calculated owing to the small number of bottles showing any growth.

All but two species (63 from BALB and 52 from BAB) were correctly identified using the Sepsityper™ and matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS). The two missed identifications were an *H. influenzae*, which showed no growth in BALB bottles, and a *Porphyromonas sp.* which could not be directly identified by MALDI-TOF MS because it was not included in the database, and was eventually identified by sequencing of the 16S rRNA gene (Table 2). High confidence identification (scores 2-3) was achieved in 73% of the BAB bottles vs. 59% in BALB bottles, and in overall identification (scores 1.7-3), these values were 90% vs. 74%, respectively. Differences were not statistically significant. In the second stage of the study, the positivity rates of both all bacteria and obligate anaerobic bacteria in anaerobic blood culture bottles were significantly higher in 2017 with BALB than in 2016 with BAB (Table 3). The numbers of bottles that grew obligate anaerobic bacteria were 42 (17 different species) and 70 (25 different species) in 2016 and 2017, respectively (Table 4).

DISCUSSION

Sepsis and bacteremia are life-threatening conditions for patients. The most sensitive and effective method to detect and identify the causative microorganism(s) is by blood culture. It is crucial

Table 1. Detection rate and time-to-detection of facultative and obligatory anaerobic bacteria from BALB and BAB blood culture bottles.

			$10^{\wedge 2}$ CFU/mL				**$10^{\wedge 1}$ CFU/mL**			
			BALB		**BAB**		**BALB**		**BAB**	
			Growth (n/n)	**TTD in hours (SD)**	**Growth (n/n)**	**TTD in hours (SD)**	**Growth (n/n)**	**TTD in hours (SD)**	**Growth (n/n)**	**TTD in hours (SD)**
Facultative anaerobic	*Escherichia coli*	ATCC25922	2/2	12.9 (5.2)	2/2	14.3 (13.9)	2/2	12.7 (5.6)	1/2	13.4 (4.9)
	Klebsiella pneumoniae	ATCC13883	2/2		2/2		2/2		2/2	
	Proteus mirabilis	Wild	2/2		2/2		2/2		2/2	
	Citrobacter koseri	Wild	2/2		2/2		2/2		2/2	
	Enterobacter cloacae	Wild	2/2		2/2		2/2		1/2	
	Haemophilus influenzae	Wild	0/2		2/2		0/2		2/2	
	Staphylococcus aureus	Wild	2/2		2/2		1/2		2/2	
	Streptococcus pyogenes	ATCC195615	2/2		2/2		2/2		2/2	
	Enterococcus faecalis	Wild	2/2		2/2		1/2		2/2	
	Streptococcus pneumoniae	Wild	2/2		2/2		1/2		2/2	
	Staphylococcus epidermidis	Wild	2/2		2/2		2/2		0/2	
Strict anaerobic	*Bacteroides fragilis*	Wild	2/2	19.4 (8.0)	1/2	38.3 (19.3)	2/2	N/A	0/2	N/A
	Porphyromonas spp	Wild	2/2		0/2		2/2		0/2	
	Clostridium septicum	Wild	2/2		1/2		0/2		0/2	
	Fusobacterium necrophorum	Wild	2/2		2/2		2/2		1/2	
	Actinomyces odontolyticus	Wild	2/2		2/2		2/2		2/2	

Table 1 continued..

			$10^{\wedge 2}$ CFU/mL				**$10^{\wedge 1}$ CFU/mL**			
			BALB		**BAB**		**BALB**		**BAB**	
			Growth (n/n)	**TTD in hours (SD)**	**Growth (n/n)**	**TTD in hours (SD)**	**Growth (n/n)**	**TTD in hours (SD)**	**Growth (n/n)**	**TTD in hours (SD)**
	Clostridium perfringens	Wild	1/2	19.4 (8.0)	2/2	38.3 (19.3)	1/2	N/A	0/2	N/A
	Veillonella atypica	Wild	2/2		1/2		0/2		1/2	
	Peptoniphilus harei	Wild	2/2		2/2		2/2		0/2	
	Prevotella bivia	Wild	2/2		0/2		2/2		0/2	
Total (n/N) detection rate (%)			**37/40***		**33/40***		**30/40****		**22/40****	

*p = 0.17 (n.s.), **p = 0.06 (n.s.). BALB, BACTEC™ Anaerobic Lytic/F bottles; BAB, BACTEC™ anaerobic/F bottles.

Table 2. Direct identification of facultative and obligatory anaerobic bacteria from positive bottles of blood culture by MALDI-TOF Sepsityper™.

MALDI-TOF score	BALB n (%)	BAB n (%)	p-value
≥ 2[a]	31 (59)	46 (73)	ns
1.70 - 1.99[a]	13 (25)	11 (17)	ns
≤ 1.69	8 (15)	6 (9)	ns

[a]Score >1.7 was considered acceptable for species level identification. MALDI-TOF, matrix-assisted laser desorption ionization-time of flight; BALB, BACTEC™ Anaerobic Lytic/F bottles; BAB, BACTEC™ anaerobic/F bottles.

Table 3. Comparison of positivity rates of anaerobic bottles between two annual periods, 2015/6 and 2017/8, with BAB and BALB, respectively.

	2015/6	2017/8	p-value
Total anaerobic bottles	9058	9586	
Positive anaerobic bottles (all bacteria)	842	913	
Positive anaerobic bottles (strictly anaerobic bacteria)	42	70	
Positive rate in anaerobic bottles (all bacteria)	9.29%	9.52%	n.s.
Positive rate of strictly anaerobic bacteria among all anaerobic bottles	0.46%	0.73%	0.018

BAB, BACTEC™ anaerobic/F bottles; BALB, BACTEC™ Anaerobic Lytic/F bottles.

that the time that has elapsed between the point at which BCs are drawn and the identification of bacterial species and delivery of antimicrobial susceptibility testing results should be as short as possible, to guide the most appropriate antimicrobial therapy, especially when the causative organism is an obligate anaerobe [9-11].

The detection/recovery rate of obligate anaerobic bacteria from anaerobic BCs is usually low. This can be explained by the fact that most of these bacteria grow slowly, are difficult to culture, and may require very strict conditions for growth [12]. Therefore, the goal of this study was to evaluate the DR, TTD, and clinical performance of BALB, as compared with BAB blood culture bottles.

As shown in Table 1, in the first stage of the study, the differences in DR in spiked bottles with both concentrations were not statistically significant; however, lytic bottles still showed the more favorable tendency. There was a 10% improvement in performance at the higher concentration that became stronger at the lower concentration (20%) with a p-value of 0.06. We can note that at this stage of the study, the lower concentration resembled the low levels of bacteria in the blood of most adult patients presenting with sepsis. In addition, in the second stage of the study performed on samples from patients with suspected bacteremia in the emergency room, a significant improvement was observed in the positivity rate for strictly anaerobic bacteria between 2016 and 2017. In addition, the number of different obligate anaerobic species was also considerably higher (25 vs. 17). These findings all suggest that the performance of BALB may be superior to that of BAB for the isolation of strictly anaerobic bacteria in a clinical setting.

The use of MALDI-TOF technology to shorten the time to identification directly from positive BCs has been described in previous studies [13]. Rapid identification on the same day, instead of 24-48 h after sampling, can improve antimicrobial stewardship, especially when a dedicated infectious disease consultant team is present [14, 15], and

Table 4. Obligate anaerobic species isolated in 2015/6 and 2017/8.

	2015/6	**2017/8**
Actinomyces odontolyticus	0	1
Actinomyces sp.	0	1
Anaerobic Gram pos bacilli	0	2
Anaerobic Gram pos cocci	1	4
Bacteroides distasonis	1	1
Bacteroides fragilis	11	12
Bacteroides ovatus	2	0
Bacteroides sp.	2	1
Bacteroides vulgatus	2	0
Bacteroides thetaiotaomicron	1	2
Bifidobacterium sp.	1	1
Clostridium paraputrificum	3	1
Clostridium clostridioforme	0	1
Clostridium perfringens	5	2
Clostridium ramosum	1	1
Clostridium sordellii	0	1
Clostridium sp.	0	1
Fusobacterium varium	1	0
Fusobacterium nucleatum	1	2
Peptostreptococcus sp.	1	0
Peptostreptococcus anaerobius	0	1
Peptostreptococcus parvulus	0	1
Peptostreptococcus prevotii	0	1
Prevotella bivia	0	1
Prevotella buccae	1	1
Cutibacterium acnes	7	26
Propionibacterium avidum	0	1
Propionibacterium granulosum	0	1
Veillonella sp.	1	0
Staphylococcus saccharolyticus	0	3
Total	**42**	**70**

support more favorable antibiotic treatment, better outcomes, and prevent the development of multi-resistant organisms. The results of this study show that the use of BALB does not interfere with Sepsityper method by MALDI-TOF allowing the rapid identification of bacteria and could contribute to all these objectives in patients presenting with anaerobic bacteremia.

Overall, very few studies have been published on the performance of BACTEC™ Lytic Anaerobic bottles. In a previous evaluation of BALB by Rocchetti *et al*. [16], the growth of *H. influenzae* in these bottles was not tested. One surprising finding of the present study was the fact that BALB does not seem to support the growth of this species. In the first stage of our study, we were unable to detect three different strains (two ATCC and one wild type) of *H. influenzae*, with which the BALB bottles were spiked at two concentrations. Consequently, we can conclude that the detection of *H. influenzae* could be missed with BALB. This could be a minor problem, as BCs are always performed using both anaerobic and aerobic bottles. According to our results, the number of strictly anaerobic bacteria detected by BALB, which were missed by BAB, and would not grow in the aerobic bottle of the same set, is more important than the lower sensitivity for *H. influenzae,* which would still grow in the aerobic bottle of the same set.

This study has some limitations. The inclusion of a greater number of obligate anaerobic bacteria in the first stage of the study would have been more favorable. In addition, theoretically, a more accurate comparison between lytic and anaerobic blood culture bottles in a clinical setting could be done by drawing the blood culture sample in both anaerobic BALB and BAB at the same time as the aerobic bottles. This was not done, because we were concerned about the relatively large volume of blood required.

Overall, the findings of this study show a higher DR for strictly anaerobic bacteria, and on average, a shorter TTD. These findings are similar to those already published in a previous evaluation, which was performed in strictly anaerobic species [17] and a recently published retrospective comparison by Bottino *et al*. [18]. In the present study, we also present new data showing that the use of BALB does not interfere with the MALDI-TOF-based rapid identification method (Sepsityper™).

CONCLUSION

In conclusion, the use of BACTEC™ Anaerobic Lytic/F bottles, instead of BACTEC™ Plus Anaerobic/F bottles, seems to be a better choice for the detection of anaerobic bacteremia.

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The authors did not receive any external funding for this study.

ETHICAL APPROVAL

The study was approved by the Independent Review Board of Emek Medical Center in compliance with the Helsinki declaration.

INFORMED CONSENT

The approval by the IRB included exemption of informed consent.

CONFLICT OF INTEREST STATEMENT

All authors have no conflicts of interest to declare.

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