High-Volume Extraction of Nucleic Acids by Magnetic Bead Technology for Ultrasensitive Detection of Bacteria in Blood Components

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Background: Nucleic acid isolation, the most technically demanding and laborious procedure performed in molecular diagnostics, harbors the potential for improvements in automation. A recent development is the use of magnetic beads covered with nucleic acid–binding matrices. We adapted this technology with a broad-range 23S rRNA real-time reverse transcription (RT-PCR) assay for fast and sensitive detection of bacterial contamination of blood products.

Methods: We investigated different protocols for an automated high-volume extraction method based on magnetic-separation technology for the extraction of bacterial nucleic acids from platelet concentrates (PCs). We added 2 model bacteria, Staphylococcus epidermidis and Escherichia coli, to a single pool of apheresis-derived, single-donor platelets and assayed the PCs by real-time RT-PCR analysis with an improved primer–probe system and locked nucleic acid technology. Co-amplification of human \(\beta_2\)-microglobulin mRNA served as an internal control (IC). We used probit analysis to calculate the minimum concentration of bacteria that would be detected with 95% confidence.

Results: For automated magnetic bead–based extraction technology with the real-time RT-PCR, the 95% detection limit was \(29 \times 10^3\) colony-forming units (CFU)/L for S. epidermidis and \(22 \times 10^3\) CFU/L for E. coli. No false-positive results occurred, either due to nucleic acid contamination of reagents or externally during testing of 1030 PCs.

Conclusions: High-volume nucleic acid extraction improved the detection limit of the assay. The improvement of the primer–probe system and the integration of an IC make the RT-PCR assay appropriate for bacteria screening of platelets.

Real-time nucleic acid amplification has become a standard application in the clinical laboratory. The superior sensitivity allows rapid diagnosis of infectious diseases and detection of bacterial contamination of blood products; however, the prerequisite to benefit from this technique is efficient nucleic acid extraction protocols. Overall sensitivity is determined by nucleic acid yield, purity, and the amount of sample equivalents that can be transferred into the amplification reaction. Conventional manual sample-preparation methods are labor intensive and susceptible to contamination, handling variation, and errors (1). The goals of automation are to avoid human error, improve precision, obtain reproducible results, and permit analysis of large numbers of samples. Most recent developments use magnetic beads that bind nucleic acids to their silica surfaces; the bound nucleic acids are then transferred through the steps of the extraction process (2). This extraction technology has successfully been evaluated to screen for viral nucleic acids in routine blood donations (3, 4).

The application of nucleic acid amplification technology for testing blood donations for viruses has reduced the risk of such transfusion-transmitted infections; however, bacterial contamination of blood platelets and the incidence of illness and fatalities caused by bacterial contamination are known to greatly exceed those of viruses (5). Bacterial contamination of blood components is the most frequent infectious complication linked to transfusion, and its incidence exceeds that of transmissible viral infections by more than 2 orders of magnitude.

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Platelet concentrates (PCs) are the most important in this context because their storage conditions support bacterial growth. The reported prevalence of bacterially contaminated PCs varies from 0.08% to 0.7% in countries that perform prospective testing, depending on the technology, testing protocols, and other methods. Furthermore, the British Hemovigilance System Serious Hazards of Transfusion report and the American Bacterial Contamination study have documented the importance of transfusion-transmitted infections. Reflecting these concerns, the American Association of Blood Banks implemented a directive stating that blood banks and transfusion services shall have methods to limit and detect bacterial contamination in all platelet components after March 2004. The different methods for bacterial screening can be categorized into culture and rapid-detection methods. Culture methods, which require a long time to demonstrate the presence of bacteria, have remained the preferred way for platelet bacteria screening, although bacteria in PCs have been detected with other approaches. A bacterial-detection test should be rapid, affordable, adequately sensitive, specific, and simple to perform. Although no single approach now meets all these criteria, molecular biological approaches offer promising opportunities for detecting contaminating organisms in blood components.

The introduction of real-time PCR analysis has led to considerable progress in automating the amplification and detection steps, but nucleic acid isolation remains laborious when performed manually. Compared with standard separation procedures, magnetic-separation techniques have several advantages, including simplicity in handling and a high automation potential.

In this study, we adapted magnetic bead technology for extracting nucleic acids in the screening of platelet donations for bacteria. Our focus was on minimizing sampling error and increasing the sensitivity of our broad-range 23S rRNA real-time reverse transcription (RT)-PCR assay. We enhanced the sensitivity of the 23S rRNA RT-PCR assay for detecting bacterial contamination of PCs by modifying sample volume, nucleic acid input, probe technology, and the nucleic acid extraction method. We also implemented an internal control (IC) and, for the first time, used probit analysis to systematically determine detection limits.

**Materials and Methods**

**BLOOD COLLECTION**

Apheresis-derived platelets from individual donors were obtained from healthy blood donors via the OWL transfusion service, Bad Oeynhausen, Germany, after standard processing with the Hemonetics MCS+ (Hemonetics) and stored at 20 °C to 24 °C with agitation. All PC experiments were carried out 1–3 days after donation.

**MICROBIOLOGIC STERILITY CONTROL**

Sterility of tested PCs was confirmed with the BacT/Alert 3D continuous-monitoring system (bioMérieux). Aerobic and anaerobic-culture bottles (40-mL bottles) were inoculated with 5-mL aliquots of platelet samples and incubated at 37 °C for up to 7 days in the automated culture system.

**BACTERIAL STRAINS AND CULTURE CONDITIONS**

We subcultured the bacterial strains used in specificity testing (see Table 1 in the Data Supplement that accompanies the online version of this article at http://www.clinchem.org/content/vol53/issue1) in trypticase soy broth (bioMérieux) at 37 °C for 24 h under aerobic conditions. Aliquots (100 μL) of serial dilutions of logarithmically growing cultures were plated on tryptase soy agar to determine the bacterial titer. Bacteria of each strain were added to PC samples negative for bacterial contamination to achieve a final concentration of 10³ colony-forming units (CFU)/L before nucleic acid extraction.

To test analytical sensitivity, we used Paul-Ehrlich-Institut (PEI) bacterial calibrators *Staphylococcus epidermidis* PEI-DSM 3269 (1.58 × 10¹¹ CFU/L) and *Escherichia coli* PEI-B-19-03 (1.66 × 10¹⁰ CFU/L). An impedance-monitoring system was used to characterize bacterial growth kinetics; bacteria were removed during the logarithmic phase and frozen in human albumin at −80 °C.

**DETERMINATION OF OPTIMAL TEST SAMPLE VOLUME**

To determine the influence of sample volume, we added bacteria to 2.4, 4.8, or 9.6 mL of PCs and tested 3 different protocols for the Chemagic viral DNA/RNA reagent set (Chemagen). For each protocol, we added *S. epidermidis* to bacteria-free PCs to a final concentration of 10⁰ CFU/L, serially diluted the PCs with pooled PC down to 10³ CFU/L, and extracted and amplified each sample.

**AUTOMATED NUCLEIC ACID EXTRACTION**

We performed high-volume, simultaneous extraction of total DNA and RNA with the Chemagic viral DNA/RNA reagent set and Magnetic Separation Module I (Chemagen). The magnetic beads used in this method consist of an iron oxide core surrounded by a matrix that binds total nucleic acids. Nucleic acids bound to magnetic beads after cell lysis were separated with Chemagic Magnetic Separation Module I (Chemagen). The Chemagen magnetic-separation system features an electromagnet and magnetizable metal rods, which are immersed into the magnetic bead suspension and connected to a stirring motor. Up to 12 samples were processed in parallel. In a modification of the manufacturer’s instructions, we mixed 2.4 mL of PC with 2.4 mL of lysis buffer. Following the addition of 20 μL of protease and vortex mixing for 10 s, we incubated the mixture for 10 min at 55 °C. We subsequently
mixed the lysate with 7.5 mL of binding buffer containing 100 μL of magnetic beads. The Chemagic Magnetic Separation Module I automatically performed the complete isolation process, including binding, washing, and elution. A 30-min binding time and continual mixing ensured magnetic capture of the nucleic acids. After 2 washing steps, we eluted the nucleic acids in 100 μL of elution buffer.

REAL-TIME RT-PCR

Extracted nucleic acid samples were analyzed by a 1-step RT-PCR method incorporating Tth DNA polymerase (Eurogentec). The probe and primers for amplifying a 122-base pair fragment of bacterial 23S rRNA were based on a previously published alignment of 23S rRNA sequences (14). As an IC, we coextracted and coamplified human β2-microglobulin (B2-MG) mRNA with each reaction to avoid false-negative results due to PCR inhibition. RT-PCRs were carried out on the Rotor-Gene 3000 cycler system (Corbett Research) in 0.2-mL tubes containing 40 μL of reaction mix and 10 μL of extract. The reaction mix consisted of 1× Eurogentec Tth buffer, 3.5 mmol/L MgCl2, 400 μmol/L of each deoxynucleoside triphosphate, 200 nmol/L of each 23S forward primer, 600 nmol/L 23S reverse primer, 200 nmol/L of each IC primer (see Table 2 in the online Data Supplement), 200 nmol/L 23S fluorescent probe, 200 nmol/L IC fluorescent probe, and 2.5 U Tth polymerase. Nucleotide positions of primers and probes refer to the E. coli 23S rDNA sequence (GenBank accession no. AF053966). The sequences of the 3 forward primers were 5′-CTKC-CCAGAAACGCTCTA-3′, 5′-CTKCAGAAAAAGCYTCTA-3′, and 5′-CTKCAGAAAAGCYTCTA-3′ (nucleotides 1560–1579); that of the reverse primer was 5′-CCATTTTGCCAGCTTCTT-3′ (nucleotides 1663–1682). The sequence of the locked nucleic acid (LNA) TaqMan probe from Eurogentec [5′-ACTaCcTgTGCtCGgTTT-3′ (nucleotides 1624–1607)] included the LNA bases (lower case). The probe was synthesized with the fluorescent reporter 6-carboxyfluorescein (FAM) covalently coupled to the 5′ end and a dark quencher to the 3′ end. The IC was detected with a primer–probe system (see Table 2 in the online Data Supplement) (23). Eurogentec synthesized the IC probe with the fluorescent reporter 2′,7′-dimethoxy-4′,5′-dichloro-6-carboxyfluorescein (JOE) covalently coupled to the 5′ end and a dark quencher to the 3′ end. Cycling conditions for the 23S rRNA RT-PCR were 60 °C for 20 min and 95 °C for 4 min, followed by 40 cycles of denaturation at 95 °C for 10 s, annealing at 60 °C for 35 s, and extension at 72 °C for 10 s. Negative controls (sterile water, no template) and positive controls [PCs with low bacterial titers (30–50 × 103 CFU/L)] were included in each run.

We tested 2 other probe systems in the evaluation study—the conventional TaqMan probe (14) and a minor groove binder (MGB) group (see LNA sequence) probe (Applied Biosystems)—to detect the bacterial nucleic acids at the same high temperature (60 °C). In contrast to conventional TaqMan probes, MGB probes contain an MGB with a nonfluorescent quencher.

PROBIT ANALYSIS

We used probit analysis and SPSS software, version 11.0, to calculate the 95% detection limit.

ASSAY DEVELOPMENT

We optimized our method for detecting bacterial contamination in PCs with abundant 23S rRNA to obtain the highest achievable assay sensitivity, specificity, and rapidity (14). The simultaneous extraction of total nucleic acids should impart increased sensitivity. To determine the influence of sample volume on sensitivity, we tested 3 different protocols with PCs contaminated with 103 to 107 CFU/L of S. epidermidis. Negative controls included in each run revealed no false-positive results (Fig. 1). The 9.6-mL protocol showed the worst mean threshold-crossing point (CP) values for 23S rRNA, the worst detection rates for low bacterial loads, and the worst analytical precision (SDmean, 1.28). The best CP 23S rRNA values were obtained for the 4.8-mL extraction protocol (SDmean, 1.02), but the 2.4-mL protocol contributed to better analytical precision (SDmean, 0.57; see Table 3 in the online Data Supplement). Therefore, these data and a reduced loss of product were the bases for our choice of the 2.4-mL protocol for further studies.

To increase specificity and to investigate the sensitivity at higher melting temperatures, we compared modified TaqMan probes with higher melting temperatures with conventional TaqMan probes (24). Adjusting the PCR protocols to the different probe technologies revealed that an annealing temperature of 60 °C increased assay specificity. Therefore, we used the same PCR protocol to compare the performance of 3 different probe technolo-

Fig. 1. Determination of the optimal sample volume.

Three different protocols of the nucleic acid extraction reagent set were tested with 2.4, 4.8, or 9.6 mL of PCs with added bacteria (S. epidermidis). Treated PCs were then extracted and amplified by the RT-PCR as described in Materials and Methods. Data are presented as the mean with the corresponding SD.
gies. First, we designed a TaqMan probe conjugated with a MGB ligand at the 3′ end and then an LNA probe. To investigate the performance of the LNA, MGB, and conventional TaqMan probes, we used the same PCR protocol to compare the CP 23S rRNA values and fluorescence intensities of the amplification curves (Fig. 2). The mean CP 23S rRNA values for the 3 probes were different: LNA, 15.60 (0.19); MGB, 18.39 (0.41); conventional TaqMan probe, 21.72 (0.23). Furthermore, the LNA probe produced higher fluorescence intensity. Therefore, the LNA probe yields a slight increase in assay sensitivity.

After determining the optimal sample volume, establishing the most efficient probe technology, and implementing the IC, we used real-time RT-PCR to screen PCs that tested negative for bacterial cultures. Fig. 3 shows a representative real-time RT-PCR screening for bacterial contamination.

**SPECIFICITY TESTING OF THE REAL-TIME RT-PCR ASSAY FOR 23S rRNA**

To determine the ability of the universal probe and primers set to detect a broad range of bacteria, we extracted total nucleic acids from 57 bacterial strains (15 genera and at least 28 species; see Table 1 in the online Data Supplement) within a platelet matrix and assayed them for 23S rRNA by real-time RT-PCR. The negative controls yielded no false positives in any of the assays for 1030 PCs uncontaminated by bacterial cultures. We also investigated the risk of false-positive results caused by high-titer samples cross-contaminating negative samples during the analysis. We created 2 matrices (3 × 4) of PC samples with added bacteria (10^8 CFU/L) alternating with samples without added bacteria and processed the samples simultaneously on the Magnetic Separation Module. We detected no false-positive results (data not shown).

**ANALYTICAL SENSITIVITY AND PRECISION TESTING**

For the first systematic evaluation of this assay’s analytical sensitivity (14), we calculated the 95% detection limit by probit analysis. Five apheresis-derived, contamination-free PCs were pooled. S. epidermidis PEI-DSM 3269 was added to PC to 10^9 CFU/L (as a positive control). Extracted nucleic acids were analyzed in triplicate by RT-PCR as described in Materials and Methods. CP mean values (SD) for 23S rRNA (upper panel) were as follows: TaqMan probe, 21.72 (0.23); MGB probe, 18.39 (0.41); LNA probe, 15.60 (0.19). CP mean values (SD) for coamplified B2-MG mRNA IC (lower panel) were as follows: TaqMan probe, 25.40 (0.23); MGB probe, 24.13 (0.15); LNA probe, 24.33 (0.25). Thick solid line indicates negative control (water).
each dilution. We repeated this experiment with *E. coli* PEI-B-19-03. The number of bacteria present at the time of inoculation was verified by a plating assay (114 × 10^3 CFU/L for *S. epidermidis* and 122 × 10^3 CFU/L for *E. coli*). The 95% detection limit was 29 × 10^3 CFU/L for *S. epidermidis* and 22 × 10^3 CFU/L for *E. coli*. The bacterial load for which all replicates were positive was 32 × 10^3 CFU/L for both *S. epidermidis* and *E. coli* experiments (Table 1). Intraassay and interassay variation and SDs calculated for CP values were low (Table 2).

**Implementation of the IC in Clinical Assays**

For implementation of an IC reaction, which would be useful for continuous monitoring of the sample-preparation and amplification process, we coamplified the B2-MG mRNA sequence. To ensure that the IC did not suppress amplification of 23S rRNA in routine screening tests, we added increasing amounts of bacteria to contamination-free PCs containing B2-MG mRNA and extracted the nucleic acids as described above. We evaluated the detection of low target concentrations with and without B2-MG coamplification and confirmed that the IC had no effect on assay sensitivity. We also found that the use of different PC sample volumes had no influence on the amount of extracted human mRNA. Therefore, coamplification of B2-MG mRNA did not decrease the detection limit of the RT-PCR (data not shown).

**Discussion**

Bacterial screening of PCs has the potential to increase the safety of the blood supply. Several automated detection systems have become available to test for contaminated PCs, but their utility is often restricted by long assay times or the lack of sensitivity for samples with low bacterial loads (25). In this study, we optimized our broad-range 23S rRNA RT-PCR assay for the detection of bacterial contamination of PCs (14).

To increase the assay’s sensitivity, specificity, and suitability for routine screening and to minimize sampling error, we evaluated the feasibility of automating the nucleic acid extraction procedure. Classic methods of DNA and RNA isolation are based on column or precipitation methods. These techniques require centrifugation or vacuum steps and often have lengthy processing times, as well as volume limitations. Covering magnetic beads with nucleic acid–binding matrices is the basis of a different methodologic approach (26). Binding to magnetic beads allows the generic (not sequence-specific) isolation of nucleic acids. Magnetically facilitated isolation with Chemagic Magnetic Separation Module I allows automated nucleic acid extraction from large PC volumes with high sensitivity. The instrument comprises a moveable separation head with 12 magnetizable metal rods that

### Table 1. Analytical sensitivity.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Bacterial load, ×10^3 CFU/L</th>
<th>Samples, no. PCR positive/total no.</th>
<th>Percent positive</th>
<th>C_P 23S rRNA</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em> PEI-B-19-03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>64</td>
<td>16/16</td>
<td>100</td>
<td>28.82</td>
<td>0.51</td>
</tr>
<tr>
<td></td>
<td>32</td>
<td>15/15</td>
<td>100</td>
<td>28.92</td>
<td>0.59</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>13/15</td>
<td>87</td>
<td>29.19</td>
<td>1.15</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>14/22</td>
<td>64</td>
<td>30.55</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>8/13</td>
<td>62</td>
<td>30.73</td>
<td>1.05</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>9/19</td>
<td>47</td>
<td>30.84</td>
<td>1.02</td>
</tr>
<tr>
<td><em>S. epidermidis</em> PEI-DSM 3269&lt;sup&gt;c&lt;/sup&gt;</td>
<td>64</td>
<td>22/22</td>
<td>100</td>
<td>30.50</td>
<td>0.96</td>
</tr>
<tr>
<td></td>
<td>32</td>
<td>22/22</td>
<td>100</td>
<td>30.73</td>
<td>1.21</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>26/35</td>
<td>74</td>
<td>31.16</td>
<td>1.34</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>16/26</td>
<td>62</td>
<td>31.24</td>
<td>0.77</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>15/26</td>
<td>58</td>
<td>32.82</td>
<td>0.80</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>11/21</td>
<td>52</td>
<td>33.12</td>
<td>0.81</td>
</tr>
</tbody>
</table>

<sup>a</sup> CP 23S rRNA, mean C_P in channel FAM (23S rRNA RT-PCR).

<sup>b</sup> For *E. coli* PEI-B-19-03, the 95% detection limit was 22 × 10^3 CFU/L.

<sup>c</sup> For *S. epidermidis* PEI-DSM 3269, the 95% detection limit was 29 × 10^3 CFU/L.

### Table 2. Precision testing of the 23S RT-PCR assay.

<table>
<thead>
<tr>
<th>Strain</th>
<th>23S rRNA, C_P (SD)</th>
<th>IC, C_P (SD)&lt;sup&gt;d&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. epidermidis</em>&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PEI-DSM 3269</td>
<td>26.97 (0.39)</td>
<td>26.62 (0.30)</td>
</tr>
<tr>
<td>Intra-assay variability&lt;sup&gt;b&lt;/sup&gt;</td>
<td>26.89 (0.39)</td>
<td>26.61 (0.28)</td>
</tr>
<tr>
<td>Interassay variability&lt;sup&gt;c&lt;/sup&gt;</td>
<td>28.24 (0.43)</td>
<td>27.59 (0.36)</td>
</tr>
</tbody>
</table>

<sup>a</sup> To PC was added *E. coli* PEI-B-19-03 or *S. epidermidis* PEI-DSM 3269 (10^6 CFU/L). Nucleic acid extraction and RT-PCR were performed as described in Materials and Methods.

<sup>b</sup> Eight nucleic acids for replicates were extracted and assayed in quadruplicate.

<sup>c</sup> Interassay variability was calculated from 8 independent RT-PCR runs.
are able to rotate on their long axes. As the rotation mixes and resuspends the magnetic beads, a transport system horizontally moves the tubes with samples and buffers under the separation head. To avoid contamination, the metal rods pick up the plastic disposables before the isolation protocol (3). The module automates the entire extraction process except for the pretreatment of sample with protease. The advantages of this extraction technology are the absence of the precipitation step (which often compromises yield and purity), the simultaneous extraction of both RNA and DNA, and the capability of high sample volumes to minimize sampling error (21). Therefore, we investigated the suitability of this separation technology, which had successfully been evaluated for the extraction of viral nucleic acids in routine blood donation screening, for serum and plasma samples (3, 4). Our results demonstrated this technique’s sensitivity to be in the same range as or better than that of the conventional spin-column technique (14). We adapted the protocol for PCs, and a comparison of the CP values for the 23S rRNA RT-PCR analyses demonstrated that a higher sample volume did not improve nucleic acid extraction efficiency. We propose that an excess of human nucleic acids saturates the binding sites of the magnetic beads; competition between human and bacterial nucleic acids for these binding sites cannot be excluded.

We modified the primers and a universal fluorescent LNA probe for detecting a 122-base pair 23S rRNA product to optimize the detection of extracted nucleic acids. That this target is present in multiple copies in the genome of all known human bacterial pathogens increases the probability of detecting small numbers of bacteria. Depending on the growth rate, microorganisms can synthesize between 1000 and 50 000 RNA copies per cell. This fact contributes to the assay’s increased sensitivity.

New forward primers were designed to reduce the size of the amplification product to allow improved PCR efficiency. Furthermore, the introduction of an LNA into a DNA oligomer improves the hybridization affinity for complementary sequences, increases the melting temperature by 3 °C to 8 °C per modified base, and therefore improves the specificity of the assay. LNA probes are ribonucleotides with a methylene bridge that connects the 2' oxygen of ribose with the 4' carbon. This bridge results in a locked 3' endoconformation that reduces the conformational flexibility of the ribose. The increased stability allows the use of shorter probes that are more sensitive to single-base mismatches. The shorter length gave LNA probes better sequence specificity and a lower fluorescence background than conventional probes (27). This observation may be attributed to improved PCR efficiency with shorter detection probes because of lower interference during the amplification process (28).

The presence of bacterial DNA in preparations of DNA polymerases is a well-known problem when broad-range primer systems are used for bacterial detection (29). Contaminating nucleic acids copurified during enzyme production are difficult to remove with downstream purification methods. No false-positive results were obtained after implementing the LNA probe, optimizing the temperature profile, and testing different available DNA polymerases.

Moreover, real-time amplification procedures must be able to differentiate a true negative result from a false negative caused by amplification inhibitors. Many substances present in clinical samples or used in the extraction process are potent amplification inhibitors. A simple way to detect such inhibition is coamplification of an IC. In real-time PCR analysis, the target and the control target can be recognized in the same reaction vessel by means of 2 differently labeled probes (2, 30). Therefore, supplemental amplification of a coextracted nucleic acid serves as an IC. An IC is an important quality control and is demanded for blood screening tests by the Paul Ehrlich Institute, the federal licensing agency of Germany. An IC for diagnostic RT-PCR assays should be easy to produce and standardize. In gene expression analysis and virus screening, so-called housekeeping genes such as β-actin or glyceraldehyde 3-phosphate dehydrogenase are often used as ICs. This approach is suitable for specimens containing large amounts of human nucleic acids, such as PCs (31).

We used defined bacterial calibrators to demonstrate sensitivity. The use of CFU calibrators facilitates comparison of different methods’ sensitivities because the standardization of bacterial nucleic acid amplification techniques, common for viral nucleic acid amplification techniques, is required to detect bacterial contamination of blood products, as Montag (22) and Saldanha (32) have previously proposed.

The time of sample drawing is one of the crucial issues in the application of bacterial-screening methods for platelets. Sampling too early can lead to sampling error because the number of bacteria contaminating blood components is initially very low. Consequently, the screening sample may not contain bacteria and may test as sterile. Therefore, different variables have to be balanced against one another: If a larger sample volume is taken, sensitivity is improved, but the product is depleted. Previous culture studies have shown that culture on the day of collection invariably misses bacterial contamination (33). From our own study, we concluded that a PC unit storage time of 24 h is indispensable (submitted for publication). This screening procedure is a justifiable compromise among competing factors: the sensitivities of nucleic acid amplification technique assays, the diagnostic window period, and delay in delivery of the PC supply.

In conclusion, we have described our optimization of the 23S rRNA RT-PCR assay (14) for detecting bacterial nucleic acids in PCs. These improvements entailed changes in nucleic acid extraction procedures and probe technology and greater nucleic acid input with the Rotor-Gene 3000. The combination of our use of an optimized
temperature profile with LNA probes and the use of a quite pure enzyme obviated the elimination of any residual contamination. Furthermore, we systematically determined the analytical sensitivity. The turnaround time for the complete process of extraction and detection is ~4 h. The degree of automation can be increased further by implementing an automated liquid-handling system that dispenses the buffers and magnetic beads and a robotic manipulator that positions the filled buffer tubes on Chemagic Magnetic Separation Module I.

Finally, implementation of an IC facilitates detection of false negatives due to PCR inhibition. In ongoing studies, we are investigating the applicability of routine contamination screening in transfusion services.

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References