J Infect Chemother (2009) 15:49–53
DOI 10.1007/s10156-008-0664-6

NOTE

**Takashi Saito · Yoshitsugu Iinuma · Shunji Takakura
Miki Nagao · Aki Matsushima · Michinori Shirano
Satoshi Ichiyama**

Delayed insertion of blood culture bottles into automated continuously monitoring blood culture systems increases the time from blood sample collection to the detection of microorganisms in bacteremic patients

Received: May 30, 2008 / Accepted: December 18, 2008

Abstract This study examined the effects on patients with bacteremia of delaying the insertion of a blood culture bottle into an automated, continuously monitoring blood culture system. We investigated the time taken from the collection of blood samples (collection) to the insertion of blood culture bottles inoculated with blood samples into the instrument (insertion), and compared the mean detection time from collection to a positive signal from the instrument with the time between collection and insertion. The study was conducted from January 2003 to December 2004 at Kyoto University Hospital. Insertion into the system on the day of blood sample collection was defined as same-day insertion, and insertion on a different day to collection was defined as delayed insertion. The 7394 aerobic and anaerobic blood culture bottle sets obtained during the study period included 4361 sets with same-day insertion and 3033 sets with delayed insertion. For same-day insertion, 458 microorganisms were isolated from 432 positive sets in which at least one blood culture bottle was positive. For delayed insertion, 405 microorganisms were isolated from 379 positive sets in which at least one blood culture bottle was positive. The mean detection time for all microorganisms was significantly earlier for same-day insertion than for delayed insertion (28.3 h vs. 45.0 h, respectively, $P < 0.0001$). Delays from collection to insertion affect the time from collection to the detection of microorganisms.

Key words Blood culture · Bacteremia · Detection time

Automated continuously monitoring blood culture systems have increased the rate of isolation of pathogenic microorganisms and improved the time to detection of microorganisms causing bloodstream infection.[1,2] Furthermore, improved culture bottles have increased microorganism yields and further shortened the detection time for microorganisms.[3–6]

More rapid detection of the existence of microorganisms is useful in allowing doctors to initiate adequate anti-infective therapy. However, limitations to blood culture examinations exist in terms of detection rate and detection time. We have considered whether any methods are available to detect the existence of microorganisms more rapidly using currently available systems and techniques. The earlier insertion of blood culture bottles may increase the yield of microorganisms and shorten the time from the collection of a blood sample to Gram-stain results and the final reports for positive samples.

Several studies have evaluated the effects of the delayed entry of blood culture bottles using microorganisms chosen from among frequently isolated species or test strains.[7–11] However, few studies have examined the effects on patients with bacteremia of delayed entry of a blood culture bottle into the system.

We investigated the time taken from when a blood sample was collected (collection) to when a bottle inoculated with that sample was inserted into the monitoring system (insertion). We compared the mean detection time from collection to a positive signal from the instrument with the time between collection and insertion.

The study was conducted between January 2003 and December 2004 at Kyoto University Hospital, a 1100-bed tertiary care, urban teaching hospital with intensive care units, transplantation services, an emergency room, and outpatient clinics.

The BacT/Alert system (bioMérieux, Marcy l'Etoile, France) was used for this study. Both aerobic (FA) and anaerobic (FN) bottles were used. Blood cultures were collected primarily by medical doctors, who were instructed to inoculate 8–10 ml of blood into each of an FA and an FN bottle as one set. The blood samples were inoculated into the blood culture bottles immediately after drawing the blood sample. All bottles were placed immediately into the BacT/Alert instrument upon delivery to the laboratory at

T. Saito (✉) · Y. Iinuma · S. Takakura · M. Nagao · A. Matsushima · M. Shirano · S. Ichiyama
Department of Clinical Laboratory Medicine, Kyoto University Graduate School of Medicine, 54 Kawahara-cho, Shogoin, Sakyo-ku, Kyoto 606-8507, Japan
Tel. +81-75-751-3503; Fax +81-75-751-3233
e-mail: saitota@kuhp.kyoto-u.ac.jp

room temperature, and then incubated at 35°C for 5 days. If the blood sample was drawn at night or during a holiday period, the blood culture bottles were incubated at 35°C in a stationary incubator in the ward or in an emergency laboratory, and then delivered to the microbiological laboratory at room temperature after 1 or 2 days. The volume of blood inoculated into the bottles was not monitored by the laboratory. Bottles delivered to the microbiological laboratory after 1 or 2 days were examined manually by turning them upside down to inspect the sensor for color change. Insertion of the blood sample into the system on the day of collection was defined as same-day insertion. Conversely, insertion at any time after the day of collection was defined as delayed insertion. When growth was detected, the microorganisms were identified using the Vitek system (bioMérieux) and/or the API series (bioMérieux). Bottles that were not flagged by the instrument after incubation for a total of 5 days were considered to be negative bottles without subcultures, as described previously.[2]

We investigated the times at which blood samples were obtained, when the blood culture bottles were inserted into the instrument, and when the instrument signaled positive results. We also examined the recovery rate of microorganisms from all blood culture bottles, and which microorganisms were isolated. The first positive bottle in a set was used to calculate the time to positivity for that set. When two or more organisms were detected in one bottle, the time that the instrument first signaled a positive result was used as the time to positivity for all organisms obtained from that bottle.

The comparability of the numbers of microorganisms according to the time between collection and insertion was analyzed using Fisher's test. The times at which the instrument signaled a positive result and the times taken between collection and insertion were analyzed using Student's *t*-test. All analyses were performed using StatView version 5.0 software (SAS Institute, Cary, NC, USA). Values of $P < 0.05$ were considered to be statistically significant.

A total of 7394 blood culture sets were obtained during the study period, with same-day insertion for 4361 sets and delayed insertion for 3033 sets. For same-day insertion, 458 microorganisms were isolated from 432 sets in which at least one blood culture bottle was positive. For delayed insertion, 405 microorganisms were isolated from 379 sets in which at least one blood culture bottle was positive. The mean time to insertion was 3.1 h (median, 3.0 h; range, 0–11 h) for same-day insertion, and 28.1 h (median, 21.0 h; range, 11–84 h) for delayed insertion.

Table 1 shows the numbers of microorganisms according to the time between collection and insertion (i.e., same day or delayed). Microorganisms which were frequently isolated in both insertion groups were coagulase-negative staphylococci, *Staphylococcus aureus*, *Enterococcus* species, and *Escherichia coli*. Delayed entry had no significant effect on recovery rates for almost all microorganisms. A comparison of mean detection time for microorganisms from collection to a positive signal from the instrument according to the time between collection and insertion (i.e., same day or delayed) is shown in Table 2. The mean detection times for *S. aureus* (23.8 h vs. 43.8 h), coagulase-negative staphylococci (30.1 h vs. 44.7 h), all isolated Gram-positive bacteria (28.4 h vs. 43.4 h), *Enterobacter* species (21.4 h vs. 31.7 h), *Klebsiella* species (13.2 h vs. 36.2 h), *E. coli* (14.2 h vs. 35.0 h), *Serratia marcescens* (22.1h vs. 45.7 h), *Enterobacteriaceae* (16.2 h vs. 35.6 h), nonfermentative bacteria (20.5 h vs. 38.2 h), obligate anaerobes (46.2 h vs. 86.4 h), and all isolated microorganisms (28.3 h vs. 45.0 h) were significantly earlier for same-day insertion than for delayed insertion.

Despite the introduction of new anti-infective agents and progress in supportive therapy, bacteremia is involved in up to 50% of hospital mortalities.[12] Hautala et al.[13] reported that Gram-stain results from positive blood cultures would allow early accurate targeting of antimicrobial therapies for bloodstream infections. To perform adequate early antibiotic therapy for a bloodstream infection, certain minimum information must be obtained from the blood sample regarding the microorganisms involved, such as Gram-staining results and the identification of rod, coccus, or yeast.

Automated continuously monitoring blood culture systems have increased the rate of isolation of pathogenic microorganisms and reduced the time to the detection of microorganisms causing bloodstream infections.[14,15] With the use of automated continuously monitoring blood culture systems, blood culture bottles sometimes cannot be inserted into the system immediately after collection, particularly at night and during holiday periods. Furthermore, since many hospitals and clinics without a microbiology laboratory submit blood culture bottles to the clinical testing industry for automated blood culture, we suspect that even more time may often elapse between collection and insertion. Therefore, clinicians and other involved staff need to be aware that the time from blood collection to the detection of microorganisms will increase if blood culture bottles cannot be inserted into the system immediately after collection.

In this study, if the sample was taken at night or during a holiday period, blood culture bottles were incubated at 35°C in the ward or in an emergency laboratory and then delivered to the microbiological laboratory after 1 or 2 days. Even if blood culture bottles cannot be inserted into the system immediately after taking the blood sample, the circumstances of incubation will be similar to those for a rapidly inserted blood culture bottles because of the incubation temperature of 35°C. However, a recent study reported that the median time to detect bacterial growth is longer at 22°C incubation temperature than at 35°C for *E. coli*, *E. faecalis*, *Haemophilus influenzae*, *S. aureus*, *S. epidermidis*, and *Streptococcus pneumoniae*.[7] Furthermore, Seegmüller et al.[8] have reported that to detect *Pseudomonas aeruginosa* in delayed-entry samples, pre-incubation at room temperature is superior to pre-incubation at 36°C. We consider that it is necessary to estimate the time from sample collection to the detection of microorganisms when blood culture bottles are incubated at 22°C under conditions in which the bottles cannot be inserted into the system immediately after the sample collection.

Table 1. Number of microorganisms according to day between collection and insertion into the system

Microorganism	No. of isolates (%)		*P* value
	same-day insertion[a] *n* = 458	delayed insertion[b] *n* = 405	
Aerobe and Facultative anaerobe			
Gram-positive bacteria			
Coagulase-negative staphylococci	110 (24.0)	78 (19.3)	0.0910
Staphylococcus aureus	73 (15.9)	72 (17.8)	0.4709
Enterococcus species	38 (8.3)	34 (8.4)	0.9585
Streptococcus species	19 (4.1)	23 (5.7)	0.2970
Bacillus species	9 (2.0)	7 (1.7)	0.7970
Corynebacterium species	3 (0.7)	4 (1.0)	0.7119
Gram positive rod (unidentified)	0 (0.0)	2 (0.5)	0.2199
Gram positive cocci (unidentified)	1 (0.2)	0 (0.0)	>.9999
Pediococcus species	1 (0.2)	0 (0.0)	>.9999
Subtotal	254 (55.5)	220 (54.3)	0.7375
Gram-negative bacteria			
Enterobacteriaceae			
Escherichia coli	35 (7.6)	32 (7.9)	0.8870
Klebsiella species	27 (5.9)	18 (4.4)	0.3387
Enterobacter species	22 (4.8)	13 (3.2)	0.2363
Citrobacter species	3 (0.7)	10 (2.5)	0.0462
Serratia marcescens	6 (1.3)	9 (2.2)	0.3062
Salmonella species	9 (2.0)	0 (0.0)	0.0043
Proteus mirabilis	1 (0.2)	1 (0.2)	>.9999
Morganella species	1 (0.2)	1 (0.2)	>.9999
Pantoea agglomerans	0 (0.0)	1 (0.2)	0.4693
Subtotal	104 (22.7)	85 (21.0)	0.5644
Nonfermentative bacteria			
Pseudomonas species	20 (4.4)	11 (2.7)	0.1934
Stenotrophomonas maltophilia	13 (2.8)	6 (1.5)	0.1752
Acinetobacter species	3 (0.7)	6 (1.5)	0.3187
Burkholderia cepacia	2 (0.4)	5 (1.2)	0.2627
Sphingomonas paucimobilis	0 (0.0)	2 (0.5)	0.2199
Alcaligenes xyl. xylosoxidase	0 (0.0)	2 (0.5)	0.2199
Subtotal	38 (8.3)	32 (7.9)	0.8317
Other gram-negative bacteria			
Campylobacter species	1 (0.2)	4 (1.0)	0.1924
Aeromonas species	0 (0.0)	2 (0.5)	0.2199
Subtotal	1 (0.2)	6 (1.5)	0.0557
Subtotal	143 (31.2)	123 (30.4)	0.7867
Obligate anaerobe			
Bacteroides species	13 (2.8)	6 (1.5)	0.1752
Fusobacterium varium	3 (0.7)	1 (0.2)	0.6270
Propionibacterium acnes	1 (0.2)	4 (1.0)	0.1924
Eubacterium limosum	1 (0.2)	1 (0.2)	>.9999
Clostridium perfringens	2 (0.4)	0 (0.0)	0.5013
Lactobacillus species	2 (0.4)	0 (0.0)	0.5013
Bifidobacterium species	0 (0.0)	1 (0.2)	0.4693
Porphyromonas asaccharolytic	1 (0.2)	0 (0.0)	>.9999
Prevotella loescheii	0 (0.0)	1 (0.2)	0.4693
Subtotal	23 (5.0)	14 (3.5)	0.2574
Yeast			
Candida species	38 (8.3)	44 (10.9)	0.1993
Cryptococcus neoformans	0 (0.0)	4 (1.0)	0.0481
Subtotal	38 (8.3)	48 (11.9)	0.0819

[a]The day of blood sample collection and insertion into the system is same
[b]The day of blood collection and insertion into the system is diffrernent

Our main objective was to investigate whether the delayed insertion of blood culture bottles into the system would increase the time from the collection of the blood sample to the detection of microorganisms. We therefore compared the mean detection time from collection to a positive signal of the presence of microorganisms being received from the instrument with the time elapsing between collection and insertion, since we considered that an increased time from collection to insertion, as seen with delayed-insertion samples, would lead to even more time elapsing from collection to the detection of microorganisms. Since signal-positive bottles are not moved to the next examination process in the absence of a laboratory which is open 24 h per day, we were unable to compare the time from sample collection to the report of Gram-stain results or final results. Furthermore, we could not compare yields

Table 2. Comparison of mean detection time according to the number of days between blood sample collection and insertion into the system

	Mean (range) detection time[a] (h)		*P* value
	Same-day insertion[b]	Delayed insertion[c]	
Aerobe and facultative anaerobe			
Gram-positive bacteria			
Coagulase-negative staphylococci	30.1 ± 15.9 (3–115)	44.7 ± 24.5 (19–177)	<.0001
Staphylococcus aureus	23.8 ± 13.7 (3–70)	43.8 ± 21.2 (17–129)	<.0001
Enterococcus species	26.7 ± 38.7 (3–189)	34.1 ± 16.1 (16–70)	0.3017
Streptococcus species	26.9 ± 23.3 (2–106)	35.5 ± 17.4 (16–80)	0.1760
Bacillus species	49.8 ± 27.0 (14–87)	48.5 ± 33.0 (16–104)	0.9289
Corynebacterium species	32.0 ± 13.8 (20–47)	90.3 ± 40.0 (35–127)	0.0638
Gram-positive cocci (unidentified)	53.0		–
Gram-positive rod (unidentified)		120.0 ± 60.2 (60–180)	–
Pediococcus species	13.0		–
Subtotal	28.4 ± 21.7 (3–189)	43.4 ± 25.0 (16–180)	<.0001
Gram-negative bacteria			
Enterobacteriaceae			
Escherichia coli	14.2 ± 6.3 (1–40)	35.0 ± 20.1 (16–86)	<.0001
Klebsiella species	13.2 ± 3.6 (1–22)	36.2 ± 19.0 (16–72)	<.0001
Enterobacter species	21.4 ± 11.3 (4–50)	31.7 ± 12.4 (17–52)	0.0174
Citrobacter species	17.7 ± 1.5 (16–19)	34.8 ± 18.1 (14–68)	0.1389
Serratia marcescens	22.1 ± 12.9 (10–47)	45.7 ± 14.4 (27–74)	0.0071
Salmonella species	17.0 ± 10.7 (3–45)		–
Proteus mirabilis	13.5	27.3	–
Morganella species	11.0	24.0	–
Pantoea agglomerans		38.0	–
Subtotal	16.2 ± 8.5 (1–50)	36.1 ± 17.7 (14–86)	<.0001
Nonfermentative bacteria			
Acinetobacter species	32.0 ± 33.9 (9–71)	27.0 ± 7.2 (22–40)	0.7279
Burkholderia cepacia	61.5 ± 0.5 (61–62)	57.5 ± 22.2 (29–82)	0.8184
Pseudomonas species	26.1 ± 23.0 (13–126)	34.4 ± 14.9 (12–54)	0.3057
Stenotrophomonas maltophilia	35.1 ± 24.0 (16–111)	52.7 ± 29.7 (32–111)	0.1861
Alcaligenes xyl. xylosoxidase		41.5 ± 4.5 (37–76)	–
Sphingomonas paucimobilis		52.0 ± 1.7 (50–54)	–
Subtotal	20.5 ± 16.1 (9–126)	38.2 ± 21.3 (12–111)	<.0001
Other Gram-negative bacteria			
Campylobacter species	37.7	77.3 ± 52.3 (48–155)	–
Aeromonas species		17.5 ± 0.5 (17–18)	–
Subtotal	37.7	57.3 ± 20.8 (17–155)	–
Subtotal	20.4 ± 16.1 (1–189)	38.2 ± 21.3 (12–180)	<.0001
Obligate anaerobe			
Bacteroides species	35.0 ± 13.2 (19–63)	46.8 ± 20.2 (27–76)	0.1438
Fusobacterium varium	34.3 ± 22.4 (10–13)	41.0	–
Propionibacterium acnes	173.0	145.3 ± 9.6 (136–155)	–
Eubacterium limosum	113.8	42.0	–
Clostridium perfringens	14.0 ± 1.0 (13–15)	–	
Lactobacillus species	49.5 ± 0.5 (49–50)		–
Bifidobacterium species		94.0	
Porphyromonas asaccharolytica	90.0		–
Prevotella loescheii		106.0	–
Subtotal	46.2 ± 36.8 (10–63)	86.4 ± 50.6 (27–155)	0.0054
Yeast			
Candida species	47.5 ± 31.4 (2–193)	54.7 ± 29.6 (13–151)	0.2910
Cryptococcus neoformans		102.8 (47–160)	
Subtotal	47.5 ± 31.4 (2–193)	58.7 ± 35.3 (13–160)	0.1299
Total	28.3 ± 23.6 (1–193)	45.0 ± 27.9 (12–180)	<.0001

[a]Values are means ± standard deviations
[b]The day of blood sample collection and insertion into the system is the same
[c]The days of blood collection and insertion into the system are different
Statistical analysis was performed for two or more microorganisms which were recovered from both insertion groups

or speeds of detection for microorganisms isolated from each type of blood culture bottle, which was our main purpose.

We conclude that delays in the time taken from blood collection to insertion affect the time from collection to the detection of microorganisms. Blood culture bottles should be submitted to the laboratory and entered into the continuously monitoring blood culture system as quickly as possible.

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