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# Effects of Preincubating Blood Culture Bottles at 37°C during the Night Shift and of Collected Blood Volume on Time to Detection and Days to Final Report

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**Background:** By varying the collected blood volume and storage temperature of the blood culture bottles prior to entry in an automated blood culture system, growth of organisms will be affected.
**Methods:** Blood culture bottles with a 20 mL blood volume per set were stored at 37°C (1st period) and room temperature (RT, 2nd period) upon arrival at the laboratory after working hours compared to baseline period (10 mL, RT). The time to detection (TTD) for all strains and the number of days until the final report after bottle entry were compared among the three periods.
**Results:** The median TTD for all strains was 13.5 h, 10.6 h, and 11.3 h in the baseline (N=268), 1st (N=454), and 2nd period (N=370), respectively ($P<0.001$). The final identification report was available within two days of bottle entry for 12.3%, 30.6% and 15.1% of bottles in the three different periods, respectively ($P<0.001$).
**Conclusion:** Collecting an adequate blood volume is critical to reduce TTD. The preincubation of blood culture bottles at 37°C during the night shift might enable earlier final reports than storage at RT for samples with the same collected blood volume. **(Ann Clin Microbiol 2014;17:14-19)**

**Key Words:** Blood culture, Detection, Storage

## INTRODUCTION

Blood culture is the gold standard for the diagnosis and management of bloodstream infections. After the inoculation of an optimal volume of blood into the blood culture bottles, the bottles should be transferred immediately to a microbiology laboratory and placed into an automatic blood culture instrument [1]. Room temperature is the appropriate transport temperature for blood culture media. The majority of retrieved references [1,2] suggest a maximal interval of 2 h. However, a delay of more than 2 h in either transport or insertion into the instruments is inevitable in certain situations. In particular, this delay might be more common during the night shift or on weekends. Many institutions do not operate their microbiology laboratories on a 24-h basis. A nationwide survey in Korea showed that 39.2% of institutions did not insert blood culture bottles into the culture instruments during the night shift [3]. According to that survey, the bottles were stored at room temperature (RT) in 16.2% and at 37°C in 23.0% of cases. There are no specific guidelines for the optimal storage of blood culture bottles that are delayed by more than 2 h in either transport or insertion into the instruments. Because the principles of microorganism detection are mostly based on the delta color change of the pH indicator or the fluorescence change at the bottom of the bottle, it is suggested that the blood culture samples should be stored at RT because bacterial overgrowth prior to entry may cause a false negative result. However, most bacteria grow much faster at 37°C than at RT; a rapid blood culture report has been associated with a decreased length of stay and hospital charges [4]. If the false negative rate is negligible, the blood culture bottles may be stored at 37°C when direct insertion into the instruments is not possible. Time to detection (TTD) is defined as the period

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from the entry of the bottles to the detection of organisms. The effect of the blood culture bottle preincubation temperature during the night shift on TTD has rarely been reported.

A sufficient volume of inoculated blood is critical for an optimal blood culture procedure. The standard volume for blood collection for adults is 20-30 mL per set. However, the recommended blood volume per set was reported as 10 mL in the most of institutions by a survey [3].

The objective of this study was to evaluate the effects of the blood culture bottle storage temperature, either RT or 37°C, with a time interval of greater than 2 h prior to entry into an automated blood culture system and the different collected blood volumes per set, either 10 or 20 mL, on the TTD and days to final report.

## MATERIALS AND METHODS

### 1. Blood volume and storage temperature

This study was conducted in a tertiary care 800-bed hospital. Adult patients (≥18 years old) who required blood culture were included in the data analysis. This study was approved by an institutional review board. A disinfectant composed of 0.5% chlorhexidine-alcohol was used for skin decontamination. The collected blood was divided into SA and SN bottles (bioMerieux Inc., Durham, NC, USA) equally. All blood culture bottles were transferred to the laboratory as quickly as possible. However, the microbiology laboratory did not operate during the night shift between 6 PM - 9 AM.

During the baseline period (January-April 2011), blood culture bottles with 10 mL of blood volume per set were stored at RT upon arrival at the laboratory during the night shift. During the 1st (January-April 2012) and 2nd (November 2012-Feburary 2013) experimental periods, blood culture bottles with 20 mL blood per set were placed in a 37°C incubator located in the stat laboratory or were stored at RT, respectively.

All of the preincubated bottles that arrived during the night shift were inserted into the BacT/Alert 3D system (bioMerieux Inc.) between 9-10 AM the following morning and were incubated for 5 days. A final subculture of bottles without signal was not performed.

### 2. TTD and final report days

The TTD was recorded using the BacT/Alert 3D software program. The growth of organisms throughout the working day (until 6 PM) was compared among the three study periods. Once the growth of an organism was detected, the bottle was subjected to gram staining, and the results were reported to the attending physician by SMS within an hour. The TTD and the percentage of organism grown within the same working day were compared among the baseline period (RT, 10 mL), 1st experimental period (37°C, 20 mL), and 2nd experimental period (RT, 20 mL). The time elapsed between the entry of the bottles and the final microbiological results (identification and susceptibility test) were analyzed. The positive rate was investigated for each period to evaluate the quality of the blood culture procedure and the possibility of false negatives. The time elapsed and the microbiological results data were retrieved from the hospital's electronic medical record system.

### 3. Frequency of isolates

The microbiological identification and susceptibility tests were performed using Vitek-2 Systems (bioMerieux Inc.). The frequencies of the isolates were compared among the three different study periods.

### 4. Statistical analysis

Statistical significance was evaluated for the TTD using the Kruskal-Wallis test and the percentage of gram stain reports on the same day of incubation or days from bottle entry to final report using $\chi^2$ test in SPSS version 20 (Chicago, IL, USA). A $P$ value $<0.05$ indicated statistical significance.

## RESULTS

### 1. TTD

The TTD is presented in Table 1. Because the numbers of organisms were different in each period, we compared the median and interquartile range (IQR) values. The median TTD was 13.5 hours (8.6-24.9 hours) in the baseline period (RT, 10 mL) (N=268), 10.6 hours (5.5-19.6 hours) in the 1st experimental period (37°C, 20 mL) (N=454) and 11.3 hours (7.2-18.1 hours) in the 2nd experimental period (RT, 20 mL) (N=370) ($P<0.001$). The median TTD for gram-positive organisms was 18.4, 13.7, and 14.3 hours in the baseline, 1st and 2nd experimental periods, respectively ($P=0.001$). There was also a significant difference in the median TTD among the periods for gram-negative organisms (8.8, vs. 7.3, vs. 8.8 hours, respectively, $P=0.003$).

### 2. Detection of organisms within the same working day

The percentages of growth by 6 PM in the same day of in-

**Table 1.** Median time to detection (TTD) and interquartile range (IQR) according to the storage conditions of blood culture bottles and collected blood volume during the night shift

| Strains | RT 10 mL | | 37°C 20 mL | | RT 20 mL | | *P* value* |
|---|---|---|---|---|---|---|---|
| | N | Median TTD (IQR) | N | Median TTD (IQR) | N | Median TTD (IQR) | |
| All strains | 268 | 13.5 (8.6-24.9) | 454 | 10.6 (5.5-19.6) | 370 | 11.3 (7.2-18.1) | <0.001 |
| Gram-positives | 142 | 18.4 (11.4-27.5) | 207 | 13.7 (7.8-20.7) | 152 | 14.3 (9.9-21.9) | 0.001 |
| Gram-negatives | 109 | 8.8 (6.9-11.6) | 230 | 7.3 (4.1-13.8) | 200 | 8.8 (6.1-12.7) | 0.003 |
| Yeasts | 17 | 40.5 (19.0-68.1) | 17 | 29.9 (26.3-39.8) | 18 | 38.8 (16.8-75.4) | 0.592 |

*Kruskal-Wallis test.
Abbreviation: RT, room temperature.

**Table 2.** Time elapsed between bottle entry and blood culture report for bottles preincubated at room temperature (RT) and 37°C during the night shift

| Days | RT 10 mL/set | | 37°C 20 mL/set | | RT 20 mL/set | |
|---|---|---|---|---|---|---|
| | N | % | N | % | N | % |
| 2 | 33 | 12.3 | 139 | 30.6 | 56 | 15.1 |
| 3 | 140 | 52.2 | 179 | 39.4 | 212 | 57.3 |
| 4 | 58 | 21.6 | 83 | 18.3 | 47 | 12.7 |
| ≥5 | 37 | 13.8 | 53 | 11.7 | 55 | 14.9 |
| Total | 268 | 100 | 454 | 100 | 370 | 100 |

$P<0.001$ by $\chi^2$ test.

cubation were 23.2% (56/241) in the baseline period, 41.6% (189/454) in the 1st experimental period and 34.6% (128/370) in the 2nd experimental period ($P<0.001$).

### 3. Time elapsed before final report

The final report of identification and susceptibility was available within two days from bottle entry for 12.3% in the baseline period, 30.6% in the 1st experimental period and 15.1% in the 2nd experimental period ($P<0.001$) (Table 2).

### 4. Positive rate

The positive rates for specimens arriving after working hours were 9.1% (268/2,960) in the baseline period, 11.0% (454/4,139) in the 1st experimental period and 10.0% (370/3,701) in the 2nd experimental period ($P<0.001$). The total positive rates for all specimens arriving during the day or night showed a similar pattern, with 8.3% (614/7,417), 10.1% (792/7,817) and 10% (739/7,360) for the baseline, 1st and 2nd periods, respectively ($P<0.001$). Approximately 40%, 53%, and 50% of the samples had arrived for three periods during the night shift.

### 5. Frequency of isolates

The identification of gram-positive organisms was more common in the baseline period (53%) compared to the 1st (45.6%) and 2nd experimental periods (41.1%) (Table 3, $P=0.012$). In contrast, the identification of gram-negative organisms was significantly more common in the two experimental periods (50.7% and 54.1%) compared to the baseline period (40.7%) ($P=0.003$). Among gram-positive organisms, coagulase-negative staphylococci and *Staphylococcus aureus* were more common in the baseline period. Among gram-negative organisms, *Acinetobacter* spp., *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Salmonella* Typhi were more common in the two experimental periods. The prevalence of *Candida albicans* was 4.1% in the baseline period, whereas that of *Candida* non-albicans was 3.8% in the 1st experimental period (Table 3).

## DISCUSSION

Preincubation time is necessarily extended during weekends or overnight in institutions where microbiology laboratory serv-

**Table 3.** Frequency of isolates from blood culture distributed by storage temperature and blood volume collected

| | RT 10 mL/set | | 37°C 20 mL/set | | RT 20 mL/set | | *P* value† |
|---|---|---|---|---|---|---|---|
| | N | % | N | % | N | % | |
| Gram (+) | 142 | 53 | 207 | 45.6 | 152 | 41.1 | 0.012 |
| CoNS* | 31 | 11.6 | 31 | 6.8 | 24 | 6.5 | |
| *Enterococcus* spp. | 10 | 3.7 | 24 | 5.3 | 24 | 6.5 | |
| *Staphylococcus aureus* | 48 | 17.9 | 54 | 11.9 | 32 | 8.6 | |
| *Staphylococcus epidermidis* | 21 | 7.8 | 36 | 7.9 | 28 | 7.6 | |
| *Streptococcus agalactiae* | 2 | 0.8 | 9 | 2 | 0 | 0 | |
| *Streptococcus pneumoniae* | 8 | 3 | 10 | 2.2 | 8 | 2.2 | |
| Viridans group streptococci | 19 | 7.1 | 28 | 6.2 | 3 | 0.8 | |
| Other gram-positives | 3 | 1.1 | 15 | 3.3 | 33 | 8.9 | |
| Gram (-) | 109 | 40.7 | 230 | 50.7 | 200 | 54.1 | 0.003 |
| *Acinetobacter* spp. | 3 | 1.1 | 14 | 3.1 | 13 | 3.5 | |
| *Escherichia coli* | 57 | 21.3 | 116 | 25.6 | 80 | 21.6 | |
| *Klebsiella pneumoniae* | 21 | 7.8 | 57 | 12.6 | 48 | 13 | |
| *Pseudomonas aeruginosa* | 1 | 0.4 | 14 | 3.1 | 10 | 2.7 | |
| *Salmonella* Typhi | 0 | 0 | 8 | 1.8 | 5 | 1.4 | |
| Other gram-negatives | 27 | 10.1 | 21 | 4.6 | 44 | 11.9 | |
| Yeasts | 17 | 6.3 | 17 | 3.8 | 18 | 4.9 | 0.283 |
| *Candida albicans* | 11 | 4.1 | 0 | 0 | 8 | 2.2 | |
| *Candida* non-albicans | 6 | 2.2 | 17 | 3.8 | 10 | 2.7 | |
| Total | 268 | 100 | 454 | 100 | 370 | 100 | |

*CoNS, coagulase-negative staphylococci other than *S. epidermidis*.
†*P* value by $\chi^2$ test.
Abbreviation: RT, room temperature.

ice is not available 24 hours per day. We noticed that many small hospitals in Korea that lack microbiology facilities stored blood culture bottles for manual detection at RT until sending them to reference laboratories far away. We believe that blood culture bottles for manual detection should always be stored at 37°C to reduce the TTD. This guideline might be applicable even for bottles that are to be analyzed using a continuous monitoring system, as was the case in our study.

The median TTD was reduced from 11.3 hours (RT) to 10.6 hours (37°C) by preincubating the 20 mL per set bottles collected during the night shift (*P*=0.035). The detection rate of organisms within the same working day was 41.6% in the 1st experimental period (37°C) vs. 34.6% in the 2nd experimental period (RT) (*P*=0.044, data not shown). Thus, changing the preincubation temperature enabled the earlier detection of organisms in 7% of cases. The difference in the percentage of final reports available within 2 days between the two experimental periods was 15.5%. There have been very few studies investigating the effect of preincubation on the same day gram stain report or on the time elapsed until the final report. Kerremans et al. [5] compared the intervals between the blood culture collection and the growth detection and susceptibility testing results for cultures stored at different temperatures prior to analysis during the night shift. These values for RT storage were 39 and 70 hours, longer than 29 and 62 hours for 37°C storage. van der Velden et al. [6] also noted that the gram stain results were available within 1 day of blood collection for 47% of samples at RT storage and 85% of 37°C preincubated samples. The data obtained in these studies showed a better performance with the 37°C preincubation. In a previous study [6], the preincubation of blood culture bottles at 37°C resulted in a 15-hours reduction in the median time to reporting gram stain results, allowing the initiation of either appropriate antimicrobial therapy (12%) or streamline therapy (24%). Although we used time of entry of bottles instead of blood collection time for the analysis, our study revealed that the 37°C preincubation has an advantage over blood culture detection than RT storage.

Unfortunately, other time parameters, such as the sample collection time, the sample arrival time at the laboratory, and the gram stain report time, could not be recorded precisely in this study. Therefore, we analyzed only the detection time from the entry of the bottles. It was assumed that the average pre-

incubation time was similar between all three periods.

We did not perform final blind cultures after 5 days of incubation due to a high workload. Therefore, it was not possible to determine the rate of false negative results of the continuous monitoring system. The major concern of preincubation at 37°C is the possible increase in false-negative rates. Theoretically, the presence of fully grown bacteria may cause a false negative when the bottles are preincubated at 37°C. All bottles preincubated at 37°C should be carefully observed for any sign of bacterial growth by laboratory personnel prior to insertion into the machine. The routine subculture of these bottles could be considered to avoid false negatives [7], although this procedure requires significant laboratory resources. In a previous study [6], during a 6-month period, only 3 cases (4%) were not detected using the Bactec 9240 system (Becton Dickinson Microbiology Systems, Cockeysville, MD, USA), suggesting that the false negative rate was negligible. Chapin and Lauderdale [8] also reasoned that Bactec 9240 vials may be incubated at 35°C for up to 24 h with a minimal loss (2.1%) of detection.

However, in a clinical study of terminal subcultures of bottles analyzed using the Bactec 9240 blood culture system, Lemming et al. [9] reported that 76 positive bottles went undetected by the instrument, of which 68 were preincubated at 35°C and 8 were preincubated at RT. Although there was a significant difference in TTD (7.2 hours for 35°C vs. 13.4 hours for RT), the high false negative rate observed when the bottles were preincubated at 35°C could not be ignored. Akan and Yildiz [10] insisted that the risk of false negatives increases 2.5 times at 35°C compared with 22°C and that this number may differ according to the type of instrument used. Although there has been a report of false negatives when detecting *P. aeruginosa* by BacT/Alert-FA in medium preincubated at 36°C [11], we did not find this phenomenon. The frequency of *P. aeruginosa* and *Acinetobacter* spp. in cultures preincubated at 37°C was not inferior to those stored at RT in our study. Also, we could not observe decline of positive rate during the prestorage at 37°C (10.1%) compared to prestorage at RT (10%), suggesting the risk of false negative does not seem high by the prestorage at 37°C.

While a similar TTD was found between the two experimental periods, the availability of a final report within 2 days of entry for bottles stored at 37°C was almost twice that of bottles stored at RT. The distribution of gram-positives and gram-negatives was similar between the two study periods and, therefore, may not be the reason for the large gap between the availability of the 2-day reports. Instead, we assumed that during the 2nd experimental period, there were more cases exceeding 8 hours of TTD that may have been reported on day 3.

We used different protocols for the blood culture procedures between the baseline (10 mL blood volume collected) and the two experimental periods (20 mL blood volume collected). The median TTD for all strains was reduced significantly in the samples with 20 mL of collected blood compared to those with 10 mL. The positive rate increased by approximately 2% in the two experimental periods compared to the baseline; this difference may be due to either the increased blood volume or the different patient groups.

The different time periods and patient groups in each study period may have substantially affected the culture results. A double-blinded prospective study of bottle storage at either RT or 37°C within the same period might give more objective data. Although this study has several limitations, we may conclude that collecting adequate blood volume is critical to reduce TTD and that there is the significant benefit of receiving earlier final reports when preincubating the bottles at 37°C when delayed entry is inevitable.

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=국문초록=

# 야간 근무 시 혈액배양 병의 보관 온도와 채혈량이 균 검출시간과 최종 보고시간에 미치는 영향

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**배경**: 자동화장비에 혈액배양 검체를 투입하기 전 검체 보관 온도와 채혈량에 따라 균 검출시간이 달라질 수 있다.
**방법**: 야간에 도착한 혈액배양 검체를 37°C에 보관(20 mL, 1차 연구기간) 했을 때와 실온에 보관(20 mL, 2차 연구기간)했을 때를 비교하였다. 채혈량이 적은 검체(10 mL)를 실온에 보관한 것을 대조군으로 하였다. 세 가지 연구기간 동안 균 검출시간과 최종 보고일을 비교하였다.
**결과**: 균 검출 중앙값은 연구기간에 따라 각각 13.5시간(N=268, 대조군), 10.6시간(N=454, 1차 연구기간), 11.3시간(N=370, 2차 연구기간) 이었다($P<0.001$). 혈액배양 검체를 장비에 투입한 후 2일 이내 최종 동정 및 감수성결과 최종 보고가 가능했던 경우는 각각 12.3%, 30.6%, 15.1%였다($P<0.001$).
**결론**: 충분한 양의 혈액채취는 균 검출시간을 줄이는데 중요하였다. 야간에 도착한 혈액배양 검체를 실온보다 37°C에 보관함으로써 최종보고를 좀 더 신속히 할 수 있었다. [Ann Clin Microbiol 2014;17:14-19]

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