Decreased Mortality Associated With Prompt Gram Staining of Blood Cultures

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Abstract

Gram stains of positive blood cultures are the most important factor influencing appropriate therapy. The sooner appropriate therapy is initiated, the better. Therefore, it is reasonable to expect that the sooner Gram stains are performed, the better. To determine the value of timely Gram stains and whether improvement in Gram stain turnaround time (TAT) is feasible, we compared data for matched pairs of patients with cultures processed promptly (<1 hour TAT) with data for patients with cultures not processed promptly (≥1 hour TAT) and then monitored TAT by control charting.

In 99 matched pairs, average difference in time to detection of positive blood cultures within a pair of patients was less than 0.1 hour. For the less than 1 hour TAT group, the average TAT and crude mortality were 0.1 hour and 10.1%, respectively; for the 1 hour or longer TAT group, they were 3.3 hours and 19.2%, respectively (P < .0001 and P = .0389, respectively). After multifaceted efforts, we achieved significant improvement in the TAT for Gram stains.

The Institute of Medicine has recommended that medical practices become more patient-centered.1 Two notable accomplishments are in the care of myocardial infarction and stroke. In patients with acute myocardial infarction, reduced door-to-balloon time for primary angioplasty decreases mortality.2 Likewise, in the setting of stroke, studies suggest that the maximum benefit of thrombolytic therapy occurs in the first 3 hours after onset of symptoms.3 In each disease, acceleration of the provision of appropriate care results in improved outcomes, ie, patients who receive appropriate therapy faster have decreased morbidity and mortality.

Pathologists and clinical microbiologists may have a similar opportunity to improve patient outcomes by more timely processing of Gram stains for positive blood cultures. However, it is possible that delayed processing of positive cultures may have little impact on the care of patients because up to 75% of patients who had blood drawn for culturing have already begun to receive presumptive antibiotics before their specimens yielded an organism.4 Nevertheless, there is ample evidence that delays in initiation of appropriate therapy have adverse consequences.5-8 In addition, Beckmann et al9 found that increased time to notification of positive blood culture results was associated with increased length of stay in the hospital. Other benefits of early notification (such as the use of more narrow-spectrum antibiotics and improved antibiotic stewardship) would also be expected.10

This study evaluated the timely processing of positive cultures (ie, rapid Gram staining and notification of caregivers) compared with delayed processing by using matched control subjects. We then evaluated whether efforts to accomplish effective 24-hour, 7-day-per-week coverage of this duty were achievable in a hospital laboratory.
Materials and Methods

Study Population

This study received approval from an institutional review board. Patients with bloodstream infections were matched for disease severity, infecting organism, and time to detection of the first positive blood culture. Their clinical and financial data were then analyzed. Figure 1 depicts the design of the study.

Matching Patients

Each patient in the less than 1 hour turnaround time (TAT) group (group 1) was matched with a patient in the TAT of 1 hour or longer group (group 2). Only patients with 1 organism causing a bloodstream infection per admission were considered in this study, ie, patients who had polymicrobial bacteremia or fungemia were excluded. (If patients had a probable contaminant such as coagulase-negative Staphylococcus isolated from only one of multiple cultures, they were still considered for the study.) Data from a patient were used only once per admission. Patients in a given pair had an infection with the same organism, had similar times to detection, were hospitalized within 1 year of each other, and had identical disease severity. The computer program All Patients Refined DRG Software Program (3M Health Information Systems, Murray, UT) was used to assess disease severity. This system uses a scale of 1 to 4 to rank patients on the basis of complications and comorbidities from minor complications to major problems. The classifications are based on age, procedures (surgical and nonsurgical), multiple diagnoses, and combinations of these factors.

The process for matching patients follows. (1) Patients were grouped by infecting organism; within this group, they were grouped by time to detection. (2) An index patient with a TAT of 1 hour or longer was selected and then matched with a patient who had the closest time to detection to the index patient, a TAT of less than 1 hour, and identical disease severity. During the matching process, other parameters such as length of stay, costs, mortality, and antibiotic history were not accessed or considered.

Definitions

Time between drawing of blood and incubation: the time elapsed between the time written on the blood culture bottles noting when the blood was drawn and the time the bottles were placed into the blood culture incubator. This portion of the study was performed at a time different (January 2006) from when the study population was hospitalized (2001-2005). The policies regarding blood cultures have been unchanged since 2001 (Figure 1).

Time to detection: the time elapsed between the time the blood was loaded into the incubator and the time the culture became positive (ie, when the incubator or instrument detected growth and sounded an alarm to alert a technologist). Only the first episode of bacteremia or fungemia and the first bottle that became positive for a patient were considered in time to detection (Figure 1).

* Staphylococcus aureus, Streptococcus pneumoniae, Streptococcus pyogenes, Streptococcus groups B and G, Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae, Enterobacter cloacae, Serratia marcescens, Pseudomonas aeruginosa, and Candida albicans.

![Diagram of a study of turnaround time (TAT) of Gram stains.](https://example.com/diagram.png)
Gram stain TAT: the time elapsed between the time the first blood culture was detected by the incubator as positive and the time the bottle was unloaded from the incubator. (This was used as a surrogate marker for Gram stain TAT and the time the report is telephoned to the physician. The Gram stain was performed as soon as possible after the bottle was unloaded from the incubator, and the result of the Gram stain was then telephoned to the physician caring for the patient, regardless of time of day. Our policy on all shifts was to call the physician first. If the physician was unavailable, we notified the nurse caring for the patient.) (Figure 1).

Notification time: the time between the bottle being unloaded from the incubator and the time noted in the medical record when the physician (or nurse) was notified (Figure 1).

Length of stay: the number of days of hospitalization.

Positive length of stay: the number of days between the date the culture became positive and the date of discharge.

Shifts: Day shift, 7:00 AM to 3:00 PM; evening shift, 3:00 PM to 11:00 PM; night shift, 11:00 PM to 7:00 AM.

Variable costs: As opposed to fixed costs (which include costs such as depreciation of the building and salaries for staff), variable costs fluctuate with each individual patient, eg, laboratory and radiologic tests, nursing care, and consumables such as pharmaceuticals, medical and surgical supplies, and rehabilitation costs.

Assessment of antibiotic therapy: The antibiotic was deemed appropriate if the pathogen tested was susceptible to it; if no susceptibility data were available (eg, no susceptibility testing was done on Candida), information from the hospital’s current antibiogram or from standard textbooks was used.

Laboratory Techniques

Blood cultures were performed using standard methods. When a bloodstream infection was suspected, generally, 2 blood cultures were obtained from 2 separate venipunctures. Approximately 20 mL per venipuncture was drawn from the patient, inoculated into 2 bottles (1 with aerobic broth and 1 with anaerobic broth), and then incubated in a continuous monitoring blood culture instrument, BacT/ALERT (bioMérieux, Durham, NC). When the blood culture became positive, the instrument sounded an alarm. As soon as possible, a technologist unloaded the bottle from the instrument, performed a Gram stain on the broth from the bottle, and notified the physician of the results. Organisms were subsequently identified and tested for antibiotic susceptibilities using standard methods. The data on organism isolated, time to detection, Gram stain TAT, and patient name were retrieved electronically from the BacT/ALERT.

Statistical Analysis

The clinical data management department supplied clinical and financial data (such as length of stay, costs, and mortality). Data on antibiotic therapy was obtained electronically from individual medical records. Statistical analysis was performed by using the paired t test and McNemar χ² test.

Process Improvement for Gram Stain TAT

In a separate period (April 2006 to March 2007), efforts were made to decrease TATs of Gram stains for all positive blood cultures, regardless of whether they were the first positive specimen for a patient. Efforts included an educational approach with in-service sessions for night staff, recruitment of a person designated to perform Gram stains at night, ongoing feedback to the night staff when TATs were unacceptable, and installation of a baby monitor–type speaker between the microbiology laboratory and the core laboratory to facilitate staff hearing the audio alert of a positive specimen from the BacT/ALERT. TATs were transferred electronically from the BacT/ALERT to an Excel spreadsheet (Microsoft, Redmond, WA); weekly averages were monitored on a control chart.13

Results

Time Between Drawing of Blood and Incubation

Of 100 blood cultures studied to determine time between drawing of blood and the time the bottles were put into the incubator, all 100 had the time the culture was drawn written on the bottles. Of these, 64% were loaded into the incubator within 1 hour of being drawn, and 92% were loaded into the incubator within 2 hours of being drawn. The average time between drawing blood and incubation was 1.0 hour.

Notification Time

To determine notification time, 52 paired charts were examined. In more than 62% of the charts examined, the notification time was 1 hour or less after the culture was unloaded; in 81% of the charts, notification time was within 2 hours of unloading. The average notification time was 1.2 hours for group 1 and 1.5 hours for group 2.

Time to Detection

There were 99 matched pairs of patients. For all pairs, the range of differences in time to detection between matched patients was 0 to 6.8 hours. For 97% of the pairs, the matched patients within the pair had times to detection within 4.6 hours of each other. The average of the differences in time to detection for matched patients was less than 0.1 hour. Overall, the differences between patients within a pair essentially nullified each other. For example, one pair had a time to detection of 9.3 hours for the patient in group 1 and 10.5 hours for the patient in group 2, giving the latter patient a 1.2-hour longer time to detection. This difference was assigned a +1.2 hour
difference. Another pair had a time to detection of 14.6 hours for the patient in group 1 and 13.3 hours for the patient in group 2, giving the latter patient a 1.3-hour shorter time to detection. This was considered a −1.3 hour difference.

The average difference in Gram stain TAT was 3.2 hours ($P < .0001$) [Table 1]. The smallest difference between TAT for 2 patients in a single pair was 0.7 hour. Only 3 pairs of patients had TATs within 1 hour of each other; the other 96 pairs had TATs that were more than 1 hour apart.

The average TAT for the Gram stain, crude mortality rate, length of stay in the hospital, positive length of stay, and variable costs for group 1 were 0.1 hour, 10.1%, 11.0 days, 7.9 days, and $9,543$, respectively; for group 2, they were 3.3 hours, 19.2%, 10.5 days, 7.7 days, and $9,361$, respectively (Table 1). Only TAT and mortality had statistically significant differences ($P < .0001$ and $P = .0389$, respectively).

Of the TATs less than 1 hour, 86% TAT occurred on the day and evening shifts and 14% were on the night shift; of TATs 1 hour or longer, 1% occurred on the day and evening shifts and 99% on the night shift. The average time to detection of all bloodstream infections in this study was 13.6 hours. Of the cultures, 50 became positive on the day shift, 36 on the evening shift, and 112 on the night shift [Figure 2]. Approximately 43% of the cultures became positive on the day and evening shifts, and approximately 57% of the cultures became positive on the night shift. Further analysis revealed the following trend. Among the 86 patients whose cultures became positive on the day and evening shifts, 8 (9%) died. However, on the night shift, 112 patients had blood cultures that became positive, and 21 died (18.8%; analysis on unpaired data, night vs day/evening, $P = .0624$; Figure 2). There were 12 pairs of patients in whom the cultures became positive only at night. Of these, 6 patients in group 2 died, whereas only 2 patients in group 1 died ($P = .1039$).

Of 86 pairs of patients for whom sufficient antibiotic data could be retrieved, 53 patients in group 1 received appropriate antibiotic therapy before the time the blood culture was obtained and 49 patients in group 2 received appropriate antibiotics at this time ($P = .5553$). Within 24 hours of the Gram stain report, 77 patients in group 1 received appropriate antibiotic therapy and 79 patients in group 2 received appropriate antibiotic therapy ($P = .5930$). Within 24 hours of the susceptibility report, 83 patients in group 1 received appropriate antibiotic therapy and 84 patients in group 2 received appropriate antibiotic therapy ($P = .5637$) [Table 2].

Of 99 matched pairs of patients, 34% had a bloodstream infection with *Staphylococcus aureus*; 22% with *Escherichia coli*; 18% with *Pseudomonas aeruginosa*; 7% each with *Klebsiella pneumoniae* and *Enterococcus faecalis*; 5% with *Serratia marcescens*. The breakdown of mortality by infecting organism is shown in [Table 3].

In a subsequent period, TAT was monitored to determine whether efforts to significantly decrease TAT could be accomplished. [Figure 3] shows the average TAT by week. Minimal effects on TAT were seen after in-service sessions to night staff and feedback to the night staff when TATs were

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**Table 1**

Differences in 99 Pairs of Patients by TAT for Laboratory Results

<table>
<thead>
<tr>
<th>Variable</th>
<th>&lt;1 h</th>
<th>≥1 h</th>
<th>Difference</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time to detection (h)</td>
<td>13.7</td>
<td>13.6</td>
<td>0.1</td>
<td>.7860</td>
</tr>
<tr>
<td>Gram stain TAT (h)</td>
<td>0.1</td>
<td>2.3</td>
<td>-2.2</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Mortality rate (%)</td>
<td>10.1</td>
<td>19.2</td>
<td>-9.1</td>
<td>.0389</td>
</tr>
<tr>
<td>Length of stay (d)</td>
<td>11.0</td>
<td>10.5</td>
<td>0.5</td>
<td>.6936</td>
</tr>
<tr>
<td>Positive length of stay (d)</td>
<td>7.9</td>
<td>7.7</td>
<td>0.2</td>
<td>.7920</td>
</tr>
<tr>
<td>Variable costs ($)</td>
<td>9,543</td>
<td>9,361</td>
<td>182</td>
<td>.9150</td>
</tr>
<tr>
<td>Male sex (% of group)</td>
<td>47</td>
<td>49</td>
<td>2</td>
<td>.7773</td>
</tr>
<tr>
<td>Age (y)</td>
<td>69.2</td>
<td>66.6</td>
<td>2.6</td>
<td>.3054</td>
</tr>
</tbody>
</table>

TAT, turnaround time.

* The number of days between the date the culture became positive and the date of discharge.
unacceptable. The most notable impact came after 2 changes: (1) recruitment of a person designated to perform Gram stains on night specimens and (2) directly after another in-service session, feedback combined with the installation of a baby monitor–type device between the microbiology laboratory and the core laboratory to facilitate staff hearing the audio alert of a positive specimen from the BacT/ALERT. Having 7 or more points below the mean in a control chart indicates significant improvement or change; decreased range of variation indicates improved consistency in the process. In our study, 14 points were below the mean, signifying that efforts to significantly improve TAT were successful, and a markedly decreased range of variation was achieved (Figure 3).

Table 2
Differences in Antibiotic Therapy Between 86 Pairs of Patients With TAT of Less Than 1 Hour vs 1 Hour or More*  

<table>
<thead>
<tr>
<th>TAT</th>
<th>Appropriate Antibiotic Therapy Initiated</th>
<th>Before time blood culture became positive</th>
<th>Within 24 h of Gram stain</th>
<th>Within 24 h of susceptibility report</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;1 h</td>
<td>53 (62)</td>
<td>77 (90)</td>
<td>83 (97)</td>
</tr>
<tr>
<td></td>
<td>≥1 h</td>
<td>49 (57)</td>
<td>79 (92)</td>
<td>84 (98)</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.5553</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.5930</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.5637</td>
</tr>
</tbody>
</table>

* Data are given as number (percentage).

Table 3
Mortality Rate of Patients by Infecting Organism  

<table>
<thead>
<tr>
<th>Organism</th>
<th>No. of Patients</th>
<th>Mortality*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Candida albicans</td>
<td>2</td>
<td>1 (50)</td>
</tr>
<tr>
<td>Enterococcus faecalis</td>
<td>14</td>
<td>3 (21)</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>44</td>
<td>5 (11)</td>
</tr>
<tr>
<td>Enterobacter cloacae</td>
<td>2</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>14</td>
<td>2 (14)</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>10</td>
<td>2 (20)</td>
</tr>
<tr>
<td>Serratia marcescens</td>
<td>2</td>
<td>1 (50)</td>
</tr>
<tr>
<td>Staphylococcus aureus (oxacillin-resistant)</td>
<td>22</td>
<td>6 (27)</td>
</tr>
<tr>
<td>Staphylococcus aureus (oxacillin-susceptible)</td>
<td>46</td>
<td>5 (11)</td>
</tr>
<tr>
<td>Streptococcus spp (hemolytic)</td>
<td>6</td>
<td>3 (50)</td>
</tr>
<tr>
<td>Streptococcus pneumoniae</td>
<td>36</td>
<td>1 (3)</td>
</tr>
<tr>
<td>All organisms</td>
<td>198</td>
<td>29 (14.6%)</td>
</tr>
</tbody>
</table>

* Mortality data are given as number (percentage).

Previously, periodic in-services and ongoing feedback to staff on the importance of performing Gram stains STAT on positive blood cultures

Sept Week 4: Addition of a person specifically to perform Gram stains on blood cultures as soon as they became positive

Oct Week 4: Installation of speakers between core lab and microbiology so that alerts notifying technologists about new positive cultures can be easily heard

Figure 3
Control chart for turnaround time (TAT, h).
Discussion

This study evaluated the impact of timely performance of Gram stains on blood cultures when they first became positive. Savinelli et al\textsuperscript{14} documented that patients whose blood cultures were stained in a timely manner had orders written for appropriate antibiotics 3 hour earlier than patients who had cultures with delayed TAT. Herein we have documented a statistically significant increase in the mortality rate for patients who had blood cultures processed after a delay (ie, Gram stain performed ≥1 hour after being detected as positive; $P = .0389$). The vast majority of the patients in group 2 had cultures that became positive on the night shift. Of the 112 patients whose blood cultures became positive on the night shift, 21 died (18.8%), but of the 86 patients whose cultures became positive on the day or evening shift, only 8 died (9%; $P = .0624$).

The finding of increased mortality in patients with delayed Gram staining is expected in view of the findings of other studies that examined closely related aspects of patients with bloodstream infections.\textsuperscript{4,8,10,14-17} In a study of bacteremic patients in the intensive care unit, Ibrahim et al\textsuperscript{12} documented a statistically significant difference in mortality among patients who received appropriate antibiotic therapy and those who did not. Mackenzie et al\textsuperscript{16} audited various approaches to guide empirical therapy in patients with bacteremia. They found poor adherence to the antibiotic policy recommended by local experts, with resulting inadequate ($P = .005$) or excessive ($P \leq .001$) antibiotic treatment and a trend toward increased mortality. Their results confirmed the usefulness of Gram staining positive blood cultures to guide antibiotic therapy in a useful time frame. In the study by Schonheyder and Hojbjerg,\textsuperscript{4} notification of the Gram stain on positive blood cultures elicited changes in antibiotic therapy in 45% of episodes, including commencement of antibiotic therapy in 18%. Thus, results of blood culture have a measurable impact on antibiotic therapy. Weinstein et al\textsuperscript{17} found increased mortality in patients with bloodstream infections whose antibiotics were not switched to appropriate drugs when the Gram stain results were available. Cunney et al\textsuperscript{10} determined that early reporting of Gram stain results from blood cultures, combined with early clinical liaison, resulted in more rational and cost-effective treatment.

Night coverage (11:00 PM to 7:00 AM) for microbiology services (such as prompt staining and reporting of blood cultures) may be inconsistent or unavailable in some locales. Although the College of American Pathologists has recommended immediate notification of caregivers about test results that require prompt management decisions (such as growth in a blood culture), this service may not be provided, even in many accredited hospitals.\textsuperscript{12} These data indicate that delay in obtaining Gram stain results was associated with significantly higher rates of mortality in septic patients and, hence, offer a compelling reason to remove operational barriers to efficient Gram staining on all shifts.

The possibility was also considered that, even after a positive result was called, time to administration of antibiotics differed, affecting patient outcomes. At Memorial Medical Center, Springfield, IL, nursing has 38% of its staffing during days, 35% during evenings, and 28% during nights. The time it takes for pharmacists to process an order for medication differs on shifts, ranging from a low of 13.6 minutes on the evening shift to a high of 27.5 minutes on the night shift (data not shown). It is possible that the difference in order processing accounts for some of the difference in outcomes between days and nights. However, these differences in medication processing times should be the same on the night shift for patients whose cultures were reported less than 1 hour from becoming positive as for patients whose cultures were reported 1 hour or longer after becoming positive. Our data showed that for paired patients who had cultures become positive only at night, the mortality was lower in group 1 (2 deaths in 12 patients vs 6 deaths in 12 patients), although this was not a statistically significant difference. Therefore, we do not believe this to be a significant confounding variable in the current study. More detailed data acquisition in future studies would be required to establish whether this is a significant variable in our institution.

A previous study found a statistically significant decrease in mortality associated with rapid reporting of data from microbiology,\textsuperscript{18} and the present study is consistent with the findings of that study, suggesting that high quality microbiology services positively impact patient care. Obstacles to providing continuous quality services and staffing in microbiology (ie, on evening and night shifts) include the lack of experienced, trained personnel during the evening shift and even fewer on the night shift. Inexperienced technologists have difficulty in interpretation of Gram stains on fluid obtained from bottles (especially bottles with a charcoal additive, which is used widely). Possible solutions include the following: (1) periodic education coupled with regular monitoring with feedback to evening and night staff on the TAT for Gram stains of positive cultures; (2) potential technical advances to decrease difficulty in interpretation of Gram stains from blood culture bottles; (3) ensuring that the cultures become positive as soon as possible by loading them into the instrument as soon as possible after collection; and (4) as last resort, if resources and staffing are limited, adopting a policy that mandates that the first positive blood culture be treated with a higher priority than blood culture(s) that become positive later for an individual patient.

Compared with patients with 1 hour or longer between the time the culture was first detected as positive and the present study is consistent with the findings of that study, suggesting that high quality microbiology services positively impact patient care. Obstacles to providing continuous quality services and staffing in microbiology (ie, on evening and night shifts) include the lack of experienced, trained personnel during the evening shift and even fewer on the night shift. Inexperienced technologists have difficulty in interpretation of Gram stains on fluid obtained from bottles (especially bottles with a charcoal additive, which is used widely). Possible solutions include the following: (1) periodic education coupled with regular monitoring with feedback to evening and night staff on the TAT for Gram stains of positive cultures; (2) potential technical advances to decrease difficulty in interpretation of Gram stains from blood culture bottles; (3) ensuring that the cultures become positive as soon as possible by loading them into the instrument as soon as possible after collection; and (4) as last resort, if resources and staffing are limited, adopting a policy that mandates that the first positive blood culture be treated with a higher priority than blood culture(s) that become positive later for an individual patient.
quality coverage of blood cultures as soon as they become positive may be in the best interests of patients; this study supports constant “24/7” coverage of these instruments. We also have documented that with sufficient effort, changes in processing and staffing can result in significant improvements in TATs, even during times that are difficult to staff.

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