Determination of the degree of bacterial contamination of whole-blood collections using an automated microbe-detection system

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BACKGROUND: The prevalence of bacterial contamination in whole-blood collections, either with immediate sampling or sampling after overnight storage as whole blood at 20°C, is determined.

STUDY DESIGN AND METHODS: Whole blood was collected under blood bank conditions in special five-bag systems, allowing sampling in a closed system for culture bottles. Samples were taken within 2 hours after collection (Group 1) or after overnight storage of the whole blood at 20°C (Group 2). Culture bottles were incubated for 7 days, and positive samples were entered on agar plates for confirmation and determination.

RESULTS: In Group 1, 9219 units were tested; 27 units were positive with positive subculture, that is, 0.29 percent with a 95% CI of 0.19 to 0.42 percent. In Group 2, 9038 units were tested; 36 units were positive with positive subculture, that is, 0.39 percent with a 95% CI of 0.28 to 0.55 percent. No significant difference could be found between the two test groups. The majority of bacteria were either Staphylococcus (all coagulase-negative) or Propionibacterium species.

CONCLUSION: For a total of 18,257 units, 0.34 percent (CI, 0.25-0.44) of whole-blood collections appeared to have bacterial contamination (mainly skin-derived). Overnight storage of whole blood at 20°C did not have a significant effect on the prevalence of bacterial contamination.

ABBREVIATIONS: PC(s) = platelet concentrate(s); SAGM = saline-adenine-glucose-mannitol; TTBI(s) = transfusion-transmitted bacterial infection(s).

Since the implementation of programs for reducing the contamination of the blood supply by bloodborne viruses, transfusion-transmitted bacterial infections (TTBIs) have become one of the major hazards of blood transfusion. Sazama estimated that 10 percent of blood transfusion-associated deaths are caused by bacterial contamination of the transfused component. The exact prevalence of bacterial contamination of blood components is unknown, but it can be estimated as about 0.4 percent for single-donor RBCs and single-donor platelet concentrates (PCs) and up to 2 percent for pooled PCs from multiple donors. The incidence of HCV and HIV infection through blood donation in Western Europe and the United States is estimated to range from 1 in 50,000 to 1 in 700,000 units, much lower than the prevalence of bacterial contamination. The risk that PCs will be contaminated with bacteria is 50- to 250-fold that of viral contamination.

Not all contaminated blood components will lead to TTBIs in the recipient. Literature data on the frequency of TTBIs are still quite rare, but reported values are around 0.1 percent. Because of the lack of precise criteria for TTBIs, and the fact that clinicians are not always alert for TTBIs in the case of transfusion complications, transmission of bacteria by transfusion is underestimated, and literature figures are acknowledged to be "the tip of the iceberg."
effect of whole blood when stored overnight instead of for 2 hours at 20°C.

**MATERIALS AND METHODS**

**Blood collection**

Whole blood (500 ± 50 mL) was collected under standardized blood bank conditions in five-bag systems (Compoflex CPD/SAGM, Fresenius Hemocare, NPBI International, Emmer-Compascuum, the Netherlands). These blood-collection systems were especially designed for this study (Fig. 1), and they allow the sampling of whole blood in a closed system, with the only aseptic handling being the transfer in a laminar airflow cabinet to anaerobic and aerobic culture bottles (BacT/Alert, Organon Teknika, Boxtel, the Netherlands).

At all different collection sites of the Sanquin Blood Banks, De Meierij and CLB, standardized methods for disinfection (double swab method with isopropyl alcohol swabs), blood collection, and blood storage were used. The only difference was the storage time on butane-1,4-diol cooling plates® (Fresenius Hemocare, NPBI International) between collection and sampling (2 hours in Group 1, De Meierij Blood Bank; overnight in Group 2, CLB Blood Bank). Butane-1,4-diol cooling plates were used to rapidly cool the blood to 20°C (within 2 hours) and to hold the blood at this temperature during subsequent storage.®

**Automated microbe-detection system**

After mixing, an aliquot of about 25 mL of whole blood was transferred to the integrated sampling bag within 2 hours after collection (Group 1) or after overnight storage of the whole blood at 20°C (Group 2).

Both aerobic and anaerobic culture bottles were inoculated with a 10-mL whole-blood sample under aseptic conditions in a laminar airflow cabinet, by use of the integrated sampling needle of the sampling bag. Culture bottles were incubated at 35°C in an automated microbe-detection system (the BacT/Alert system, Organon Teknika) based on the colorimetric detection of CO₂® until a positive reaction is detected or for 7 days if negative. Culture bottles that produce a positive result were subcultured on blood agar plates for confirmation to identify false-positive results due to instrument errors. False-positive results inadvertently due to environmental contamination during the introduction of samples into culture bottles are not identified in this way. However, in control studies under similar conditions in our laboratory, it was shown that the frequency of this type of false-positive result was less than 0.05 percent. Identification of bacteria was done using bacteriologic methods as described by the National Committee for Clinical Laboratory Standards (Villanova, PA).

**Statistical analysis**

Statistical analysis was performed with a computer program (Instat 2.03, GraphPad Software, San Diego, CA). The difference in proportions between the two groups was tested with a two-sided chi-square test; p values <0.05 were considered significant.

**RESULTS**

**Validation of five-bag blood-collection system**

The Compoflex five-bag system (Fig. 1) was used for the collection of whole-blood units and validated for this purpose by characterization of the prepared components. Measurement of plasma factor VIII immediately after collection and after overnight storage showed a decrease from 1.06 ± 0.37 U per mL to 0.90 ± 0.29 (n = 35) in about 16 hours, which is comparable to the decrease found with the original Compoflex four-bag system (1%/hour®).

Compared to results with that system, no significant differences were found for volume and composition (RBC, WBC, and platelet counts; Hct) of blood components (RBCs, PC, plasma, and buffy coat) prepared from units collected in the five-bag system (results not shown). PCs, prepared from whole-blood units collected in the five-bag system and stored for 6 days at 20 ± 2°C, showed decreases in quality values like swirling, blood gases, and concentration similar to those found with the regular system (n = 12; results...
not shown). RBC concentrates (after buffy coat removal and resuspension in saline-adrenaline-glucose-mannitol [SAGM]) prepared from whole-blood units collected in the five-bag system were stored for 42 days at 4°C. Hemolysis was 0.2 ± 0.11 percent after 21 days, 0.5 ± 0.28 percent after 35 days, and 0.7 ± 0.40 percent after 49 days (results as mean ± SD; n = 28). These figures are quite comparable to data found for storage of RBCs in SAGM in other studies with the standard Compoflex four-bag system.8,11

Part of the validation was also a check on the composition of the sample in the sample bag after overnight storage, mixing, and filling. The hematologic values (WBC count, platelet count, RBC count, Hb content, and Hct) for this sample were quite similar (94-99% of the values; n = 20) to the values of a sample taken out of the blood bag immediately after the collection.

Degree of bacterial contamination

In Group 1 (sampling within 2 hours after whole-blood collection), a total 9219 units were collected and tested with the BacT/Alert system. Of these 9219 units, 27 were positive with positive subculture, that is, 0.29 percent with a 95% CI of 0.19 to 0.42 percent. In Group 2 (sampling after overnight storage), a total of 9038 units were collected and tested. In this group, 36 units were found positive with positive subculture, that is, 0.39 percent with a 95% CI of 0.28 to 0.55 percent. No significant difference was found between the two test groups (chi-square test, p = 0.3). Combining these results, a total 18,257 units were tested, with a bacterial contamination prevalence of 0.34 percent (95% CI, 0.25-0.44).

Identification of positive samples

Table 1 shows the identification results of the subcultures derived from the BacT/Alert bottles that screened positive.

<table>
<thead>
<tr>
<th>TABLE 1. Differentiation of positive samples*</th>
<th>Group 1</th>
<th>Group 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard skin flora</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Staphylococcus</em> species‡</td>
<td>8</td>
<td>17</td>
</tr>
<tr>
<td><em>Propionibacterium</em> species‡</td>
<td>10</td>
<td>17</td>
</tr>
<tr>
<td>Diphtheroids, <em>Corynebacterium</em> species</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Transient skin flora</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Bacillus</em> species</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td><em>Micrococcus</em> species</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Gastrointestinal flora</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Peptostreptococcus</em> species</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Not identified§</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

* Standard skin flora: bacteria normally living on the skin; transient skin flora: bacteria normally in air, but transient on skin; gastrointestinal flora: bacteria normally present in gastrointestinal system.
† Coagulase-negative, that is, no case of *Staphylococcus aureus*; mainly identified as *Staphylococcus epidermidis*.
‡ Mainly identified as *Propionibacterium acnes*.
§ Growth curve in BacT/Alert is suggestive of *Propionibacterium*

The majority of bacteria were identified as *Staphylococcus* or *Propionibacterium* species, that is, common skin flora. One *Peptostreptococcus* species was found, not belonging to the normal skin flora. For most of the bacteria, BacT/Alert produced a positive signal within 62 hours; only *Propionibacterium* species was an exception, with a positive signal usually after 4 to 6 days (data not shown). For 15 samples found to be positive, we were able also to test the RBC concentrate (n = 15) and/or the PC (n = 7). A positive signal was detected for 11 of 15 RBC concentrates and 6 of 7 PCs. After subculturing, this contamination was shown to be caused by the same bacterium as identified in the whole-blood sample.

**DISCUSSION**

The characterization of components prepared from whole-blood units collected in the Compoflex five-bag system with an integrated sampling bag showed that the quality of the collected blood was not affected by the changes in design. The prevalence of bacterial contamination with sampling after overnight storage of whole blood at 20°C was the same as with immediate sampling. Earlier inoculation studies showed a reduction in bacterial contamination for RBC concentrates prepared after overnight storage from that with immediate preparation,12-14 but, in those studies, the buffy coat was removed, whereas we analyzed whole blood. Therefore, our results indicate that the reported effect is likely to be due to removal of bacteria (taken up by WBCs during the overnight storage) with buffy coat during component preparation.

For a total of 18,257 units, 0.34 percent of whole-blood collections appeared to have a bacterial contamination (mainly skin-derived), as detected by the BacT/Alert system. The 95% CI of this result (0.25-0.44%) is rather small compared to other published data on bacterial contamination of blood and/or blood components. However, results for whole-blood donations were from very limited studies, showing a contamination degree of 0.6 percent (in retrospect, 500 units7 and 2000 units of whole blood15). In a limited number of cases, we were able to check components derived from whole-blood donations that produced a positive screen result. In most of these cases, we were able to confirm the bacterial contamination in the component after culturing in the BacT/Alert.

Because most bacteria were skin flora, it seems likely that this contamination was introduced during venipuncture. There are theories that contamination is caused by a skin plug punched by the needle.16,17 Part of the contamination may be prevented by discarding the first volume of blood (containing the skin plug) during the collection, as suggested by the in vitro results of Wagner et al.18 A study of the effect of this removal has recently been initiated at our institution, because we now have a reliable baseline of contamination for the current collection method.
A similar distribution of species was found in both groups, mainly with skin-associated flora. It is unknown whether the origin of the Peptostreptococcus species was the gastrointestinal tract of the donor, because this bacterium could easily be attributed to transient skin flora, from donor or phlebotomist, that were not properly removed by hygienic measures. Many authors described serious morbidity and mortality due to infections with gram-negative rods, such as Yersinia or Pseudomonas grown in blood components\textsuperscript{19,22} (bacteria species not detected in our study). Initial numbers of these bacteria in fresh whole blood as tested in our study are probably below the detection limit: for Yersinia, an initial decline has been described\textsuperscript{20} with a necessary storage period before the theoretical detection limit of 1 CFU per sample volume\textsuperscript{9} is reached. Moreover, on the basis of the low frequency of contamination with gram-negative rods, such cases are not likely to be included in our study.

However, normal skin flora are not equivalent to harmless contaminants. In immunocompromised patients\textsuperscript{17} or patients with intravascular devices, prosthesis valves, or vascular grafts (especially nonhomografts), these bacteria can induce life-threatening complications. In many cases, the graft has to be removed to cure the patient.\textsuperscript{23}

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REFERENCES