Growth of bacteria in platelet concentrates obtained from whole blood stored for 16 hours at 22°C before component preparation

C. Sanz, A. Pereira, J. Vila, A.-I. Faundez, J. Gomez, and A. Ordinas

BACKGROUND: Previous studies have shown that cooling whole blood to 22°C immediately after collection allows it to be held for up to 16 hours before component preparation (overnight-hold method) without a significant decrease in the quality of components obtained. A study was designed to evaluate the effect of the overnight-hold method on the growth of bacteria in experimentally contaminated blood units.

STUDY DESIGN AND METHODS: Twenty whole-blood units were inoculated with Staphylococcus epidermidis (300 colony-forming units [CFU]/mL; n = 10) or Escherichia coli (50 CFU/mL; n = 10) immediately after collection. Half of the units of each group were fractionated 6 hours after collection and the other half after storage for 16 hours at 22°C. Twenty additional whole-blood units were divided in two equal parts, one of which was white cell reduced before inoculation. These 40 half-units were inoculated with S. epidermidis or E. coli and processed by the overnight-hold method. The growth of bacteria was assessed in platelet concentrates on the second and fifth days of storage, in packed red cells on Day 35, and in fresh-frozen plasma after 60 days.

RESULTS: No bacteria growth was detected in plasma or red cell units. On the second day of storage, both bacteria strains grew more slowly in platelet concentrates obtained from blood processed by the overnight-hold method. This difference disappeared for S. epidermidis on the fifth day. When white cell-reduced and non-white cell-reduced whole-blood units were compared, platelet concentrates from the latter showed a delayed growth of both bacterial strains on the second and fifth days of storage.

CONCLUSION: Prolonged storage of whole-blood units at 22°C before component preparation delays bacteria growth. This effect seems to be mediated by white cells.

RAPID COOLING OF WHOLE BLOOD (WB) TO 22°C AFTER COLLECTION HAS GAINED ACCEPTANCE, MAINLY IN EUROPE, BECAUSE IT ALLOWS BLOOD TO BE HELD OVERNIGHT AT ROOM TEMPERATURE BEFORE COMPONENT PREPARATION. THIS EXTENDED STORAGE AT 22°C DOES NOT IMPAIR THE QUALITY OF BLOOD COMPONENTS—WITH THE EXCEPTION OF 2,3 DPG CONCENTRATION—AND IT GIVES BLOOD BANKS MANY LOGISTIC ADVANTAGES. HOWEVER, THE POSSIBILITY THAT THE OVERNIGHT-HOLD METHOD MIGHT FAVOR THE GROWTH OF BACTERIA IN PURPOSELY CONTAMINATED WB UNITS HAS NOT BEEN FULLY INVESTIGATED. IN THIS STUDY, WE COMPARED BACTERIA GROWTH IN COMPONENTS OBTAINED FROM DELIBERATELY CONTAMINATED WB UNITS THAT WERE PROCESSED 6 HOURS AFTER COLLECTION OR HELD AT 22°C FOR 16 HOURS IN PLATES OF BUTANE 1,4-DIOL BEFORE COMPONENT PREPARATION.

MATERIALS AND METHODS

Blood collection, inoculation, storage, and samples

WB (465-495 mL) was collected from random donors in triple-bag systems with 63 mL of CPD (FL-124, Fresenius Laboratories, Deerfield IL). Sample-site couplers (Fenwal) were placed in each bag to allow inoculation and sampling. All procedures were performed under sterile laminar air flow conditions.

For the first series of experiments, we inoculated 20 WB units with either 300 colony-forming units (CFU) of Staphylococcus epidermidis per mL or 50 CFU of Escherichia coli per mL immediately after collection. Half the units inoculated with each microorganism were fractionated 6 hours thereafter, and the other half was processed after storage for 16 hours at 22°C in cooling plates of butane 1,4-diol. All of the WB units

ABBREVIATIONS: CFU = colony-forming units; PC(s) = platelet concentrates; WB = whole blood; WBC(s) = white cell(s).

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were fractionated by the "soft-spin" platelet-rich plasma method. WB was centrifuged (Sorvall RC-3B, DuPont, Wilmington, DE) at 1600 × g for 7 minutes at 22°C. Platelet-rich plasma was pressed into a satellite bag and centrifuged at 5000 × g for 5 minutes at 22°C. All but 55 g of the supernatant platelet-poor plasma was removed, and the platelets were resuspended after a 1-hour rest. Platelet concentrates (PCs) were stored at 22°C in a horizontal continuous agitator (Heller Laboratories, Fort Wayne, IN) for 5 days. Red cell concentrates were stored at 4°C for 35 days, and plasma was frozen to -60°C and stored at -30°C for 60 days.

For the second series of experiments, the content of each of another 20 WB units was divided in two equal parts, one of which was passed through a white cell (WBC)-reduction filter (RC-50, Pall Newquay LTD, Newquay, UK), whereas the other was held as a control. Thereafter, the units were inoculated with either 100 CFU of S. epidermidis per mL or 50 CFU of E. coli per mL. All of the WB units were fractionated after being held for 16 hours in cooling plates at 22°C.

Microbiologic methods

The strains of S. epidermidis and E. coli used in this study were clinically isolated during routine diagnostic processes conducted in our hospital's clinical microbiology laboratory. The bacteria suspension was prepared in phosphate-buffered saline, and the density of bacteria was adjusted to a McFarland turbidity standard of 1, which corresponds to an inoculum of 3 × 10⁸ cells per mL. However, this suspension was further quantified to ascertain the true concentration of bacteria. A series of dilutions was prepared from this bacterial suspension, and WB samples were inoculated with 1 mL of the diluted bacterial suspension to obtain a final concentration in the WB unit of 300 or 140 CFU of S. epidermidis per mL and 50 CFU of E. coli per mL.

 Cultures were performed on samples from red cell concentrates after 35 days of storage, from frozen plasma after 50 days, and from PCs on the second and fifth days of storage. Visible counts were made by spreading 100 μL of sample on blood agar plates, so that the limit of detection was 10 CFU per mL. To detect a level of growth below 10 CFU per mL, 900 μL of sample was inoculated in thioglycolate broth. The media were incubated at 37°C for 24 hours and left for an additional 24 hours if there was no growth. After incubation, the number of colonies was counted, and the concentration of bacteria per mL of sample was calculated.

RESULTS

In the first series of experiments, no growth of bacteria was detected in plasma units. In red cell concentrates, bacteria were recovered from only 3 units that were inoculated with S. epidermidis (1 processed within 6 hours and 2 that were held overnight), but the numbers of CFU were lower than the initial inocula (10, 20, and 60 CFU/mL, respectively). No bacteria were recovered from the 10 units inoculated with E. coli.

In samples obtained from PCs on the second day of storage, the growth of both E. coli and S. epidermidis was less in the units held overnight (Table 1). On the fifth day, E. coli continued to grow less in the units held overnight, whereas no differences were found for S. epidermidis (Table 1).

Results of the second series of experiments are shown in Fig. 1. Recovery of S. epidermidis from PCs after 2 days' storage was less than that of the inocula in the concentrates obtained from unfiltered blood. In nine such PCs, no bacteria were found. On the fifth storage day, the growth of S. epidermidis was either below (in 2 PCs) or only slightly above the initial inoculation level (median, 800 CFU/mL). In PCs prepared from WB that had been WBC-reduced before inoculation, the median recovery on the second storage day was 133.5 CFU per mL (range, 4-430). In five of these latter PCs, the number of CFU per mL on the second storage day was lower than that in the inocula. On the fifth day, there were always >10⁴ CFU per mL of S. epidermidis in PCs from WBC-reduced blood.

In PCs from blood units inoculated with E. coli, the concentration of organisms on the second storage day was only slightly above the inoculation level in WB-containing units, but it was >10⁴ CFU per mL in previously WBC-reduced units. After 5 days, all units contained more than 10⁴ CFU per mL. It should be noted that filtered units yielded macroscopically visible bacterial colonies on the fifth storage day, which nonfiltered units did not. This strongly indicates that E. coli grew better in WBC-reduced PCs, despite >10⁴ CFU per mL of E. coli in both filtered and nonfiltered units.

TABLE 1. Growth of S. epidermidis and E. coli in PCs

<table>
<thead>
<tr>
<th>Platelets obtained 6 hours after collection</th>
<th>Platelets obtained after 16-hour hold at 22°C</th>
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</thead>
<tbody>
<tr>
<td>Number</td>
<td>Second day</td>
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<td>--------</td>
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</tr>
<tr>
<td>S. epidermidis</td>
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<tr>
<td>1</td>
<td>100</td>
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<tr>
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<td>5</td>
<td>100</td>
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<tr>
<td>E. coli</td>
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<td>11</td>
<td>&gt;10⁵</td>
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<td>12</td>
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DISCUSSION

Holding blood at room temperature for long periods has traditionally been perceived as a risky condition for bacteria growth, should the blood units be unexpectedly contaminated. Therefore, concern may arise about an increasing risk of transfusion sepsis when the overnight-hold method is used before component preparation. Our results show that such a method actually delays the growth of bacteria, an effect that
BACTERIA GROWTH IN PCs

![Graphs showing bacterial growth over time](image)

Fig. 1. Growth of *S. epidermidis* (A) and *E. coli* (B) in platelets. Ten WB units were divided into two parts: one was WBC reduced with an RC-50 filter (---) and the other was held as a control (---). Half of the WB units were inoculated with *S. epidermidis* (140 CFU/mL; n = 20) and half with *E. coli* (50 CFU/mL; n = 20) and stored in a cooling plate at 22°C for 16 hours before component preparation.

...seems to be mediated by WBCs. Hagman et al. were the first to show that the presence of WBCs results in a reduction in the bacterial content of purposely contaminated blood units stored at room temperature. However, other authors did not find that WBCs play an important role in clearing bacteria from contaminated blood units. There are methodological differences that may account for these and other contradictory results when the effect of interventions on bacterial growth in intentionally contaminated blood units is studied. Among these, the dose of inoculum, the holding period before component preparation, and the time of WBC reduction in relation to that of inoculation. The latter factor may be an important confounder, because bacteria may be cleared by the filter, as a result of direct adhesion, either to the filter or within the retained WBCs. The species and strains of bacteria used may also contribute to conflicting results, perhaps because of differences in sensitivity to phagocytosis or plasma resistance. Bacteria may also show differences in the pattern of growth, depending on the blood component assayed, as recently pointed out by Wagner et al.

The doses of bacteria used in our study were probably much higher than would be expected in accidentally contaminated blood units. We chose such doses because, in previous experiments, they were the lowest ones that consistently produced bacteria growth, thus allowing the measurement of the effect of interventions, such as prolonged storage at 22°C or WBC reduction. Doses closer to those expected in clinical practice would make necessary the statistical analysis of a much higher number of blood units in order to deal with confounders, such as individual donors’ factors influencing bacterial growth. In addition, strains of *S. epidermidis* and *E. coli* were used, because they were found in previous studies to grow well in blood. Both bacteria are frequent pathogens in immunocompromised patients. *S. epidermidis* is a common saprophyte in skin and may contaminate blood units during venipuncture, while *E. coli* has also been reported to contaminate blood components.

In our study, prolonged storage at 22°C before component preparation resulted in a delay in the growth of *E. coli* in PCs, on both the second and fifth days of storage. For *S. epidermidis*, the initially lower growth was followed by a multiplicative phase, so that the number of CFU per mL at the fifth storage day was similar in PCs obtained after the holding method and in controls. This pattern of growth is similar to that found by Hagman et al. for *S. aureus*. As suggested by these authors, it is possible that bacteria were initially phagocytosed but not killed, and, therefore, they might reappear and multiply upon WBC death. In fact, both species of *Staphylococci* can survive in the phagocytes for long periods.

The filtration of WB units before bacterial inoculation resulted in accelerated growth of both *S. epidermidis* and *E. coli*. It has been suggested that WBC-reduction filters can adsorb plasma proteins, possibly including opsonins and growth factors. Some filters can also activate complement, so that filters theoretically might affect bacteria growth by mechanisms other than WBC reduction. However, the latter is the most conspicuous consequence of blood filtration, and, in the absence of studies on other hypothetical effects of filters, it is reasonable to ascribe the accelerated growth of bacteria that occurs in filtered units to the reduction of WBC content.

In recent years, considerable discussion has addressed the potential risk of bacteria growth in prestorage-filtered blood components, as well as the most appropriate timing for filtering. Our work was not designed to address these questions of the hypothetical risks of bacteria growth in WBC-reduced blood components or the best timing for such a maneuver. We used WBC-reduced blood units only to assess the role of WBCs on the delay of bacteria growth that we observed in PCs prepared by the overnight-held method. Therefore, blood units were filtered before bacterial contamination to avoid possible removal of bacteria by the filter. Certainly, this
is not the sequence that one can expect in clinical practice, where the contamination of blood units, either by unexpected bacteremia in the donor or by a poor venipuncture technique, would always precede WBC reduction.

REFERENCES

AUTHORS
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