Clinical Impact of Preincubation of Blood Cultures at 37°C

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Received 15 March 2010/Returned for modification 16 April 2010/Accepted 2 November 2010

The effect of immediate incubation of blood cultures at 37°C on the turnaround time and the impact of Gram stain results on antimicrobial management were investigated. During a 6-month period, blood cultures collected at the emergency department outside laboratory operating hours were preincubated at 37°C until transport to the laboratory. Upon the arrival of blood cultures at the laboratory, Gram stains and subcultures were made from all bottles prior to further incubation in the automated system (Bactec 9240). Data from 1 year earlier, when all blood cultures were stored at room temperature, were used for comparison. In the study period, 79 episodes of bacteremia were detected for 75 patients, compared to 70 episodes for 67 patients in the control period. Preincubation of blood cultures at 37°C resulted in a 15-h reduction in the median time to reporting of Gram stain results, from 34 to 19 h (P < 0.001). With preincubation, 3 episodes (4%) of bacteremia were not detected by the Bactec 9240 system. Based on the reporting of the Gram stain results, appropriate antimicrobial therapy was initiated for 12% of all patients with positive blood cultures, while for 24% the therapy was streamlined. Thus, immediate incubation of blood cultures reduced the time to reporting of Gram stain results. However, not all episodes of bacteremia were detected by the Bactec 9240 system after preincubation at 37°C. Blood culture results contributed importantly to appropriate antimicrobial management.

Appropriate antibiotic therapy initiated in a timely manner has been shown to reduce mortality in patients with bloodstream infections (5–7). Therefore, if a bloodstream infection is suspected, blood should be collected for culture as soon as possible, preferably before antimicrobial therapy is initiated. Ideally, blood culture results lead either to the streamlining of broad-spectrum empirical therapy or to the initiation of appropriate therapy (3). Blood culture results may also help to confirm or identify the site of infection.

To optimize the clinical use of blood culture results, the interval between the collection of blood and the entry of the bottles into an automated blood culture system should be kept to a minimum. However, many microbiology laboratories do not provide 24-h service, and cultures obtained outside operating hours are often stored at room temperature before entry into the system. Aside from laboratory operating hours, the use of satellite laboratories referring blood cultures to their reference laboratory can cause a significant delay in bottle entry into the system. This delayed entry has been shown to result in increased times to detection of bacteremia and longer hospital stays (1, 9).

To overcome this problem, an automated blood culture system can be placed outside the laboratory, and blood cultures may be entered by health care workers. Such an approach resulted in a 10-h reduction in the interval between blood culture collection and growth detection (6). Since it is not always feasible to place a monitoring system outside the laboratory, an alternative may be to preincubate blood cultures in a regular incubator at 37°C before their entry into the automated system. Such an incubator could be placed either in the wards or in the laboratory reception area. With this approach, a proportion of positive cultures may remain undetected. If the increase in CO2 production by the bacterial growth occurs prior to entry of the bottle into the automated blood culture system, such an increase may go unnoticed (2, 4).

One study has reported on preincubation of blood cultures (8). This procedure showed a reduction in the time to detection (TTD), but the authors did not investigate the clinical significance of the earlier detection of bacteremia.

The purpose of our study was to determine the effect of immediate preincubation of blood cultures at 37°C on the time to detection of bacteremia and the occurrence of false-negative results in the automated blood culture system. In addition, we evaluated the clinical impact of the detection of bacteremia on antimicrobial therapy.

MATERIALS AND METHODS

Setting and sample collection. The study was conducted in the Radboud University Nijmegen Medical Centre, a 950-bed tertiary-care university hospital in the Netherlands. The laboratory operating hours were from 8:00 am to 5:00 pm on weekdays and from 8:00 am to 11:30 am on weekends and public holidays. A 37°C incubator was installed at the emergency department (ED) of the hospital for a 6-month study period, from March to September 2006. The ED staff was instructed to place all blood cultures collected from patients visiting the ED outside laboratory operating hours in this incubator. The collection of 2 sets of blood cultures, consisting of 1 aerobic (Bactec Plus Aerobic/F) and 1 anaerobic (Bactec Plus Anaerobic/F) bottle each, for adult patients and 1 or 2 pediatric bottles (Bactec Peds Plus/F) for pediatric patients was recommended. All bottles were transferred to the microbiology laboratory by the routine transport service on the following morning.

Upon the arrival of the blood cultures in the laboratory, Gram staining and subcultures on chocolate agar were performed for all preincubated bottles before they were entered into the Bactec 9240 automated blood culture system (Becton Dickinson Microbiology Systems, Cockeysville, MD). Gram stains were evaluated immediately, and the subcultures were incubated overnight under 5% CO2 at 37°C. Blood cultures collected at the ED during laboratory operating hours were entered into the Bactec 9240 system directly upon arrival in the laboratory.
FIG. 1. Timeline. The transport time was calculated for all bottles, the time to detection for all positive bottles, and the time to reporting of the Gram stain result for each episode of bacteremia. During the study period, the positive Gram stain could be available before the positive Bactec 9240 signal, and for other bottles, signals remained absent altogether.

Blood culture results obtained between 1 March and 1 October 2005, when all blood cultures collected at the ED were stored at room temperature, were used as controls. All cultures without a collection date or time were excluded from analysis.

Data collection. The collection date and time were retrieved from the culture request form. The times of bottle entry and removal were recorded by the Bactec 9240 system. The following parameters were determined (Fig. 1): transport time (calculated as the time of arrival at the laboratory minus the time of blood culture collection; this is equivalent to the preincubation time, where applicable); loading time (the time of entry into the Bactec 9240 system minus the time of arrival at the laboratory); TTD (the time of a positive signal for growth by the Bactec 9240 system minus the time of bottle entry into the system); and time to reporting (TTR) (the time of removal of the first positive bottle from the Bactec 9240 system minus the time of collection).

If the Gram stain that was made on arrival was positive, the TTR was defined as the interval between collection and bottle entry. If the subculture that was prepared on arrival was positive before the bottle in the Bactec 9240 system was detected as positive, the TTR was defined as the interval between collection and the time at which the subculture was investigated for growth. This was recorded in the laboratory information system.

To distinguish between contamination and true infection, all positive blood cultures with isolates known as potential skin contaminants were assessed by the investigators for clinical significance. This assessment included a review of the patients’ clinical history, physical findings, number of positive bottles, results of cultures from other sites, and response to therapy. Cultures deemed to be contaminated were excluded from analysis.

Effect of preincubation on antimicrobial treatment. The clinical impact of Gram stain results from positive blood cultures on antimicrobial treatment was investigated by examining the changes in antimicrobial treatment at the time when Gram stain results were reported for all bacteremia episodes. The antimicrobial prescriptions were collected from the medical files of all patients with bacteremia. Impact on treatment was categorized as either (i) initiation of appropriate antimicrobial therapy, (ii) streamlining of antimicrobial therapy, or (iii) no treatment alterations. Antimicrobial therapy was considered appropriate when the pathogen isolated was susceptible to the prescribed therapy in vitro. Therapy was considered inappropriate when at least one of the pathogens isolated was intrinsically resistant or resistant in vitro to the prescribed therapy. Streamlining of antimicrobial therapy was defined as the cessation of one of the antimicrobial agents in case of combination therapy or a switch to a drug with a narrower spectrum.

Statistical analysis. Differences in transport times, TTD, TTR, and the numbers of contaminated bottles were analyzed using nonparametric tests (the Mann-Whitney U test or the chi-square test where appropriate). A two-sided P value of <0.05 was considered statistically significant. All statistical analyses were performed with SPSS, version 16.0 for Windows (SPSS Inc., Chicago, IL).

RESULTS

Comparability of the study period and control period. The study and control periods were comparable with respect to the number of patients visiting the ED, the number of patients from whom blood cultures were collected, the number of bottles collected per patient, and the number of episodes of bacteremia (Table 1). In the study period, fewer blood cultures needed to be excluded because of incomplete data collection. This was probably due to the strict instructions to the ED staff to fill out the collection time and date. Blood cultures with incomplete collection data corresponded to 21 episodes of bacteremia in the study period and 33 in the control period. After the exclusion of cultures with incomplete collection data, 2,598 bottles from 779 patients were available for analysis in the study period, as opposed to 1,947 bottles from 589 patients in the control period. In the study and control periods, 62 (2.4%) and 32 (1.6%) bottles were considered contaminated (P = 0.102). Isolates representing contamination were predominantly coagulase-negative staphylococci (n = 72) and Gram-positive rods (n = 17). After the exclusion of contaminated blood cultures, 235 true-positive bottles from 79 episodes of bacteremia in the study period and 182 true-positive bottles from 70 episodes of bacteremia in the control period (P, 0.635) remained for analysis.

Transport time and TTD. The median transport times in the study and control periods were comparable—14 h (range, 0 to

### TABLE 1. Characteristics of the study and control periods

<table>
<thead>
<tr>
<th>Period</th>
<th>No. of ED&lt;sup&gt;a&lt;/sup&gt; visits at which a BC&lt;sup&gt;b&lt;/sup&gt; was obtained/total visits (%)</th>
<th>Total no. of bottles collected (no. per visit)</th>
<th>No. (%) of ED visits with incomplete collection data</th>
<th>No. (%) of ED&lt;sup&gt;a&lt;/sup&gt; visits with incomplete collection data</th>
<th>No. of evaluable BC bottles</th>
<th>No. of true-positive bottles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study</td>
<td>2,598 (3.3)</td>
<td>192 (20)</td>
<td></td>
<td></td>
<td>779</td>
<td>235</td>
</tr>
<tr>
<td>Control</td>
<td>1,947 (3.4)</td>
<td>291 (33)</td>
<td></td>
<td></td>
<td>589</td>
<td>182</td>
</tr>
</tbody>
</table>

<sup>a</sup> ED, emergency department.

<sup>b</sup> BC, blood culture.
46 h) and 15 h (range, 0 to 57 h)—but for unknown reasons, more bottles had transport times of >24 h in the control period (P < 0.05) (Table 2). In the study period, the preincubated bottles had a longer median transport time than nonpreincubated bottles (16 h [range, 0 to 46 h] versus 4 h [range, 0 to 44 h]; P < 0.001), confirming that the majority of bottles for which the transport time was expected to be long were successfully preincubated at 37°C. The median transport times for positive bottles were similar in the study and control periods (14 h [range, 0 to 33 h] and 13 h [range, 0 to 36 h], respectively; [P, 0.843]). The median transport time for 71% of the positive bottles in the study period was 8 h (range, 1 to 339 h). In contrast, the median transport time was 12 h (range, 1 to 121 h) (P, <0.001) (Table 3).

In the study period, the median TTD of all positive bottles was 7 h (range, 1 to 339 h). The median TTD in the control period was 5 h longer, i.e., 12 h (range, 1 to 121 h) (P, <0.05) (Table 3).

Controls within the study period. To evaluate the impact of preincubation within the study period, we compared the TTDs of preincubated and nonpreincubated positive bottles with transport times of >8 h. Ideally, all these bottles would have been preincubated; however, 21 of the 153 positive bottles detected by the Bactec 9240 system with a transport time of >8 h had not been preincubated and therefore could serve as controls within the study period.

The median TTD of the 132 bottles that were preincubated and detected by the Bactec 9240 system was 3 h (range, 1 to 339 h). In contrast, the median TTD was 8 h (range, 4 to 44 h) for the 21 bottles without preincubation (P, <0.001). The median TTD of the 130 positive bottles with a transport time of >8 h in the control period was 12 h (range, 1 to 121 h). There was no significant difference between the TTD of the 21 bottles without preincubation and the TTD for the control period (P, 0.11 by the Mann-Whitney U test). This suggests that the control period is comparable to the study period for these bottles with long transport times.

![FIG. 2. Time interval between blood culture collection and the reporting of the Gram stain result to the clinician. Each horizontal line represents one episode of bacteremia. The time of arrival at the laboratory is designated 0 h. The left side of each graph represents the time between the collection of the blood culture and its arrival at the laboratory. Results for preincubated bottles (red lines) were reported significantly earlier than those for nonpreincubated bottles (black lines).](http://jcm.asm.org/)
TABLE 3. Time to detection and time to reporting of Gram stain results for positive blood cultures in the study and control periods

<table>
<thead>
<tr>
<th>Period and transport time</th>
<th>No. of true-positive bottles</th>
<th>Median (range) TTD (h)</th>
<th>Median (range) TTR per episode (h)</th>
<th>No. of episodes of bacteremia for which the Gram stain results were reported at the following timea</th>
<th>% of all episodes of bacteremia for which the Gram stain results were reported at the following timea</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Study</td>
<td>235</td>
<td>7 (1–339)</td>
<td>19 (1–133)</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>182</td>
<td>12 (1–121)</td>
<td>34 (4–134)</td>
<td>1</td>
</tr>
<tr>
<td>≦8 h</td>
<td></td>
<td></td>
<td></td>
<td>5</td>
<td>14</td>
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<tr>
<td>9–16 h</td>
<td></td>
<td></td>
<td></td>
<td>15</td>
<td>71</td>
</tr>
<tr>
<td>17–24 h</td>
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<td></td>
<td></td>
<td>2</td>
<td>9</td>
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<td>&gt;24 h</td>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td>6</td>
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<tr>
<td></td>
<td>Study</td>
<td>235</td>
<td>7 (1–339)</td>
<td>19 (1–133)</td>
<td>14</td>
</tr>
<tr>
<td></td>
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<td>1</td>
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<tr>
<td>≦8 h</td>
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<td>5</td>
<td>14</td>
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<td>9–16 h</td>
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<td>15</td>
<td>71</td>
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<td>17–24 h</td>
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<td>9</td>
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<td>&gt;24 h</td>
<td></td>
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<td>2</td>
<td>6</td>
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</table>

a TTR, time to reporting of the Gram stain results.

b Day 0, the day on which the blood culture was collected; day 1, the first day after collection; day 2, the second day after collection.

**TTR and clinical impact.** The median time to reporting (TTR) was significantly shorter in the study period (19 h [range, 1 to 133 h]) than in the control period (34 h [range, 4 to 134 h]) (P < 0.05) (Table 3). In the study period, 85% of the positive blood cultures were reported on the day of collection or on the following day, compared to 47% in the control period. The TTR for each episode of bacteremia in each period is shown in Fig. 2. Time zero corresponds to the arrival of the blood culture in the laboratory.

The impact of Gram stain results for positive blood cultures on antimicrobial management could be determined for 134 of all 149 episodes of bacteremia. The reporting of Gram stain results led to the initiation of appropriate antimicrobial treatment, the streamlining of antibiotic therapy, and no change in antimicrobial management in equal percentages during the study period and the control period (P, 0.826). Appropriate antimicrobial therapy was initiated in 20 episodes of bacteremia (15%); therapy was streamlined in 26 episodes (19%); and the Gram stain of the positive blood culture did not contribute to the choice of therapy in 88 episodes (66%). The microorganisms that were isolated from the blood cultures were comparable in the study and control periods (Table 4).

**False-negative Bactec 9240 results after preincubation.** Gram stains prepared on arrival at the laboratory were positive for 88 bottles from 33 episodes of bacteremia. Of these, 85% turned positive in the Bactec 9240 system, but 13 bottles (6 aerobic, 5 anaerobic, and 2 pediatric) remained negative. Despite these 13 false-negative results obtained by the Bactec 9240 system after preincubation, only 2 episodes with a positive Gram stain remained undetected by the blood culture system, because another bottle of the same blood culture set or another bottle of a set collected at the same time did turn positive in most episodes. Subcultures made on arrival from bottles with negative Gram stains were positive the next day for 35 bottles from 10 episodes. However, 9 of these 10 episodes were detected by the Bactec 9240 system before the result of the subculture was known; thus, these subcultures did not contribute to faster reporting of positive results. Four bottles with positive subcultures (2 aerobic and 2 anaerobic) remained negative in the Bactec 9240 system, and 1 bacteremia episode would have been missed if subcultures had not been made. Thus, 3 episodes of bacteremia remained undetected by the Bactec 9240 system. The median transport time of the bottles that were positive on arrival but remained undetected by the Bactec 9240 system was the same as the median transport time of all preincubated bottles, i.e., 16 h (range, 10 to 36 h).

We also calculated what the TTR would have been if Gram stains and subcultures had not been made. Based on the positive signal of the Bactec 9240 system, the median TTR would have increased from 19 to 22 h (range, 1 to 133 h). This would have resulted in the reporting of Gram stain results on the day of collection in 12% of the episodes of bacteremia, and 1 day, 2 days, or more than 2 days after collection in 62%, 16%, and 11% of the episodes. Moreover, 3 episodes of bacteremia would have been missed by the Bactec 9240 system.

**DISCUSSION**

We investigated the clinical impact of preincubation of blood cultures at 37°C when a long delay before entry into the automated blood culture system was expected. Compared to that for storage at room temperature, the median TTD of bacteremia decreased from 12 h to 7 h, and therefore, the median TTR decreased from 34 h to 19 h. This resulted in reporting of the Gram stain results on the day of blood culture collection or 1 day after collection for 85% of the patients with...
bacteremia compared to 47% without preincubation. The reporting of Gram stain results of positive blood cultures resulted in the initiation of appropriate antimicrobial therapy or the streamlining of therapy for 36% of the patients.

The effect of preincubation on the TTD of bacteremia has been studied in a clinical setting only once before. Lemming et al. described a comparable TTD of 7.2 h with preincubation at 35°C (8). As in our study, a significant reduction in the TTD was shown, but the true advantages of preincubation for patient management, i.e., the time to reporting of positive results and the impact on antimicrobial therapy, were not investigated. In our study, the median TTR without preincubation was 34 h, compared to 19 h \((P, <0.001)\) with preincubation. This is in keeping with the median time interval of 21.5 h between blood culture collection and the reporting of Gram stain results reported by Bengtsson et al., who examined the loading of a blood culture system 24 h a day in the Infectious Diseases department of their hospital (2).

The downside of preincubation with Gram staining and subculture on arrival at the laboratory was the labor intensiveness and the cost of supplies involved. In addition, a larger number of preincubated positive cultures were interpreted as contaminated, possibly as a result of the extra handling of the cultures in the laboratory.

Preincubation without Gram staining and subculture would be less costly and would not entail the risk of contamination in the laboratory. However, studies with experimentally inoculated blood culture bottles have shown a higher occurrence of false-negative results after preincubation at 37°C for at least 24 h than after storage at room temperature (4, 10). False-negative results can occur when the system fails to detect bacterial growth, because significant bacterial growth and the concomitant CO₂ production have taken place before the entry of the bottles into the automated system. Indeed, in our study, with a median preincubation time of 14 h, 9% of the positive bottles remained negative in the Bactec 9240 system, and 3 of the 79 episodes of bacteremia (4%) would have been missed without the preparation of Gram stains and subcultures before the entry of the bottles into the Bactec 9240 system. In the previous study that investigated preincubation, 15% of the positive bottles remained negative in the Bactec 9240 system (8). This higher proportion may be explained by the longer median preincubation time of 18 h, compared to 14 h in our study.

As in previous studies, anaerobic subcultures from the bottles were not performed in our study, and false-negative results due to preincubation in case of bacteremia with anaerobes may have been missed.

Based on our results and the experimental studies, preincubation at 37°C for as long as 12 h may be considered without Gram staining and subculture on arrival to reduce the costs and workload while minimizing the risk of false-negative results. In our study, this would have obviated Gram staining and subculture on arrival for 56% of the bottles, while 2 positive bottles and 1 episode of bacteremia would have been missed. The remaining 15 false-negative bottles had been preincubated for more than 12 h. However, performing Gram stains and subcultures for a large proportion of the blood cultures submitted would probably still be unacceptable in many laboratories.

Alternatively, if the laboratory is located in the hospital, a continuously monitoring blood culture system could be placed outside the laboratory. Two studies have investigated the effect of rapid placement of blood culture bottles directly into the blood culture system on the time to reporting of positive results. The study by Bengtsson et al. showed a TTR similar to that found in our study (2). In the second study, by Kerremans et al., a continuously monitoring incubator was installed outside the laboratory (6). The latter study reported a longer TTR of 29 h. The shorter TTRs in our study and the study by Bengtsson et al. were probably due to the availability of the incubator in the department where the blood cultures were collected, as opposed to the study by Kerremans et al., where the incubator was located near the laboratory, and blood cultures from the entire hospital had to be transported to this location. Thus, if immediate incubation is offered 24 h a day by the microbiology laboratory, either by preincubation or by the availability of a blood culture system outside the laboratory, it is important that blood cultures be transported to the incubator immediately after collection in order to obtain the greatest possible benefit. The hospital staff should be informed of the importance of rapid incubation of blood cultures. Twenty-four-hour service for Gram staining and subculturing from positive bottles could further improve diagnostic speed, since a substantial proportion of positive bottles were detected outside laboratory operating hours. When a true-positive bottle was detected by the Bactec 9240 system outside laboratory operating hours (n = 224), the median time between the Bactec 9240 positive signal and the removal of the first positive bottle was 8 h, similar to the 8.4 h reported previously (2). However, 24-h service is not available in most Dutch laboratories.

The impact of microbiology results on antimicrobial therapy has been described previously (1, 3, 6). In our study, we confirm the importance of Gram stain results for the antimicrobial management of patients with bacteremia. For 12% of patients with bacteremia, antimicrobial treatment was initiated or changed from inappropriate to appropriate therapy, and for 24%, antimicrobial treatment could be streamlined. The actual impact of blood culture results on patient management was probably even more substantial, because changes to therapy based on the results of bacterial identification and susceptibility testing were not investigated. Studies that did investigate therapy changes based on all blood culture results reported changes in 64 to 88% of cases (3, 6). However, the contribution of microbiology results to antimicrobial management differs for different settings and depends, among other factors, on local antimicrobial policies and the local epidemiology of resistance.

The most important limitation of our study was its nonrandomized character and the use of a historical control period. However, the study and control periods were similar with respect to the number of ED visits, the number of blood cultures collected, the transport time, and the number of positive blood cultures. During the control period, Gram stains and subcultures were not prepared on arrival at the laboratory, and it is not known to what extent this procedure, rather than preincubation, contributed to the decreased TTR during the study period. However, besides the reduced TTR, the TTD by the Bactec 9240 system was also reduced by 5 h, supporting the conclusion that preincubation at 37°C contributed to the de-
creased TTR. The finding that blood cultures with a transport time of >8 h that inadvertently were not preincubated in the study period had a TTD similar to that of blood cultures with a transport time of >8 h in the control period (P = 0.11) further supports the comparability of the two periods. Investigator bias could potentially have resulted in more-rapid handling of bottles in the laboratory during the study period. However, this was not the case, since the interval between the arrival of bottles in the laboratory and their entry into the Bactec 9240 system was the same during the study and control periods. The time between a positive signal and removal from the Bactec 9240 system was shorter during the study period than during the control period (4.4 h versus 6.0 h [data not shown]). This difference may have resulted from the enthusiasm of the technicians during the study period. However, this time difference is relatively small compared to the 15-h reduction in the TTR in the study period.

Although nowadays clinical microbiology laboratories are equipped with instruments that can produce results 24 h a day, most laboratories do not keep staff on hand to operate these instruments 24 h a day. The results from our study are likely to be representative for all laboratories with limited operating hours and storage of blood cultures at room temperature before their entry into the blood culture system. In our study, approximately two-thirds of all blood cultures were collected outside laboratory working hours. We demonstrated that with immediate incubation of these bottles at 37°C by hospital staff, the TTR could be decreased to less than 24 h for the majority of patients. It should be noted that our results were obtained with the Bactec 9240 system and may not be valid for other automated systems.

The automated blood culture systems have decreased the workload in the laboratory. However, to make optimal use of the system, the time to the entry of blood culture bottles into the system should be minimal. Where possible, laboratories should endeavor to make an automated blood culture system available outside the laboratory so as to enable immediate incubation.

REFERENCES