Bacterial contamination of ex vivo processed PBPC products under clean room conditions

Markus Ritter, Joachim Schwedler, Jörg Beyer, Kamran Movassaghi, Reinier Mutters, Andreas Neubauer, and Nimrod Schwella

BACKGROUND: Patients undergoing high-dose radio- and/or chemotherapy and autologous or allogeneic PBPC transplantation are at high risk for infections owing to profound immunosuppression. In this study, the rate of microbial contamination of ex vivo processed PBPC products was analyzed, comparing preparation under clean room conditions to standard laboratory conditions.

STUDY DESIGN AND METHODS: After implementation of good manufacturing practice conditions in the two participating institutions, the microbial contamination rate of 366 PBPC harvests from 198 patients was determined under certified clean room conditions (Group A) from 2000 until 2002. To investigate influence of improved environmental conditions along with other parameters, this set of samples was compared with a historical control set of 1413 PBPC products, which have been processed ex vivo under a clean bench in a regular laboratory room and were harvested from 626 patients (Group B) from 1989 until 2000.

RESULTS: In Group B microbial contamination was found in 74 PBPC products (5.2%) from 57 patients. In Group A microbial growth was detected in 3 leukapheresis products (0.8%) from 3 patients. After exclusion of PBPC products, which were probably contaminated before manipulation, statistical analysis showed a significant difference ($\chi^2 = 10.339; p < 0.001$).

CONCLUSION: These data suggest an impact of clean room conditions on the bacterial contamination rate of PBPC products. To identify confounding variables, variables like technique of leukapheresis, culture methodology, and microbial colonization of central venous catheters were taken into account. Further variables might be identified in following studies.

PBPCs, transfused for hematopoietic rescue in patients undergoing high-dose radio- and/or chemotherapy can be contaminated by microorganisms. Several studies have been published analyzing the contamination rate of PBPC products. The investigators found a microbial contamination rate of 0.2 percent to 18 percent. Because the patients are heavily immunosuppressed following the conditioning regimen, microbial contamination of blood products may threaten PBPC recipients even more compared to patients not receiving radio- and/or chemotherapy. In a previous study, data were published concerning the clinical sequelae of reinfused contaminated autologous marrow. No significant differences regarding engraftment and febrile days could be observed. Nevertheless, only bacteria from the standard skin flora were detected in this relatively small group of patients, and all of them received an antibiotic prophylaxis. Assuming that the graft could be contaminated by highly pathogenic bacteria, the immunosuppressed recipient might be threatened even under antibiotic treatment. Thus, during the processing of PBPC products any possible source of contamination should be avoided.

ABBREVIATIONS: AMG = German law of drug manufacturing (“Arzneimittelgesetz”); CVC(s) = central venous catheter(s); EU = European Union; GMP = good manufacturing practice; SOPs = standard operating procedures.

From the Department of Haematology, Oncology and Immunology, Philipps-University Hospital, and the Institute for Medical Microbiology and Hospital Hygiene, Philipps-University, Marburg, Germany; and the Institute of Transfusion Medicine, Charité University Hospital/Virchow Clinic, Humboldt-University, Berlin, Germany.

Address reprint requests to: Department of Haematology, Oncology and Immunology, Hospital of the Philipps-University, Baldinger Strasse, 35043 Marburg, Germany; e-mail: markus.ritter@mailer.uni-marburg.de.

Received for publication November 14, 2002; revision received July 6, 2003, and accepted July 14, 2003.

TRANSFUSION 2003;43:1587-1595.
The collection and processing of PBPCs are regulated by the German law of drug manufacturing ("Arzneimittelgesetz," AMG). According to §4 AMG, blood preparations are defined as medical products either prepared from blood or containing blood components. Therefore, the manufacturing of PBPC products requires a production license according to §13 of the AMG. Thus, the regulations of the AMG and the Guidelines of the European Union (EU) for Good Manufacturing Practice (GMP) must be followed.

The whole processing should be performed in a closed-bag system. No disconnection of tubing sets is allowed. Nevertheless, the processing of PBPCs consists of many different techniques, which complicate the use of standardized closed-bag systems. For open handling of blood components, the GMP guidelines demand the installation of a clean room laboratory with an air lock system.

The purpose of this study was the evaluation of manufacturing of PBPC products under GMP conditions. One group of PBPC products was manipulated under clean room conditions Grade A (GMP), and the other group according to our former laboratory standards, which means that a clean bench was placed in a laboratory without special air conditioning system. The results of sterility testing, which were taken at the end of manipulation before cryopreservation, were compared.

**MATERIALS AND METHODS**

**Patients**
In total 1830 leukapheresis products from 863 patients were processed ex vivo at the Institute of Transfusion Medicine, Charité University Hospital/Virchow Clinic, Humboldt-University Berlin, Germany (Center 1); and the Department of Haematology, Oncology and Immunology, Philipps University Marburg, Germany (Center 2).

Two groups of patients and donors were analyzed. In the time period from October 1989 until May 2000 PBPCs were processed under a lamina air flow clean bench placed in a laboratory without special air conditioning systems (Group B). In the following time period from June 2000 until June 2002 PBPCs were processed under clean room conditions Grade A with background conditions Grade B (Group A).

Table 1 shows the number of products and patients characteristics. As outlined in Table 1, during the first time period (Group B), PBPC manipulation was only performed in the Institute of Transfusion Medicine, Berlin (Center 1). In the second time period (Group A), in both study centers PBPCs were processed following GMP conditions.

**Mobilization of PBPCs**
Patients underwent mobilization by disease-specific cytotoxic chemotherapy plus G–CSF (Neupogen, Amgen, Munich, Germany) at 5 to 10 μg per kg of body weight per day. Healthy subjects received G–CSF alone at 10 μg per kg per day. G–CSF was administered subcutaneously until completion of PBPC collection.

**Leukapheresis**
For the collection of PBPCs the several cell separators were used (Fresenius AS104 or COM.TEC, Fresenius HemoCare, Bad Homburg, Germany; COBE Spectra, Gambro BCT.

**TABLE 1. Patient characteristics**

<table>
<thead>
<tr>
<th>Number of patients (n = 824)</th>
<th>Group B: Berlin</th>
<th>Group A Total</th>
<th>Berlin</th>
<th>Marburg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men (n = 583)</td>
<td>457</td>
<td>126</td>
<td>90</td>
<td>36</td>
</tr>
<tr>
<td>Women (n = 241)</td>
<td>169</td>
<td>72</td>
<td>54</td>
<td>18</td>
</tr>
<tr>
<td>Median age (years)*</td>
<td>36 (4-69)</td>
<td>48 (2-70)</td>
<td>47 (2-69)</td>
<td>49 (11-70)</td>
</tr>
<tr>
<td>Number of leukapheresis procedures (n = 1779)</td>
<td>1413</td>
<td>366</td>
<td>268</td>
<td>98</td>
</tr>
<tr>
<td>Median number of leukapheresis procedures per patient*</td>
<td>2 (1-12)</td>
<td>1 (1-7)</td>
<td>1 (1-7)</td>
<td>2 (1-5)</td>
</tr>
</tbody>
</table>

**Disease**
- Testicular cancer: 237 (35.6) 21 (12.6) 4
- Non-Hodgkin’s lymphoma: 147 (22.1) 38 (19.2) 28 10
- Multiple myeloma: 75 (11.2) 43 (21.7) 32 11
- Hodgkin’s disease: 37 (5.5) 17 (8.6) 12 5
- Acute myeloid leukemia: 33 (4.9) 19 (9.6) 15 4
- Acute lymphoblastic leukemia: 14 (2.1) 4 (2.0) 4 0
- Chronic myeloid leukemia: 48 (7.2) 20 (9.6) 19 1
- Chronic lymphocytic leukemia: 0 (0) 2 (1.0) 1 1
- Breast cancer: 20 (3.0) 0 (0) 0 0
- Soft tissue sarcoma: 18 (2.7) 5 (2.5) 4 1
- Other tumors: 36 (5.4) 10 (4.5) 8 2
- Healthy donors: 0 (0) 15 (7.6) 0 15

* Number in parentheses is range.
Planegg-Martinsried, Germany; and/or Amicus, Baxter, Unterschleisheim, Germany). The regular duration of leukapheresis was 150 to 300 minutes by processing a median total blood volume of 11 L (range, 6-16 L) at a blood flow of 40 to 70 mL per min.

Venous access was performed in approximately 80 percent of patients by puncture of cubital veins. Patients with insufficient venous conditions received double-lumen central venous catheters (CVC) (GamCath catheter, JOKA GmbH, Hechingen, Germany; or a Baxter-CVVH-catheter, MEDCOMP, Harleysville, PA). Insertion of CVC was performed under strict adherence to aseptic technique.

**Cryopreservation**

The PBPC products were frozen in cryopreservation bags (Cryocyte, Baxter Biotech, Unterschleisheim, Germany; or Hemofreeze, Gambro, Hechingen, Germany) under standard conditions with human cell cryopreservation systems (Biofreeze BV50, Consarctic, Schöllkrippen, Germany; or Cryo10, Messer Griesheim, Düsseldorf, Germany). According to the directives of the German law and the guidelines of the EU, the PBPC products were tested for viral, bacterial, and fungal contamination. In case of questionable or positive results, the bags were stored in a laboratory freezer (HFU586 Sorvall, Heraeus, Hanau, Germany) or a −80°C laboratory freezer (HFU586 Sorvall, Heraeus, Hanau, Germany). From October 1989 through June 1997, cryopreservation bags were purchased from Gambro and from July 1997 through June 2002 Cryocyte bags were purchased from Baxter Biotech.

Sterility testing of DMSO was made before release for cryopreservation use. In Marburg, 10 percent of each charge was tested for microbial contamination. DMSO was injected into two bottles (Bact/ALERT FAN, Organon Teknika, Eppelheim, Germany), and the samples were analyzed by the Institute for Medical Microbiology and Hospital Hygiene, Philipps-University. In Berlin, the Institute of Pharmacy of the Humboldt University supplied the Department of Transfusion Medicine with a DMSO solution of 33 percent. The DMSO solution, prepared by the Institute of Pharmacy, was produced according to the German law of sterile drug manufacturing and delivered after sterility testing. From 1989 to 1994, DMSO was purchased in small volumes and was declared as sterile by the supplier (Wak-Chemie, Bad Soden, Germany). After opening of the vial, the DMSO solution was directly injected into the cryopreservation bag. During this time period no sterility testing was made.

**Clean room conditions**

In both study centers, clean room laboratories with a comparable design were established in June 2000 (Berlin) and July 2000 (Marburg). In both laboratories, the PBPC products were processed under environmental conditions Grade A in a lamina air flow clean bench (HS120 Heraeus, Kendro Laboratory Products, Hanau, Germany) placed in a clean room with background condition Grade B. This clean room quality corresponds to US Federal Standard 100 (US FS 209E 100/ft³). Table 2 shows a comparison of the classification of clean room according the US and the EU standards. Separate air locks for personnel and material lead to a clean room Grade C, which was used as quality control laboratory. A consecutive air lock, representing clean room condition Grade D, led to a laboratory corridor with restricted access.

Entering the first clean room Grade D, clothes and shoes had to be changed and a hair cap had to be worn. Before entering the clean room Grade B special clean room clothes were put on in the personnel air lock (GRS, Frankfurt, Germany). The clean room clothes consisted of overall, shoes, cap, gloves, and face mask. The leukapheresis bag and the material were transferred into the clean room Grade B through a separate material lock.

**Monitoring of clean area environmental conditions**

Each PBPC processing was monitored by two settle plates during and five finger glove prints of each hand at the end

---

**TABLE 2. Comparison of maximum particulate count values as defined by US Federal Standard 209E (US FS 209E) and EU guidelines**

<table>
<thead>
<tr>
<th>Classification</th>
<th>US FS 209E</th>
<th>EU (at rest)</th>
<th>EU (operational)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total particulate count</td>
<td>M 3.5 (100)</td>
<td>A and B</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>3,500/m² = 0.5 μm</td>
<td>3,500/m² = 0.5 μm</td>
<td>3,500/m² = 0.5 μm</td>
</tr>
<tr>
<td></td>
<td>100/ft³</td>
<td>0/m³ &gt; 5.0 μm</td>
<td>0/m³ &gt; 5.0 μm</td>
</tr>
<tr>
<td>Classification</td>
<td>M 5.5 (10,000)</td>
<td>C</td>
<td>B*</td>
</tr>
<tr>
<td>Total particulate count</td>
<td>353,000/m² = 0.5 μm</td>
<td>350,000/m² = 0.5 μm</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td>10,000/ft³</td>
<td>2,000/m³ &gt; 5.0 μm</td>
<td>3,500,000/m³ = 0.5 μm</td>
</tr>
<tr>
<td>Classification</td>
<td>M 6.5 (100,000)</td>
<td>D</td>
<td>Not defined</td>
</tr>
<tr>
<td>Total particulate count</td>
<td>3,530,000/m² = 0.5 μm</td>
<td>3,500,000/m² = 0.5 μm</td>
<td></td>
</tr>
<tr>
<td></td>
<td>100,000/ft³</td>
<td>20,000/m³ &gt; 5.0 μm</td>
<td></td>
</tr>
</tbody>
</table>

* Clean room class Grade B is used as background for Grade A.
of the procedure. The results of microbial environmental monitoring of each preparation with settle plates were within the limits of the EU guidelines.\textsuperscript{11,12} The five-finger glove prints exceeded in 4 percent the limits of less than 1 CFU. Nevertheless, in none of these cases could a contamination of the PBPC product be detected. As outlined in our previously published data, the elevated number of CFUs in five-finger glove prints might be caused by the contaminated outer side of the leukapheresis bag.\textsuperscript{11,12} As recommended by the supplier, the leukapheresis bag may not be disinfected because damage to the PBPCs may occur.

Additional comprehensive environmental monitoring of the clean room laboratories was performed with particle counter, air sampler, settle agar plates, contact agar plates, and five-finger glove prints to identify critical steps in operation. This qualification procedure was executed according to GMP standards. As shown in Tables 3 and 4, results of the environmental monitoring in operation of the laboratory in Marburg and Berlin revealed values within the limits of the EU guidelines.

### Processing of leukapheresis products

After the clean room laboratories were established, the standard operating procedures (SOPs) formerly used for the standard laboratory were adopted. Beside the SOPs for the introduction of the product into the clean area, many additional SOPs dealing with cleaning procedures, with sterile gloves, and with wearing clean room clothes were added. The direct manipulation of the product was not changed. Thus, procedures related to the environmental conditions were changes or added, when establishing the clean room, but not the direct processing of the PBPC product.

In Group B, 100 percent autologous leukapheresis products were processed. In Group A, 96 percent autologous leukapheresis products and 4 percent allogeneic leukapheresis products were processed. If necessary the leukapheresis products were concentrated by centrifugation. Coupling of tubes was done with a sterile tubing welder (TSCD, Terumo, Frankfurt am Main, Germany). DMSO solution was added to the PBPC suspension under a clean bench Grade A.

### Microbiologic analysis

At the Philipps University Hospital, sterility of the leukapheresis products was tested after the final manipulation just before cryopreservation. With the aliquot method, 2 mL of the final product was aspirated and injected into two bottles (Bact/ALERT FAN) for microbiologic testing (1 mL per bottle). At the Humboldt University Berlin, sterility testing was performed by eluting the preparation bag with 20 mL of autologous plasma after transfer of the final suspension to the cryopreservation bag. Each aerobic and anaerobic bottle was inoculated with 10 mL of the elution. From October 1989 to April 1996, bottles (BBL-S Septi-CHEK BHI-S and BBL SEPTI-CHEK Thio, Becton Dickinson, Heidelberg, Germany) were used and from May 1996 to June 2002 an aerobic and anaerobic system (Bact/ALERT FAN) was used. Analysis of the bottles was made by the Departments of Hygiene and Microbiology in Berlin and Marburg with standardized media and following SOPs.

### Statistical analysis

Statistical analysis was performed with the “Web chi-square calculator” of the Department of Linguistics, Georgetown University, Washington, DC. The difference in proportions between the two groups was tested with a two-sided chi-square test; p values of less than 0.05 were considered significant.

## RESULTS

### Sterility testing of leukapheresis products

A total of 1830 leukapheresis products from 863 patients were processed ex vivo. Thirty-nine patients with 51 leuka-
Cultured. Sixty out of 76 cultured microorganisms (79%) contaminated PBPC products, two different bacteria were revealed 18 different microorganisms. In 2 of these 74 culture-positive products were from 57 patients and products, 74 (5.2%) showed positive microbial growth. The 74 culture-positive products were from 57 patients and revealed 18 different microorganisms. In 2 of these 74 contaminated PBPC products, two different bacteria were cultured. Sixty out of 76 cultured microorganisms (79%) belonged to the physiologic skin flora (Table 6). In 7 patients (12%), all products showed microbial contamination (2-6 products per patient). Two leukapheresis products from 1 patient revealed different microorganisms. In the remaining 49 patients (86%), only 1 product from 1 to 6 PBPC preparations was found to be contaminated.

<table>
<thead>
<tr>
<th>TABLE 5. Patients, leukapheresis products, and sterility testing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of leukapheresis products</td>
</tr>
<tr>
<td>----------------------------------</td>
</tr>
<tr>
<td>Total</td>
</tr>
<tr>
<td>Number with sterility testing</td>
</tr>
<tr>
<td>Number of products processed in the laboratory</td>
</tr>
<tr>
<td>Number of products processed in the clean area</td>
</tr>
</tbody>
</table>

Comparison of microbial contamination rate

To prove the hypothesis of a significant difference in the analyzed Groups A and B, chi-square test was used. In several patients more than one leukapheresis procedure was performed and in some of these all products were contaminated. Therefore, we calculated with 626 patients of whom 57 had a contaminated PBPC product in Group B versus 198 patients of whom 3 had a contaminated PBPC product. Results of our calculations showed a highly significant difference ($\chi^2 = 12.836; p < 0.001$). Nevertheless, assuming that the same reason, for example, a colonized CVC, led to the microbial burden in several patients, these products were probably not contaminated by inoculation during laboratory manipulation and should therefore be excluded. Furthermore, it must be considered that the difference in sample size (1413 to 366 PBPC products) might have influenced the statistical results.

Exclusion of PBPC products with assumably intrinsic contamination

To analyze the impact of laboratory conditions on the contamination of PBPC products, all products with probably intrinsic contamination, caused by the bacteremic donor and not environmental conditions, should be excluded. Therefore, patients with two or more PBPC products from different leukapheresis procedure, which were contaminated with the same microorganism were classified as catheter-related infections (see Table 7). The retrospective analysis of the patients’ records confirmed that all of these patients had a CVC at the time of leukapheresis. Twenty-three catheter-infected PBPC products from seven patients were excluded, because the contamination probably occurred before manipulation under either clean room or standard laboratory conditions. One PBPC product of an additional patient was contaminated by *Salmonella enteritidis*. This product was also excluded because *S. enteritidis* is probably an intrinsic contamination.

We recalculated with 618 patients with 49 having a contaminated PBPC product in Group B versus 198 patients with 3 having a contaminated PBPC product in Group A. Results of this calculation showed a highly significant difference ($\chi^2 = 10.339; p < 0.001$).
Contamination rate according to the type of cell separator
We analyzed the influence of the type of cell separator on the contamination rate. With COBE Spectra, 661 leukapheresis products were collected with 29 being contaminated. Two types of cell separators (AS104 or COM.TEC, Fresenius) showed 44 contaminated leukapheresis products of 1084 in total. The Baxter Amicus cell separator revealed 1 contaminated leukapheresis product of 34. Comparison of the three groups showed no significant differences.

Variation of the contamination rate over time
To assess whether the contamination rate varies over time we wanted to know whether there was any decline in the contamination rate even before preparation under clean room conditions. Table 7 shows the number of contaminated PBPC products per year. In parentheses, the ratios of contaminated products compared to all products within 1 year are shown. There is no continuous decline of the contamination rate before manipulation under clean room conditions, thus indicating that the differences are probably not caused by stepwise improvement of the PBPC preparation technique.

Comparing data of Berlin and Marburg
We compared the results of both institutions, because microbiologic samples were obtained in a different manner. In Marburg none of the analyzed 98 leukapheresis products were contaminated. Of the 268 PBPC products in Group A from Berlin, 3 were contaminated. In Marburg the stem cell laboratory was established as a clean room. Therefore, no data of a standard laboratory were available. Group B comprises only data from Berlin institution. Thus, we calculated with 0 contaminated of 98 products (Marburg) versus 3 contaminated of 268 products (Berlin).

The Pearson-Clopper 95 percent CI for the frequency of contaminated PBPC products under clean room conditions in Marburg and Berlin was 0 to 0.037 and 0.002 to 0.032, respectively. No significant differences were found. More PBPC products would be necessary to identify a potential difference.

Because the PBPC products processed in Marburg showed no single bacterial contamination, we wanted to know whether exclusion of Marburg data still reveals a significant decrease of the contamination rate in Berlin. We excluded Marburg data and compared the number of Berlin patients in Group B (49 contaminated after exclusion of intrinsic contamination/569 not contaminated) with those of Group A (3 contaminated/144 not contaminated). This analysis also revealed a significant difference in the contamination rate ($\chi^2 = 6.276; p < 0.012$).

### DISCUSSION
In a retrospective analysis, we investigated the microbial contamination of ex vivo processed PBPC products under either standard laboratory or clean room conditions. A total of 1413 PBPC products (Group B) were processed under standard laboratory conditions and 366 (Group A) under clean room conditions. The overall microbial contamination rate decreased significantly from 5.2 to 0.8 percent. Excluding PBPC products with probably intrinsic contamination of the leukapheresis products, the contamination rate also dropped from 3.6 to 0.8 percent.

Several studies have been published by other groups who did not perform PBPC preparation under clean room conditions. In these studies the rate of microbial contamination of PBPC products showed a wide range from 0.2 to 18 percent.\(^1\)\(^-\)\(^5\) In this study, the rate of microbial contamination of PBPC products processed under standard laboratory conditions was within this range. Nevertheless, the contamination rate of PBPC products processed under clean room conditions was within this range.

<table>
<thead>
<tr>
<th>Year</th>
<th>Number of leukapheresis products</th>
<th>Number of contaminated products (%)</th>
<th>Number of patients with catheter infections (number of products)</th>
<th>Number of contaminated products, but without catheter infections (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1989</td>
<td>22</td>
<td>1 (4.5)</td>
<td>0 (0)</td>
<td>1 (4.5)</td>
</tr>
<tr>
<td>1990</td>
<td>56</td>
<td>8 (14.3)</td>
<td>1 (5)</td>
<td>3 (5.4)</td>
</tr>
<tr>
<td>1991</td>
<td>43</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>1992</td>
<td>87</td>
<td>5 (5.7)</td>
<td>1 (3)</td>
<td>2 (2.3)</td>
</tr>
<tr>
<td>1993</td>
<td>114</td>
<td>4 (3.5)</td>
<td>0 (0)</td>
<td>4 (3.5)</td>
</tr>
<tr>
<td>1994</td>
<td>148</td>
<td>13 (8.7)</td>
<td>1 (2)</td>
<td>11 (7.4)</td>
</tr>
<tr>
<td>1995</td>
<td>189</td>
<td>7 (3.7)</td>
<td>1 (2)</td>
<td>5 (2.6)</td>
</tr>
<tr>
<td>1996</td>
<td>216</td>
<td>6 (2.8)</td>
<td>0 (0)</td>
<td>6 (2.8)</td>
</tr>
<tr>
<td>1997</td>
<td>209</td>
<td>7 (3.3)</td>
<td>0 (0)</td>
<td>7 (3.3)</td>
</tr>
<tr>
<td>1998</td>
<td>139</td>
<td>4 (2.9)</td>
<td>0 (0)</td>
<td>4 (2.9)</td>
</tr>
<tr>
<td>1999</td>
<td>141</td>
<td>11 (7.8)</td>
<td>2 (5)</td>
<td>6 (4.3)</td>
</tr>
<tr>
<td>Jan-May 2000</td>
<td>49</td>
<td>8 (16.3)</td>
<td>1 (6)</td>
<td>2 (4.1)</td>
</tr>
<tr>
<td>Total</td>
<td>1413</td>
<td>74 (5.2)</td>
<td>7 (23)</td>
<td>51 (3.6)</td>
</tr>
</tbody>
</table>
range, too. This seems to indicate that a clean room is not necessary to achieve reduced contamination rates. The wide range of contamination rates in the published studies might be explained by different standards in PBPC collection, processing, and culture method before implementation of clean room and GMP conditions. There is only one publication investigating the microbial contamination of PBPC products processed under clean room conditions, showing similar results according to the contamination rate (1.32%), when compared to our data.\textsuperscript{14}

Perez and colleagues,\textsuperscript{15} who performed a multivariate analysis of determinants of bacterial contamination of whole-blood donations, identified multiple parameters that were not standardized and documented in the cited studies and might have influenced the rate of contamination in blood products. Perez and coworkers\textsuperscript{15} identified in the multicenter study the following parameters as significantly associated with bacterial contamination: 1) blood bank, 2) period of collection, 3) time since last meal, 4) donor age greater than 35 years, 5) the presence of hairs at the phlebotomy site, and 6) lack of repetition of the antisepsis scrub. While Perez and associates\textsuperscript{15} analyzed whole-blood donations, leukapheresis is a more complex harvesting technology and might therefore result in even more relevant parameters considering bacterial contamination.

Especially patients undergoing autologous PBPC harvest after mobilization chemotherapy and consecutive cytopenia are at high risk for bacteremia. Most of these patients have a CVC for parenteral nutrition and drug application. The detailed analysis of our study showed that in a subgroup of patients (n = 7), who had all PBPC products contaminated with the same bacteria, a CVC was used for leukapheresis. Assuming that the contamination of these PBPC products was caused by a colonized CVC, the seven patients were excluded from statistical analysis. The difference between the two analyzed groups was still significant.

Additionally, insufficient topical disinfection of skin or the technique of phlebotomy might be a source for microbial contamination of blood products.\textsuperscript{16} Mobilization of small skin fragments harboring bacteria in sebaceous glands as described by Gibson and Norris\textsuperscript{17} may contribute to bacterial contamination.\textsuperscript{17} Therefore, de Korte and coworkers\textsuperscript{18} suggested the use of the first 10 to 30 mL of the aspirated blood for diagnostic purposes. With this technique they found a significant reduction of bacterial contamination of the tested blood products. Bruneau and colleagues\textsuperscript{19} tested two initial portions of peripheral blood after phlebotomy and found the first portion being significantly more often contaminated. In our study with a large historical control group, the first portion of blood was not routinely used for diagnostic purposes. Thus, we cannot exclude insufficient topical skin disinfection or the technique of phlebotomy as a confounding factor.

Microbiologic culturing might be a further potentially significant parameter. Many different blood culture systems are in use for the detection of microorganisms in human blood or body fluids. Others tested the blood culture systems used in our study and found comparable abilities of recovering aerobic and anaerobic organisms.\textsuperscript{20-22} Therefore, we believe that the blood culture systems used in our study does not contribute to the differences in contamination rate.

Nevertheless, the volume of material inoculated in the blood culture bottles might influence the detection rate.\textsuperscript{20} Weinstein and colleagues,\textsuperscript{23} who performed a study to evaluate 5 versus 10 mL of blood cultured in aerobic BacT/Alert blood culture bottles, found a significant increase in the yield and speed of detection of blood pathogens.\textsuperscript{23} In our study 95 percent of microbial testing was performed by eluting the preparation bag with 20 mL of autologous plasma. On the one hand, the high volume of specimen might increase the sensitivity of bacterial detection, and on the other hand, plasma could be an additional source of contamination. Therefore, we compared the contamination rate of all products processed under clean room conditions analyzed either with the aliquot technique or with the autologous plasma method. No significant differences were found. Nevertheless, the number of PBPC products manipulated under clean room conditions with different techniques of microbial testing was too small to draw a conclusion.

Recently Cassens and associates\textsuperscript{14} published a retrospective study comparing PBPC manufacturing under clean room conditions to standard laboratory conditions. In contrast to our data, Cassens and associates\textsuperscript{14} found no difference comparing PBPC manufacturing under clean room conditions to standard laboratory conditions. The contamination rate in Group I was 1.03 percent (regular laboratory) versus 1.32 percent in Group II (clean room). Cassens and coworkers\textsuperscript{14} concluded that the rate of bacterial contamination in PBPC components could not be reduced by processing in a clean area.

Cassens and colleagues\textsuperscript{14} used the aliquot technique for sterility testing. One to three mL of specimen was inoculated into bottles designed for pediatric patients (the BACTEC Peds, Becton Dickinson Diagnostic Systems, Sparks, MD). The possibility of obtaining small volumes of blood is advantageous; nevertheless, the BACTEC Peds media are specialized media for the detection of common pediatric pathogens as recommended by the supplier. The use of this culture system in adult patients might therefore lead to different results. Furthermore, to enhance the growth of fastidious bacteria Cassens and coworkers\textsuperscript{14} added NADH and hemin to the BACTEC Peds bottles after inoculating the sample.
With regard to the above-mentioned aspects we compared the results of our study with the results of Cassens et al.\(^1\) and found contamination rates under clean room conditions of 0.8 and 1.32 percent, respectively. As far as we know no other studies have been published analyzing the impact of clean room conditions on the manufacturing of PBPC products. The contamination rate of both studies seems to be similar, but a direct comparison is complicated owing to the different blood culture systems and the different patient or donor age.

Nevertheless, even when the PBPC products are produced under strict aseptic conditions, and following GMP standards, there is still a certain risk for microbial contamination. Considering the situation of the individual patient who suffers from a malignant disorder, the high-dose radio- and/or chemotherapy with PBPC rescue may be the only chance to overcome the disease. Recently we have described that retransfusion of contaminated autologous marrow grafts in patients with antibiotic prophylaxis did not result in serious infectious complications.\(^7\) Nevertheless, each infection could be deleterious to the patient and should therefore be omitted.

In this study manufacturing of PBPCs under GMP conditions was compared to standard laboratory conditions as used before the implementation of a clean room area. Keeping in mind that confounding variables in the historical control group might have influenced the results, we found a significant reduction of microbial contamination when ex vivo processing was performed in a clean area. Because microbial contamination can already occur before processing owing to nonantiseptic skin disinfection, bacterial colonization of CVCs, asymptomatic bacteremia of the donor, or nonsterile medical solutions, the reduction of microbial contamination in our study might be only partially caused by manufacturing in the clean room area. Ongoing monitoring of critical steps of PBPC manufacturing is necessary to avoid contamination; otherwise, patients might be threatened by infections in the posttransplant neutropenic phase owing to PBPC products.

ACKNOWLEDGMENTS

We thank Mrss Cordula Loechelt, Almut Wachtel, Margit Paulus, and Ira Trehkopf and Mr Herbert Rüdiger for expert technical assistance. We appreciate the help of the whole staff participating in this study.

REFERENCES

18. de Korte D, Marcelis JH, Verhoeven AJ, et al. Diversion of first blood volume results in a reduction of bacterial...


