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ORIGINAL ARTICLE

Evaluation of the blood volume effect on the diagnosis of bacteremia in automated blood culture systems

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Background: Blood culture volume is the most important variable in detecting bacteremia and fungemia. However, the majority of hospitals in Taiwan do not meet the criteria for an ideal blood culture volume (8–10 mL per bottle, two bottles per set) during collection.
Methods: The object of this study is to initiate an educational program for healthcare workers to increase blood volume collection and to evaluate the relationship between blood volumes and bacteremia recovery rate for detecting bacteremia and fungemia effectively by using the BD BACTEC 9240 blood culture system.
Results: After education, the blood sample volume $\geq$5 mL group increased from 2.93% to 71.24%. For a total of 4,844 bottles, the relative improvement in recovery rate for detection has increased by 17.81% between the $<$5 mL group and the $\geq$5 mL group. The recovery rates for the low-volume ($<$3 mL), mid-volume (3–7 mL), high-volume (8–10 mL) and extreme high-volume ($>$10 mL) groups are 13.31%, 15.02%, 17.68%, and 14.96%, respectively.
Conclusion: With good blood collection practice, our study found that blood volume obtained was in direct proportion to recovery rate for the detection of bacteremia and fungemia.

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Introduction

Among many kinds of infection, bloodstream infection is systemic and can result in life-threatening sepsis, leading to high morbidity and mortality. Therefore, early and accurate detection is important to improve patient care.[1] Positive blood culture can help to diagnose bacteremia and fungemia in patients with fever, because the number of microorganisms present in an adult's blood is typically of a low level, usually fewer than 10 colony-forming units (CFUs)/mL, and sometimes less than 1 CFU/mL.[2] The volume of blood for culture is therefore the most important variable in determining the recovery rate of blood culture.[2]

Due to limitations in terms of collection devices, many hospitals in Taiwan cannot, despite the importance of this, meet the recommended blood volume, which should be 20—30 mL per venepuncture.[3] Our preliminary study shows that the average blood volume taken for blood culture in China Medical University Hospital is low, less than 3 mL per bottle for 89.1% of venepunctures. The major reason is that blood culture usually needs collection into multiple tubes; therefore, one 10-mL syringe can only deliver about 2—3 mL blood into blood culture bottles. Other reasons may relate to the traditional beliefs of Taiwan people, most Asian people thinking that blood is too precious to be drawn.

The current method for blood culture is automatic detection, which should be more sensitive than traditional manual methods.[4] This might mask the effect of a low blood sample volume on the blood culture-positive rate in our hospital. Therefore, we conducted a study to evaluate the importance of blood volume and recovery rate for detection, and the relative detection time in an automatic blood culture system.

Our study design involved instituting a series of educational programs for healthcare workers on adequate blood collection practice. Afterwards, we calculated the blood volume and organism distribution, and then compared the recovery rate for detection and the species of microorganisms between the low-, mid- and high-volume groups. We wanted to determine whether there was any clinical significance and thereby convince patients who need blood cultures that a sufficient volume of blood is of clinical importance.

Methods and materials

Educational program

During July 2010, several courses were held in the emergency room (ER unit) focusing on adequate blood collection. This included disinfection and the prevention of needle-stick injuries by using blood collection safety sets (BD Safety-Lok, Becton, Dickinson and Company, Sumter, South Carolina, USA), which can allow serial blood collection and let each tube or bottle reach an ideal volume. Inoculation of the blood volumes collected into blood culture media sets was performed according to manufacturer's instructions (BD BACTEC Standard aerobic/F medium, BD BACTEC lytic/Anaerobic/F medium).

Collection of samples

A total of 4,844 samples were collected in the ER unit over a 6-month period. Before measuring the volumes of the blood specimens, we set up 10 reference bottles in which 1—10 mL of red-colored solution were injected into 10 separate blood culture bottles. The volumes of the unknown blood specimens were then measured by performing a comparison with the reference bottles. All of the bottles were placed into BD BACTEC 9240 instruments.

Detection of samples

BD BACTEC 9240 blood culture instruments are designed for the rapid detection of microorganisms in clinical blood specimens. The sample to be tested is inoculated into the vial, which is entered into the BD BACTEC instrument for incubation and periodic reading. Each vial contains a sensor that responds to the concentration of carbon dioxide produced by the microorganisms' metabolism. The sensor is monitored by the instrument every 10 minutes for any increase in its fluorescence, which is proportional to the increasing amount of carbon dioxide present in the vial. A positive reading indicates the presumptive presence of viable microorganisms in the vial. The protocol length was a total of 5 days before the response for a vial was determined to be negative.

Identification of samples

All the positive vials were stained and identified by BD Phoenix Automated Microbiology System (BD Diagnostic Systems). The BD Phoenix Automated Microbiology System is intended for the *in vitro* rapid identification and quantitative determination of antimicrobial susceptibility using the minimal inhibitory concentration of frequently isolated clinically relevant microorganisms.

Analysis of data

The paired *t* test was used to verify the effects of educational programs. The elative improved recovery (RIR) was used to analyze the relative recovery rate for detection between the <5 mL group and the ≥ 5 mL group in different circumstances. For instance, the recovery rates of the <5 mL and ≥ 5 mL groups in Table 1 (see below) are 13.21% and 17.30%, respectively, so the RIR would be $[(17.30\% - 13.21\%)/13.21\%] * 100\% = 30.96\%$, which indicates the relative increment of recovery. The Chi-square test and analysis of variance were used to compare separate pair groups and the overall recovery difference between the low (<3 mL), mid- (3—7 mL), high- (8—10 mL), and extreme high- (>10 mL) volume groups.

Results

Of the 4,844 samples submitted during the study period, we first compared blood volume distribution before and after education. It was found that the blood sample volume ≥ 5 mL group had increased from 2.93% to 71.24%, whereas the <5 mL group had decreased from 97.07% to 28.76% (Fig. 1), a statistically significant difference ($p < 0.05$).

To further analyze the volume effect on blood culture, we divided all the samples into low-volume (<3 mL), mid-

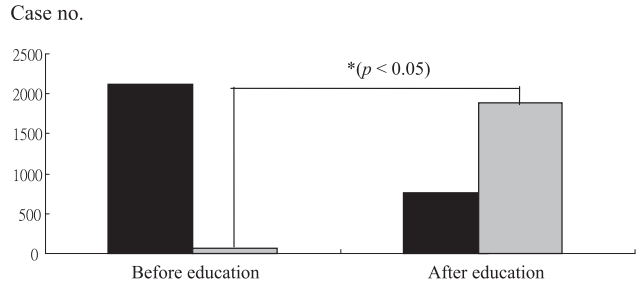

Figure 1. Blood volume analysis. Comparison was made between the <5 mL(■) group and the ≥5 mL(■) group. After education, the blood sample volume ≥5 mL group had increased from 2.93% to 71.24%, and the <5 mL group has decreased from 97.07% to 28.76%.

volume (3—7 mL), high-volume (8—10 mL), and extreme high-volume groups (>10 mL). The recovery rates for detection for these four groups were 13.31%, 15.02%, 17.68%, and 14.96%, respectively (Fig. 2). By pairing comparison, we found a significant difference between the low- and high-volume groups ($p < 0.05$); by overall comparison, there was also a significant difference among all four groups ($p < 0.05$).

Next, in order to identify more precisely the recovery rate for detection in the two groups, we excluded either duplicated or contaminated samples, or both, to see where there was any difference in the RIR. From our data, we found that the RIR for total samples, samples excluding duplication, samples exclude contamination, and samples excluding both duplication and contamination were 30.96%, 36.37%, 12.91% and 17.81%, respectively (Table 1).

As shown in Table 2, the positive culture rate increased from 13.21% to 17.30% if the blood volume increased from <5 mL to ≥5 mL. In terms of various bacterial species, the increase in positive rate occurred for *Staphylococcus aureus*, *Streptococcus* spp., *Enterobacteriaceae* spp., glucose nonfermenting species., multiple isolates, and contaminants; however, a decrease in detection rate occurred with *Enterococcus* spp., yeasts and anaerobe groups.

When analyzing the detection time for the <5 mL and ≥5 mL groups (Table 3), detection time was seen to decrease for *Streptococcus* spp., *Enterobacteriaceae* spp., multiple isolates, and contaminant groups. The interval time, however, increased for *S. aureus*, *Enterococcus* spp., glucose nonfermenting species, and anaerobes.

Discussion

Obtaining an adequate blood volume is important for the detection of bloodstream infections,[5] and we continue to educate our associates with this concept. Through the educational program, the blood volume collected during this period showed significant increases in the ER unit, which demonstrated that our program was effective. Training will therefore be continued since adequate volume collection in Asian countries is not easy due to traditional beliefs. Our analytical data also proved that a collected blood volume ranging from 8 mL to 10 mL per bottle demonstrated the best recovery rate for detection, compared with a 3—7 mL group and a <3 mL group. We may use these data to convince our patients to give a higher volume of blood.

In addition, we found that blood volume measurement by visual comparison may be somewhat subjective as there may be deviation when judging with the naked eye. Weighting the bottle before and after sample collection would be a more precise method.[6] According to a previous study, more significant yields could be reached if the sample number falling into the low-volume group (<3 mL) could be lowered; it was shown that, as a result, the yield of blood cultures in adults increased by approximately 3% per milliliter of blood collected.[7]

In Table 1, the recovery difference between the <5 mL and the ≥5 mL group seemed to be less significant when removing contamination factors, which means that our contamination rate was high. One reason for such a finding may relate to syringe usage: nurses often change needles while injecting blood into different bottles, a practice that increases not only the chance of a needle-stick injury, but also the chance of introducing any contaminating microorganism carried on the nurse's gloves. To minimize man-made contamination, we are encouraging our colleagues to use blood collection sets during the blood-drawing process, so that there is no need to change needles.

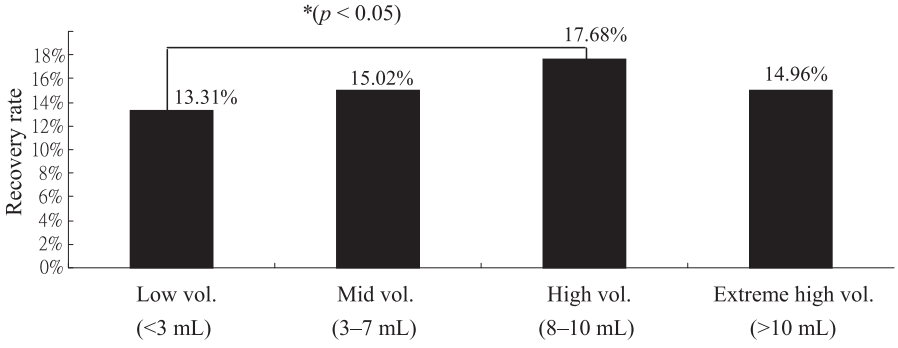

Figure 2. Recovery rate analysis. Comparison of recovery rate for detection was made between the low-volume (<3 mL), mid-volume (3—7 mL), high-volume (8—10 mL) and extreme high-volume (>10 mL) groups. The positive number/total number in the low-, mid-, high-, and extreme high-volume groups was 251/1886, 241/1604, 172/973, and 57/381, respectively.

Table 1 Relative improved recovery rate analysis between the <5 mL group and the ≥5 mL group

Volume	Recovery rate			
	Total	Exclude duplicated isolates	Exclude contaminated isolates	Exclude contaminated and duplicatedisolates
<5 mL	13.21%	14.60%	11.39%	12.07%
≥5 mL	17.30%	19.91%	12.86%	14.22%
Relative improved recovery	30.96%	36.37%	12.91%	17.81%

When it comes to multiple-tube blood collection combined with blood culture collection, we also encourage the use of a safety collection device, which again not only prevents needle-stick injury and ensures that an adequate volume is collected,[8] but also eases patients' worry related to large-volume syringe use. However, those isolates found may be true pathogens because we did not verify whether only one bottle had been identified in a series of blood culture specimens.[9]

As for the recovery for detection, we found that bottles that had been inoculated with higher volumes of blood showed a higher recovery rate, which echoes the results of previous publications.[5,6,10,11] From previous studies, we know that blood culture set number is also important for recovery for detection, and we may include this factor in our future investigations. Increasing the number of blood culture sets used will also increase the volume of blood collected, which should result in a further increase in positivity rates. Further analysis to verify correlation with patient severity and recovery rate could be done, because there are studies addressed that the recovery rate of blood culture among severe patients had positive correlation of blood volume; while mild patients did not.[6]

In Table 2, a decreased detection rate was observed for *Enterococcus* spp., yeasts, and anaerobe groups. This might have resulted from the small sample sizes in each group.

The data in Table 3 show that there was no significant difference in average detection time between the <5 mL and ≥5 mL groups; however, by organism type, the detection of the genus *Enterobacteriaceae* in the ≥5 mL group was faster than in the <5 mL group (11 vs 7.2 hours). Because *Enterobacteriaceae* are the microorganisms most frequently isolated from blood culture, we view it as clinically important that higher blood volumes contribute to a quicker time to obtain results.

However, the isolation time for *S. aureus*, *Enterococcus* spp., and anaerobes in the high-volume group became longer. For *S. aureus*, this might be due to a lower number of microorganisms per volume in some samples, which was the portion of increased isolation rate in high-volume group (8—10 ml). For *Enterococcus* spp. and anaerobic groups, small sample sizes might be the reason for the lack of decrease in detection time. In our hospital in 2010, the top five organisms isolated in blood culture were coagulase-negative staphylococci (CoNS), *E. coli*, *Klebsiella pneumoniae*, *S. aureus*, and methicillin-resistant *S. aureus*. In conclusion, increasing the blood volume collected has a benefit in terms of both recovery for detection, and early detection.

There is one other interesting finding from our study: the recovery rate for detection was decreased for the extreme high-volume (>10 mL) group, compared with the high-volume (8—10 mL) group. A possible cause for this may be the dilution effect, which means that failure to maintain a blood to broth ratio of between 1:5 and 1:10 may result in a false-negative result,[12] because of the inhibitory effect of complement, lysozyme, phagocytes, antibodies, and antimicrobial agents.[13,14] This problem can be solved by using neutralizing agents such as resin-containing culture media, which inactivate the inhibitory substances present in blood.

The development of automated continuous-monitoring blood culture systems during the 1990s accelerated the trend from conventional manual methods. However, the

Table 2 Isolation rate of organisms for the <5 mL group and the ≥5 mL group before and after education

Organism	<5 mL, number (%)			≥5 mL, number (%)		
	n = 2,885			n = 1,959		
	Before education	After education	Total	Before education	After education	Total
Staphylococcus aureus	37	8	45 (1.56)	3	29	32 (1.63)
Streptococcus spp.	19	10	29 (1.01)	2	25	27 (1.38)
Enterococcus spp.	4	1	5 (0.17)	—	3	3 (0.15)
Enterobacteriaceae spp.	125	44	169 (5.86)	6	121	127 (6.48)
Glucose nonfermenting spp.	30	8	38 (1.32)	—	29	29 (1.48)
Yeasts	5	—	5 (0.17)	—	2	2 (0.10)
Anaerobes	8	9	17 (0.59)	—	8	8 (0.41)
Contaminants	38	21	59 (2.05)	2	98	100 (5.10)
Multiple isolates	11	3	14 (0.49)	—	11	11 (0.56)
Total	277	104	381 (13.21)	13	326	339 (17.30)

Table 3 Detection time of organisms in the <5 mL group and the ≥5 mL group before and after education

Organism	<5 mL			≥5 mL		
	Before education (h)	After education (h)	ADT (h)	Before education (h)	After education (h)	ADT (h)
Staphylococcus aureus	12.49	10.95	12.1	27.05	14.06	14.9
Streptococcus spp.	12.92	12.31	12.6	24.3	9.24	10.3
Enterococcus spp.	10	6.1	12.7	—	11.9	13.1
Enterobacteriaceae spp.	12.75	7.64	11	9.2	6.46	7.2
Glucose nonfermenting spp.	20.27	18.93	19.4	—	21.47	20.7
Yeasts	—	—	-	—	43.3	43.3
Anaerobes	13.2	72.73	62.7	—	80.34	80.3
Contaminants	33.5	35.15	34.3	43.4	28.69	28.8
Multiple isolates	10.46	19.63	12.8	—	8.55	8.6
Average	16.31	20.67	17.9	19.21	17.54	17.6

ADT = average detection time, i.e. the sum of all the detection times divided by all the positive samples.

effect of blood volume on the positive rate of blood culture remains important.

In conclusion, the collection of an adequate blood volume is correlated with increased recovery rates of blood culture. Therefore, we must continue educating phlebotomists on the concept of collecting an adequate volume of blood in order to improve the recovery rate for detection. In this way, infections in patients with sepsis can be rapidly and correctly detected, benefiting both patients and hospitals.

Acknowledgments

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