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Research Article

Does the Sample Fill Volume of BacT/ALERT Bottles Impact the Detection of Positive Blood Cultures?

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Abstract

The fill volume of 460 blood culture samples were assessed for compliance across two Australian metropolitan hospitals. Results were analysed according to collecting ward and patient demographics, showing only 22.6% of bottles were correctly filled. Additionally, the rates of false positives and false negatives were analysed compared to sample fill volumes. Overfilled bottles had higher rates of false positives (2.2%), whereas falsely flagging bottles did not show a strong correlation with under filling. Despite the high number of underfilled bottles, no clinically significant organisms were missed.

Keywords: Laboratory Information System (LIS); Blood Culture

Introduction

Blood cultures are the key to diagnosis of bloodstream infections. The BacT/Alert® VIRTUO® (bioMérieux) (BAV) system is an automated blood culture instrument that detects microbial growth via the production of CO_2 causing a change in pH. The Instructions For Use (IFU) specifies a sample volume of 8-10ml for anaerobic (BFN+) and aerobic bottles (BFA+); growth should be detected within the optimal window of 4-5 days [1-3]. If this sample volume cannot be achieved, a paediatric (BPF+) bottle is recommended [4]. There is no minimum volume for BPF+ bottles; the media is designed to enrich low sample volumes for bacterial growth to be detected in the same timeframe as the BFN+ and BFA+ bottles [1,2,5]. The IFU further specifies that BFN+ bottles must be inoculated last, to account for any air in the collection system to maintain an anaerobic environment [2]. When inoculated correctly, the chances of positive samples being detected are 99.9% [6]. A lower volume can reduce chances of detection in the standard 5-day period, while a higher volume can increase the chance of false positives due to the respiration of the abundance of white blood cells [1,2].

Correct sample collection directly impacts patient care and when testing such a critical sample type it is important to monitor compliance. A survey of blood culture quality practices by the Royal College of Pathologists of Australasia in 2019 found that only 2 of 71 laboratories actively monitored fill compliance and suggested that Australasian laboratories should monitor compliance as part of their accreditation requirements [7]. Companies such as Becton Dickinson and bioMérieux have produced middleware to track metrics pertaining to sample adequacy and demographic tracking to make this task more efficient [8]. The lack of fill monitoring can impact the optimal performance of this test and should be considered an important factor in laboratory quality control [7,9-11].

Aim

This purpose of this study is to investigate the fill volumes of BacT/Alert bottles received in the laboratory from two metropolitan hospitals to ascertain adherence to the manufacturers optimal fill range and identify any impacts improperly filled bottle volumes may have on the true positivity rate. The study will further analyse results by hospital ward to identify any contributing factors to adherence.

Methods

Sample recruitment

Samples recruited were retrospective, consecutive blood cultures received by the laboratory in a 12-day period from the two hospitals during June - July 2023. Samples were collected using usual methods based on guidelines provided in the Statewide NSW Health Pathology handbook.

Processing of blood cultures

Blood culture samples received in the laboratory were registered in the Laboratory Information System (LIS) and loaded into the BAV instrument for a 5-day incubation period. Bottles are scanned at intervals looking for changes to the indicator. When detection criteria are met, the instrument flags the bottle for processing. Positive bottles underwent usual processing, including Gram stain microscopy and subsequent acridine orange stain if no bacteria were seen. Bottles were sub-cultured onto horse blood agar (HBA) and chocolate agar (CHOC) and incubated 5% CO_2; HBA in anaerobic jars using gas packs; and MacConkey agar incubated in O2, all at 35C for 16 – 24 hours. Supplementary plates were included depending on the Gram stain findings. Organism identification was performed using matrix-assisted laser desorption ionization – time of flight mass spectrometry (Bruker MALDI Biotyper) and aided by various biochemical, agglutination and antigen testing techniques; antibiotic susceptibilities were performed within 24-48 hours if clinically relevant.

Detection of sample fill volumes

The BAV records sample fill volume for the BFA+ and BFN+ bottles using a photographic detection system that can read bottles that have correctly positioned barcode LIS labels attached. BPF+ bottles are not supported for fill volume data by the system and not included in this study. The sample fill volume is found using the search function to check each bottle individually, further data also collected includes load/unload time and time to positivity.

Data collected using LIS

Data collected using the LIS system Cerner includes the sample statistics; collecting ward, collection date/time, Gram stain results and the organism(s) isolated.

Determination of false positives

False positive bottles were defined as those that flag by the instrument as positive but have a negative Gram and acridine orange stain. These bottles were sub-cultured onto standard media described above for the processing of positive samples, with the addition of a Sabouraud dextrose agar plate, before being re-loaded into the instrument for a further 5 days incubation. If no bacterial or fungal growth was observed in this incubation period, the bottle was considered a false positive.

Determination of false negatives

Terminal sub-cultures were performed on all bottles that completed their 5-day incubation period with no growth detected by BAV; but bottles that demonstrated organism growth were classified as false negatives. These bottles were inoculated onto CHOC and incubated in 5% CO_2 at 35C for 48 hours and anaerobically at 35C for 72 hours. Colonies were identified using the MALDI-TOF. The sample was then again sub-cultured onto CHOC agar to reproduce and confirm the result.

Statistical analysis

The bottles were divided into 4 categories based on instrument criteria; critically under filled bottles were filled 0-4mls and flag as incorrectly filled. Under filled bottles were those filled 5-7mls, these bottles are filled less than recommended but are accepted without alarm; over filled bottles are those filled more than 10mls. Bottles that were filled according to the IFU were 8-10mls. The differences between fill volumes between different wards and patient age and gender were analysed using paired or unpaired T-tests, using GraphPad Prism software, where $P < 0.05$ was considered statistically significant. The fill volumes were similarly compared to their respective rates of positivity for all bottles, false positives and false negatives.

Results

The fill volumes from 460 bottles are shown in Figure 1. There were 22.6% of total bottles within the correct range. In the other categories there were, 19.1% critically under filled bottles, 38.4% under filled bottles and 20% over filled bottles. There is a larger peak at 6mls and a smaller one at 11mls with a total range between 0 and 23mls (Figure 1).

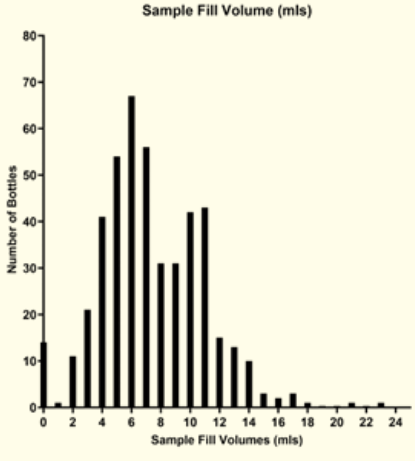

Figure 1: Sample fill volumes in blood culture bottles showing the largest peak at 6mls and a smaller peak at 11mls.

Figure 2 compares fill volumes in BFN+ and BFA+ bottles. The means of sample fill are plotted comparing the bottle types with error bars showing 95% confidence intervals. A two tailed, unpaired T-test was performed (p-value of 0.0144), indicating significantly more BFA+ bottles were under filled than BFN+.

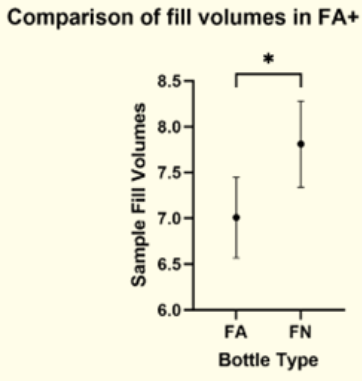

Figure 2: Comparison of sample fill volumes in BFA+ and BFN+ bottles * shows significance p=0.0144, aerobic bottles were under filled significantly more than anaerobic bottles.

Pairwise t-tests were performed on all categories against the within range category and found that there were significantly more under filled bottles than within range (p = 0.0051). There was no significant difference in bottles critically under filled or over filled compared to the within range category (p=0.6553 and p=0.3494 respectively).

Analysing wards individually found that some had a greater difference in sample fill volume than others, shown in figure 3. Wards 1 and 2 from figure 3 were emergency wards, wards 3-11 were medical wards and 12-17 surgical. Wards which had less than ten bottles collected during the study were omitted from this graph.

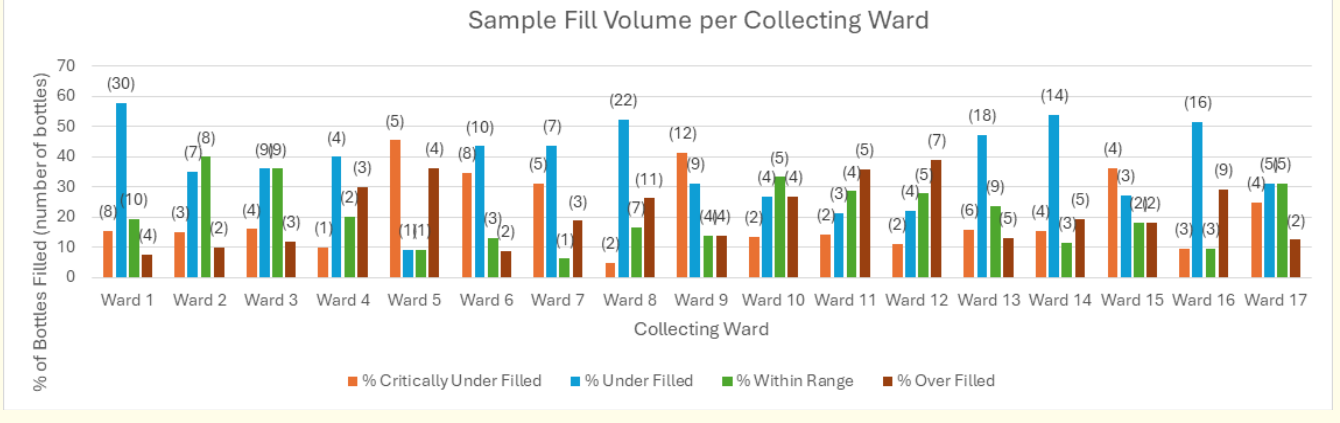

Figure 3: Sample fill volume showing the percentage of bottles filled in each category per ward. Wards 1 and 2 were emergency wards, wards 3-11 were medical and wards 12-17 were surgical.

Emergency ward 1 had the most under filled bottles of all the wards analysed during this study, close to 3 times more than those filled correctly. The medical wards also had significant under filling of bottles, particularly ward 5 which had a large percentage of critically under filled bottles compared to correctly filled ones. Wards 8, 14 and 16 had very high percentages of under filled bottles compared to other categories. The only wards which had more over filled bottles than other categories were wards 11 and 12. The highest percentage of correctly filled bottles was collected by emergency ward 2 at 40%.

Positive samples were analysed to find the time it took for the bottles to flag as positive compared to the volume in the bottle (Figure 4). A simple linear regression with 95% confidence interval was performed on the data set and found no significant trend.

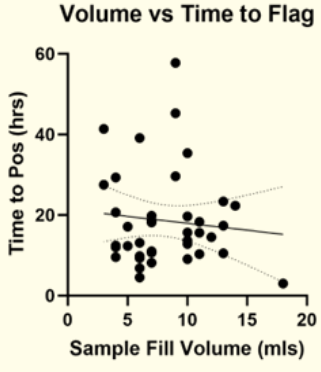

Figure 4: Volume vs time to flag for positive samples. Simple linear regression does not show a strong correlation between the volume of sample filled and the amount of time taken to flag positive.

The organism isolation rate for a bottle filled correctly was 2%. The isolation rates for critically under filled bottles was 1.7%, under filled bottles was 3% and overfilled bottles was 2%. When comparing the rates of improperly filled categories to the rate for bottles filled within the correct range, there is no significant difference.

Through culturing negatives, 3 out of 420 negative samples grew organisms; 2 of these samples grew on the anaerobic plates only and one sample grew on both aerobic and anaerobic plates (Table 1). The false negative results span three different fill categories as seen in table 1. All three samples had exponential growth curves such as that seen in figure 5 showing the curve of sample 2.

False positives were similarly analysed; there were two for the duration of the study with fill volumes of 17mls and 18mls. These bottles were cultured onto plate media as per protocol and did not grow any organisms after 5 days.

Discussion

This review conducted on the fill volumes of the BFA+ and BFN+ bottles found a significant number were under filled (Figures 1, 3). It has been found that the chance of organism detection by automated systems increases up to 3% per ml of sample added, so it can be inferred that for over 70% of samples, detection may be decreased by 3-18%. These results are more divergent from recommendations than a study conducted over three institutions across North America in 2017 [12] but does more closely match a study conducted in Sweden [13]. A multitude of other studies suggest that this may be a systemic issue throughout healthcare systems [9-

Table 1: Negative samples which grew organisms after culture.

Sample	Sample Fill Volume (mls)	Fill Category	Organism ID	Plates with growth
Sample 1	10	Within Range	*Staphylococcus saccharolyticus*	Anaerobic only
Sample 2	5	Under filled	*Cutibacterium acnes*	Anaerobic only
Sample 3	13	Over filled	*Cutibacterium acnes*	Anaerobic and aerobic

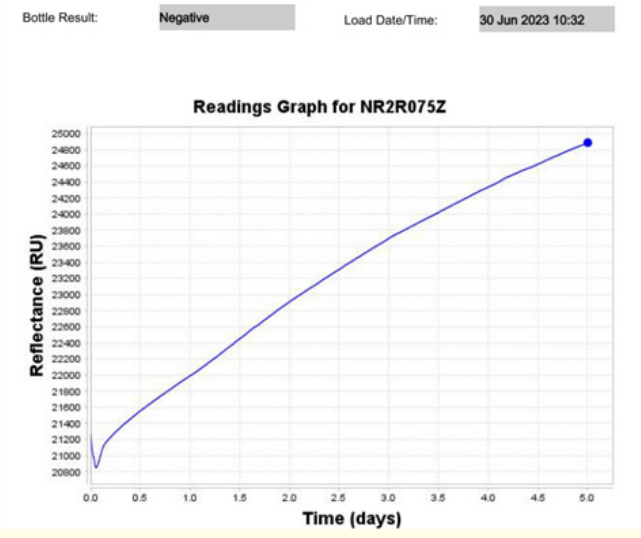

Figure 5: Reflectance of sample 2 (false negative) from BAV, showing an exponential curve with a negative result.

11,14,15]. The results show that the majority of bottles had 6mls of blood collected, but the range could vary from less than 1ml to 23mls, more than double the IFU (Figure 1).

BFA+ bottles were unexpectedly under filled significantly more than the BFN+ bottles (Figure 2). It was hypothesised that the BFN+ bottles would more likely be under filled due to the necessity for them to be filled last [2], however the opposite is true.

Across most wards, there were significantly more bottles under filled than any other category (Figure 3); the average percentage of underfilled bottles was 57.5%, compared to other studies where approximately 30% are underfilled [9-13]. The wards which were mostly contributing to the problem are spread across the two hospitals without significant staffing overlap. This may suggest that a lack of information and training may be a contributor to the poor compliance of blood culture bottle collections [11].

There was no clear trend in volume vs time to flag (Figure 4); however, a larger sample size would aid trend analysis, as only 40 bottles flagged positive during this review. The samples tend to cluster around the 10–20-hour mark which correlates with the IFU for time to detection [2]. However, it is not possible to know if added sample volume would have captured scarce bacteria within the bloodstream.

The false positives did not impact patient care as bottles are cultured and monitored closely for growth. As expected, the false positives were over filled bottles due to the overabundance of white cells [1]. There were 2 false positives during the study and 92 over filled bottles, not all bottles that were over filled flagged positive, but every false positive was over filled.

The false negatives found by this study grew coagulase negative staphylococci (CNS) and *Corynebacterium acnes* (Table 1), which underwent clinical review and was found to be non-significant; these are usually considered to be skin contaminants and not treated [3]. If there was a higher bacterial load by having a greater sample volume, these samples likely would've reached the detection threshold within the 5-day incubation window, as shown by the exponential curve for the *C. acnes* sample (Figure 5). Another study conducted found that incubating samples for longer than 5-days found more detected positives, but all the additional positives that were detected after 5 days had a tendency to be skin contaminants [3]. This suggests that increasing the incubation window to capture the low volume positive samples may not be an effective strategy in terms of laboratory time and resources. Despite the significant numbers of under filled bottles, there were only 3 samples out of 420 negative bottles that grew organisms, suggesting that it is unlikely for an organism to go undetected after five days, even at low volumes.

Many studies looking at blood culture fill volumes have concluded that the best solution is re-education and training for those collecting the samples. Research conducted found that re-education and refreshed training improved sample fill volumes from 31% compliance to 43% compliance [10,11]. Supplying additional information and training resources to ward staff and additional training sessions may benefit sample adequacy and compliance.

Conclusions

The blood culture samples collected at the two hospitals included in this study have low compliance with collection volume. As evidenced by this review, wards are generally under filling the bottles and additional or refresher training may benefit the quality of samples collected. However, the non-compliance had a low clinical impact. Despite having a low impact on patient care, correct procedures and the manufacturer's IFU should still be followed to optimise results.

Acknowledgements

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Conflicts of Interest and Sources of Funding

The authors state that there are no conflicts of interests to disclose.

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