

Blood culture is an important laboratory investigation in cases of suspected bacteraemia; however, often little thought is given to the size of the sample taken. Here, Michelle Lingwood, Mohammed Abba, Devadas Pillay and Keith Burrows look at the importance of volume.

Link between inoculum size and blood culture positivity rates

Owing to the high morbidity and mortality associated with bacteraemia, the rapid detection and subsequent identification of microorganisms from blood remain critical functions of the clinical microbiology laboratory.¹ Numerous blood culture methods are available, but modern systems have been improved by automation. This, together with advances in the use of liquid media, has enhanced the ability of laboratories to provide faster blood culture results.^{2,3} However, sensitivity is affected by inoculum size, and manufacturers make specific recommendations about the volume of blood that should be added to their culture bottles.

The Good Hope Hospital microbiology laboratory uses BACTEC Lytic 10 Anaerobic/F and Plus Aerobic/F bottles (BD Diagnostics) for its anaerobic and aerobic cultures, respectively. These bottles require a minimum inoculum size of 5 mL, although this should ideally be 8–10 mL according to the manufacturer's instructions.

‘To eliminate the potential for biased results, random blood cultures were chosen and performed at different times and on different days’

‘Sensitivity is affected by inoculum size, and there are specific recommendations about the volume of blood that should be added to culture bottles’

Clearly, inoculum size is important so staff in microbiology at Good Hope decided to evaluate compliance with trust guidelines among doctors and other hospital staff involved in collecting blood culture samples, as well as to analyse the yield from optimal and suboptimal samples.

A matter of culture

Blood cultures (n=304) received in the Good Hope microbiology department between 2 February and 17 December 2005 were studied. Each pair of Lytic 10 Anaerobic/F and Plus Aerobic/F bottles (BD Diagnostics) was weighed to two decimal places using an electronic balance. The blood cultures were incubated at 35°C on the BACTEC 9240 system, which monitors the cultures over a five-day period.

When growth was detected by the system in an individual blood culture bottle it was examined by microscopy and inoculated on appropriate agar plates. Blood cultures that showed no evidence of bacterial growth on microscopy were repeated. If a second examination proved negative then the blood culture was returned to the automated culture system.

Positive isolates were subjected to appropriate biochemical/microbiological identification. To eliminate the potential for

biased results, random blood cultures were chosen and performed at different times and on different days. In addition, the clinician or member of staff taking blood for culture was not aware that the audit was in progress.

Determining blood volume in a weighed culture bottle

Using an electronic balance, 10 uninoculated blood culture bottles were weighed. From these values mean weights for the uninoculated anaerobic and aerobic blood culture bottles were calculated. Each bottle type was then inoculated with 10 mL lysed whole horse blood, and weighed. From the data above, the mean weight of 1 mL blood was determined as 1.01 g and the volume of blood (mL) in each blood culture bottle in relation to weight was calculated as: overall total weight of inoculated blood culture bottle minus mean weight of the uninoculated bottle, divided by 1.01.

Analysis of results

A total of 152 paired blood culture samples were analysed. In the aerobic blood cultures, 45 (29.6%) of the samples were inoculated with less than 5 mL blood, 86 (56.6%) contained 5–10 mL and 21 (13.8%) contained over 10 mL blood (Fig 1a).

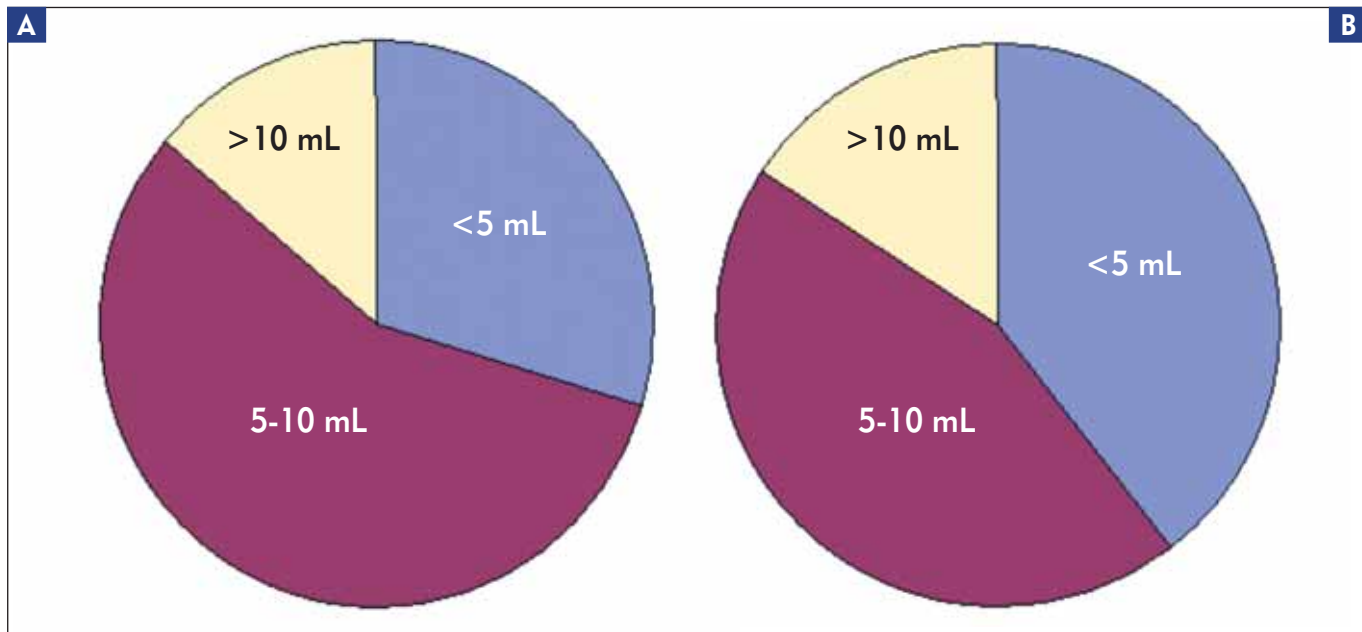


Fig 1. Volume of randomly selected blood cultures received by Good Hope Hospital microbiology department between 2 February and 17 December 2005: a) aerobic bottles, b) anaerobic bottles.

In the anaerobic culture samples, 60 (39.5%) were inoculated with less than 5 mL blood, 68 (44.7%) contained 5–10 mL, and 24 (15.8%) contained over 10 mL blood (Fig 1b).

Forty (13.1%) of the blood cultures were found to be positive. There was a higher number of positive anaerobic bottles compared to aerobic bottles (ratio of 3:2, respectively). Of the 40 positive cultures, 11 (27.5%) were from suboptimal inocula while 29 (72.5%) were from those regarded as containing optimal volumes. Positivity yield from the suboptimal cultures was significantly different (Fisher's exact test [two tailed] $P=0.375$) to that obtained from optimal cultures.

Importance of volume

A significant proportion (34.5%), equivalent to 105 of the 304 blood culture bottles received by the microbiology department, contained less than the optimal volume of blood. There was also a significant difference in the inoculated volume between the anaerobic and aerobic bottles (Fig 1). This resulted in a low percentage yield of positive

Table 1. Yield for blood culture type as a percentage of the total in each category.

BOTTLE TYPE	VOLUME OF BLOOD CULTURE		
	<5 mL	5–10 mL	>10 mL
Aerobic	29.6%	56.6%	13.8%
Anaerobic	39.5%	44.7%	15.8%

results from those cultures that contained less than optimal blood volumes.

The Good Hope audit emphasises the importance of using an optimal volume in a blood culture and supports the findings of other studies.⁴⁻⁶ The latest recommendation from the manufacturer is that all blood culture bottles should be inoculated with 8–10 mL blood.

From the Good Hope study, it is reasonable to conclude that all culture bottles containing less than 5 mL blood are suboptimal and, depending on the clinical situation, may need to be repeated. Thus, all junior doctors and other hospital staff involved in collecting blood culture samples should be encouraged to familiarise themselves with the blood culture sampling protocol that is available in their respective trust policies. ■

- Muller-Serieys C, Bergogne-Berezin E. Blood culture update. *Presse Med* 2002; **31**: 27–32.
- Weinstein MP, Reller LB. Remarks concerning testing parameters for blood cultures. *Clin Infect Dis* 2005; **40**: 202.
- Archibald LK, Dobbie H, Kazembe P *et al.* Utility of paired BACTEC MYCO/F LYTIC blood culture vials for detection of bacteraemia, mycobacteraemia and fungaemia. *J Clin Microbiol* 2001; **39**: 1960–2.
- Cockerill FR 3rd, Wilson JW, Vetter EA *et al.* Optimal testing parameters for blood cultures. *Clin Infect Dis* 2004; **38**: 1724–30.

‘It is reasonable to conclude that all culture bottles containing less than 5 mL blood are suboptimal’

REFERENCES

- Mensa J, Almela M, Casals C *et al.* Yield of blood cultures in relation to the cultured blood volume in Bactec 6A bottles. *Med Clin* 1997; **108**: 521–3.
- George BJ, Horvath LL, Hospenthal DR. Effect of inoculum size on detection of *Candida* growth by the BACTEC 9240 automated blood culture system using aerobic and anaerobic media. *J Clin Microbiol* 2005; **43**: 433–5.

Michelle Lingwood, Mohammed Abba, Devadas Pillay and Keith Burrows work in microbiology at Good Hope Hospital, Sutton Coldfield, West Midlands B75 7RR. Further information can be obtained from Dr Devadas Pillay (email: devadas.pillay@goodhope.nhs.uk). The authors acknowledge the assistance of the biomedical scientists at Good Hope Hospital who contributed to this study.