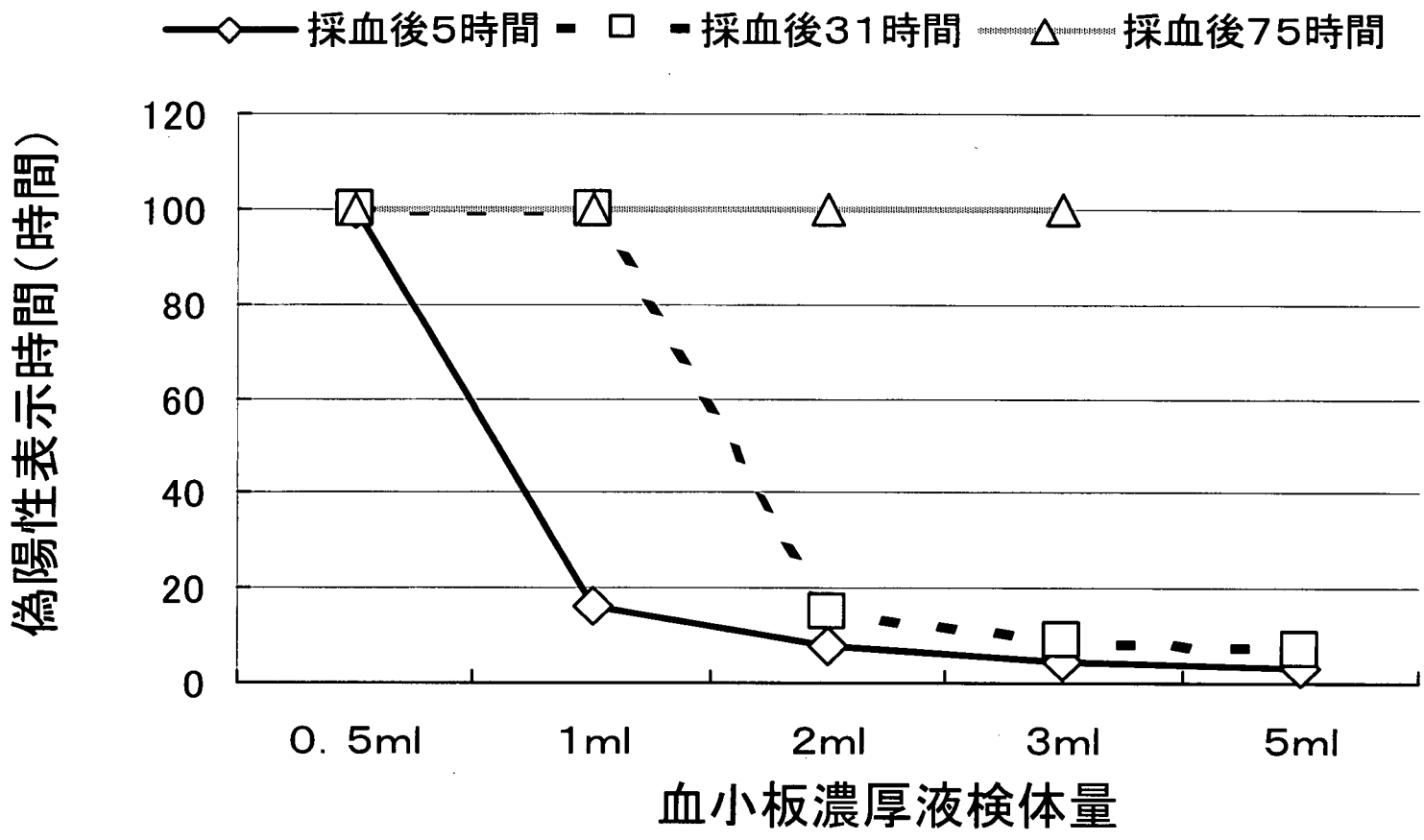
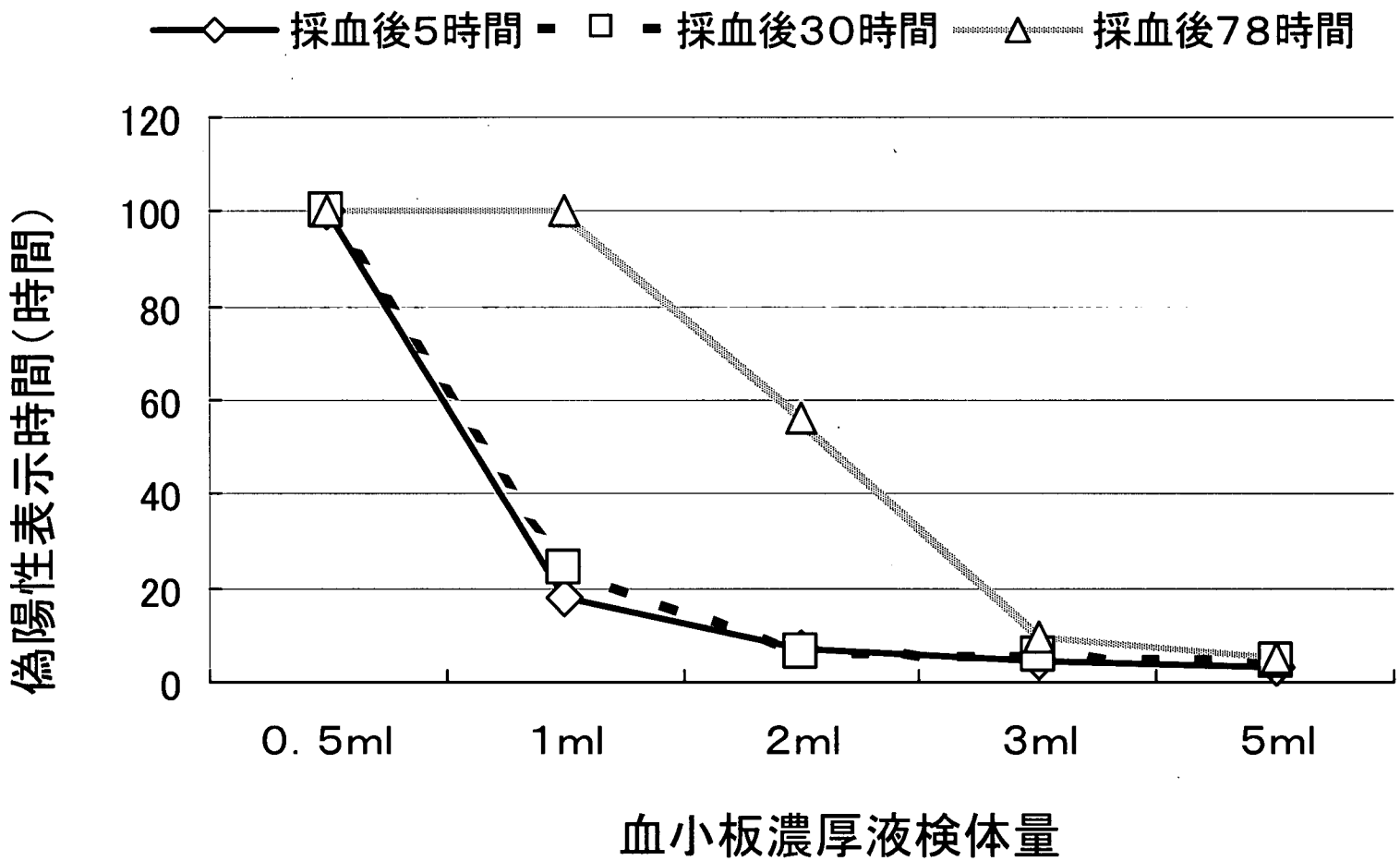
# 図5.　SensiMedia測定における血小板代謝由来CO2の影響
## －POバッグ保存－

血小板濃厚液の血小板濃度：143万／cmm（POバッグに40ml保存）

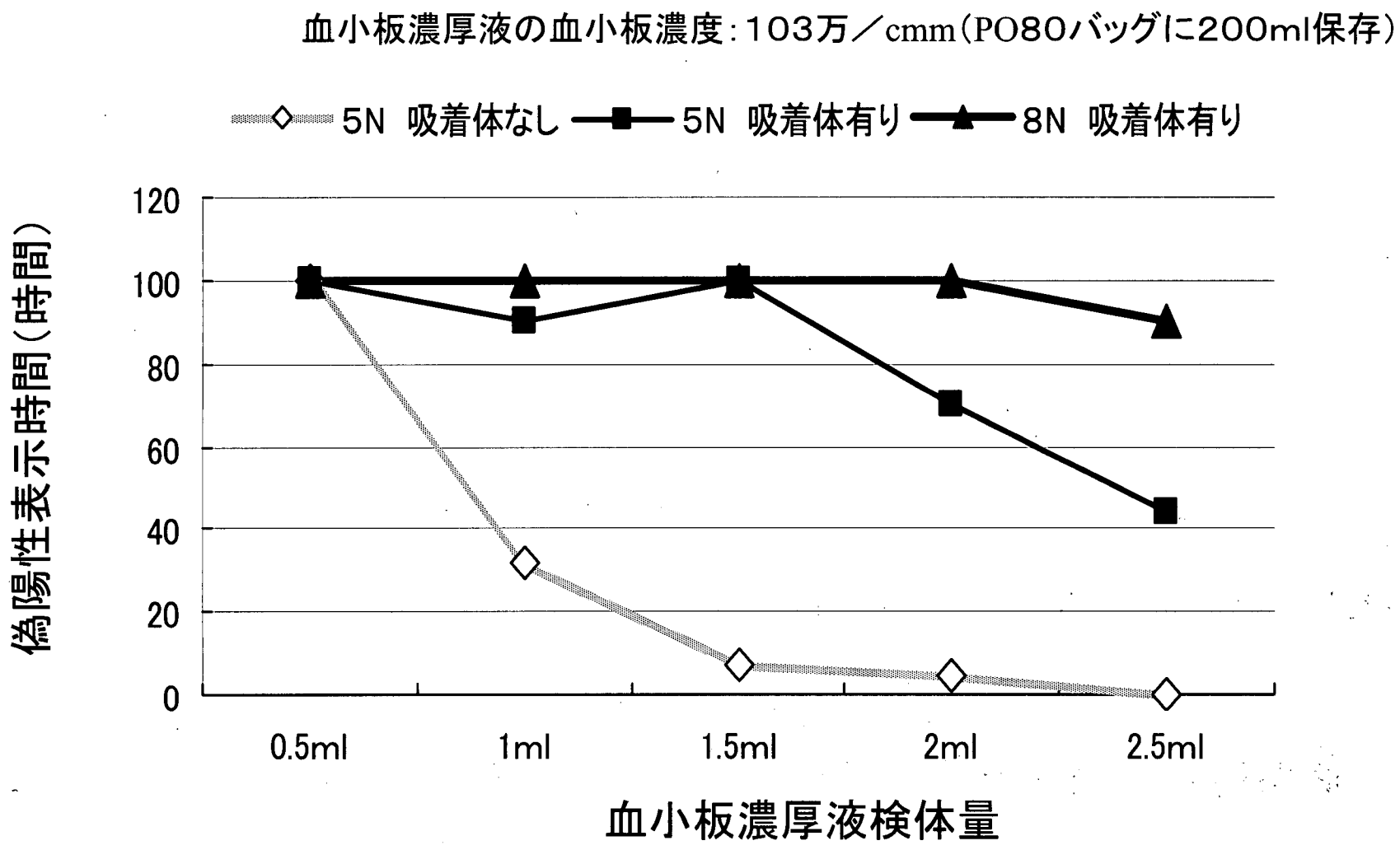


# 図6.　SensiMedia測定における血小板代謝由来CO2の影響
## －PO80バッグ保存－

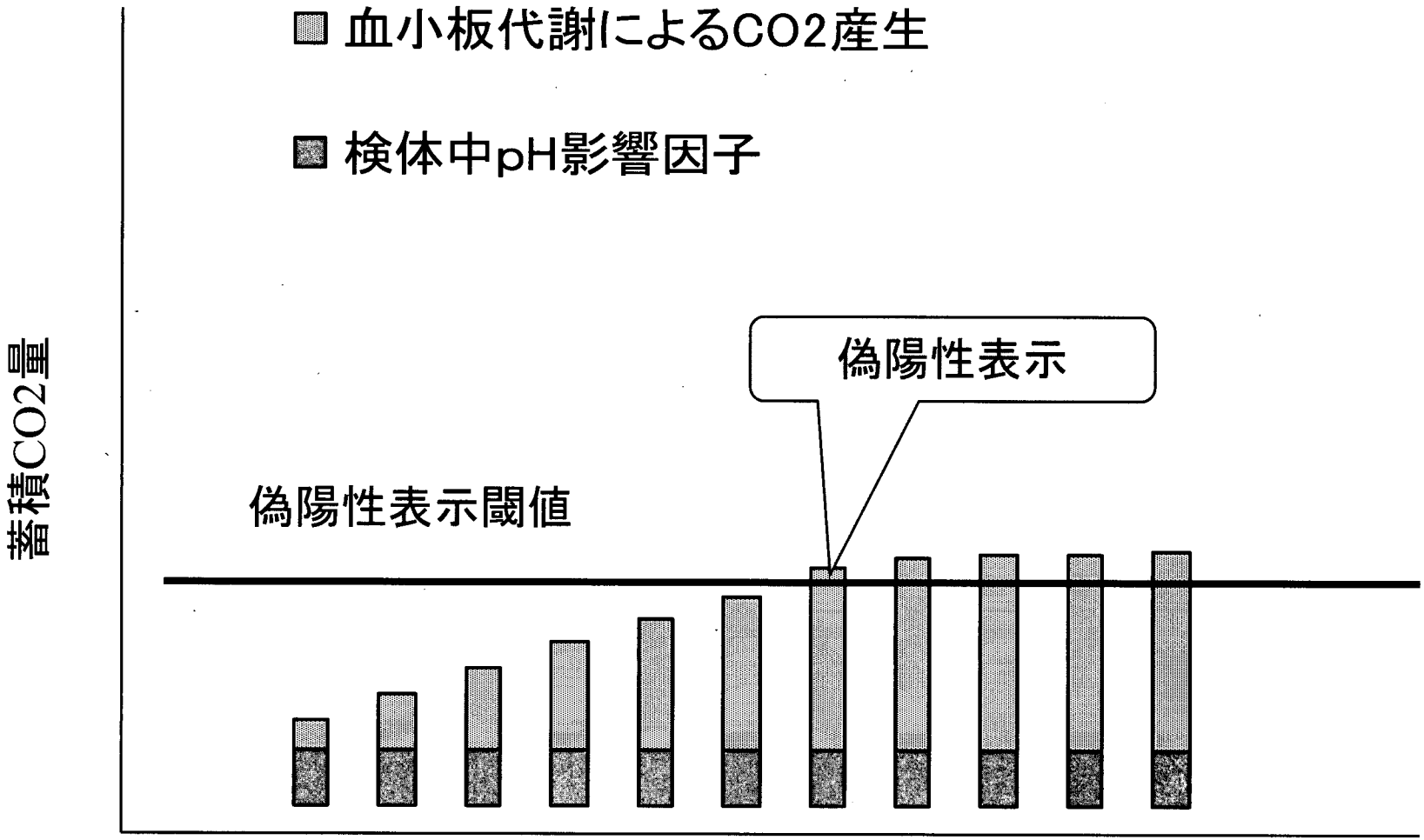
血小板濃厚液の血小板濃度：124万／cmm（PO80バッグに200ml保存）



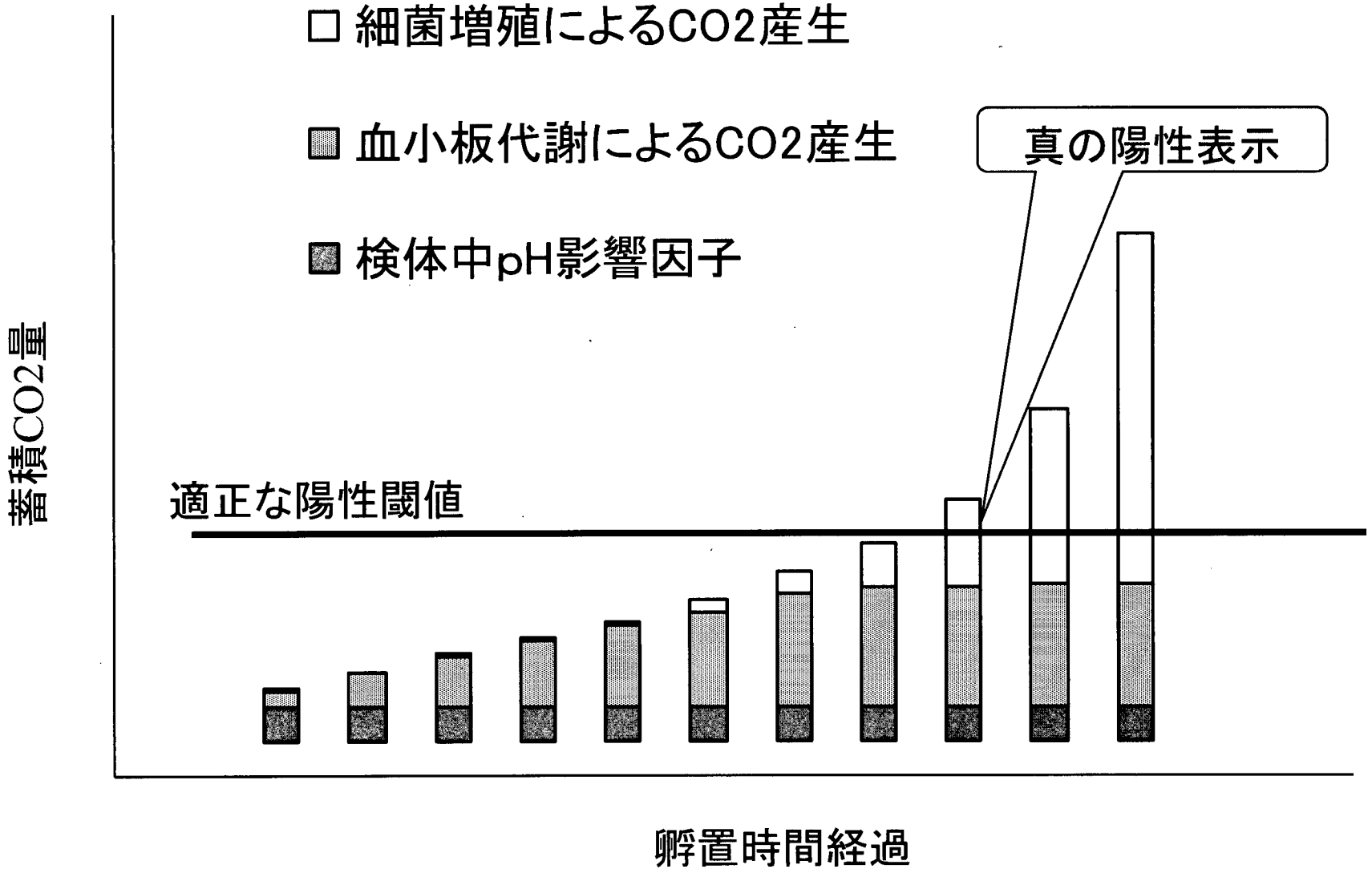
# 図7.　CO2センサー感度の調整と血小板産生CO2による偽陽性表示の影響



# 図8.　血小板代謝CO2による偽陽性表示の可能性



# 図9.　陽性表示適正閾値の設定に関する考え



# Ⅲ．研究成果の刊行に関する一覧表

別紙５

書籍

| 著者氏名 | 論文タイトル名 | 書籍全体の編集者 | 出版社名 | 出版年 | ページ |
|---|---|---|---|---|---|
| 浜口 功、山口一成 | 輸血・移植と感染症．小児感染症学特殊な状況下での感染症 | | 診断と治療社 | 2007 | 155-161 |

雑誌

| 発表者氏名 | 論文タイトル名 | 発表誌名 | 巻号 | ページ | 出版年 |
|---|---|---|---|---|---|
| Ezuki S, Kawabata K, Kanno T, Ohto H. | Culture-based bacterial detection systems for platelets: the effect of time prior to sampling and duration of incubation required for detection with aerobic culture. | Transfusion | 47 | 2044-2049, | 2007. |
| Ezuki S, Kanno T, Ohto H, et al. | Survival and recovery of apheresis platelets stored in a polyolefin container with high oxygen permeability. | Vox Sanguinis | | In press | 2008 |
| 藤井康彦、宮田茂樹、浅井隆善、他 | ABO 不適合輸血の発生原因による解析. | 日本輸血細胞治療学会誌 | 53(3) | 374-382 | 2007 |
| 山口一成 | 輸血医療・医学の新展開 はじめに | 医学のあゆみ 第１土曜特集 | 218 No. 6 | 555 | 2006 |

# Ⅳ. 研究成果の刊行物・別冊

# Culture-based bacterial detection systems for platelets: the effect of time prior to sampling and duration of incubation required for detection with aerobic culture

*Shoji Ezuki, Kinuyo Kawabata, Takahiro Kanno, and Hitoshi Ohto*

**BACKGROUND:** Bacterial contamination of platelet (PLT) products occurs at low concentrations requiring a period of incubation for growth to minimize sampling error. Culture-based detection methods also need sufficient incubation time; together these periods may limit the useful life of PLTs. This study characterizes the impact of sampling and detection times with two commercially available bacteria detection products.

**STUDY DESIGN AND METHODS:** Apheresis PLTs inoculated with nine bacterial species at low concentrations were sampled immediately and 24 hours after inoculation. Test results were analyzed after incubation at 16, 20, and 24 hours after sampling with two bacterial detection systems.

**RESULTS:** When sampled immediately after inoculation, two commercially available bacterial detection systems (BacT/ALERT, bioMérieux; and eBDS, Pall Corp.) failed to detect some PLTs inoculated with *Staphylococcus epidermidis*, *Serratia liquefaciens*, or *Pseudomonas aeruginosa* and *S. epidermidis*, *S. liquefaciens*, *Bacillus cereus*, or *P. aeruginosa*, respectively. The BacT/ALERT was better at 20 hours ($p < 0.02$), but not at 16 or 24 hours for Time 0 sampling. When sampling occurred 24 hours after inoculation, there were no difference between the two systems.

**CONCLUSION:** Results suggest that for either bacteria detection system, holding PLTs for 24 hours before sampling improves the detection sensitivity for PLTs contaminated with low concentrations of bacteria, and longer incubation periods improve detection.

Bacterial contamination is a major problem with transfusion of platelet (PLT) products that can lead to serious morbidity and mortality. The bacterial contamination of PLTs has been estimated to occur at frequencies of 1 in 2000 to 1 in 3000 PLT transfusions.[1] Several countries have a national surveillance system for collecting and monitoring data on the occurrence of adverse effects of transfusion. The French Blood Agency Haemovigilance Surveillance System has attributed 18 deaths to blood components contaminated by bacteria in the period from 1994 to March 1998.[2] In the United States, the BaCon Study revealed six fatal transfusion-related PLT transfusions between 1998 and 2000.[3] The UK surveillance system SHOT (Serious Hazards of Transfusion) reported 22 incidents, including 6 fatalities, caused by the bacterial contamination of blood components between 1995 and 2002.[4]

AABB has since March 2004 required all transfusion services to implement methods to limit and detect bacterial contamination in PLT components.[5] For culture screening systems, there is a choice between the semiautomatic system BacT/ALERT (bioMérieux, Marcy I'Etoile, France)[6] and the nonautomated system eBDS (Pall Corp., East Hills, NY).[7] These two detection methods have been cleared for the quality control (QC) of PLTs by the Food

**ABBREVIATIONS:** PC = platelet concentrate; TSA = trypticase soy agar.

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Received for publication February 5, 2007; revision received April 16, 2007, and accepted April 26, 2007.

doi: 10.1111/j.1537-2995.2007.01428.x

**TRANSFUSION** 2007;47:2044-2049.

and Drug Administration (FDA). An ideal screening system for detecting bacterial contamination should be rapid and sensitive. The above two methods require, in accordance with the manufacturers' instructions for use, a holding period of 24 hours before sampling. The methods of detection are based on growing the sampled bacteria to sufficient levels during a period of incubation such that surrogate markers of growth will signal the presence of bacteria. The Pall eBDS system detects the reduction of oxygen in air above the incubated sample, whereas the BacT/ALERT system monitors the carbon dioxide produced by respiring bacteria. Therefore, in addition to the prerequisite incubation before sampling and the duration of incubation of the sample to effect changes in the surrogate marker required for detection, PLTs may be released and used before results are available. In contrast, if these products are used outside the recommendations of manufacturers as a release criterion, these requisite durations of time may impact upon the shelf life of the products. Recent data are available suggesting further characterization of incubation time before sampling and duration of bacteria testing is warranted.[8-10] If either or both of the studied bacterial detection systems are able to detect in a shorter time, effective use of PLTs can be performed. We have evaluated PLTs spiked with common bacteria. The efficacy of detecting bacterial contamination after a reduced holding time and/or shorter bacteria detection incubation period was tested with the two bacterial culture methods.

## MATERIALS AND METHODS

### Study design

After inoculation of PLTs with bacteria, samples were taken for incubation and detection in either bacteria detection system and for durations of incubation including 16, 20, and 24 hours. The remaining volume of contaminated PLTs was stored at room temperature (20-24°C) with agitation. After 24 hours, sampling from the contaminated PLTs was repeated for incubation at 16, 20, and 24 hours of incubation with each bacteria detection system.

Postinoculation quantitative culture was performed by standard streak plate method with incubation on trypticase soy agar (TSA) at 37°C for 20 to 24 hours. The culture bottles and pouch samples after sampling were tested to confirm true positivity or true negativity for the presence of bacteria by plating 0.5 to 1 mL on TSA. The bacterial growth in PLTs, including extinct bags, was quantified every day up to 5 days.

### Apheresis PLTs

PLTs have a shelf life of 3 days in Japan. Expired (>72 hr after collection) apheresis PLTs, that were collected by Haemonetics (Braintree, MA) machines (70%) with polyolefin storage bags, Terumo (Tokyo, Japan) machines (20%) with polyolefin bags, or by Gambro BCT (Lakewood, CO) machines (10%) with polychloridevinyl bags, 4 and 5 days old, were aseptically preserved in an appropriate condition until use and shipped to our laboratory from Red Cross Blood Centers in PLT preservation boxes. PLT sterility was assured by sampling before inoculation with bacteria and testing sterility of the PLTs with the standard streak plate method.

### Bacteria

Nine bacterial species were obtained from the American Type Culture Collection (KWIK-STIKT DuoPak, Kanto Reagents, Tokyo, Japan; Table 1). They were revived from frozen (–80°C) vials by growing in TSA ( Becton Dickinson, Franklin Lakes, NJ). To quantify bacteria, undiluted samples were serially diluted 1 in 10 with saline up to a dilution $10^{-9}$. A 100-µL aliquot from dilution of $10^{-6}$ to $10^{-9}$ bacteria were plated, in duplicate, to obtain a dilution that gave more than 20 and less than 200 colony-forming units (CFUs) per plate from which calculations of concentration were made. Dilutions (0.5-5 mL) were used such that targeted concentrations of 1 to 10 CFUs per mL were made in each of four PLT units for each that bacteria species were studied. One milliliter of sample from each PLT was drawn, and a 100-µL aliquot for each plate was spread onto each of 10 TSA plates. The final bag inoculation density was determined after incubation of plates at 37°C for 20 to 24 hours.

### Bacteria detection systems

**BacT/ALERT.** This system consists of aerobic and anaerobic culture bottles (BPA and BPN culture bottles, bioMérieux), and the BacT/ALERT 3D system

**TABLE 1.** Bacterial species used bacterial inoculum density

| Species | Bacterial inoculum level (CFU/mL)† | ATCC order number |
|---|---|---|
| Gram positive | | |
| *Staphylococcus aureus* | 6.5 (3-10) (n = 4) | 29,213 |
| *Staphylococcus epidermidis* | 12.11 (7-15) (n = 4) | 49,134 |
| *Bacillus cereus* | 1.25 (1-2) (n = 4) | 10,876 |
| Gram negative | | |
| *Escherichia coli* | 3.25 (1-9) (n = 4) | 25,922 |
| *Serratia marcescens* | 20.13 (2-48) (n = 4) | 43,862 |
| *Serratia liquefaciens* | 1.25 (1-2) (n = 4) | 27,592 |
| *Pseudomonas aeruginosa* | 6 (1-13) (n = 4) | 27,853 |
| *Enterobacter cloacae* | 6.25 (1-22) (n = 4) | 13,047 |
| *Klebsiella oxytoca* | 18.75 (1-56) (n = 4) | 43,086 |

† Data are denoted as mean (range).
The actual number of bacterial cells inoculated was determined by the standard culture method.

(bioMérieux). The BacT/ALERT automated microbial detection system uses a colorimetric sensor at the bottom of the culture bottles that changes color in the presence of carbon dioxide produced during bacterial proliferation. The bottles are examined approximately every 10 minutes; the computer software monitors the rate of color change produced by sensor. This is nearly a closed system except for the transfer of sample from the PLTs to the sample bottle with a syringe and needle used in a clean workstation. We used an aerobic culture with 4 mL of sample each. Although readings are taken every 10 minutes throughout incubation, we recorded the state of bacteria detection after 16, 20, and 24 hours of incubation.

**eBDS.** The Pall eBDS system consists of a disposable sample set, in which a pouch contains a readily dissolvable tablet of sodium polyanethol sulfonate in trypticase soy broth, a flatbed agitator and an incubator (Helmer, Noblesville, IN), and an oxygen analyzer (PBI-Densensor, Ringsted, Denmark). This system is based on the principle that growing aerobic and facultative anaerobic bacteria consume oxygen in plasma that will equilibrate with air in the space within a sample pouch. After collection of PLTs, the pouch is heat-sealed and incubated at 35°C before measuring oxygen concentration with an oxygen analyzer. The sample set was attached to a segment from a platelet concentrate (PC) unit with a sterile connecting device (TSCD, Terumo Corp., Tokyo, Japan). Pouches of eBDS were incubated for 24 hours at 35°C with agitation on a horizontal shaker and evaluated 16, 20, and 24 hours after inoculation. Oxygen in the headspace was measured by the attached oxygen analyzer. A positive result was determined as any $O_2$ reading less than 9.4 percent.

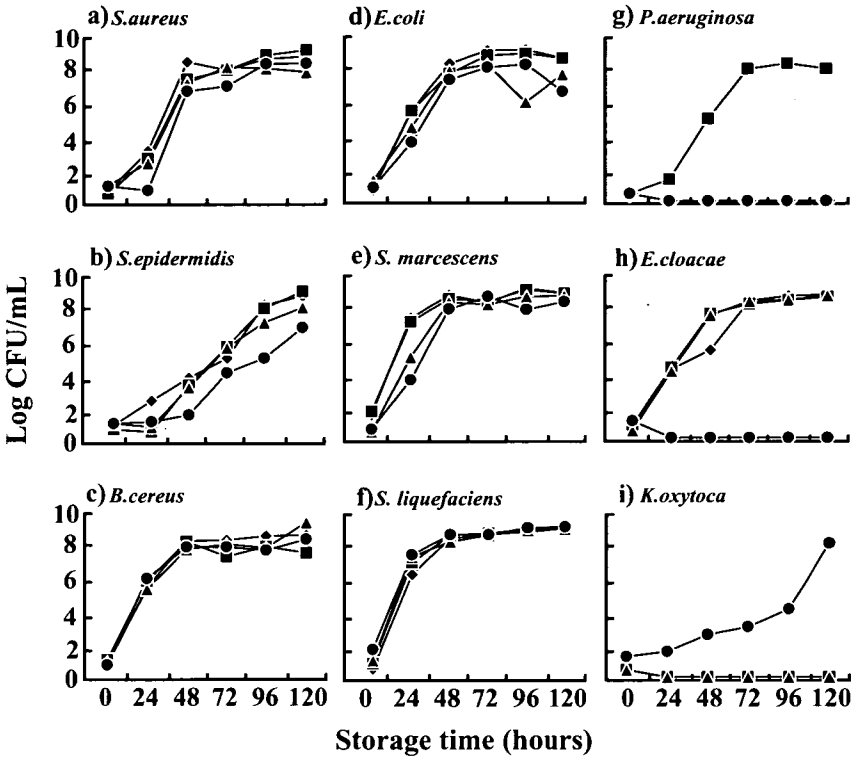


**Fig. 1. Growth curves for nine bacterial species in PLTs. (A) *S. aureus;* (B) *S. epidermidis;* (C) *B. cereus;* (D) *E. coli;* (E) *S. marcescens;* (F) *S. liquefaciens;* (G) *P. aeruginosa;* (H) *E. cloacae;* and (I) *K. oxytoca.* Each species was inoculated into four PLT bags at 1.25 to 20.13 CFUs per mL on Day 0. Data are shown as means ± SD (n = 4). *P. aeruginosa* became extinct after a 24-hour holding in one of four PLT units and *S. liquefaciens* and *E. cloacae* in three of four PLTs.**

### Statistical analysis

A Fisher's exact test was used to compare the measurements. A p value of less than 0.05 is considered to indicate significant difference.

## RESULTS

The mean initial bacterial inoculum densities for the PLTs ranged from 1 to 20 CFUs per mL (Table 1). All PLTs inoculated with *Staphylococcus aureus, Bacillus cereus, Serratia marcescens, Escherichia coli,* and *Klebsiella oxytoca* showed increased growth to concentrations exceeding 5 log CFUs per mL at 48 hours after inoculation. *Staphylococcus epidermidis* reached this level at 72 hours (Fig. 1). These bacteria were extinct in one of four PLT units inoculated with *Pseudomonas aeruginosa* at 24 hours, and in three of four PLTs inoculated with *Serratia liquefaciens* and *Enterobacter cloacae,* at 5 days. The remaining three PLT units with *P. aeruginosa* and one PC with *S. liquefaciens* reached a density of at least 5 log CFUs per mL at 24 hours. *E. cloacae* required 120 hours to reach this level.

### Time 0 study

As shown in Table 2, BacT/ALERT needed mean times of 9.6 to 18.2 hours for detection when sample were taken immediately after inoculation. None of the samples inoculated with *S. epidermidis* or *S. liquefaciens* and only one of four samples with *P. aeruginosa* were detected at 16 hours of incubation. When incubated for 20 or 24 hours, all the negative samples became positive.

With the eBDS system, a positive result was determined in all specimens with an oxygen reading of less than 9.4 percent. There were no false-positive results. No specimens inoculated with *S. epidermidis, S. liquefaciens,*

**TABLE 2.** Results of two systems for inoculum detection in PLTs

| | BacT/ALERT | | | | | | | | eBDS | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Time 0 | | | Time 24 | | | | Time 0 | | | Time 24 | | |
| Species | Mean detection time (hrs) | 16 hr | 20 hr | 24 hr | Mean detection time (hrs) | 16 hr | 20 hr | 24 hr | 16 hr | 20 hr | 24 hr | 16 hr | 20 hr | 24 hr |
| *S. aureus* | 12.1 | 4/4 | 4/4 | 4/4 | 7.4 | 4/4 | 4/4 | 4/4 | 4/4 | 4/4 | 4/4 | 4/4 | 4/4 | 4/4 |
| *S. epidermidis* | 17.9 | 0/4 | 4/4 | 4/4 | 15.9 | 3/4 | 4/4 | 4/4 | 0/4 | 4/4 | 4/4 | 4/4 | 4/4 | 4/4 |
| *B. cereus* | 9.6 | 4/4 | 4/4 | 4/4 | 4.8 | 4/4 | 4/4 | 4/4 | 3/4 | 3/4 | 4/4 | 4/4 | 4/4 | 4/4 |
| *E. coli* | 11.7 | 4/4 | 4/4 | 4/4 | 6.4 | 4/4 | 4/4 | 4/4 | 4/4 | 4/4 | 4/4 | 4/4 | 4/4 | 4/4 |
| *S. marcescens* | 11.2 | 4/4 | 4/4 | 4/4 | 5.2 | 4/4 | 4/4 | 4/4 | 4/4 | 4/4 | 4/4 | 4/4 | 4/4 | 4/4 |
| *S. liquefaciens* | 18.2 | 0/4 | 4/4 | 4/4 | 12.4 | 1/1 | 1/1 | 1/1* | 0/4 | 3/4 | 4/4 | 1/1 | 1/1 | 1/1* |
| *P. aeruginosa* | 16.9 | 1/4 | 4/4 | 4/4 | 9.4 | 3/3 | 3/3 | 3/3** | 0/4 | 0/4 | 1/4 | 2/3 | 3/3 | 3/3** |
| *E. cloacae* | 12.3 | 4/4 | 4/4 | 4/4 | 13.2 | 1/1 | 1/1 | 1/1* | 4/4 | 4/4 | 4/4 | 1/1 | 1/1 | 1/1* |
| *K. oxytoca* | 11.8 | 4/4 | 4/4 | 4/4 | 3.7 | 4/4 | 4/4 | 4/4 | 4/4 | 4/4 | 4/4 | 4/4 | 4/4 | 4/4 |

Spiked PLTs were sampled immediately after inoculation (Time 0) and after a 24 hour room temperature holding (Time 24).
* *S. liquefaciens* and *E. cloacae* became extinct in 3 of 4 PLTs after 24 hours of holding.
** *P. aeruginosa* became extinct in 1 of 4 PLTs after 24 hours of holding.

**TABLE 3.** Bacterial detection total positive

A. Time 0 sampling

| | at 16 hr | at 20 hr | at 24 hr |
|---|---|---|---|
| BacT/ALERT | 25/36 | 36/36 | 36/36 |
| eBDS | 23/36 | 30/36 | 33/36 |
| P value# | 0.8 | 0.02 | 0.24 |

B. Time 24 sampling

| | at 16 hr | at 20 hr | at 24 hr |
|---|---|---|---|
| BacT/ALERT | 28/29 | 29/29 | 29/29 |
| eBDS | 28/29 | 29/29 | 29/29 |
| P value# | 1.00 | 1.00 | 1.00 |

# Fisher's exact test.

or *P. aeruginosa* were detected at 16 hours of incubation. Seventy percent of the specimens inoculated with *B. cereus* were detected at 16 hours. When incubated for 20 hours, *B. cereus* and *S. liquefaciens* were detected in three of four samples. Specimens inoculated with *P. aeruginosa* were not detected at 20 hours of incubation; however, 25 percent of those specimens were detected at 24 hours of incubation. The BacT/ALERT was superior at 20 hours overall ($p < 0.02$), but not at 16 or 24 hours for those inoculations for the time 0 sampling (Table 3A).

### Time 24 study

As shown in Table 2, BacT/ALERT needed mean times of 3.7 to 15.9 hours when samples were taken 24 hours after inoculation. All the samples, except those inoculated with *S. epidermidis* in which only one of four PLTs was detected, were detected efficiently at 16 hours of incubation. At 20 and 24 hours, there were no false-negative samples, as confirmed by repeated samples up to 5 days.

In the eBDS system, there were no false-positive results. Although specimens inoculated with *P. aeruginosa* had a 67 percent detection rate at 16 hours of incubation, the specimens inoculated with the other bacteria had a 100 percent detection rate. After a 24-hour hold and subsequent sampling, there were no differences in sensitivity between the two systems (Table 3B).

## DISCUSSION

The principal objectives of the detection of bacterially contaminated PLTs are the prevention of transfusion-related sepsis and the extension of PLT shelf life. Among various bacterial detection schemes, the FDA of the United States has approved two bacterial culture systems for use in QC testing to monitor PLT contamination: BacT/ALERT and Pall eBDS.[6,7] At present, bacterial detection in PLTs is routinely performed on all PC products in Belgium, the Netherlands, Wales, and Hong Kong and on most PLTs in the United States[11] and some European countries.[12,13]

In 2005, the FDA, sanctioned the use of leukoreduced apheresis PLTs stored for 7 days in an approved storage

container provided that aerobic and anaerobic release cultures are procured from all units 24 to 36 hours after collection with an additional set of study cultures procured from all outdated units after 7 days of storage. Although recent advances in screening systems and their ability to detect bacteria are improving the safety of blood supply,[6,7] a perfect screening system does not exist yet. Culture systems are very sensitive but they still require several days to detect a positive unit and case reports of false-negative samples have appeared.[8-10]

PLTs require shaking at room temperature for preservation to maintain their optimum functions.[14] Coincidentally, bacteria can proliferate under these conditions from low concentrations (<1 CFU/mL) at the time of collection to very high concentrations (>8 log CFUs/mL) throughout storage.[15] Our results highlight that some bacteria grow rapidly under the conditions of PLT storage and would be captured if sampling occurs at 24 hours (*B. cereus, E. coli, S. marcescens, P. aeruginosa,* and *K. oxytoca*) whereas others grow more slowly and their concentrations are increased at 24 hours but not greatly (*S. epidermidis, S. aureus, S. liquefaciens,* and *E. cloacae*). These data are applicable only to the ATCC strain tested and should not be generalized to all strains and certainly not to different species of the same genus. More importantly, these data highlight the issue that some bacteria grow slowly and their concentrations at the time of sampling may be too low to capture. Longer storage durations before sampling clearly reduce the risk of sampling error.

The eBDS has been reported to be ineffective in detecting two of nine (22.2%) samples inoculated with *B. cereus* at 18 hours of incubation by the 24-hour-holding sampling.[13] All four samples inoculated with *B. cereus* were detected at 16 hours of incubation by the 24-hour-holding sampling; however, only three of four of the same samples were detected at 16 hours of incubation by immediate sampling. Only two of three samples inoculated with *P. aeruginosa* were detected at 16 hours of incubation by the 24-hour-holding sampling. By use of BacT/ALERT, none of the four samples inoculated with *S. epidermidis* was detected at 16 hours of incubation by the immediate sampling, three of four of the same samples were detected at 16 hours of incubation by the 24-hour-holding sampling. Therefore, both systems are less sensitive when specimens are sampled immediately after inoculation with *S. epidermidis, S. liquefaciens, P. aeruginosa, E. cloacae,* and *B. cereus.*

Some bacteria find it difficult to grow when the inoculum densities are very low (below 1 CFU/mL). It seems that some bacteria have the property of being susceptible to self-sterilization or so-called autosterilization in PLTs. This could be due to killing by preformed antibodies, complement proteins, lysozymes, or some lipoproteins in plasma. No live bacteria were observed in the PLTs after 24 hours of incubation in one or more PLT units inoculated with three bacterial species (i.e., *P. aeruginosa, S. liquefaciens,* and *E. cloacae*) at room temperature. A contamination level less than 5 CFU per mL of contamination has shown a high frequency of autosterilization during 24-hour storage at room temperature.[7] In contrast, previous studies have suggested that slow-growing organism with lower inocula may escape detection with decreased chance of detection.[16,17] We believe that self-sterilization in this study was involved in negative detection in some PLTs inoculated with *P. aeruginosa, S. liquefaciens,* or *E. cloacae*; postinoculation bags held for up to 5 days were negative for the presence of bacteria.

Two systems can detect a sample inoculated with *B. cereus* and *S. liquefaciens* at a sensitivity of 1 CFU per mL. In the Time 0 sampling, the two systems detected specimens inoculated with three bacteria (i.e., *P. aeruginosa, S. liquefaciens,* and *E. cloacae*). Bacterial screening technology is useful for blocking PLTs that contain high levels of bacteria contamination, but cannot reliably detect low levels of bacterial contamination at Time 0. To reduce sampling error (below 1 CFU/ mL), in accordance with the manufacturer's recommendations for eBDS that PC sampling should be carried out with a 24-hour holding after collection,[7] BacT/ALERT specifies the same sampling time in the United States. In European countries, BacT/ALERT sampling is performed 2 to 18 hours after the collection of apheresis PLTs and immediately after the production of a buffy coat derived from PLTs, which means 22 hours after the collection of whole-blood units.[18] To improve detection sensitivity, the sampling volumes used are 7 to 10 mL (the aerobic and anaerobic bottles used had sampling volumes of 14-20 mL). In the United States, 4- to 6-mL samples are generally used.[18] Moreover, bacteria that grow after 24 hours may be overlooked. There have been three published reports on false-negative results with BacT/ALERT.[19-21] Yomtovian and colleagues[22] revealed that active surveillance by culture at time of issue detected 38 of 39 contaminated PLT units with high sensitivity (0.97) and good positive predictive value (0.83).

In conclusion, our results have shown the reduced sensitivity of early sampling for culture-based bacterial screening systems to detect bacteria that grow in PLTs and cause sepsis. Therefore, the sampling errors (i.e., false negative) of the two systems mentioned above are major constraints to the early release of PLTs with any culture-based technique. Ideally, at-issue screening methods with required sensitivity and specificity are hoped for and under development. Alternatively, a point-of-use bacteria detection system may be of value. Results of this study indicate that a 24-hour holding and a 20-hour incubation are required to allow the low level of bacterial contamination to increase sufficiently to be able to detect bacteria in PLTs.

**ACKNOWLEDGMENTS**

This work was supported by a grant from the Ministry of Health, Labour and Welfare of Japan and funded partially by Kawasumi Laboratories, Inc. We appreciate the critical reading by Ludo Muylle MD, PhD from Belgian Federal Agency for Medicines and Health Products and University of Antwerp, Belgium, and Girolamo A. Ortolano PhD from Pall Corp.

## REFERENCES

1. Hillyer CD, Josephson CD, Blajchman MA, Vostal JG, Epstein JS, Goodman JL. Bacterial contamination of blood components: risks, strategies, and regulation: Joint ASH and AABB Educational Session in Transfusion Medicine. Hematology Am Soc Hematol Educ Program 2003:575-89.
2. Debeir J, Noel L, Aullen J, Frette C, Sari F, Mai MP, Cosson A. The French haemovigilance system. Vox Sang 1999;77: 77-81.
3. Kuehnert MJ, Roth VR, Haley NR, Gregory KR, Elder KV, Schreiber GB, Arduino MJ, Holt SC, Carson LA, Banerjee SN, Jarvis WR. Transfusion-transmitted bacterial infection in the United States, 1998 through 2000. Transfusion 2001; 41:1493-9.
4. Stainsby D, Cohen H, Jones H, on behalf of the SHOT Steering Group. Haemovigilance in the UK–the SHOT scheme. Blood Banking Transfus Med 2004;2:27-30.
5. Standards for blood banks and transfusion services. 22nd ed. Bethesda: American Association of Blood Banks; 2003.
6. Brecher ME, Hay SN, Rothenberg SJ. Evaluation of a new generation of plastic culture bottles with an automated microbial detection system for nine common contaminating organisms found in PLT components. Transfusion 2004;44:359-63.
7. Holme S, McAlister MB, Ortolano GA, Chong C, Cortus MA, Jacobs MR, Yomtovian R, Freundlich LF, Wenz B. Enhancement of a culture-based bacterial detection system (eBDS) for platelet products based on measurement of oxygen consumption. Transfusion 2005;45:984-93.
8. Schmidt M, Karakassopoulos A, Burkhart J, Deitenbeck R, Asmus J, Müller TH, Weinauer F, Seifried E, Walther-Wenke G. Comparison of three bacterial detection methods under routine conditions. Vox Sang 2007;92:15-21.
9. Burkhart J, Wittmann G, Howe J, Michel P, Schramm W, Weinauer F. Klebsiella pneumoniae in platelet concentrates—a case report [abstract P8.6]. Transfus Med Hemother 2005;32(Suppl 1):64.
10. Dickmeiss E, Bidstrup M, Salado J. The importance of the sampling time after preparation of platelet concentrates for detection of bacterial contamination [abstract]. Vox Sang 2006;91(Suppl 3):10.
11. Siva MA, Gregory KR, Carr-Greer MA, Holmberg JA, Kuehnert MJ, Brecher ME; Task Force. Summary of the AABB Interorganizational Task Force on Bacterial Contamination of Platelets: Fall 2004 impact survey. Transfusion 2006;46:636-41.
12. Ness PM, Braine HG, King K, Barrasso C, Kickler T, Fuller A, Blades N. Single-donor platelets reduce the risk of septic platelet transfusion reactions. Transfusion 2001;41:857-61.
13. Fournier-Wirth C, Deschaseaux M, Defer C, Defer C, Godreuil S, Carrière C, Bertrand X, Tunez V, Schneider T, Coste J, Morel P. Evaluation of the enhanced bacterial detection system for screening of contaminated platelets. Transfusion 2006;46:220-4.
14. Holme S, Sawyer S, Heaton A, Sweeney JD. Studies on platelets exposed to or stored at temperatures below 20 degrees C or above 24 degrees C. Transfusion 1997;37:5-11.
15. Goodnough LT, Shander A, Brecher ME. Transfusion medicine: looking to the future. Lancet 2003;361:161-9.
16. Brecher ME, Holland PV, Pineda AA, Tegtmeier GE, Yomtovian R. Growth of bacteria in inoculated platelets: implications for bacteria detection and the extension of platelet storage. Transfusion 2000;40:1308-12.
17. Blajchman MA, Ali AM, Lyn P. Bacterial surveillance of platelet concentrates: quantitation of bacterial load [abstract]. Transfusion 1997;37(Suppl):74S.
18. Blajchman MA, Beckers EA, Dickmeiss E, Lin L, Moore G, Muylle L. Bacterial detection of platelets: current problems and possible resolutions. Transfus Med Rev 2005;19:259-72.
19. Blajchman MA, Lyn P, Rosenberg E, Richardson H, Ali M. Bacterial surveillance of platelet concentrates: a comparison of day 1 versus day 3 bacterial cultures. [abstract] Blood 1996;88(Suppl 1):286a.
20. Merten G, Muylle L. False-positive and false-negative results of sterility testing of stored platelet concentrates. Transfusion 1999;39:539-40.
21. Larsen CP, Ezligini F, Hermansen NO. Six years' experience of using the BacT/ALERT system to screen all platelet concentrates, and additional testing of outdated platelet concentrates to estimate the frequency of false-negative results. Vox Sang 2005;88:93-7.
22. Yomtovian RA, Palavecino EL, Dysktra AH, Downes KA, Morrissey AM, Bajaksouzian S, Pokorny MA, Lazarus HM, Jacobs MR. Evolution of surveillance methods for detection of bacterial contamination of platelets in a university hospital, 1991 through 2004. Transfusion 2006;46:719-30.