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PERFORMANCE OF BC120 SYSTEM IN SIMULATED BLOOD CULTURES: SENSITIVITY AND EFFECTS OF EXPERIMENTAL VARIABLES

Desempenho do Sistema BC120 em Hemoculturas Simuladas: Sensibilidade e Efeitos das Variáveis Experimentais

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ABSTRACT

Bacteremia detection through blood culture is essential for diagnosing infections and determining appropriate treatment. This study evaluated the performance of the BC120 system from a public hospital in the Federal District, comparing it with automated technologies such as Virtuo BacT/Alert, VersaTrek, and BD Bactec. We used aeróbic culture bottles FA and PF from the same lots and ATCC reference strains, ensuring sample consistency. Various sample volumes were tested to assess the time to positivity (TTP) and false positive rates. The results indicated that the BC120 had TTPs comparable to other systems, though slightly higher in some cases. For example, Staphylococcus aureus had a TTP of 12 hours with BC120, compared to 11.4 hours with Virtuo BacT/Alert and 11.8 hours with VersaTrek. The BC120's TTP for Escherichia coli was consistently around 9 hours, regardless of volume. Larger volumes generally increased TTP for Candida albicans but had minimal effect for Enterococcus faecalis. Tests for false positives, including pH variations, indicated that acid and alkaline solutions could impact results, highlighting the need for rigorous sample handling. Sensitivity, specificity, and precision of the BC120 were all 100%, demonstrating its effectiveness in detecting microorganisms without false positives or negatives. In conclusion, the BC120 system proved to be a reliable tool for detecting bacteria and fungi in blood cultures. It matched the performance of leading automated systems, though careful attention to sample handling is crucial to avoid false positives due to pH changes.

KEY-WORDS: Blood culture; Autobio BC120; Sensitivity e specificity.

RESUMO

A detecção de bacteremia por hemocultura é vital para diagnosticar infecções graves e orientar tratamentos. Métodos rápidos e precisos são essenciais devido ao impacto na mortalidade e morbidade. Este estudo avalia a eficácia dos sistemas BC120 e BC60 da Autobio Diagnostics em comparação com outras tecnologias, focando na questão de falsos positivos. Metodologia: Utilizou-se frascos de hemocultura aeróbica pediátricos e adultos, inoculados com cepas de referência. Foram preparadas suspensões em diferentes volumes e realizadas incubação e monitoramento dos tempos para positividade (TTP). Testes complementares avaliaram a influência de alterações do pH na precisão dos resultados. Resultados: O BC120 apresentou TTP's geralmente comparáveis ou ligeiramente superiores aos de outras tecnologias para diversas cepas. Especificamente, para Staphylococcus aureus com 1 ml de sangue, o TTP do BC120 foi de 12 horas, superior ao Virtuo BacT/Alert (11,4 horas) e VersaTrek (11,8 horas), mas inferior ao BD Bactec (13,4 horas). A sensibilidade e a especificidade do BC120 foram de 100%, evidenciando alta precisão. Alterações no pH resultaram em alguns resultados falso-positivos. Discussão: O BC120 demonstrou eficácia e precisão na detecção de microrganismos, com desempenho competitivo em relação a métodos tradicionais. Durante análise foi observada possível influência do volume de amostra e a alteração de pH nos resultados. Conclusão: Os sistemas BC120 e BC60 mostraram-se robustos e confiáveis na detecção de bacteremia, com sensibilidade e especificidade elevadas. A influência do pH e a necessidade de práticas padronizadas para coleta e manipulação são essenciais para garantir resultados precisos e evitar falsos positivos. A pesquisa confirma a eficácia dos sistemas automatizados, destacando sua aplicabilidade em ambientes clínicos.

PALAVRAS-CHAVE: Hemocultura; Sensibilidade e especificidade; Automação.

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1 INTRODUCTION

Accuracy in blood culture is essential to prevent incorrect diagnoses that can compromise treatment. False positives and negatives pose significant challenges, often caused by contamination during collection or inadequate blood volume. In addition, the patient's clinical conditions, such as metabolic acidosis and leukocytosis, can influence the results, making data interpretation more complex (THOMPSON, 2009).

Recently, advances in automated methods have significantly improved the detection of microorganisms. Equipment such as the BC120 and BC60 from Autobio Diagnostics offer a more efficient and continuous approach to sample incubation and analysis. However, it is necessary to investigate possible influences that could lead to incorrect diagnoses of bacteremia or sepsis.

This study aims to explore the effectiveness of these automated systems, assessing their ability to detect microorganisms accurately and identifying possible sources of error, such as false positives. Through a comparative analysis and specific tests, we aim to better understand the limitations and strengths of current methods, contributing to a more robust and reliable approach to detecting bacterial infections.

2 MATERIALS AND METHODS

In this study, commercial blood culture media were used: Aerobic Culture Bottle FA - Batch: 20230515Q7-5 (Ref. MC0301) and Aerobic Culture Bottle PF - Batch: 20230530 (Ref. MC0302), all from the same production batch. ATCC test strains were employed, following the internal quality control document for determining MIC and disc diffusion (Comitê Brasileiro de Testes de Sensibilidade aos Antimicrobianos, 2023). The ATCC strains used were *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Candida albicans* ATCC 90028.

Sixty-milliliter solutions of sterile water were prepared for *Escherichia coli* and *Staphylococcus aureus*, adjusted to a turbidity of 0.01, and a solution with a turbidity of 0.09 for *Candida albicans*, based on the preparation guidelines in the document *Reference Method for Antifungal Susceptibility Testing of Yeasts by Broth Dilution* (CLSI, 2017). From these solutions, 10 µL were taken, and the following volumes were completed: 5, 8, 10, and 13 mL for *Escherichia coli* and *Staphylococcus aureus*; 3, 8, 10, and 13 mL for *Candida albicans* in adult vials (FA); and 1.5 and 8 mL for *Candida albicans* in pediatric vials (PF).

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The approximate concentration of each strain at different volumes was calculated using the McFarland standard. For *Escherichia coli* and *Staphylococcus aureus*, the concentrations were as follows: 1 mL (3.0×10^4), 5 mL (6.0×10^3), 8 mL (3.75×10^3), 10 mL (3.0×10^3), and 13 mL (2.31×10^3). For *Candida albicans*, the concentrations were: 3 mL (9.0×10^4), 5 mL (5.4×10^4), 8 mL (3.38×10^4), and 13 mL (2.08×10^4).

The negative control consisted of 11 bottles (4 pediatric and 7 adult) inoculated with sterile water in volumes of 1, 3, 5, 8, 10, and 13 mL. These were prepared under strict sterile conditions to avoid contamination. Simultaneously with the dilution, the samples were plated according to the methodology described by Klaerner *et al*. (2000) to confirm bacterial growth. This procedure was repeated after 5 days.

The vials were labeled with adhesive tags containing information about the microorganism and the dilution. The samples were inoculated using sterile needles and syringes and incubated in the equipment configured for a 5-day protocol, following the study by Yarbrough *et al*. (2021), which compared microorganism detection and time to positivity in pediatric and standard media using large commercial continuous monitoring systems.

Additionally, a complementary test was conducted to verify the influence of pH. Two solutions were prepared for adult vials (FA): an acidic solution, made with 0.15 mL of 0.8% sulfanilic acid in 9.85 mL of sterile water (final volume of 10 mL), and a basic solution, made with 0.15 mL of 40% potassium hydroxide in 9.85 mL of sterile water (final volume of 10 mL).

3 RESULTS AND DISCUSSION

This study analyzed the efficiency of the IHB BC120 system in pediatric vials, comparing it with other automated blood culture technologies such as Virtuo BacT/Alert, VersaTrek and BD Bactec (YARBROUGH *et al.* 2021). The analysis focused on the mean times to positivity (TTP) for the microorganisms *Staphylococcus aureus* and *Escherichia coli* with varying blood volumes (Table 1).

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Table 1: Time to Positivity (TTP) in hours for Escherichia coli in adult aerobic vial (FA).

Organism	Sample Volume (mL)	RESULTS							
		BC120 (time to positivity[h])		Virtuo Bact/Alert (time to positivity[h])		VersaTrek (time to positivity[h])		BD BACTEC (time to positivity[h])	
		Pediatric aerobic	*p*	Pediatric aerobic	*p*	Pediatric Aerobic	*p*	Pediatric aerobic	*p*
Staphylococcus aureus	1	12	0,196	11,4	0,673	11,8	0,001	13,4	0,046
	3								
	5	13,2	0,04	11	0,099	12,4	3,78	12	0,005
Escherichia coli	1	11,52	0,196	9	1	11	0,286	10,5	0,094
	3								
	5		0,0001		0,887		0,097		0,842

Source: Prepared by the author (2024).

For *Staphylococcus aureus* at a volume of 1 mL, BC120 had a TTP of 12 hours. This value was slightly higher than the TTP obtained by Virtuo BacT/Alert (11.4 hours) and VersaTrek (11.8 hours), but lower than BD Bactec (13.4 hours). Statistical analyses (P) indicated that the difference between BC120 and VersaTrek was significant (P = 0.001), while the difference with respect to BD Bactec was also significant (P = 0.046). At a volume of 5 mL, BC120 had a TTP of 13.2 hours, compared with 11 hours for Virtuo BacT/Alert, 12.4 hours for VersaTrek, and 12 hours for BD Bactec. Again, the differences with Virtuo BacT/Alert (P = 0.004) and BD Bactec (P = 0.005) were statistically significant, demonstrating a lower efficiency of BC120 at larger volumes for this microorganism.

For *Escherichia coli* with 1 ml of blood, the BC120 showed a TTP of 11.52 hours, while the Virtuo BacT/Alert had 9 hours, the VersaTrek 11 hours, and the BD Bactec 10.5 hours. Statistical analysis showed a significant difference only between the BC120 and the Virtuo BacT/Alert (P = 1.000), indicating a significant advantage of the latter. For volumes of 5 ml, the efficiency of the BC120 decreased (P = 0.0001), showing a longer TTP compared to the other automated systems.

3.1 Time To Positivity (TTP)

Time to Positivity (TTP) indicates the time required for a sample to be identified as positive for the presence of the microorganism. For the *Escherichia coli* in an adult aerobic vial (AF), the TTP remained consistent across all volumes tested: 5 mL (8.64 hours), 8 mL (9.12 hours), 10 mL (9.12 hours) and 13 mL (9.36 hours), with all points indicating approximately 9 hours for detection of positivity (n=8, p=0.0001). This suggests that within the range, the sample volume does not significantly impact the time to positivity for E. coli.

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For *Staphylococcus aureus*, the TTP ranged from 10 to 12 hours, depending on the final sample volume. The 5 mL volume showed a slightly lower TTP of 10.8 hours. Intermediate volumes (8 mL and 10 mL) showed a TTP of approximately 11 hours, while the larger volume (13 mL) resulted in a TTP of approximately 12 hours (n=8, p=0.004). It was observed that when increasing the volume from 5 mL to 8 mL, there was a reduction in TTP, suggesting that a larger sample volume may lead to faster detection, possibly due to the greater amount of bacteria present.

For *Candida albicans*, the volumes of 3 mL, 5 mL and 8 mL showed a constant TTP of approximately 18 hours (n=8, p=0.01). However, in the largest volume (13 mL), there was a slight increase in TTP to 18.96 hours, indicating a possible dilution of the microorganisms (n=4, p=0.0035).

Regarding *Pseudomonas aeruginosa* in adult aerobic flasks (AF), a general trend of decreasing TTP was observed with increasing volume, ranging from 11.76 to 10.92 hours. The variation in the results for volumes of 8 mL and 13 mL suggests the need for a more detailed evaluation of the factors that may be influencing these detection times, such as the measurement technique or experimental conditions. However, at 13 mL, the TTP decreased slightly to 10.92 hours (n=8, p=0.0044), showing a significant association between sample volume and TTP.

For *Escherichia coli* in a pediatric aerobic (PF) bottle, all volumes tested (1 mL, 5 mL, and 8 mL) resulted in an identical TTP of 11.52 hours (n=4, p=0.19). This consistency indicates that, within the range of volumes tested, the amount of sample did not affect the time required to detect the presence of the microorganism.

The time to positivity (TTP) for *Staphylococcus aureus* in pediatric bottles was relatively consistent, ranging from 10 to 12 hours. A slight reduction in TTP was observed when the volume increased from 5 mL to 8 mL, suggesting faster detection with larger volumes due to the higher bacterial load.

For *Enterococcus faecalis*, the TTP ranged from 8.16 to 9.12 hours in volumes of 3 mL, 8 mL, 10 mL, and 13 mL (n=8, p=0.00419), with a slight decrease in the time to positivity observed in the 13 mL volume. Data analysis revealed a nonlinear relationship between sample volume and TTP, indicating that larger volumes may lead to faster detection, attributed to the greater amount of bacteria present.

Significantly relevant results were observed using an initial solution of 60 mL of sterile water and only 10 µL of different volumes of strains, reaching concentrations close to 100 CFU/mL, with $p<0.05$ for the isolates, except for pediatric aerobic vials. These findings corroborate the results of

Yarbrough, Wallace and Burnham (2021), who, in a comparative study between pediatric and adult vials, did not identify significant differences in the TTP for pediatric vials.

The performance of the BC-120 in detecting yeast is particularly noteworthy. A study by Choe, Lim and Lee (2023) showed that, even with higher concentrations than those used in this study, it took 24.6 hours for detection. The BC-120, with a concentration of 1.03×10^3 CFU/mL, lower than that of the Choe study, was able to detect the presence of yeast in approximately 18 hours, demonstrating the efficiency of the platform under lower concentration conditions.

All samples that tested positive on the equipment showed bacterial growth on the culture plates. The negative control samples showed no growth on the plates. Among the samples prepared to verify pH interference, only the vial with potassium hydroxide showed bacterial growth on the plate.

In the negative control, all eleven samples produced a true negative result, with no sign of contamination.

The main causes of false positivity in blood cultures include contamination during collection, inadequate blood volume, inadequate collection technique, and improper handling and processing (ARAUJO, 2012). To investigate possible causes of false positivity, literature sources suggest that metabolic acidosis does not directly result in false-positive results in blood cultures, but may influence the growth of specific microorganisms, especially those that thrive in environments with lower pH (RUSCHEL; RODRIGUES; FORMOLO, 2017).

It was observed that the introduction of the acid solution into the bottle caused an immediate color change from brown to pale yellow, as illustrated in Figure 1. The color change indicated a probable positivity for the sample, since the color variation is associated with the detection processes of the platform. Positivity was identified approximately 36 hours after inoculation with sulfanilic acid.

The sample inoculated with potassium hydroxide showed positivity in approximately 25 hours, with turbidity and increased viscosity characteristic of bacterial growth. The blood agar plate confirmed the growth of *Pseudomonas aeruginosa*, indicating possible contamination.

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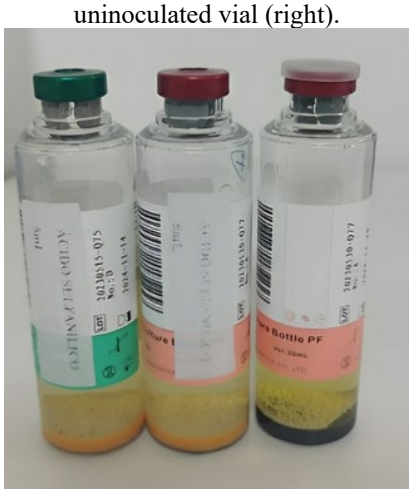
Figure 1: Demonstration of positive vials after 36 hours of sulfanilic acid inoculation (left), compared to the uninoculated vial (right).



Source: Prepared by the author (2024).

3.2 Test Performance

The BC120 system performance evaluation was based on key metrics including sensitivity, specificity, false positive rate, false negative rate, and accuracy. The results, as shown in Table 2, indicated a sensitivity, specificity, and accuracy of 100%, with confidence intervals of 90.75% to 100%, 71.51% to 100%, and 92.75% to 100%, respectively.

These results demonstrate that the BC120 has an excellent ability to correctly detect positive and negative cases, eliminating false positives and negatives. The true positive rate (PPV) and true negative rate (NPV) were also 100%, ensuring that all positive and negative diagnoses were accurate.

Table 2: Statistical table of the Method.

Bacteria detection	**Negative**	**Positive**	**Total**
Present	0	38	38
Absent	11	0	11
Total	11	38	49
Statistic	**Value**		**IC 95%**
Sensitivity:	100%		90,75% to100,00%
Specificity:	100%		71,51% to 100,00%
Precision:	100%		92,75% to 100,00%

Source: Prepared by the author (2024).

The BC120 system was assessed as extremely reliable, with an accuracy of 100%, with a 95% confidence interval ranging from 86.77% to 100%. This range confirms the system's high accuracy, even in the worst-case scenario. These metrics highlight the BC120's robustness and reliability in clinical applications, where accuracy is crucial.

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4 CONCLUSION

The research conducted aimed to evaluate the efficiency and accuracy of the IHB BC120 system in blood cultures, using pediatric and adult bottles, in comparison with other automated technologies such as Virtuo BacT/Alert, VersaTrek and BD Bactec. The methodology was carefully structured, covering preparation of dilutions, inoculation, incubation and complementary tests to verify the presence of false positive results.

Aerobic culture bottles FA and PF from the same batches were used, ensuring consistency of materials. ATCC reference strains were used, ensuring viability through two subcultures. Standard suspensions were prepared in different volumes for CFU/mL analyses. Inoculation and incubation followed strict protocols with negative control to ensure the absence of microbial contamination.

The results of the study indicate that BC120 presents times to positivity (TTP) generally comparable to other technologies, although, in some cases, slightly longer. For example, for *Staphylococcus aureus*, the BC120 demonstrated a TTP of 12 hours with 1 ml of blood. This time is slightly longer compared to the Virtuo BacT/Alert and VersaTrek, but shorter than the BD Bactec. It was also observed that increasing sample volume tended to prolong the TTP, although this relationship was not consistent for all microorganisms analyzed.

In addition, insufficient blood volumes can increase the contamination rates of blood cultures, resulting in false positives. This occurs because a low concentration of blood may not adequately neutralize the antimicrobial agents present in the culture medium, allowing the growth of contaminating microorganisms (THOMPSON, 2009).

Another relevant factor is the influence of pH changes in the samples. Visual changes after the addition of sulfanilic acid and potassium hydroxide suggest that pH can impact detection, underlining the importance of maintaining sterile conditions to avoid false-positive results (NUNES; SANTOS; ROSÁRIO, 2020) highlight that an excessive volume of blood can alter the proportion of blood in relation to the culture medium, which can be beneficial by reducing the interference of external contaminants, but can also affect the detection of slow-growing bacteria.

We observed that errors in the volume of blood collected and the presence of metabolic acidosis can influence the interpretation of the results, potentially contributing to false positives. Following the recommended guidelines for collecting and processing blood cultures is essential for accurate diagnoses.

The BC120 demonstrated excellent performance, with 100% sensitivity and specificity. This means that it correctly identified all positive and negative cases without generating false positives. The accuracy of the system was nearly to 100%, confirming its reliability. In summary, the BC120 proved to be an effective tool for blood cultures, offering consistent and accurate results.

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