

Growth of bacteria in inoculated platelets: implications for bacteria detection and the extension of platelet storage

Mark E. Brecher, Paul V. Holland, Alvaro A. Pineda, Gary E. Tegtmeier, and Roslyn Yomtovian

BACKGROUND: Recent reports from Europe have advocated the use of bacterial culturing of platelets on Day 2 or 3 of storage to extend the shelf life of platelets to 7 days, thereby reducing the outdating of platelets and preserving a limited medical resource. To assess the optimal timing, the necessary sensitivity, and the possible efficacy of bacterial detection, the bacterial growth characteristics were reviewed in 165 platelet units, each inoculated on the day of collection with one of the following organisms: *Bacillus cereus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Serratia marcescens*, *Staphylococcus aureus*, and *Staphylococcus epidermidis* from four previously published studies.

STUDY DESIGN AND METHODS: Quantitative culture data from inoculated platelet concentrates from five sites and four studies were combined into one database and analyzed for bacterial concentration thresholds ($\geq 10^1$, $\geq 10^2$, $\geq 10^3$, $\geq 10^4$, $\geq 10^5$ CFU/mL) by day of storage.

RESULTS: All examples of *B. cereus*, *P. aeruginosa*, *K. pneumoniae*, *S. marcescens*, and *S. aureus* had concentrations $\geq 10^2$ CFU per mL by Day 3 after inoculation. By Day 4, all units with these organisms contained $\geq 10^5$ CFU per mL. Units contaminated with *S. epidermidis* showed slower and more varied growth. By Day 3 after inoculation, 81.3 percent had 10^2 CFU per mL. By Day 4 after inoculation, 46 (95.8%) of 48 units had concentrations $\geq 10^2$ CFU per mL.

CONCLUSION: These experiments suggest that an assay capable of detecting 10^2 CFU per mL on Day 3 of storage would detect the vast majority of bacterially contaminated platelet units, prevent many cases of platelet-associated bacterial sepsis, and provide a scientific basis for the extension of the current platelet storage time. It would be expected that a rare, slow-growing organism could escape such a detection scheme.

In the United States, platelet storage at room temperature was approved for 7 days in the early 1980s, but the time was reduced to the current 5 days because of the increasing risk of bacterial proliferation over time. Recent reports from Europe have advocated the use of bacterial culturing of platelets on Day 2 or 3 of storage to extend the shelf life of platelets to 7 days, thereby reducing the outdating of platelets and preserving a limited medical resource.^{1,2} Such a strategy is considered cost-effective.³

To assess the optimal timing, the necessary sensitivity, and the possible efficacy of bacteria detection, we reviewed the bacterial growth characteristics of 165 platelet units, each inoculated on the day of collection with one of six bacteria (*Bacillus cereus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Serratia marcescens*, *Staphylococcus aureus*, and *Staphylococcus epidermidis*) from four previously published studies.⁴⁻⁷

MATERIALS AND METHODS

Study design

Quantitative culture data from inoculated PCs (PCs) from five sites—the University of North Carolina at Chapel Hill (n = 69), the University Hospitals of Cleveland (n = 24), the Community Blood Center of Greater Kansas City (n = 24),

ABBREVIATION: PC(s) = platelet concentrate(s).

From the University of North Carolina at Chapel Hill, Chapel Hill, North Carolina; the Institute of Pathology, Case Western Reserve University School of Medicine and University Hospitals of Cleveland, Cleveland, Ohio; the Community Blood Center of Greater Kansas City, Kansas City, Missouri; Mayo Clinic, Rochester, Minnesota; and the Sacramento Medical Foundation, Sacramento, California.

Address reprint requests to: M.E. Brecher, MD, Transfusion Medicine Service, CB 7600, University of North Carolina Hospitals, 101 Manning Drive, Chapel Hill, NC 27514; e-mail: brecher@med.unc.edu.

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the Sacramento Blood Center (n = 24), and the Mayo Clinic (n = 24)—were combined into one database and analyzed for bacterial concentration thresholds ($\geq 10^1$, $\geq 10^2$, $\geq 10^3$, $\geq 10^4$, $\geq 10^5$ CFU/mL) by day of storage. Inoculated units that remained sterile through Day 7 of storage or units that had been irradiated before storage were excluded from study. All units were inoculated on the day of collection.

Blood collection

Each PC was obtained after standard processing of whole-blood units from healthy, volunteer blood donors. After preparation, PCs were stored at 20 to 24°C with agitation.

Inoculation, sampling, and storage of units

Inoculation of units was performed through sampling-site couplers by one of two methods. Fresh whole-blood units were inoculated to obtain a final concentration of 1 to 5 CFU per mL and then processed into PCs (Method 1: 12 units inoculated with *S. epidermidis*), or fresh PCs were inoculated to obtain a final concentration of 10 to 50 CFU per mL or 1000 CFU per mL (Method 2: 153 units). Although actual bacterially contaminated blood components may initially contain less than 1 CFU per mL, because of inconsistent growth seen with such low inoculums, it is common practice to perform in vitro experiments with an inoculum that will ensure growth (typically, 10-1000 CFU/mL). In some cases, 2-unit pools of ABO-identical whole blood or multi-unit pools of PCs were inoculated and then split and returned to their original bags before any further processing or storage (n = 11; 9 of the units were derived from whole blood and 2 units from different platelet pools).

Before inoculation of the units, samples were drawn through a sampling-site coupler to ensure baseline sterility of the units. All units were inoculated with bacterial suspensions obtained from several colonies placed overnight on 5-percent sheep blood agar plates or in broth. The turbidity of the suspension was adjusted to match McFarland turbidity standards (0.5 or 1.0 McFarland standards). Serial dilutions of these suspensions yielded concentrations from which small aliquots (0.2-1.5 mL) were inoculated. The actual inoculum dose was confirmed from quantitative cultures of duplicate aliquots of the inoculum.

PCs were aseptically sampled daily (n = 69) for 6 to 9 days of storage or on weekdays (Monday through Friday) (n = 96) until Day 7 after inoculation or until testing with a chemiluminescent universal rRNA probe assay exceeded 500,000 RLU (equivalent to approx. 10^5 CFU/mL).⁵ Sample volume was 1.5 to 2 mL per day.

Quantitative bacterial culture

Quantitative cultures were performed by serial 1-in-10 dilutions of 0.1 mL of platelets. We then plated 0.1 mL of sample (and dilutions thereof) on sheep blood agar and incubated it at 35 to 37°C for 24 to 48 hours. Colonies were

then counted from plates with 25 to 300 colonies and the CFU per mL were calculated. In some cases if the final concentration exceeded 3×10^8 CFU per mL, the concentration was recorded as $>3 \times 10^8$ CFUs per mL. The limit of the culture sensitivity was 10 CFU per mL.

RESULTS

The cumulative percentage above incremental thresholds of bacterial concentrations is summarized in Table 1 for Days 1 to 3 following inoculation. Platelet growth curves for these six organisms are summarized in Fig. 1.

All examples of *B. cereus*, *P. aeruginosa*, *K. pneumoniae*, *S. marcescens*, and *S. aureus* had concentrations $\geq 10^2$ CFU per mL by Day 3 after inoculation. By Day 4, all units with these organisms contained $\geq 10^5$ CFU per mL. Units contaminated with *S. epidermidis* showed slower and more varied growth (as evident in the wide SD seen in Fig. 1F). By Day 3 after inoculation, 91.7 percent and 81.3 percent had $\geq 10^1$ and 10^2 CFU per mL, respectively. By Day 4 after inoculation, 47 (97.9%) of 48 units were at concentrations $\geq 10^1$ CFU per mL and 46 (95.8%) of 48 units had concentrations $\geq 10^2$ CFU per mL.

DISCUSSION

Skin saprophytes such as *B. cereus* and *S. epidermidis* are most frequently isolated from PCs; however, gram-negative organisms such as *Enterobacteriaceae* species and *S. aureus* account for most of the reported fatalities. From 1987 to 1991, 19 fatalities due to bacterial contamination of platelets were reported to the FDA.⁸ The most commonly implicated organisms were gram-positive *Staphylococcus* species (6 *S. aureus*, 1 *S. epidermidis*, 1 *S. Warneri*, and 2 *Streptococcus* species) and gram-negative *Enterobacteriaceae* species (2 *K. pneumoniae*, 1 *Klebsiella oxytoca*, 1 *Escherichia coli*, 2 *S. marcescens*, 1 *Salmonella cholerae Livingstone*, 1 *P. aeruginosa*, and 1 *Enterobacter aerogenes*).

Unlike viral contamination of blood components, which can usually be detected at the time of donation, bacterial contamination of blood components frequently requires time for the organisms to proliferate before being detectable. For example, Blajchman et al.,⁹ using an automated detection system (BacTec, Becton Dickinson, Cockeysville, MD), cultured random-donor PCs on Days 1 (n = 16,290 PCs) and 3 (n = 10,065 PCs) after preparation. Of the 16,290 PCs cultured on Day 1, 4 were found to be culture-positive on Days 1 and 3, but an additional 3 that were culture-negative on Day 1 were culture-positive on Day 3. On the basis of these results, the authors concluded that cultures from Day 1 samples may be inadequate to detect all contaminated PC units.

Our study assessed the growth characteristics of *B. cereus*, *P. aeruginosa*, *K. pneumoniae*, *S. marcescens*, *S. aureus*, and

TABLE 1. Proportion (%) of PCs above threshold CFU per mL (n = 165) on Days 1 to 3 of storage

Culture results (CFU/mL thresholds)	<i>B. cereus</i> (n = 33)	<i>K. pneumoniae</i> (n = 9)	<i>P. aeruginosa</i> (n = 30)	<i>S. marcescens</i> * (n = 7)	<i>S. aureus</i> (n = 38)	<i>S. epidermidis</i> † (n = 48)
Day 1						
≥10 ¹	100	100	100	71.4	84.2	56.4†
≥10 ²	100	100	100	28.6	68.4	41.0†
≥10 ³	100	88.9	96.7	0	29.0	23.1†
≥10 ⁴	97.0	33.3	83.3	0	18.4	0†
≥10 ⁵	97.0	0	56.7	0	5.3	0†
Day 2						
≥10 ¹	100	100	100	85.7	100	66.7†
≥10 ²	100	100	100	85.7	100	56.4†
≥10 ³	100	100	100	71.4	100	46.2†
≥10 ⁴	100	100	96.7	71.4	92.1	18.0†
≥10 ⁵	100‡	100	93.3§	71.4	76.3	15.4†
Day 3						
≥10 ¹	100	100	100	100	100	91.7
≥10 ²	100	100	100	100	100	83.3
≥10 ³	100	100	100	85.7	100	68.8
≥10 ⁴	100	100	100	85.7	100	50.0
≥10 ⁵	100‡	100	100§	85.7	100¶	33.3

* By Day 4, all *S. marcescens* units were ≥10⁸ CFU per mL.

† Culture data were not available from 12 *S. epidermidis* units from Days 1 and 2 (6 inoculated at 1000 CFU/mL and 6 at 10-50 CFU/mL); however, 2 of these units showed no growth on Day 3 and 1 unit had a concentration of 10 CFU per mL on Day 3. Thus, the growth characteristics of 9 units were not evaluable (leaving 39 units) on Days 1 and 2. Three of the 4 units (2 inoculated to 1-5 CFU/mL and 2 inoculated to 50 CFU/mL) that showed no growth on Day 3 were ≥40 CFU per mL by Day 4; one unit had demonstrable growth only on Day 7 (100 CFU/mL, original inoculum of 10-50 CFU/mL).

‡ Data from 32 evaluable *B. cereus* units.

§ Data from 29 evaluable *P. aeruginosa* units.

|| Data from 37 evaluable *S. aureus* units.

¶ Data from 36 evaluable *S. aureus* units.

S. epidermidis in 165 inoculated units of platelets. By Day 3 after inoculation, concentrations exceeding 10² CFU per mL were present in 100 percent of units inoculated with *B. cereus*, *P. aeruginosa*, *K. pneumoniae*, *S. marcescens*, and *S. aureus* (Table 1). The rapid growth of these organisms is consistent with the rapid growth that Wagner and Robinette found when they inoculated PCs with *E. coli* (also a species of *Enterobacteriaceae*).¹⁰ In their experiments, 36 PCs were inoculated (12 each inoculated to 0.1, 1.0 or 10 CFU/mL); by 24 hours, all 36 of the PCs had a concentration of ≥2 CFU per mL (sampling volumes were 0.5, 1.0, or 2.0 mL, corresponding to minimal detectable concentrations of 2, 1, or 0.5 CFU/mL, respectively).¹⁰

Unlike the rapid (by Day 3) and complete detection of moderate- to fast-growing organisms, the rapid and complete detection of a slow growing organism such as *S. epidermidis* remains more problematic. In this study, 91.7 percent of the units inoculated with *S. epidermidis* were detectable 3 days after inoculation, but by Day 4, 47 (97.9%) of the 48 studied units had more than 10¹ CFU per mL. The findings are comparable to the experiments conducted by Wagner and Robinette,¹⁰ in which 36 PCs were inoculated with *S. epidermidis* (12 each inoculated to 0.1, 1.0, or 10 CFU/mL). Wagner and Robinette found that, by 48 hours (but not at 24 hours), all (36/36) of the PCs had a concentration ≥2 CFU per mL (sampling volumes were 0.5, 1.0, or 2.0, corresponding to minimal detectable concentrations of 2, 1, or 0.5 CFU/mL, respectively).

The experiments summarized in our study suggest that an assay capable of detecting 10² CFU per mL on Day 3 of storage would detect the vast majority of bacterially contaminated platelet units, prevent many cases of platelet-associated bacterial sepsis, and provide a scientific basis for the extension of the current platelet storage time. It would be expected that a rare, slow-growing organism could escape such a detection scheme. Similarly, it is possible that some organisms such as *S. epidermidis* may show dose-dependent growth and grow slower than the data in this report suggest.

As a general rule, the more rapid a bacteria-detection assay, the lower its sensitivity. Therefore, decisions regarding the implementation of platelet bacteria testing must balance the need for rapid detection versus sensitivity. The principal objectives in regard to detection of bacterially contaminated platelets are 1) the prevention of transfusion-related sepsis and 2) the extension of the platelet shelf life. The level of detection and the timing of detection to achieve these two interrelated objectives may differ. For example, a rapid test with limited sensitivity (e.g., >10² CFU/mL) performed late in storage, such as on Day 4, may be sufficient to justify the extension of the platelet shelf life but would not be effective in preventing platelet transfusion-related sepsis in units that were less than 4 days old. Similarly, a very sensitive technique such as culture performed on the day of collection may require days to detect a positive unit and would likely miss many bacterially contami-

PLATELET BACTERIA GROWTH

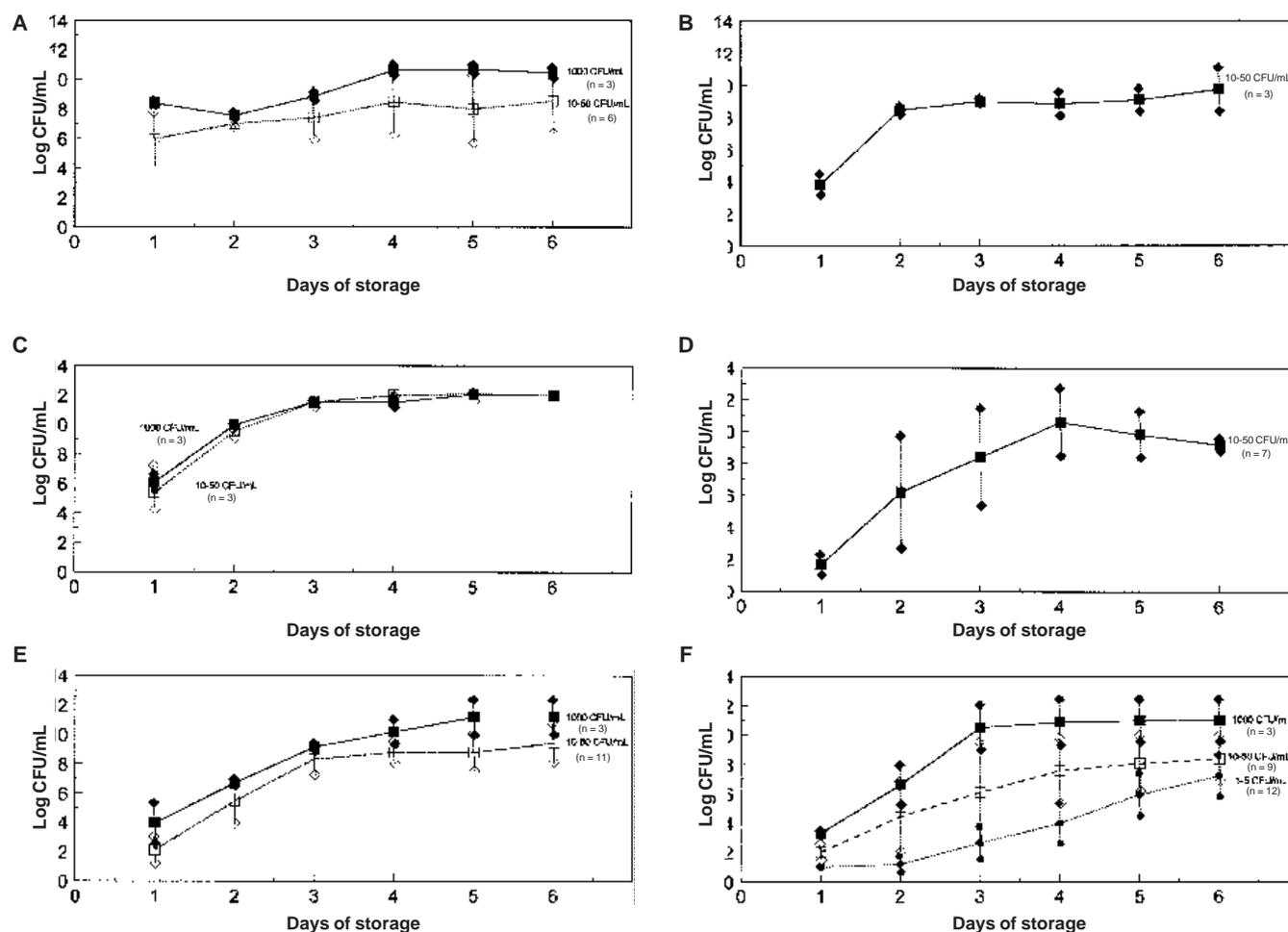


Fig. 1. Growth curves illustrating the mean \pm 1 SD of the log CFU per mL for *B. Cereus* (A), *P. aeruginosa* (B), *K. pneumoniae* (C), *S. marcescens* (D), *S. aureus* (E); and *S. epidermidis* (F). For the purposes of these plots, when no growth was detected, the concentration was taken as the limit of the culture assay sensitivity (10 CFU/mL). Only units for which a complete set of daily cultures for 6 days of storage was available are included. No growth was detected in 12, 10, and 2 units of *S. epidermidis* on Days 1, 2, and 3, respectively (all inoculated to 1-5 CFU/mL) and in 2 and 1 units of *S. marcescens* on Days 1 and 2, respectively. Growth curves were comparable at low (10-50 CFU/mL) or high (1000 CFU/mL) inocula with *B. cereus*, *P. aeruginosa*, and *S. aureus*. The growth curves for *S. epidermidis* suggest that there is a dosage effect on the rate of growth (although there are overlapping SDs).

nated units because of initial sampling error. To achieve both objectives, a combination of multiple detection strategies (e.g., at the time of collection and at 4 days of storage) may be required.

The data presented in this report provide an overview of growth characteristics of a variety of clinically significant bacteria that grow in platelets. Our analysis should allow for more informed decisions regarding the timing and the required sensitivity of testing.

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