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Original Research Article

Evaluation of five days protocol of aerobic incubation in automated blood culture system for optimal recovery of organisms

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Abstract

Background: Blood culture is the gold standard for blood pathogen detection. Automated blood culture systems such as BacT/Alert®VIRTUO® have revolutionized the diagnosis of Blood stream infections (BSI) enabling faster detection and improved patient care. However, the standard protocols rely on a 5day incubation period before deeming culture negative. This study investigates the potential for recovering organisms from BacT/Alert®VIRTUO® blood cultures flagged as negative after 5 days.
Materials and Methods: The study was conducted over 6months in a tertiary care teaching hospital. The negatively flagged bottles were sub cultured onto blood agar and Sabouraud dextrose agar at the end of 5 days, 5-7 days and 7 completed days.
Results: A total of 10,003 negatively flagged blood culture bottles were included in the study. On subculture of all those bottles on day 5, a total of 22 organisms were grown out of which 9 were pathogens and 13 were contaminants. On re-incubating the bottles again for 2 days (total incubation period- 7 completed days), some bottles flagged positive which on further subculturing on blood agar yielded total of 44 isolates out of which all were contaminants. Unflagged bottles on final blind subculturing on day 7 yielded a single pathogen and five contaminants. All the pathogens isolated were found to be insignificant after bedside discussion with the treating clinical team.
Conclusion: Prolonged incubation offers minimal clinical benefit and extending the incubation places unnecessary burden on laboratory resources increasing the cost, man power and potentially delaying the processing of other critical samples.

Keywords: Blood culture, BacT/Alert Virtuo, Negative bottle subculture, Prolonged incubation.

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1. Introduction

Blood stream infections are potentially life threatening conditions associated with increased mortality, morbidity and healthcare costs worldwide.[1,2] Prompt identification of pathogen and its reporting are crucial as this greatly assist in initiating appropriate antimicrobial therapy to improve clinical outcome of the patient.[3] However, even when there is strong clinical suspicion of bacteraemia or fungemia, blood culture system may fail to identify the pathogen due to various reasons like bacteraemia, presence of fastidious pathogens and empirical antibiotic therapy. The conventional blood culture methods are almost replaced by various automated blood culture systems worldwide.[4,5] Our laboratory uses FA® plus bottles for aerobic bacterial culture and BacT/Alert® Virtuo® blood culture detection system from bioMérieux. This system has advantage of continuous incubation and monitoring without interruption, automatic loading and unloading of bottles and real time notification of blood volume. But, the automated blood culture systems available at present including BacT/Alert Virtuo recommend only a 5day incubation period except in few conditions such as endocarditis where extended incubation time is essential. After this time period, these bottles are automatically

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signalled negative and unloaded by this system. There is need to evaluate whether pathogens actually grow after this short incubation period as the false negative reports may result in alteration of antibiotic course and patient outcome. Therefore, we have performed intensive research which involved further processing of negatively flagged bottles which were unloaded from the instrument and evaluated the number of pathogen, contaminants and sterile reports issued from such bottles.

2. Materials and Methods

Clinical setting: The study was performed in the department of Microbiology, Jawaharlal Institute of Postgraduate Medical Education & Research (JIPMER), a 3000 bedded tertiary care centre in Puducherry, South India over period of 6 months from January to June 2023 Culture and identification**:** Our blood culture laboratory is fully functional with 24x7 availability of resident microbiologist and laboratory technicians for processing positively flagged blood culture bottles. According to the laboratory`s standard procedure, blood culture bottles collected from patient with suspected bacteraemia as per clinician's order was loaded into the BacTalert virtuo instrument. Bottles were continuously monitored until positive up to 5 days of incubation at 37 0 Celsius. Whenever bottles were signalled positive by the instrument, time to positivity (TTP) was noted and sub-cultured onto blood agar and MacConkey agar and organism was identified by Gram staining and MALDI-TOF MS (Vitek MS BioMerieux). If bottles were not signalled positive after 48 hours of incubation in the instrument, the preliminary sterile report was issued. While an option for extended incubation based on the clinician`s request was available, there were no requests during the time frame of the study. After 5 days of incubation, the blood culture bottles were automatically flagged negative by the instrument and final report was issued as sterile. After this time period, negative bottles were unloaded and blindly sub-cultured onto 5% sheep blood agar and re-inserted for incubation in the instrument. If the colonies were grown on those plates, it was processed to identify the organism. If there was no growth after appropriate incubation time, bottles which was further incubated till 7 days was sub-cultured onto 5% sheep blood agar and Sabouraud's dextrose agar to appreciate the growth. For all the bottles which flagged beyond 5 days of incubation, bed side discussion with the treating clinical team was made to know the pathogenicity of the organism isolated (**Figure 1**).

2.1. Inclusion and exclusion criteria

The blood culture bottles which did not flag any signal after completion of 5 days of incubation were included in the study as depicted in the flow chart below. The blood culture bottles which were requested for fungal blood culture, anaerobic blood culture and bottles inoculated with sterile body fluids were excluded from the study.

3. Results

The total number of BacT/ALERT bottles received in blood culture laboratory for culture and sensitivity during the study period was 13,035. Out of these, 10,003 bottles were flagged negative after 5 days of incubation. On subculture of all those bottles, a total of 22 organisms were grown out of which 9 were pathogens and 13 were contaminants. On re-incubating the bottles again for 2 days (total incubation period- 7 completed days), some bottles flagged positive which on further subculturing on blood agar yielded total of 44 isolates out of which all were contaminants. Unflagged bottles on final blind subculturing yielded a single pathogen and five contaminants. The average TTP varied significantly among different organisms. The average TTP consistently rises from day 5 to day 7 and there is a clear increasing trend in average TTP over the different time intervals. The data indicates that the time interval day 5-7 has the highest number of positive cultures with an average of 2.35. The mean average TTP for Day 5 is 22.61, 141.6 for day 5-7 and 147.06 for Day 7.

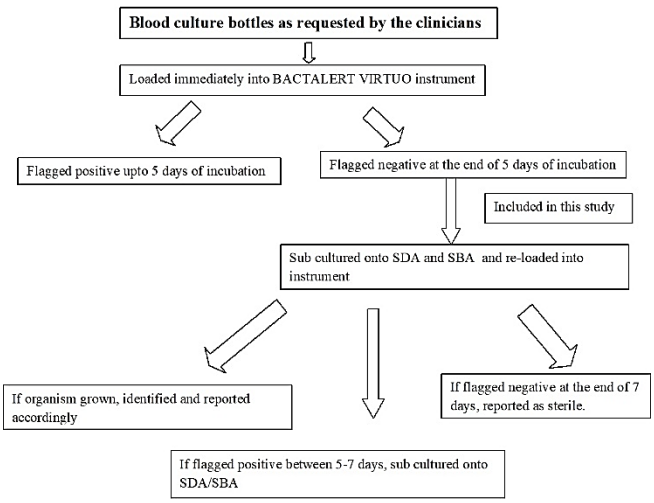

Figure 1:

Table 1: Distribution of number of positive blood cultures across different time intervals

	Pathogens grown	Contaminants grown	Total
Total no. of bottles screened (flagged negative on Day 5)	0.09% (9/10003)	0.60% (63/10003)	0.69% (72/10003)
Total organisms grown	0.07% (8/10003)	0.13% (14/10003)	0.20% (22/10003)
On 5th day subculture	0	0.40% (44/10003)	0.40% (44/10003)
Flagged between 5-7 days	0.01% (1/10003)	0.04% (5/10003)	0.05% (6/10003)
On 7th day subculture	0.08% (8/10003)	0.13% (14/10003)	0.21% (22/10003)

Table 2: Microbiological profile of the bottles sub cultured at different time periods

	Day 5	Day 5-7	Day 7	Total number (%)	Average TTP (hr)
No growth	9981	9960	9997		
Pathogens					
Staphylococcus aureus	2			2	120
Escherichia coli	1			1	120
Klebsiella pneumoniae			1	1	168
Acinetobacter baumannii	4			4	120
Pseudomonas aeruginosa	1			1	120
Contaminants					
Gram positive					
Diphtheroids		9	3	12	151
Micrococcus luteus	5	5		10	138
Micrococcus lylae	1				120
Coagulase negative staphylococci	3	11		14	142
Kytococcus schroeteri	1		1	1	120
Aerobic spore bearers	2			2	120
Exigobacterium aurantiacum		1		1	121
Microbacterium aurum		1		1	174
Enhydrobacter aerosaccus	1			1	120
Gram negative					
Other Acinetobacter spp.		7		7	
Other *Pseudomonas* spp.		8		8	
Elizabethkingia anophelis	1	1			120
Nonfermenting gram-negative bacilli (NF-GNB), unidentified		1	1	2	150

TTP: Time-to-positivity

4. Discussion

Blood culture is the gold standard for blood pathogen detection. In the acute phase of sepsis, the role of clinical microbiology is crucial as the culture and the antimicrobial susceptibility pattern of the pathogen is needed for the fine tuning of the empirical antibiotic therapy.[3,6-8] Blood culture using conventional systems typically take 24-72 hours for pathogen identification and antimicrobial susceptibility testing. In the past few years, there has been an explosion of continuous monitoring blood culture systems (CMBCS) and rapid identification of pathogens directly from the blood culture broths using various methods like MALDI TOF MS and molecular assays combined with direct susceptibility testing from the blood culture broths.[10] These automated blood culture systems have revolutionized the diagnosis of BSI, enabling faster detection and improved patient care thereby reducing the morbidity and mortality. In addition, several technical refinements in the blood culture broths and automated detection of growths have greatly improved the diagnostic performance of CMBCS.

The BacT/Alert VIRTUO is one of the most common Automated Blood Culture System used globally. This system utilises colorimetric detection of carbon dioxide produced to identify the bacterial growth. While this system has shown high sensitivity and specificity in identification of common bacterial pathogens, several factors may contribute to the false negative results.[11-15] An intrinsic limitation of the blood culture system is failure to identify slow growing or fastidious pathogens like *Nocardia* species, *Bartonella*, *Francisella tularensis* many of which may be responsible for what is labelled as culture negative bacterial endocarditis.[13] Persistent low-grade bacteraemia, where the bacterial load can be as low as 1CFU/ml, is often associated with intravascular focus of infection such as infective endocarditis. In such cases microbiological diagnosis to support the clinical diagnosis is often difficult. The empirical antibiotic therapy initiated before the collection of blood culture may result in failure to detect the organism by culture. Additionally defined incubation conditions of the various automated blood culture systems may further reduce the chance of identification of certain fastidious organisms or low levels of organism present in the blood.

Clinical and laboratory standards institute (CLSI) recommends a routine incubation period of 5 days[16] for blood cultures based on a combination of factors like vast majority of the clinically significant blood stream infections become positive within the first 5 days of incubation and while some organisms may take longer time to grow, the positivity after 5 days of incubation is often outweighed by the increased risk of contamination and the potential for delaying necessary treatment modifications based on various studies. However, the traditional practice of incubating the bottles for 7 days, a holdover from the older manual method, are still practiced

despite mounting evidence supporting a shorter 5-day incubation due to the fear of missing out the fastidious organisms. Studies have shown that extending the incubation beyond this period results in a very small increase in true positives often less than 1-2%.[17]

In this study we tried to evaluate role of extended incubation of blood culture bottles beyond the recommended 5 days to see the microbiological profile and significance of the pathogens isolated. The analysis focuses on the number of positive cultures recorded at different time intervals day 5, day 5-7 and day 7. While the presence of any microorganism in the blood warrants attention, distinguishing between pathogen and contaminant is necessary for optimal clinical management. Following definitions of contaminant was adapted from CLSI M47[16] document to characterize the organisms isolated from the subcultures. Contaminant is defined as a microorganism which was introduced into the blood culture during the specimen collection or processing and that was not pathogenic for the patient from whom the blood was collected. While CLSI provides extensive documents on blood culture collection, processing and interpretation, no strict definitions for pathogens and contaminant are given because interpretation of blood culture reports often require clinical and other laboratory test correlation. CLSI documents acknowledge that certain microorganisms, such as coagulase-negative staphylococci, *Corynebacterium* species, and *Propionibacterium acnes*, are frequently encountered as contaminants.

In the present study, blind subculture was done on Day 5 for all the bottles that were flagged negative after 5 days of incubation. After the subculture, bottles were loaded again into the machine. Whichever bottle flagged positive within 5-7 days of incubation were again subcultured and isolates were identified standard methods. The number of positive cultures shows a dramatic increase from the D5 interval to the D5-7. Subculture done on day 5 yielded 22 organisms out of which 8 were pathogens and 14 were contaminants. However, after bedside discussion with the treating clinical team, none of these organisms were considered to be significant. 5th to 7th day represents the grey zone and this time period presents the greatest challenge in interpretation in case of isolation of any organism. In our study, 43 organisms were isolated from the bottles that flagged positive between day 5 and Day 7 subculture out of none of which were pathogens. Isolates from 7th day subculture are highly likely to represent the contaminants especially coagulase negative staphylococci. However, there was a single isolate of *Klebsiella pneumoniae* at the end of 7 days of incubation. Bedside discussion with the treating clinical team found it as insignificant. Few of the bottles were incubated for up to 10 days considering the possibility of fastidious and slow growing organisms like *Brucella* species in suspected cases of brucellosis and infective endocarditis. However, we could not isolate any of these pathogens even after 10 days of aerobic incubation. In addition to this, to consider 7th day subculture as pathogen the following points need to be kept in mind. Same organism must be isolated from multiple blood cultures collected at different time intervals and the patient's clinical history and presentation should be in favor of the isolated organism.[18,19] One of the reasons for this rise could be the chance of introducing contamination while subculturing as there was not even a single pathogen isolated from the positively flagged bottles between Day 5-7. The likelihood of isolation of pathogens decreases as the incubation period increases. The data suggests that the majority of positive cultures are detected by D5, with no additional pathogen cultures being recorded in the subsequent intervals (D5-7). This is clearly depicted in **Table 2**, highlighting the significant drop in pathogens after D5. Extending the incubation and subcultures significantly increases the risk of isolating the contaminants leading to the false positive results and these results can drive inappropriate antibiotic prescriptions leading to rise in antimicrobial resistance.[18]

Clear communication between the laboratory and the clinical team is crucial while making decisions regarding the organisms isolated after the subcultures. In our study, two *Staphylococcus aureus* and an Escherichia coli was isolated which were considered not significant after bedside discussion with the clinicians.

5. Conclusion

A 5-day incubation period for routine blood cultures in automated systems strikes the optimal balance between sensitivity, specificity, and resource utilization. Prolonged incubation offers minimal clinical benefit and extending the incubation places unnecessary burden on laboratory resources increasing the cost, man power and potentially delaying the processing of other critical samples.

6. Ethical Approval

This study Ethical committee approval was obtained for the same from the Institutional ethics committee, JIPMER.

7. Source of Funding

None.

8. Conflict of Interest

None.

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