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Original Research Article

Comparison of intra-assay and inter-assay reproducibility and positive detection times of two different (BacT/Alert 3D and Autobio BC) commercial blood culture systems

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ABSTRACT

Purpose: In our study, we aimed to compare the performance of the BacT/Alert 3D (bioMerieux, France) system, which is currently used in our laboratory, and the Autobio BC (Autobio, China) system, which was newly introduced in our country, using standard and clinical isolates.
Methods: Bacterial suspension was prepared by two technicians on the same day and three consecutive days from five different standard strains with 0.5 McFarland turbidity, then serial dilution to a final concentration was adjusted and was simultaneously inoculated in aerobic blood culture bottles. The bacterial concentration was measured by making a quantitative counting plate. The same procedure was also performed for 55 clinical isolates belonging to eleven species. After simulated bacteremia with standard and clinical isolates, the growth results were confirmed by inoculation from positive blood culture bottles onto solid medium and identification was made in the next day with MALDI-TOF MS (bioMérieux). In each study, sterile saline and blood was inoculated into the bottles as a negative control to check contamination. Intra-assay and inter-assay reproducibility of recovery rates and detection times of standard strains; recovery rates and detection times of clinical isolates were compared for both systems.
Results: Recovery rates were 100 % in both systems, and when positive detection times were compared, it was found that there was no difference between the two devices in clinical isolates (p:0.262) but that Autobio BC gave significantly ($p < 0.001$) earlier results in standard strains.
Conclusions: In our simulated bloodstream infection study, Autobio BC was found to be comparable with BacT/Alert 3D, both recovery rates and growth detection time performance were found to be very good, and it can be used in routine microbiology laboratories.

1. Introduction

Sepsis is a life-threatening loss of organ function that occurs due to impaired host response to infections. It was reported that there were an estimated 48.9 million cases of sepsis worldwide in 2017, and approximately 19.7 % of global deaths were due to sepsis [1]. The World Health Organization (WHO) has designated sepsis as a global health priority because it causes approximately six million deaths every year [2]. Although the sensitivity of blood culture is low due to the low concentration of bacteria in the blood, blood culture is still used to diagnose bloodstream infections [3]. Blood culture systems keep the ideal temperature required for the growth of microorganisms constant with the shaking system, instantly detects bacterial growth with its CO_2/chemical sensor and continuously monitors the colorimetric/fluorescence change. Due to these features, it shortens the time from the beginning of incubation until a positive signal is received (time to detection). Detection time also varies depending on factors such as initial bacterial load, source of infection, amount of inoculum, presence

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of antibiotics and host serum factors [4]. Rapid and accurate detection of bacteremia and early initiation of antibiotic treatment are critical to reducing hospital length of stay, healthcare costs and mortality.

The BacT/Alert 3D (bioMérieux, France) and Autobio BC (Autobio, China) blood culture systems work on the same principle and detect the colour change of the gas-permeable sensor at the bottom of the culture bottle from green to yellow due to the presence of CO_2 after bacterial growth. The aim of our study was to compare the BacT/Alert 3D blood culture system, which is routinely used in our laboratory, and the Autobio BC blood culture system, which is newly introduced in our country, using standard strains and clinical isolates, and to evaluate the performance of the Autobio BC system.

2. Material and methods

2.1. Study design

Intra-assay (two technicians on the same day) and inter-assay (three consecutive days) reproducibility of Autobio BC and BacT/Alert 3D blood culture systems were evaluated using standard strains. Recovery rates and positive detection times of both systems were determined using 55 clinical isolates (11 different species). For this purpose, the first 3 Gram-negative (*K. pneumoniae, E. coli,* and *P. aeruginosa*) and Gram-positive bacteria (*S. aureus, S. epidermidis,* and *E. faecium*) most frequently isolated as sepsis causative agents in our center were included in the study. Aerobic bottles were used to compare the two systems; no comparison was made for anaerobic or pediatric bottles.

2.2. Microorganisms

Nine bacteria (*E. aerogenes*, *E. coli*, *E. faecalis*, *E. faecium*, *K. pneumoniae*, *P. mirabilis*, *S. aureus*, *S. epidermidis*, *P. aeruginosa*) and two yeasts (*C. albicans, C. glabrata*), isolated from various clinical samples in our laboratory and identified with the MALDI-TOF MS (bioMérieux) system were included in the study. Five isolates from each species with a total of 55 isolates were inoculated into aerobic blood culture bottles of both systems to create a simulated bloodstream infection. For the intra-assay and inter-assay reproducibility study, ATCC 25922 *E. coli*, ATCC 27853 *P. aeruginosa*, ATCC 29213 *S. aureus*, ATCC 29212 *E. faecalis* and ATCC 49619 *S. pneumoniae* standard strains were used.

2.3. Inoculation and incubation of blood culture bottles

Bacterial suspensions were prepared with colonies taken from standard strain and clinical isolate fresh cultures which were suspended in sterile saline (SF) in a 0.5 McFarland turbidity (bacteria: 1.5-2x10^8 colony forming unit; CFU/mL; fungus: 10^6 CFU/mL) by direct colony suspension method. The McFarland adjustment was performed using an automatic DensiCHEK (bioMérieux) optical reader. Serial dilutions were made from the working solutions, and 500 µL microorganism suspension (150–200 CFU/mL) with 9.5 mL of human blood (obtained from the blood bank) was added into aerobic blood culture bottles so that the final concentration in the bottle was 7.5–10 CFU/bottle (Figure-1). Inoculation procedures for both systems were carried out simultaneously and the bottles were loaded into the blood culture system without waiting. For the repeatability study, the same procedures were performed by two technicians on the same day and three consecutive days, using standard strains.

2.4. Confirmation of final bacterial concentration

In order to confirm the valid colony count from the final bacterial concentration cultivated in blood culture bottles, a quantitative counting plate of sheep blood agar and MacConkey agar (bioMérieux) medium was inoculated with 10 µL from final dilution. Colony counts were performed after 24 h of incubation (Supplementary Fig. 1).

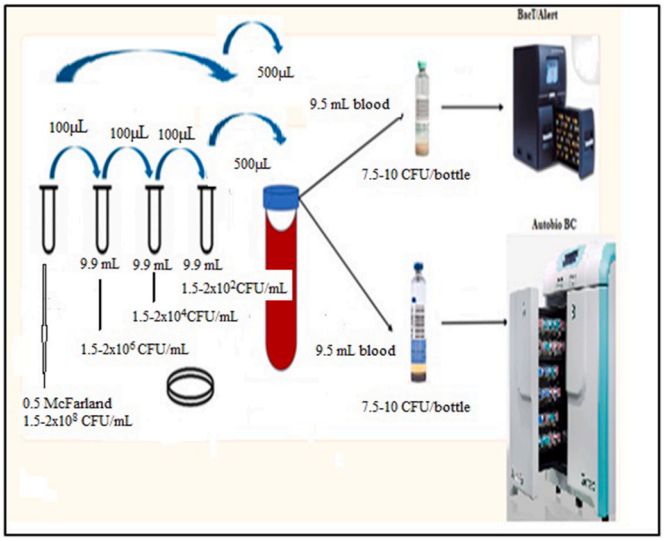

Fig. 1. Preparation of samples and inoculation of blood culture bottles.

2.5. Detection time

For all positive cultures, the time elapsed from the time of loading into the blood culture system until a positive signal was received was taken from the blood culture device software and compared for clinical isolates and standard strains. Detection times were expressed in hours and minutes.

2.6. Confirmation of recovery results

Bottles giving positive signals were inoculated onto 5 % sheep blood agar medium (bioMérieux) and the plates were incubated at 36 ± 1 °C for 24–48 h. Identification of the growing bacteria was confirmed by MALDI-TOF MS (bioMérieux) (Supplementary Fig. 2).

2.7. Statistical analysis

Intra-assay, inter-assay and inter-device compatibility of detection time were examined with intraclass correlation coefficient and repeated measures one way ANOVA. Statistical significance level was accepted as $p < 0.05$.

3. Results

3.1. Repeatability results

Intra-assay and inter-assay repeatability results of both systems with standard strains are given in Supplementary Table 1. When the results of the study performed by two technicians during the day were examined, it was seen that the intra-assay repeatability was extremely good (day 1 p: 0.037, day 2 p: 0.001, day 3 p: 0.003) in the Autobio BC system, while the intra-assay compatibility was poor (day 2 p: 0.075, day 3 p: 0.141) on the second and third day in the BacT/Alert 3D device (Table-1). When the compatibility between days (inter-assay)was examined separately for each device, it was seen that there was a significant level of compatibility ($p < 0.001$) between the days (Table-1). When the results obtained for both devices with standard strains were compared, it was determined that there was a significant level of compatibility (p:0.002). When the average detection times were compared, it was determined that the results obtained with the Autobio BC system were significantly shorter ($p < 0.001$)(Table-2).

Table-1
Intra-assay and inter-assay compatibility for each device.

Intra-assay compatibility for each device								
Device	Day	1st technician		2 nd technician		P[a]	ICC	P[b]
		Mean	SD	Mean	SD			
BacT/Alert 3D	Day 1	13.46	1.66	13.26	1.93	0.536	0.944	**0.001**
	Day 2	14.78	2.36	13.57	1.55	0.148	0.612	0.075
	Day 3	14.67	2.67	13.57	1.66	0.328	0.649	0.141
Autobio BC	Day 1	12.49	1.79	13.32	1.91	0.219	0.717	**0.037**
	Day 2	12.48	1.85	12.18	1.46	0.177	0.959	**0.001**
	Day 3	12.47	1.62	12.16	2.29	0.474	0.907	**0.003**

Inter-assay compatibility for each device									
Device	Day 1		Day 2		Day 3		P[a]	ICC	P[b]
	Mean	SD	Mean	SD	Mean	SD			
BacT/Alert 3D	13.36	1.70	14.18	1.99	14.12	2.17	0.171	0.889	**<0.001**
Autobio BC	12.90	1.80	12.33	1.58	12.32	1.88	0.340	0.865	**<0.001**

[a] Repeated measures one-way ANOVA results.
[b] intraclass correlation coefficient (ICC) results.

3.2. Recovery and detection time results

After simulated bacteremia with standard strains and clinical isolates, positivity was detected in all bottles loaded into both systems. Growth results were confirmed by inoculation of blood culture bottles giving positive signals onto blood agar media. The recovery rates for both systems were determined as 100 %. The results of valid inoculum obtained with clinical isolates, recovery rates and detection times are given in Supplementary Table 2. In the comparison study conducted for clinical isolates, it was determined that the two systems were comparable with each other ($p < 0.001$) and there was no statistical difference between the detection times of the two systems (p:0.262). These results are given in Table-2. When comparing on a bacterial basis, the Autobio BC system had a significantly shorter positive detection time for *K. pneumoniae* (p: 0.043) and *E. faecalis* (p: 0.042), and the BacT/Alert 3D system had a significantly shorter positive detection time for *C. glabrata* isolates (p: 0.043). There was no significant difference between the two systems for other species.

4. Discussion

The primary goal of blood culture, which is a very valuable laboratory test for the diagnosis of bacteremia, is to increase the bacterial growth rate and shorten the positivity detection time. Commonly used automated blood culture systems and newly developed devices for these purposes are constantly updated to improve these parameters. Increasing the culture positivity rate by taking the appropriate number of samples from the patient at the appropriate time and by improving the factors that may affect the growth rate, such as the patient's use of antibiotics, gives the clinician the chance for appropriate patient management.

Although there are many studies comparing widely used blood culture systems, there are very few comparative studies with the relatively recently introduced Autobio BC system [3].

In our study, a shorter detection time (12.52 h) was found with the Autobio BC system for standard strains and the difference between the two systems was found to be statistically significant ($p < 0.001$). In the simulated bacteremia study conducted with clinical isolates, the detection times determined by the two systems were found to be close to each other (15.25-15.02), and no difference was detected between the systems for both recovery rates and detection times (p: 0.262). Although the detection time is shorter for standard strains in the Autobio BC system, there is no difference in detection time between the devices for clinical isolates, indicating that the devices do not have any superiority over each other in the daily routine but are compatible.

In 2024, Hardy et al. [3] compared the usability and performance of four different commercial systems, using the BacT/Alert 3D system as a reference, and reported that all four systems (Autobio BC60, Mindray TDR60, Scenker Labstar50, and DL-biotech DL-60) showed good performance (sensitivity-96.7–100 %; specificity-97.5–100 %). When comparing positive detection times, the shortest detection time was found with Autobio BC (p: 0.001). The researchers obtained false negative results for *C. neoformans* in the Autobio BC system and reported the sensitivity of 96.7 %. In our study, using a relatively small number of yeast isolates (*C. albicans* and *C. glabrata*), no false negatives were found in either system. However, the BacT/Alert 3D system was found to have a significantly (p: 0.043) shorter detection time than the Autobio BC system for *C. glabrata* isolates.

In the study conducted by Ombelet et al. [5] the manual biphasic blood culture system of Autobio Diagnostics was compared with the automated BacT/Alert device. On the first day, the positivity rate of the Autobio BC system was close to the BacT/Alert system, 90.8 % and 99.3 % respectively. On the second day, the same (100 %) positivity rates were detected for both systems. According to the study results, although the efficiency of manual systems is comparable to that of automated systems, they are still inferior to automated systems in terms of detection time.

Li et al. [6] compared the recovery rates and detection times for different bottle types belonging to three different blood culture systems (BACTEC Plus, BacT/Alert, and VersaTREK). They found the recovery rates and detection times to be 100 % in the case of aerobic bacteria and fungi blood bottles for the three systems. It was determined that the VersaTREK system had a shorter detection time in 6 of the 12 bacteria tested in the absence of antibiotic effect, the BACTEC Plus system had a shorter detection time in 5 bacteria, and the BacT/Alert system did not

Table-2
Comparison of device compatibility and detection time averages for standard strains and clinical isolates.

Comparison of device compatibility and detection time averages for standard strains							
Standard strains	BacT/Alert 3D		Autobio BC		P[a]	ICC	P[b]
	Mean	SD	Mean	SD			
	13.89	1.93	12.52	1.72	**<0.001**	0.662	**0.002**

Comparison of device compatibility and detection time averages for clinical isolates							
	BacT/Alert 3D		Autobio BC		P[a]	ICC	P[b]
Clinical isolates	Mean	SD	Mean	SD			
	15.25	4.88	15.02	5.11	0.262	0.976	**<0.001**

[a] Repeated measures one-way ANOVA results.
[b] intraclass correlation coefficient (ICC) results.

have any advantage in terms of detection time compared to the other two systems. In our study, although the Autobio BC system had a significantly shorter positive detection time for *K. pneumoniae* (p: 0.043) and *E. faecalis* (p: 0.042), no significant difference was found between BacT/Alert 3D and Autobio BC system in the comparison for all clinical isolates.

In the study conducted by Sun et al. [7] in which Autobio BC blood culture results were compared with real-time PCR results in patients with suspected bloodstream infection, some bacteria (*K. oxytoca*, *E. faecium*, *P. aeruginosa*) found positive by PCR could not be detected by blood culture, whereas an *Acinetobacter baumannii* isolate was detected positive in blood culture, but it could not be detected by PCR. The researchers did not conduct a detailed analysis of this situation, and only commented that the relationship between the detection of bacterial DNA in the blood and the development of sepsis was controversial. It would not be a correct approach to evaluate the performance of the Autobio BC blood culture system with these findings.

According to the results of an unpublished study conducted by Gürler et al. [8] in our country in 2023 and presented as a poster, the aerobic and pediatric bottle recovery results of BacT/Alert 3D and Autobio BC were found to be the same. It was reported that a *P. aeruginosa* clinical isolate did not grow in the BacT/Alert anaerobic bottle, and a *H. influenzae* clinical isolate did not grow in the Autobio BC anaerobic bottle. In the study where the number of isolates was low (n: 26), the results were interpreted as acceptable. Although no statistical comparison was made in this study, it was emphasized that the detection times were similar for both systems.

5. Study limitations

Our study was conducted with simulated bacteremia samples created by inoculating blood obtained from healthy donors and different bacterial species. The effects of possible antibiotic concentrations and other interfering factors in clinical samples were not investigated, which is the main limitation of our study. We were not able to increase the size of our study sample due to limited resources. It would be possible to get more significant data by increasing the sample size.

6. Conclusion

Although there are many studies in the literature comparing the performance of the BacT/Alert 3D system with other routinely used blood culture systems, there are very few comparison studies with the Autobio BC system. In our study, Autobio BC was found to be comparable with BacT/Alert 3D. Both recovery rates and growth detection time performance of Autobio BC were found to be very good, and it can be used in routine microbiology laboratories. In this direction, we think that our study results will contribute to the literature and, but more comparative studies should be conducted on the subject.

CRediT authorship contribution statement

Nilgün Kansak: Writing – review & editing, Writing – original draft, Validation, Methodology. **Nilay Zeynep Kalender:** Visualization, Methodology. **Neslihan Arıcı:** Writing – review & editing, Methodology. **Rıza Adaleti:** Methodology. **Sebahat Aksaray:** Methodology. **Handan Ankaralı:** Data curation. **Nevriye Gönüllü:** Writing – original draft, Methodology.

Ethical board approval

This study was carried out with the approval of the Haydarpasa Numune Training and Research Hospital Clinical Research Ethics Committee Ethics Board No:(HNEAH-KAEK 2024/KK/16).

Source of support

Yaztek Foreign Trade Inc.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Nilgun Kansak reports equipment, drugs, or supplies was provided by Yaztek Foreign Trade Inc. Nilgun Kansak reports a relationship with Yaztek Foreign Trade Inc. that includes: travel reimbursement. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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The study was presented as an oral presentation at the second Azerbaijan Laboratory Medicine Congress & Lab Expo (AZLTK & LAB EXPO) and received the first prize.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijmmb.2024.100754.

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