Technical method

Earlier detection of bacteraemia using conventional microbiological techniques

ELIZABETH R YOUNGS, C ROBERTS *Public Health Laboratory, Fazakerley Hospital, Liverpool*

The rapid detection of bacteraemia and subsequent identification and determination of antimicrobial susceptibility of isolates is important in the management of patients with serious infections. The optimum time for routine subculture of blood cultures is, however, controversial. The Association of Clinical Pathologists' broadsheet on blood culture technique,¹ although giving a detailed account of laboratory methods, does not suggest specific recommendations on the timing of subcultures. Shanson² believes this should be done after the first, second, and seventh days of incubation. Although most laboratories perform the initial subculture on the day after the specimen is received, recent studies have examined the value of earlier subculture. Todd and Roe³ compared early subculture after 4-14 h incubation with the traditional 24 or 48 h Gram stain and subculture and found the former detected 85% of all significant cultures in their paediatric population. Harkness *et al*⁴ performed subcultures between 3 and 19.5 h after blood collection in a predominantly adult population and found 48% of positive blood cultures were detected by this early examination. Silva and Washington⁵ compared routine aerobic subcultures after 6-12 h incubation with those after 12-17 h incubation. These yielded 36% and 63% respectively of isolates, whereas only 10% were recovered when subculture took place within 6 h of blood collection. More recently, Ganguli *et al*⁶ identified half of their bacteraemic patients after subculture at 10 pm on the day of receipt of blood cultures.

These reports prompted this prospective study of early subculture of blood cultures on the day of receipt during normal laboratory working hours.

Material and methods

The study was conducted in a district general hospital serving mainly an adult population but including

a maternity unit and special care baby unit. All blood cultures, which were taken by the medical ward staff and received in the laboratory between 9 am and 4 pm during weekdays, were investigated. Those that had been taken after normal working hours the previous night and placed in an incubator within easy access for junior doctors and all those taken at weekends were not included in this study.

The blood culture set consisted of two media in 80 ml volumes, each in 100 ml glass screw-capped bottles. The aerobic medium was brain heart infusion broth (Oxoid CM 225) and the anaerobic medium was laboratory made cooked meat broth with ox heart and nutrient broth (Oxoid CM 67). Each bottle carried instructions that 5 ml of blood should be added.

Blood culture sets received between 9 am and 4 pm on weekdays were examined at 4 pm on the day of receipt for signs of bacterial growth—for example, turbidity, haemolysis. If present, a Gram film of the broth was made. The blood culture bottles were then subcultured (day 0) and further routine subcultures were done on the following day (day 1) and on days 3 and 7. They were examined every day. Subcultures were done in a quiet room with door and windows closed. After gentle mixing, each bottle was sampled using a sterile Pasteur pipette. A drop of broth was placed on to half a plate of blood agar (Oxoid blood agar base CM55 and 7% horse blood), chocolate agar (heated Oxoid blood agar base CM55 and 7% horse blood), MacConkey agar (Oxoid CM7b), and prereduced blood agar and spread with a wire loop. The first three plates were incubated at 37°C in air with 10% carbon dioxide and were examined after 24 and 48 h incubation. The prereduced blood agar was incubated under anaerobic conditions (Don Whitley anaerobic jar and Oxoid Gaspak) at 37°C and left for 48 h before inspection.

All organisms were identified and tested for antimicrobial susceptibility from subculture plates using routine laboratory methods. The clinical importance of isolates was determined after discussion between the medical microbiologist and clinical colleagues.

Results of subculture in relation to length of incubation

	Number (%)
Total number of blood cultures received between 9 am and 4 pm	112
Total number of significant cultures	16 (14)
Detected on solid media after ≤7 h incubation (day 0)	10 (63)
Detected on solid media after >7 h incubation	6 (37)
Total number of doubtful or contaminated cultures	16 (14)
Detected on solid media after ≤7 h incubation (day 0)	1 (6)
Detected on solid media after >7 h incubation	15 (94)

Results

In one year 112 blood culture sets had early subcultures. Sixteen (14%), from 14 patients, subsequently yielded clinically significant isolates. Of these, 10 (63%), from eight patients, were detected on solid media by the following morning. None was detected by initial visual examination. A further 16 (14%) isolates were regarded as contaminating organisms and only one of these grew on solid media as a result of the early subculture (Table). The contamination rate in the blood cultures taken between 4 pm and 9 am on weekdays and all those at weekends, which were processed by the routine method (subcultured on days 1, 3, and 7) during the same one year period, was 7%.

Discussion

Blood culture results are important to the clinician in the management of individual patients and so early recognition of positive cultures is desirable. With this aim in mind, several rapid techniques, including automation, have been examined.⁷ But for a number of reasons, including cost, the widespread adoption of automated equipment may be limited, and conventional techniques will still be necessary in some routine clinical laboratories.

Although the numbers are small during the period under review, the early detection—that is, within 24 h of receipt—of 10 (63%) significant isolates from eight bacteraemic patients made an appreciable contribution to the patients' management and compares well with the later detection of an additional four grown after subculture the day after receipt. In turn, this early detection resulted in the reporting of antimicrobial sensitivities 24 h earlier. Inevitably, the accompanying increased opening of the blood culture bottles produced a rise in contaminating organisms, thus increasing laboratory workload, although some of these would have been introduced at the time of collection of blood from patients. Silva and Washington⁵ detected only two of

20 (10%) isolates after incubation for 1–6 h and recommended routine subculture after 6–17 h. If this was followed many blood cultures received during the working day would not be subcultured until the following morning in laboratories which perform routine work only between 9 am and 5 pm. Ganguli *et al*⁶ avoided this by subculturing at 10 pm on the day of receipt. Although 119 of 237 bacteraemic patients were detected within 24 h by this practice, it may place an extra cost and burden on technical and medical staff outside the normal laboratory working day. Two other studies^{3,4} found 85% and 40% of significant cultures after 4–14 h and 3–19.5 h incubation respectively, but the authors do not state the time of day that subcultures were performed.

We believe that by subculturing both in the morning and at 4 pm—that is, during normal laboratory hours—earlier detection of bacteraemia can be achieved. This regimen employs conventional microbiological techniques without the expense of new equipment, reagents, and out of hours work.

References

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Requests for reprints to: Dr Elizabeth R Youngs, Public Health Laboratory, Fazakerley Hospital, Lower Lane, Liverpool L9 7AL, England.

Letters to the Editor

Annual and seasonal variation in the frequency of β -haemolytic streptococcal infections

Dr Millar¹ reports that in Yorkshire over the past five years there has been a significant increase in the frequency with which streptococci of Lancefield group C have been found. In Shropshire, where the

population is principally rural or semi-rural, our experience has been different. There have been only modest fluctuations from year to year with no consistent pattern of change (Tables 1–4). We have, however, in common with others in the United Kingdom, noted a seasonal incidence in outbreaks of streptococcal skin sepsis among meat handlers²; this is an

occupational hazard directly related to the peak period in the autumn for animal slaughtering and processing, and is typically associated with group A streptococci.

CA MORRIS
DM BERRY
Public Health Laboratory,
The Royal Shrewsbury Hospital,
Shrewsbury SY3 8JH