The Pan Genera Detection Immunoassay: a Novel Point-of-Issue Method for Detection of Bacterial Contamination in Platelet Concentrates

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Bacterial contamination of platelet concentrates (PCs) still represents an ongoing risk in transfusion-transmitted sepsis. Recently the Pan Genera Detection (PGD) system was developed and FDA licensed for screening of bacterial contamination of PCs directly prior to transfusion. The test principle is based on the immunological detection of lipopolysaccharide (for Gram-negative bacteria) or lipoteichoic acid (for Gram-positive bacteria). In the present study we analyzed the applicability of this method with regard to detection limit, practicability, implementation, and performance. PCs were spiked with Staphylococcus aureus, Bacillus subtilis, and five different Klebsiella pneumoniae strains, as well as eight different Escherichia coli strains. The presence of bacteria was assessed by the PGD immunoassay, and bacteria were enumerated by plating cultures. Application of the PGD immunoassay showed that it is a rapid test with a short hands-on time for sample processing and no demand for special technical equipment and instrument operation. The lower detection limits of the assay for Gram-positive bacteria showed a good agreement with the manufacturer’s specifications (8.2 × 10^3 to 5.5 × 10^4 CFU/ml). For some strains of K. pneumoniae and E. coli, the PGD test showed analytical sensitivities (>10^6 CFU/ml) that were divergent from the designated values (K. pneumoniae, 2.0 × 10^5 CFU/ml; E. coli, 2.8 × 10^4 CFU/ml). Result interpretation is sometimes difficult due to very faint bands. In conclusion, our study demonstrates that the PGD immunoassay is an easy-to-perform bedside test for the detection of bacterial contamination in PCs. However, to date there are some shortcomings in the interpretation of results and in the detection limits for some strains of Gram-negative bacteria.

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screening method based on flow cytometric detection (10), which was compared with the PGD test, among others. Preliminary results revealed that the PGD test detected Gram-positive bacteria in the given range, but Gram-negative bacterial species such as *Klebsiella pneumoniae* were detected with considerably divergent detection limits, as specified by the manufacturer. Based on these data, we have evaluated this effect systematically and in detail in the present study.

### Materials and Methods

**Bacterial strains and culture conditions.** The two strains *Escherichia coli* ATCC 35218 and *K. pneumoniae* ATCC 13882 were purchased from the American Type Culture Collection (ATCC) (LGCM Promocochm GmbH, Wesel, Germany). Strains *K. pneumoniae* PEI-B-08-08 and *Staphylococcus aureus* PEI-B-23-04 were obtained from the Paul-Ehrlich-Institute (PEI) (Langen, Germany). *E. coli* strain L01207081 and *K. pneumoniae* strain L01204084 were provided by Verax Biomedical Inc. (Worcester, MA). Bacterial spore suspensions with defined titers of *Bacillus subtilis* ATCC 35031 (SGM Biotech Inc., Bozeman, MT) were cultured in Trypticase soy broth (bioMérieux, Nürtingen, Germany) under aerobic conditions at 37°C for 24 to 48 h. All other *K. pneumoniae* and *E. coli* strains were previously isolated from patient specimens at our hospital and were characterized in our microbiological laboratory by standard methods.

**PC collection.** Apheresis-derived single-donor PCs were obtained from the transfusion service UniBlutspendenzentrale OWL (Bad Oeynhausen, Germany) and using the Haemonetics MCS+ (Haemonetics GmbH, Munich, Germany) from healthy blood donors and stored in gas-permeable containers (LN994CF-CP, Haemotest GmbH) at 20 to 24°C with agitation. Predonation sampling was performed after donor arm disinfection using a single-swab method with 70% isopropyl alcohol. The final PC volume was approximately 235 ml.

**PGD testing.** The PGD test (Verax Biomedical Inc.) was performed according to the manufacturer’s instructions. Briefly, 500 µl of PC sample was mixed with 8 drops of reagent 1 (water, methanol, surfactants, and preservative [ProClin300]) by inverting the tube three times, followed by centrifugation at 11,000 × g for 5 min. Subsequently, the supernatant was removed and 8 drops of reagent 2 (water, sodium hydrosulfide, and preservative [sodium azide]) were added to the cell pellet. Sample preparations were not vortexed before the addition of reagent 3 (Tricine buffer with surfactants, anticoagulants, protein stabilizers [bovine, mouse, and rabbit], and preservatives [ProClin300 and sodium azide]). In a change from the manufacturer’s instructions, the pellet was loosened carefully from the bottom of the tube with a disposable pipette but was not divided into three or four fragments. The maximum residence time of reagent 2 did not exceed 2 min. Afterwards, 4 drops of reagent 3 were added, the pellet was completely resuspended by vortexing, and the total sample volume was transferred to the test device. Test performance and interpretation of results were implemented as described by the manufacturer.

**Detection of bacterial proliferation in PCs.** Before bacterial inoculation, all PCs used for spiking experiments were sampled to ensure baseline sterility of the original apheresis bags. Five milliliters of sample was inoculated into both the aerobic (Bact/Alert SA; bioMérieux, Nürtingen, Germany) and anaerobic (Bact/Alert SN; bioMérieux) culture bottles and incubated for up to 7 days. The time periods until detection of bacterial contamination using the PGD test were compared during PC storage at 20 to 24°C after inoculation with <1 CFU/ml of *K. pneumoniae* PEI-B-08-08, *E. coli* ATCC 35228, *S. aureus* PEI-B-23-04, and *B. subtilis* ATCC 35031. Bacterial titers of <1 CFU/ml were achieved by 10-fold serial dilution of stationary-grown overnight cultures in phosphate-buffered saline (PBS), followed by inoculation of 1 ml of the respective dilution (serial dilution of stationary-grown overnight cultures in phosphate-buffered saline) to the test device. Test performance and interpretation of results were implemented as described by the manufacturer.

**RAPD PCR analysis and serotyping.** Bacteria were harvested from cultures grown overnight, and bacterial DNA was extracted using the QIAamp DNA blood kit (protocol D; Qiagen, Hilden, Germany) according to the manufacturer’s instructions. Nucleic acids were eluted with 200 µl elution buffer (Qiagen). Randomly amplified polymorphic DNA (RAPD) PCR analysis was performed using arbitrary primers (ERIC-1, 5'-AGTAAGTCTCTGGOATTCAC-3'; ERIC-2, 5'-AAGTAAGTCTCTGGOATTCAC-3') (36). DNA amplification was carried out in 0.2-ml tubes containing 45 µl reaction mix and 5 µl DNA extract. The reaction mixture consisted of 1× AmpliTaq buffer, including 1.5 mM MgSO4 (Applied Biosystems, Foster City, CA), 200 µM each deoxyribonucleoside triphosphate, 2000 nM each primer, and 5 U of AmpliTaq DNA polymerase (Applied Biosystems). DNA amplification was carried out with the following thermal cycling profile: preliminary denaturation at 95°C for 5 min, followed by 45 cycles of denaturation at 95°C for 30 s, annealing at 35°C for 60 s, and extension at 72°C for 120 s. DNA fragments were separated by gel electrophoresis in a 1.5% agarose gel (Carl Roth, Karlsruhe, Germany) in 1× UltraPure Tris-borate-EDTA (TBE) buffer (pH 8.0) (Gibco Technologies, Paisley, Scotland) containing 500 ng/ml ethidium bromide. After addition of 2 µl of 6× loading dye (Fermentas, St. Leon-Rot, Germany), 10 µl of PCR product was loaded onto the gel. The pUC mix 8 DNA ladder (Fermentas) was used as a molecular size marker. Electrophoresis was carried out at room temperature and at a constant voltage of 6 V/cm in 0.5× TBE buffer.

### Results

**Performance and implementation.** The PGD test is very easy to perform, with a short hands-on time. Altogether, the processing of one sample at a time took 8 min. Reading of the test device took a maximum of an additional 6 min (1 min per result interpretation, six different times in case of negative results). The PGD test procedure took 77 min before negatively tested PCs were accessible for transfusion release, whereas the earliest positive results could be obtained after 37 min. Increasing processing to up to six samples at a time did not considerably influence the hands-on time or time to result.

**Detection of bacterial proliferation in PCs.** PCs were analyzed after inoculation with 0.06 CFU/ml *K. pneumoniae* PEI-B-08-08, 0.19 CFU/ml *E. coli* ATCC 35218, 0.07 CFU/ml *B. subtilis* ATCC 35031, or 0.13 CFU/ml *S. aureus* PEI-B-23-04 at eight different times during storage (Fig. 1), under conditions which might be encountered in practice. The Bact/Alert automated culture system was used to confirm successful contamination. For each inoculated PC, bacteria were detected in a minimum of one Bact/Alert culture bottle at the time of inoculation, whereas bacterial contamination was detected for all four strains under both aerobic and anaerobic conditions after 24 h (data not shown). Subsequently, the applicability of the PGD test was analyzed with regard to sensitivity and the time period until detection of bacterial contamination. Contamination with *S. aureus* was initially detected after 48 h, with a corresponding titer of 8.4 × 106 CFU/ml (Fig. 1A). The bacterial titer of 8.4 × 103 CFU/ml at the previous sampling point was very close to the specified detection limit of 8.2 × 105 CFU/ml, resulting in a statistical contingency to obtain a positive or negative result. The PGD test revealed an initially positive result for *B. subtilis* after 72 h, with a corresponding different *E. coli* and five different *K. pneumoniae* strains and incubated at 22°C with agitation for 48 h. Samples were diluted in a 10-fold dilution series with sterile PCs, three negative controls were added randomly, and all samples were analyzed by PGD. Test devices were read by five independent experimenters in a blinded trial. Each individual result was scored (positive, 1 points; arguable, 0.5 point; negative, 0 points), and total results were evaluated as follows: (i) 0 to 1, negative; (ii) 1.5 to 2.5, arguable; and (iii) 3 to 5, positive.

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At the previous sampling point, the bacterial titer of 667 CFU/ml was too low to obtain a positive result. In summary, the results obtained for the Gram-positive strains correlated well with the analytical sensitivities reported by the manufacturer (S. aureus, 8.2 × 10^3 CFU/ml; B. cereus [representative for B. subtilis], 1.2 × 10^4 CFU/ml).

In contrast, PGD testing of PCs inoculated with Gram-negative bacteria demonstrated analytical sensitivities that were considerably divergent from the specified lower detection limits, followed by a delayed detection of bacteria. The earliest positive result for the PC unit inoculated with E. coli was obtained after 54 h, with a bacterial titer of 9.20 × 10^6 CFU/ml (Fig. 1C), whereas the detection limit reported by the manufacturer was 2.8 × 10^4 CFU/ml. For the PC inoculated with K. pneumoniae, the PGD test revealed an arguably positive result, with very faint bands after 30 h of incubation, with a corresponding bacterial titer of 2.13 × 10^7 CFU/ml (Fig. 1D). The lower detection limit was indicated by the manufacturer to be 2.0 × 10^4 CFU/ml; therefore, the PGD test should already provide a positive result after 24 h, as well as at this sampling point. Definite positivity was not achieved until sampling after 48 h, with a corresponding bacterial titer of 8.97 × 10^8 CFU/ml. Additionally, we observed difficulties in obtaining valid procedural control windows when analyzing high titers of bacteria, especially for K. pneumoniae. According to our consultation with the manufacturer, valid procedural control windows are not necessary in the case of a clearly positive result (personal communication).

To analyze the detection limits of the PGD test for K. pneumoniae and E. coli in detail, PCs were spiked with eight different E. coli and five different K. pneumoniae strains and incubated at 22°C with agitation for 48 h. The two strains E. coli L01207081 and K. pneumoniae L01204084, which were used for the FDA validation studies, were used as reference strains. Samples were diluted in a 10-fold dilution series with sterile PCs and analyzed by PGD. Test devices were read by five independent experimenters in a blinded trial (Table 1). To allow for the subjectivity of reports by each individual experimenter and to obtain an objective qualitative result, we scored each individual result and calculated the total results as described in Materials and Methods. In general, very faint bands complicated the result interpretation, which is clearly comprehensible on the basis of the test device interpretation presented in Table 1. The reference strain E. coli L01207081, as
TABLE 1. Strain characteristics and detection limits for different *E. coli* and *K. pneumoniae* strains

<table>
<thead>
<tr>
<th>Species and isolate</th>
<th>Strain characteristics</th>
<th>Titer (CFU/ml)</th>
<th>Score given by experimenter:</th>
<th>Total score&lt;sup&gt;a&lt;/sup&gt;</th>
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<td></td>
<td>RAPD cluster&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>2</td>
<td>3</td>
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<tr>
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<td>+</td>
</tr>
<tr>
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<td>+</td>
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<td>+</td>
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<tr>
<td>L01207081</td>
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<td>+</td>
<td>+</td>
</tr>
<tr>
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<td>+</td>
<td>+</td>
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<tr>
<td><em>K. pneumoniae</em></td>
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<tr>
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<tr>
<td>L01204084</td>
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<td>+</td>
</tr>
<tr>
<td>Isolate 01662</td>
<td>KP-V</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Isolate 7724</td>
<td>KP-VI</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

<sup>a</sup>  Scores were evaluated as described in Materials and Methods.

<sup>b</sup>  Strain clustering is based on identical banding pattern for both primers used in RAPD-PCR analysis.

<sup>c</sup>  NA, not applicable.

well as *E. coli* isolate A001S, were detected in the given range of 2.8 \times 10^4 CFU/ml with a maximum score of 5.0 (positive, concordant results by all experimenters). In contrast, *E. coli* isolates MS82 and MS88 were detected (score, 5.0) only with bacterial titers of 10^6 CFU/ml and 10^7 CFU/ml, respectively. *E. coli* isolates 079 and 008 were definitely detected (score, 5.0) for titers of 10^7 CFU/ml (score, 2.0 or 2.5). Only *E. coli* strain ATCC 35218 (arguable; score, 2.0) and isolate 835 (positive; score, 5.0) were detectable with bacterial titers of 10^6 CFU/ml. Accordingly, the *K. pneumoniae* reference strain L01204084 was detected (score, 5.0) with a bacterial titer of 7.95 \times 10^3 CFU/ml, which is closely below the given range of 2.0 \times 10^6 CFU/ml. However, similarly to the detection of *E. coli*, *K. pneumoniae* strains PEI-B-08-08 and ATCC 13882 and isolate 13734 were positive only at a concentration of 10^6 CFU/ml (score, 5.0). Only isolate 01662 (score, 4.0) was also detectable with a bacterial titer of 10^7 CFU/ml.

**RAPD-PCR analysis and serotyping.** To study the molecular relatedness of *K. pneumoniae* and *E. coli* strains and to infer a potential connection to the differences in detection limit, RAPD-PCR analysis was performed using two single-oligonucleotide primers with arbitrary sequences. To determine whether the composition of *E. coli* LPS influences the immunologic detection, strains were serotyped regarding their O and H side chains (Table 1). Primer ERIC-1 yielded six different banding patterns for the different *K. pneumoniae* strains and seven different patterns for *E. coli*, whereas primer ERIC-2 yielded five different patterns for *K. pneumoniae* and six different banding patterns for *E. coli* (data not shown). Regarding both primers, a molecular relatedness was detectable only for the two *E. coli* strains A001S and L01204081 (cluster EC-VI).
Both strains AS001 and L01204081 had no H antigen, whereas the O antigens are not typeable so far. Interestingly, these two E. coli strains were detected by the PGD assay at as low as 10² CFU/ml, in accordance with the detection limit reported by the manufacturer. All other strains demonstrated no molecular relatedness based on RAPD-PCR profile and serotyping results (Table 1).

**DISCUSSION**

Different strategies to optimize the detection of bacterial contamination in PCs have been discussed (8): (i) early testing after preincubation for 24 h using culture methods with PC release and negative-to-date status, (b) early testing after preincubation for 24 h using rapid methods (NAT and FACS), and (iii) late testing in combination with rapid detection methods (NAT, FACS, and PGD) (8). The consensus of several studies is a late-sampling strategy in combination with rapid detection methods (9, 18), offering the possibility of sample drawing at a later stage to overcome the risk of sampling error due to initially low bacterial count or slow-growing bacterial species. Decisions regarding bacterial testing strategies always have to balance the requirements for rapid detection and assay sensitivity (27). Considering the logistic complications involved in testing immediately before transfusion, the ideal rapid screening method for bacterial contamination of PCs should be easy to perform, should not be laborious, and should have a quick turnaround time (24). The optimal solution comprises a direct, bedside-like bacterial detection method applied directly prior to transfusion. The PGD test fulfills most of these requirements. Specialized personnel qualifications and technical equipment are not required for the application of the PGD test, and instrument operation does not considerably interrupt the work flow in the PC-providing facilities. The simplicity of performance is a promising aspect that should promote usage of the PGD test as a bedside-like test. The test requires a minimum time to result of approximately 30 min in the case of positive results; the time to result increased up to 1.5 h in the case of negative results, which is also comparatively maintainable for a bedside-like test. Therefore, the greatest advantages of the PGD test are its rapidity and easy performance.

The analytical sensitivity of the PGD test method is specified to be 10³ to 10⁵ CFU/ml for Gram-negative bacteria and 10⁷ to 10⁹ CFU/ml for Gram-positive bacteria. Detection of bacterial contamination under conditions which might be encountered in practice showed a good agreement with the given detection limits for Gram-positive bacteria. The PGD test showed analytical sensitivities for some Gram-negative strains, e.g., E. coli and K. pneumoniae strains, that were considerably divergent from the designated values (K. pneumoniae, 2.0 × 10⁴ CFU/ml; E. coli, 2.8 × 10⁴ CFU/ml), resulting in a delayed detection of bacteria during PC storage or false-negative test results. The systematic analysis of different Gram-negative K. pneumoniae and E. coli strains confirmed these results. The detection limit was mostly determined in the 10⁶ to 10⁷ range, with the exception of the reference strains and E. coli isolate A001S. We also observed this problem with some strains of other Gram-negative bacteria, such as Serratia marcescens, Pseudomonas aeruginosa, and Klebsiella oxytoca (data not shown). To analyze whether the strain-dependent detection limits observed by the PGD test also occur using nonimmunological rapid detection principles, PCs were analyzed in parallel using a recently published flow cytometric assay (BactiFlow) (10). Strain-dependent detection limits were not observed using this flow cytometric detection principle (data not shown). Molecular relatedness between RAPD-PCR patterns, as well as serotypes, of all investigated isolates was observed only for the E. coli reference strain L01204081 and isolate A001S. These two strains were the only strains which were detected in the specified detection limit of the PGD assay. A relationship between RAPD-PCR patterns or serotypes of the other strains and the detection limit of the PGD test could not be demonstrated. These results reinforce the observation that the PGD test showed differences in the efficiency of detection of different strains. The LPS and LTA components of the bacterial cell are attractive targets for the detection of bacterial contamination in blood products. However, the use of these two peptides has to ensure that all relevant bacterial species are detected with equal sensitivity (3), and it seems that the PGD test could not detect the LPS components of different E. coli and K. pneumoniae strains with equal sensitivity. Potential factors interfering with the test antigens were the capsule structure and exopolysaccharides which disguise the LPS antigen structure. In particular, Klebsiella spp. express a large capsule (K antigen) playing a significant role in pathogenicity (29). Bacteria also express two different classes of LPS: smooth LPS, which is composed of O antigen, complete core oligosaccharides and the lipid A hydrophobic domain, and rough LPS, which lacks the O antigen but possesses lipid A and progressively shorter core oligosaccharides (35). The epitopes of the utilized antigens strongly affect the detection efficiency, and the PGD test might benefit from an extended antigen detection panel. Notably, three E. coli strains tested did not possess the H antigen, including the two strains that met manufacturer specifications for the lower detection limit. This raises the question of whether the absence of the H antigen might influence the detection efficiency. However, antibodies used for immunological detection by PGD target the bacterial lipopolysaccharide, whereas the flagellum is not involved. This is further reinforced by the fact that E. coli isolate 079, which, in contrast, is detected only in the 10⁷ CFU/ml range, did not possess the H antigen either.

Previous studies have also shown that the storage time for contaminated PCs significantly differs between Gram-negative and Gram-positive organisms (median storage time, 2.5 versus 5 days; P < 0.0001) (14)). Consequently, the spectrum of bacteria accounting for the majority of transfusion-associated septic complications and fatal casualties varies. In all fatal cases, endotoxin was present in the posttested products (14). Despite the fact that Gram-positive organisms were detected most frequently in PCs, Gram-negative organisms accounted for the majority of transfusion fatalities (59.7%) (4). Kuehnert et al. also observed that the majority of transfusion fatalities were associated with Gram-negative organisms and PC transfusion within 3 days of storage, whereas PCs associated with nonfatal transfusion complications were related to Gram-positive organisms and PC transfusion after 5 days of storage (BaCon study) (14). The presence of endotoxin in PCs contaminated with Gram-negative organisms most likely explains the correlation between transfusion fatalities with Gram-neg-
ative organisms, short storage time, and death (1). In contrast, Reading and Brecher observed that fatalities show a tendency to be equally divided between Gram-positive and Gram-negative organisms (27). Remarkably, \textit{E. coli} and \textit{K. pneumoniae} were responsible for 5.8\% and 17.3\% of all PC transfusion fatalities in the United States from 1976 to 1998 (27). \textit{Klebsiella} spp. were the most commonly reported Gram-negative organisms (21). As mentioned above, we observed that the PGD immunoassay revealed some strain-dependent differences, especially regarding the efficiencies of detection of different \textit{K. pneumoniae} and \textit{E. coli} strains. All of these strains were capable of growing in PCs and therefore had the potential to induce septic transfusion reactions. The predominant proportion of Gram-negative organisms involved in transfusion fatalities emphasizes the requirement that the divergent intra- and interspecies detection efficiencies of the PGD test had to be adapted for an effective improvement of PC safety. Recently, a first study using the PGD test for screening of 7,733 whole-blood-derived PCs was published (38). Two PCs were positive for coagulase-negative staphylococci and group B streptococci, respectively, whereas 12 PCs returned false-negative results. Gram-negative bacteria were not detected. However, only PCs showing a reactive signal after application of the PGD test were tested using automated culture systems. Therefore, the frequency of PCs contaminated with bacteria which were missed by the PGD test is unknown. The authors also state that the frequency of bacterial contamination observed in this study is at the lower end of that reported in the literature, which might be explained by a lower sensitivity of the PGD test compared to the culture-based test (38).

The critical factor for evaluation of any detection method is the definition of the number of bacteria representing clinically significant contamination (24). In addition to the previously mentioned disadvantages, incubation or cultivation methods were, to date, the only methods for sterility testing of PCs, recognizing a minimum contamination of at least one viable microorganism. The lower detection limits of all available rapid detection methods, including NAT, FACS, and PGD, made them inadequate for sterility testing of PCs due to their relative lack of sensitivity for the detection of a low-level bacteremia. Nowadays, these methods need to be considered only as screening methods for bacterial contamination, not for sterility testing. The clinical significance of the bacteriological titer, and therefore the minimum detection limit required for a rapid screening method, is still questionable. However, it is commonly accepted that initial levels of bacteria need to increase around 10,000-fold to have a clinical impact. Low levels of bacteria may have no clinical significance, but transfusion reactions may also occur with as few as $10^2$ to $10^3$ CFU/ml, even with organisms usually regarded as nonpathogenic (e.g., \textit{S. epidermidis}) (24). Therefore, the sensitivities of screening methods had to be increased as much as possible (e.g., BactiFlow flow cytometry, 150 CFU/ml [10]; NAT techniques, 22 to 29 CFU/ml [33]), but sensitivities of up to $10^4$ CFU/ml, as specified for the PGD test, are commonly accepted.

A second shortcoming of the PGD assay is the interpretation of results, which is sometimes difficult due to very faint bands and a strong dependence on the experimenter. In addition, the expectations of the experimenter in routine clinical use are not comparable to the situation with spiking experiments in the present study. The average rate of contamination of PCs varies from 0.08 to 0.7\%, depending on technology, testing protocols, and additional intervention methods (34, 37). Indeed, the incidence is approximately 1:2,000 to 1:3,000 in PCs (2, 7). Therefore, the chance of missing a contaminated PC using a PGD test resulting in very faint bands is considerable, regarding the high proportion of negative tests. Of course, PCs with an at least arguable result have to be discounted immediately, but the general shortage of blood products, the high costs (particularly for apheresis products), and the ethical concerns regarding the donor demand a clear and nonambiguous result.

In this context, an improvement of the PGD test in regard to result interpretation, potentially by an automated reader, would considerably enhance test performance and practicability. An interlaboratory comparison of proficiency testing with different blood centers and transfusion facilities needs to be performed to compare experiences using the PGD immunoassay, especially for strains which are difficult to detect within the determined detection limits.

In summary, application of the PGD assay as a novel point-of-issue detection method for bacterial contamination in PCs has revealed some shortcomings demanding further improvement in order to effectively increase the safety of PCs: (i) Gram-positive bacteria were detected in the given range, but we observed a strain-dependent detection limit for different \textit{E. coli} and \textit{K. pneumoniae} strains that, in some cases, is considerably lower than the given range, and (ii) result interpretation is often complicated due to very faint bands, and the detection of positive PGD results for different \textit{E. coli} and \textit{K. pneumoniae} strains is strongly dependent on the experimenter performing the reading. Without a doubt, the PGD test is a rapid and easy-to-handle immunoassay for the detection of bacterial contamination in PCs which does not require special precautions or stringent work flow conditions.

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REFERENCES


