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# Effect of blood volume in automated blood culture of the BACT/ALERT 3D system on isolation rate and time to positivity of pathogens, in a tertiary care hospital, South India

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ABSTRACT

**Aim:** To determine the appropriateness of blood volume in automated blood culture bottles of the BACT/ALERT 3D system, its effect on isolation rate and time to positivity of pathogens**.**

**Materials and Methods:** The study was carried out in a tertiary care hospital in South India from 1$^{st}$ March 2018 to 31$^{st}$ August 2018. Automated blood culture bottles sent to Microbiology laboratory for blood culture were included in the study. The bottles were weighted by electronic weighing balance. The filled blood culture bottles received at microbiology laboratory were re-weighed and was categorized into appropriately filled and inappropriately filled bottles. Frequency of appropriately filled bottles and inappropriately filled bottles was expressed in percentage.

**Result:** A total of 8740 blood culture bottles were included in the study, out of which the blood volume was found to be suboptimum in 3978 bottles (45.5%), optimum in 3498 bottles (40%) and overfilled in 1264 bottles (14.5%). There was an increase in the number of blood culture bottles with optimum blood volume from 35.1% in March to 58.12% in August. The average TTP for true pathogens was found to be 16.72 hrs. with optimum blood volume, 17 hrs. with overfilled blood volume and 21.5 hrs. with sub-optimum blood volume. The isolation rate for true pathogens was found to be 88.62% with optimum blood volume, 86.43% with overfilled blood volume and 70.01% with sub-optimum. The false positive rate was found to be 4.1% with overfilled blood volume, 1.6%with sub-optimum and 1.4%with optimum blood volume.

**Conclusion:** Through this study, the correlation between the blood volume with the isolation rate, time to positivity and false positivity of blood culture isolates was established**.**

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## 1. Introduction

Blood stream infections (BSI) are a major cause of morbidity and mortality worldwide.[1] Early diagnosis and initiation of appropriate antimicrobial therapy can greatly reduce the risk of mortality and antibiotic overuse.[2] Blood culture is the most important diagnostic tool in the investigation of BSI as it helps in the detection of bacteremia and fungemia.[3] The blood culture results obtained help the clinicians in the management of individual patients. Therefore, early and correct interpretation of blood culture results is crucial to initiate appropriate treatment, thereby improving patient care.[4]

There are numerous factors influencing the likelihood of detecting bacteremia such as organism load, transport time to laboratory, volume of blood drawn etc. Of all these factors, the volume of blood drawn for culture is the most important variable in the recovery of microorganisms.[5–8]

The sensitivity of blood culture to a great extent depends upon the volume of blood obtained for culture due to the low density of microbial load in the blood of adult patients, which is often in the range of <1- 10 cfu/ml.[9] Mermel et al, in their study have shown that the yield of blood culture in adults increases approximately by upto 3% per

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millilitre of blood cultured.[10] The Clinical and Laboratory Standards Institute (CLSI) guidelines recommends to take four bottles with blood volume of 8 to 10-mL each (two sets) for detecting about 90–95% of patients with bacteremia. Whereas six bottles with same blood volume, i.e. 60 mL in total (three sets) have a detection rate of 95–99%.[11]

Several literature have demonstrated that an optimum volume of blood of 8-10 ml significantly increases the number of positive cultures. Studies have reported that the detection rate of bacteremia associated with a standard blood volume (mean 8.7 mL) culture to be considerably higher compared to that of low blood volume (mean 2.7 mL).[1] Blood culture bottles with inadequate blood volume are often associated with low isolation rate or negative blood culture result, falsely excluding significant bacteremia. Apart from its clinical importance, the underfilled bottles also add economic loss to the hospital.[4,12,13] On the other hand, overfilled blood culture bottles may result in false positivity. False positive refers to blood culture bottles positively flagged by automated blood culture system but show no micro-organism in Gram stained smear and show no growth on subculture. Alfa et al suggested that the carbon dioxide generated from leukocyte respiration in the large blood volume of overfilled blood culture bottles might be sufficient to trigger a positive signal by the automated blood culture system. False positive blood cultures not only cause unnecessary consumption of time and resources but can also lead to unnecessary administration of antibiotics.[6]

The volume of blood also influences the time to positivity (TTP) of bottles. Time to positivity (TTP) is defined as the time interval from the entry of blood culture bottles to the observation of a positive signal in the automated blood culture system.[7] TTP reflects the magnitude of bacterial load in blood and is often used as a prognostic marker by the clinicians to predict the clinical outcome.[14] Erroneous TTP may occur due to several factors such as delayed loading of bottles and suboptimum blood volume. An erroneous TTP cannot be reliably used as prognostic marker[15]

To the best of our knowledge, no studies are available from India on determining the blood volume drawn and its effect on isolation rate and TTP. Moreover, the automated blood culture system has been recently introduced in our hospital (BacT/Alert blood culture system). Hence this study was designed with the purpose of determining the blood volume drawn in automated blood culture bottles and its effect on isolation rate and TTP.

## 2. Aim

The purpose of this study was to determine the appropriateness of blood volume drawn in automated blood culture bottles of the BACT/ALERT 3D system and its effect on isolation rate and time to positivity of pathogens in a tertiary care hospital, South India.

### *2.1. Inclusion criteria*

It was a prospective cohort study carried out in a tertiary care hospital in South India from 1$^{st}$ March 2018 to 31$^{st}$ August 2018. Blood sample from the adult patients which were inoculated into BACT/ALERT 3D automated blood culture bottles, received in the Microbiology laboratory for blood culture were included in the study.

### *2.2. Exclusion criteria*

1. Paediatric blood samples collected for blood culture in BACT/ALERT 3D bottles were excluded from the study.
2. The bottles with inadequate blood volume were analysed separately and discriminated from the adequately filled and sub-optimally filled blood culture bottles for comparison of effect on isolation rate and TTP.

## 3. Materials and Methods

The bottles were weighed by electronic weighing balance which had a readability range of 0.01gm to 1000 gm. Upon receipt at microbiology laboratory, the filled blood culture bottles were re-weighed. The weighing information was maintained in Microsoft excel. Equations for blood volume measuring of bottles was done as given in Table 1. As after filling, bottles do not have cap & bottle barcode sticker and possess an additional patient barcode sticker; the necessary adjustment was done in the equation while calculating the blood weight (g). The measured blood weight "g" was converted in volume "mL" in consideration of blood density.[10] The blood culture bottles were verified to have adequate blood volume according to the standard recommendations (Table 2) and was categorized into appropriately filled and inappropriately filled bottles. As a part of quality improvement of laboratory, when blood culture volume was less than the recommended volume per bottle, it was cited in the culture report as "Test performed on specimen received with less than the recommended volume. Inappropriate blood volume may lead to false-negative or false positive result or a delay of blood culture positive report".

### *3.1. Parameters Studied*

Frequency of appropriately filled bottles and inappropriately filled bottles was determined. The association between appropriateness of blood volume with isolation rate, false positivity and time to positivity was determined.

### *3.2. Statistical tests used for data analysis*

Frequency of appropriately filled bottles and inappropriately filled bottles was expressed in percentage.

**Table 1:** Equations for blood volume measuring with weight components

| Parameters | Equation |
|---|---|
| Before filling weight (g) | Unfilled bottle weight + bottle cap weight + bottle bar code weight |
| After filling weight (g) | Unfilled bottle weight + patient l bar code sticker weight + blood weight |
| Blood weight (g) | After filling weight - before filling weight - patient bar code sticker + cap weight + bottle bar code weight |
| Blood volume (ml) | Blood drawn (g) X blood density (1.06 g/ml) |

**Table 2:** Appropriateness of blood culture bottles according to volume of blood drawn

| Volume | Adult Blood culture bottle |
|---|---|
| Appropriately filled bottles (optimum volume) | 8-10 ml |
| Inappropriately filled bottles | <8 or >10 ml |
| Suboptimum volume Overfilled volume | <8 ml<br>>10ml |

### *3.3. Ethical Considerations*

Ethical clearance was obtained from Institute Ethics Committee (IEC) and waiver of consent was obtained as data was collected from the laboratory without any direct or indirect patient involvement.

### *3.4. Implications*

The correlation between the blood volume with isolation rate, false positivity and time to positivity of blood culture isolates was known. This helped in quality improvement. Timely feedback of blood volume collected to the clinician helped in change of practice towards optimum blood volume collection.

## 4. Results

The study was conducted for a duration of 6 months from 1$^{st}$ March 2018 to 31$^{st}$ August 2018 in the Department of Microbiology, JIPMER. A total of 8740 blood culture bottles were included in the study, out of which the blood volume was found to be suboptimum (less than 8ml) in 3978 bottles (45.5%), optimum (8-10ml) in 3498 bottles (40%) and overfilled (more than 10ml) in 1264 bottles (14.5%). On receipt of blood culture bottles with suboptimum blood volume, a comment was cited in the culture report stating that the test was performed on specimen received with less than the recommended volume. Consequently, there was an increase in the number of blood culture bottles with optimum blood volume from 35.1% in March to 58.12% in August. Figure 1

Out of 8740 blood culture bottles, 5716 were sterile and 3024 bottles were flagged positive which comprised of 997 blood culture bottles with suboptimum volume, 1467 with optimum blood volume and 560 bottles with overfilled blood volume. Out of 3024 positively flagged blood culture bottles, 2482 samples yielded positive for true pathogens, 60 were found to be false positive, and contaminants were observed in 482 samples. The majority of pathogenic isolates comprised of *Staphylococcus* species (n= 1461), followed by members of *Enterobacteriaceae* (n=428), non-fermenters (n= 389), *Enterococcus/Streptococcus* species (n=132), and yeast (n=72).

The average time to positivity (TTP) for true pathogens was found to be shortest for blood culture bottles with optimum blood volume (16.72 hrs.),indicating earlier detection of pathogens, followed by those with overfilled blood volume (17 hrs) and maximum with sub-optimum blood volume (21.5 hrs) Table **??**. The TTP for every group of organisms was also found to be higher with suboptimum blood volume (Table 4). The isolation rate for true pathogens was found to be 88.62% (1300/1464) with optimum blood volume, 86.43% (484/560) with overfilled blood volume and only 70.01% (698/997) of true pathogens were isolated when the blood volume was sub-optimum. The false positive rate was found to be 4.1% (23/560) in blood culture bottles with blood volume more than 10ml, 1.6% (16/997) with sub-optimum and 1.4% (21/1467) with optimum blood volume.

Table 3: Comparison of average TTP and isolation rate of pathogens from blood culture bottles with appropriately and inappropriately filled blood volume.

## 5. Discussion

Blood volume has been considered to be the most important factor affecting the yield of micro-organisms from blood culture. There is a possibility that relatively only few micro-organisms ranging from less than 1 to 10 c.f.u./mL may be present in a given volume of blood, which may be missed out if inadequate blood volume is submitted in the blood culture bottles for culture, leading to false negative result (4,9).

In the present study, an attempt was made to estimate the blood volume inoculated in the automated blood culture bottles as well as to improve the frequency of appropriately filled bottles. Timely feedback of the inadequate blood volume collected in the blood culture bottles was given to

**Table 3:** Comparison of average TTP and isolation rate of pathogens from bloodculture bottles with appropriately and inappropriately filled blood volume.

| Blood volume | No. of blood culture bottles flagged positive (N) | Avg. TTP(Hrs) for pathogens | No. of true pathogens (n) | Isolation rate (n/N)% | No. of false positive (n1) | False positive rate (n1/N)% |
|---|---|---|---|---|---|---|
| Sub optimum (<8ml) | 997 | 21.5 | 698 | 70.01 | 16 | 1.6 |
| Optimum (8-10ml) | 1467 | 16.72 | 1300 | 88.62 | 21 | 1.4 |
| Overfilled (>10ml) | 560 | 17 | 484 | 86.43 | 23 | 4.1 |

**Table 4:** The average TTP and isolation rate for different group of pathogens isolated.

| Blood Volume | No. of true pathogens detected (N) | Staphylococcus | | | Enterococcus/ Streptococcus | | | Enterobacteriaceae | | | Non fermenters | | | Yeast | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | TTP (Hr) | No. of isolates (n) | Isolation rate (n/N)% | TTP (Hr) | No. of isolates (n) | Isolation rate (n/N)% | TTP (Hr) | No. of isolates (n) | Isolation rate (n/N)% | TTP (Hr) | No. of isolates (n) | Isolation rate (n/N)% | TTP (Hr) | No. of isolates (n) | Isolation rate (n/N)% |
| Sub optimum | 698 | 24.96 | 412 | 59.0 | 14.25 | 32 | 4.6 | 15.41 | 125 | 17.9 | 17.02 | 110 | 15.8 | 24.86 | 19 | 2.7 |
| Optimuml | 1300 | 18.64 | 770 | 59.2 | 12.78 | 75 | 5.8 | 12.71 | 225 | 17.3 | 14.44 | 194 | 14.9 | 21.04 | 36 | 2.8 |
| Overfilled | 484 | 18.03 | 279 | 57.6 | 12.31 | 25 | 5.2 | 13.07 | 78 | 16.1 | 16.44 | 85 | 17.6 | 23.65 | 17 | 3.5 |

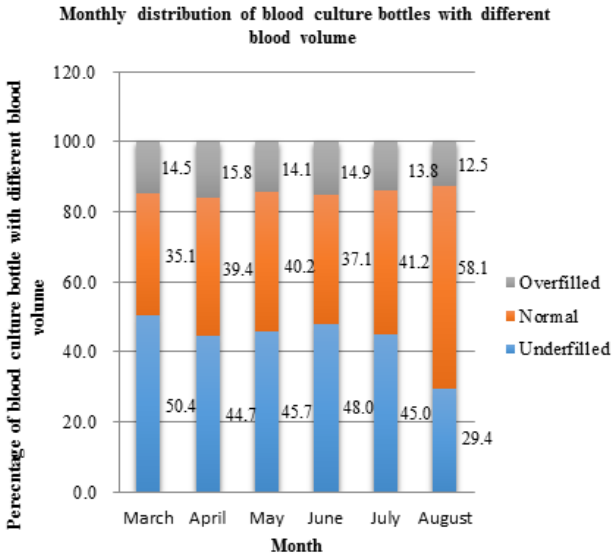


**Fig. 1:** Monthly distribution of blood culture bottles with different blood volume.

the clinician. As a consequence, the blood volume collected showed a significant increase from 35.1% in March 2018 to 58.12% in August 2018.

H-H Lin, et al (2011, Taiwan), conducted a study on evaluation of blood volume effect on diagnosis of bacteremia in automated blood culture system, where they found that the detection rate of bacteremia was best seen when the blood volume collected ranged from 8-10 ml per blood culture bottle.[3]

‘ Similarly, the isolation rate for true pathogens in this study was found to be higher (88.62%) with optimum blood volume of 8-10ml compared to that with the sub-optimum blood volume of less than 8ml ( 70.01%). This also complies with several other studies that have demonstrated a significant increase in the number of positive cultures with the collection of optimum volume of blood.(12,13)

In a study conducted by Min-Kyung So, et al, (2016, Seoul, Korea) on effect of blood volume monitoring, they found that the TTP (16.1±16.3hr) of anaerobic bottles during the post-intervention (6.5±1.7 ml of blood per bottle) was significantly shorter than the TTP (18.6±18.3hr) of bottles during the pre-intervention period (0.7±0.3 ml of blood per bottle).[1] The average TTP for true pathogens in this study was found to be shortest when the blood volume collected was optimum (16.72 hrs) and the TTP was maximum with sub- optimum or low blood volume (21.5 hrs).

Meesen et al (1998, Netherlands) evaluated the blood volume drawn in the automated blood culture bottles, where they found false positive rate to be clearly associated with overfilling of bottles. They found a false positive rate of 2% in overfilled blood culture bottles whereas no false positive result was seen in bottles with optimum blood volume.[6]

Similarly, higher false positive rate (4.1%) was observed in overfilled blood culture bottles in this study, compared to

that with sub-optimum (1.6%) and optimum blood volume (1.4%).

### *5.1. Limitations*

Our study was limited to a single medical center. Hence the findings may not be applicable for all hospitals. The study was conducted on limited number of samples spanning over 6 months, which generated limited data not sufficient to draw appropriate conclusions. Hence, it is very important to routinely monitor the blood volume inoculated into the blood culture bottles before loading into the automated instruments and continuously educate the clinicians about the recommended blood volume collection for sustained and remarkable improvement in microbiological yield and appropriate intervention at the earliest. Also the two blood culture bottles if collected from the same patient (one with adequate blood volume and other with suboptimum blood volume) were not analysed at individual level for effect on pathogen isolation rate.

## 6. Conclusion

Through this study, the correlation between the blood volume with isolation rate, false positivity and time to positivity of blood culture isolates was established. It was a quality improvement study as the continuous monitoring of the blood volume drawn in blood culture bottles and timely feedback given to the clinicians helped in significantly improving the frequency of appropriately filled bottles, which was further associated with increased isolation rate. Hence, it is very important to routinely monitor the blood volume in order to ensure that adequate blood volume is available for culture. This practice will not only benefit the patients by rapid and accurate diagnosis but will also reduce the economic loss to the hospital resulting from underfilled blood culture bottles.

## 7. Source of Funding

None.

## 8. Conflict of Interest

None.

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