

## ORIGINAL ARTICLE

## Evaluation of two detection methods of microorganisms in platelet concentrates

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### SUMMARY

**Background:** The performance of a bacterial 16S ribosomal DNA real-time polymerase chain reaction (PCR) assay was evaluated and validated with an automated culture system to determine its use for screening of platelet concentrates (PCs).

**Study Design and Methods:** PCs were spiked with suspensions of *Escherichia coli*, *Serratia marcescens*, *Staphylococcus epidermidis* and *St. aureus* at 1, 10, and 100 colony-forming units (CFUs) mL and stored for 5 days. DNA amplification was performed using real-time PCR. The BacT/ALERT was used as a reference method and samples were inoculated into an aerobic culture bottle; for the PCR assay, aliquots were drawn from all (spiked) PCs on days 0 to 5 of storage.

**Results:** Real-time PCR detected only the gram-positive bacteria in PCs spiked with low bacterial titres (1 CFU mL) after 48 h; however, it was able to detect

all positive samples in PCs spiked with 10 CFU mL of either gram-positive or gram-negative bacteria after 48 h. In addition, real-time PCR detected all positive samples in PCs spiked with high gram-positive bacterial titres (100 CFU mL) after 24 h. On the other hand, the BacT/ALERT system showed positive results in all samples within 24 h.

**Conclusion:** The BacT/ALERT method is more sensitive and should continue to be the gold standard for identifying bacterial contaminations in blood samples. The real-time PCR approach can be used for the screening of PCs for microbial detection before they are released from blood centres or shortly before they are used in blood transfusion, and thus allow an extended shelf life of the platelets.

**Key words:** BacT/ALERT, bacterial contamination, blood components, molecular testing, platelet concentrates, real-time PCR.

Transfusion of platelet concentrates (PCs) contaminated with bacteria may cause serious, and even fatal, septic reactions in patients (Depcik-Smith *et al.*, 2001; Kopko & Holland, 2001). Recently, various techniques and methods, such as pre-transfusion bacterial screening of PCs, have been adopted by blood centres to reduce the risk of transfusion-associated septic reactions (Mitchell & Brecher, 1999; Morel *et al.*, 2003; Pietersz *et al.*, 2003; Blajchman *et al.*, 2004). In the United States, the introduction of an automated microbial detection system using an aerobic culture bottle reduced the risk of septic platelet transfusion reaction to approximately

1 : 42 000 for a two-arm procedure, using single-donor PCs (Eder *et al.*, 2007). A further reduction was obtained through diversion of the initial collected blood volume, decreasing the risk for septic platelet transfusion reaction to approximately 1 : 91 000 with the use of single-donor PCs (Eder *et al.*, 2009).

Despite the implementation of preventive measures, the risk of transfusion-transmitted bacterial infection is still much higher than that of transfusion-transmitted viral infections (Brecher & Hay, 2005). In countries that have introduced PC screening, culture-based methods are used almost exclusively (Pietersz *et al.*, 2007). In Brazil, the BacT/ALERT aerobic bottle (bioMérieux, Boxtel, the Netherlands) culture method is used. This method is sensitive, as it can detect 1–10 colony-forming units (CFUs) per 5 mL, generally within 24–48 h. PCs are released as ‘negative-to-date’, i.e. they are negative for

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bacterial growth and can be transfused at the time they are issued (Wagner & Robinette, 1998; Brecher *et al.*, 2001; De Korte *et al.*, 2001; McDonald *et al.*, 2001).

According to Brazil's Agência Nacional de Vigilância Sanitária (ANVISA), PCs should be stored in a temperature-controlled environment of  $22 \pm 2$  °C with constant stirring. Under these conditions, the PC is valid for 3–5 days, depending on the type of storage bag plasticiser (Ministério da Saúde, 2010). However, cultures are continued up to 7 days, during which the culture bottles can become positive for bacterial growth. Reports of septic transfusion reactions and fatalities after transfusion of culture-negative PCs show that slow-growing bacteria or low bacterial loads are not being detected (Benjamin & Wagner, 2007; Lessa *et al.*, 2008; Eder *et al.*, 2009). Most worrying is the platelet stored at room temperature that increases the risk of bacterial contamination.

As reported by Murphy *et al.* (2008) and AuBuchon (2011), culturing of platelet units detects probably less than half of the bacterially contaminated ones, and the implementation of other detection techniques (using rapid immunological methods) has been slow. As a result, there are still probably 30–100 cases of sepsis after platelet transfusion annually in the United States and perhaps several dozen deaths (Eder *et al.*, 2007). In this sense, the purpose of this study was to validate American Type Culture Collection (ATCC)-polymerase chain reaction (PCR) when incorporated into routine Colsan and to be an alternative for the BacT/ALERT when PCs are screened on the fifth day of production. This molecular tool provides a valuable alternative to quantification standards expressed in 'copy number' or 'CFU' as defined quantities of microorganisms are generated.

A promising method for bacterial screening of PCs is the detection of bacteria by using a PCR assay (Mohammadi *et al.*, 2005; Stormer *et al.*, 2007; Rood *et al.*, 2008). PCR assays are sensitive, and in contrast to the BacT/ALERT, the results are generated relatively quickly (i.e. within 4 h). However, PCR assays cannot detect bacterial contamination of PCs immediately after preparation (Dreier *et al.*, 2008) because blood products contaminated with bacteria initially contain <1 CFU mL owing to low inoculation (Brecher *et al.*, 2000). Therefore, PCR can only be used to test PCs after an initial delay to allow time for bacterial growth.

The combination of high sensitivity and specificity, low contamination risk, ease of performance and speed has made real-time PCR technology an appealing alternative to conventional culture-based testing methods. When using real-time PCR for the detection of bacterial contamination, several points have to be considered including the choice of target gene, the assay format, the nucleic acid extraction method, the sample type and the

evaluation of an ideal sampling strategy. Moreover, cost is an important factor.

In this study, real-time PCR assay detecting the 16S ribosomal DNA (rDNA) gene was used (Nadkarni *et al.*, 2002; Mohammadi *et al.*, 2003). The most common bacterial pathogens involved in clinical cases of transfusion-related sepsis and detected by nucleic acid amplification testing (NAT) include *Staphylococcus* spp. (42%), *Streptococcus* spp. (12%), *Escherichia coli* (9%), *Bacillus* spp. (9%), *Salmonella* spp. (9%), *Serratia* spp. (8%), *Enterobacter* spp. (7%) and other microorganisms (4%) (Wagner, 2004). Most cases of septic shock are caused by gram-negative bacteria (Arnou *et al.*, 1986). The sensitivity of the assay was determined for *Staphylococcus epidermidis*, *St. aureus*, *E. coli* and *Serratia marcescens*. To explore the possible advantages of the improved real-time PCR, routinely prepared PCs were spiked with bacterial titres (1, 10 and 100 CFU mL) of four different bacterial strains, and the assay sensitivity was evaluated by simultaneously performing an automated culture system as a reference method.

## MATERIALS AND METHODS

### *Bacterial strains and culture conditions*

To determine the amount of DNA in bacteria during growth, *E. coli* (ATCC 25922), *Se. marcescens* (ATCC 274), *St. epidermidis* (ATCC 12228) and *St. aureus* (ATCC 29213) were cultured in brain–heart infusion (BHI; Probac, São Paulo, Brazil) broth at 37 °C to promote bacterial multiplication. These bacterial strains were chosen because they are known to be associated with *in vivo* platelet contamination or bacteraemia (Nadkarni *et al.*, 2002; Mohammadi *et al.*, 2003; Wagner, 2004; CDC, 2005). The ATCC strains were used in this study as a reference for technique standardisation. Overnight cultures were diluted 1000-fold in fresh BHI medium, and every hour, samples were diluted in PCs at a concentration at which DNA copies could be determined by PCR. Growth was monitored by plating samples on blood agar plates to determine the bacterial titre and by measuring the optical density at 600 nm. To determine the DNA concentration in copies per millilitre, 10-fold serial dilutions in PCs of *St. epidermidis* and *St. aureus* at  $t = 2$  h and of *E. coli* and *Se. marcescens* at  $t = 3$  h were made. At these time points, the strains were growing in a logarithmic phase, and thus all cells were viable and capable of forming colonies.

### *DNA internal positive control*

The TaqMan® exogenous non-competitive internal positive control (IPC) (VIC Probe, part no. 4308323; Applied

Biosystems, Foster City, CA, USA) was used as an IPC for real-time PCR. All samples were contaminated with the IPC to monitor the extraction and amplification of the samples. This kit contains a pre-optimised IPC with pre-designed primers and the TaqMan probe. The IPC can be spiked into samples to distinguish true target negatives from PCR inhibition. The TaqMan Exogenous IPC Reagents kit was used to amplify a low-copy target DNA in the same tube with the IPC.

#### Nucleic acid extraction

DNA was isolated from 1 mL aliquots of spiked PCs using a manual extraction system (RTP<sup>®</sup> Bacteria DNA Mini Kit; Invitex, Berlin, Germany) according to the manufacturer's instructions. Before DNA isolation, samples were centrifuged at  $9300 \times g$  for 3 min, and the supernatant of each sample was discarded. Next, 5  $\mu$ L of DNA IPC was added to each pellet, followed by 400  $\mu$ L of Resuspension Buffer R. The resultant solution was transferred into Extraction Tube L and incubated for 10 min at 37 °C. Then, the pelleted gram-positive and gram-negative bacteria were incubated for 10 min at 65 °C with continuous shaking.

#### Primer and probe design

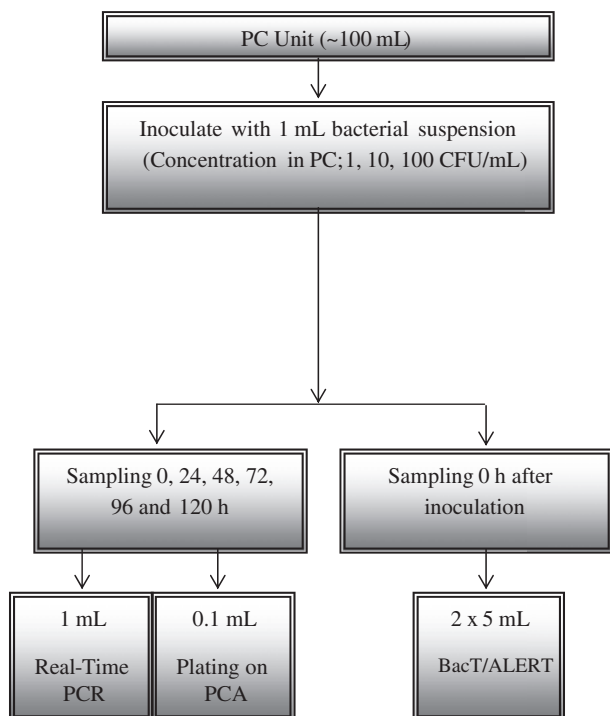
The primers and probes used for the amplification of 16S rDNA by real-time PCR were used as previously described (Nadkarni *et al.*, 2002; Mohammadi *et al.*, 2003). They were also used to design the forward primer 5'-TCC TAC GGG AGG CAG CAG T-3' (16S), the reverse primer 5'-GGA CTA CCA GGG TAT CTA ATC CTG TT-3' (16S) and probe (FAM) 5'-CGT ATT ACC GCG GCT GCT GGC AC-3' (TAMRA).

#### Real-time PCR amplification

Amplification and detection of DNA by real-time PCR was performed with the ABI-7500 Real Time PCR System (Applied Biosystems) using optical-grade 96-well plates. Triplicate samples were routinely used for the determination of DNA by real-time PCR, and mean values were calculated. The PCR reaction was performed in a total volume of 25  $\mu$ L using the TaqMan Universal PCR Master Mix (Applied Biosystems) containing 900 nM of each of the universal forward and reverse primers and 200 nM of the fluorogenic probe (Mohammadi *et al.*, 2003). The reaction conditions for the amplification of DNA were as follows: 50 °C for 2 min, 95 °C for 10 min and 40 cycles of 95 °C for 15 s and 60 °C for 1 min. Data analysis was done with the sequence detection software supplied by Applied Biosystems. Negative-template controls with water were included in each run.

#### Spiking study

Two hundred and eighty-eight routinely prepared PCs in plasma were obtained from the Colsan blood bank. One hundred and forty-four units were used for gram-negative bacteria, and the remaining 144 units were used for gram-positive bacteria, each spiked with 1 mL of the appropriate bacterial suspension (1, 10 or 100 CFU/mL) per bag (Fig. 1). Twenty-four units were used as no-template control (NTC) spiked with saline. The inoculum dose of 10 CFU/mL was chosen because from the few studies that have attempted to quantitate the degree of bacterial contamination on the day that the contamination was suspected, it can be deduced that most contaminated PCs had bacterial contamination of no more than 10 CFU/mL (Buchholz *et al.*, 1973; Cunningham & Cash, 1973; Arnow *et al.*, 1986; Blajchman *et al.*, 1997; Leiby *et al.*, 1997). Immediately after being spiked and homogenised, 5 mL samples were taken from each unit with a syringe via a sampling-site coupler under aseptic conditions and transferred into BacT/ALERT aerobic culture bottles. The aerobic culture bottles were incubated at 37 °C in the BacT/ALERT system until a positive signal was detected. The PCs were incubated at  $22 \pm 2$  °C with a horizontal shaking of 1 cycle  $s^{-1}$  for up to 5 days. Samples were taken daily until day 5 to monitor the presence of bacteria by real-time PCR and



**Fig. 1.** Flow diagram representing the inoculation and sampling strategy of the spiking study.

BactAlert (1 mL) by plating on plate count agar (PCA) plates (0.1 mL) (Fig. 1).

#### Statistical analysis

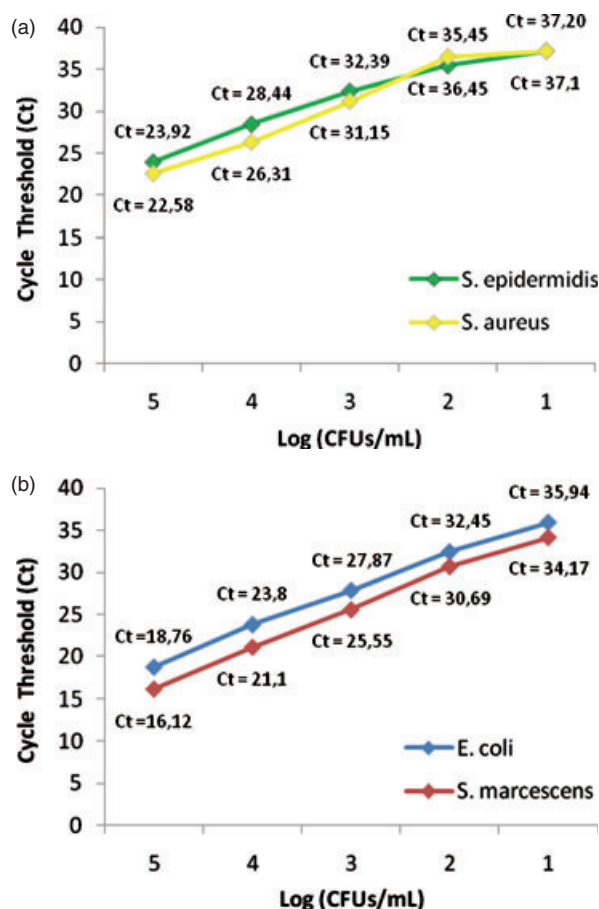
The analytical sensitivity of the two systems was checked by individually testing 24 replicates of each dilution of the gram-positive and gram-negative groups. The standards were diluted to the 95% detection limit for each assay. Amplification data were analysed with the SDS software (Applied Biosystems). The cycle threshold (Ct) value represents the PCR cycle at which the SDS software first detects an increase in reporter fluorescence above a baseline signal. For statistical analysis of the results, the confidence interval was calculated with the SIGMASTAT 2.0 programme (Sigma Plot, Chicago, Illinois, USA).

## RESULTS

A total of 24 PCs were sampled for each concentration and assessed for the presence of bacteria by both the PCR assay and the BacT/ALERT culturing system. After PCR amplification of DNA isolated from PCs, a Ct value was generated for each reaction. A Ct value is defined as the PCR cycle number at which fluorescence generated by cleavage of probe exceeds a fixed baseline threshold level. A high Ct value corresponds to a low input of target DNA. The Ct values were used to interpret the results of the PCR assay.

#### Sensitivity of the assay

CFU standards are used in the sensitivity determination of bacterial NAT (Stormer *et al.*, 2007). In addition, bacterial CFU standards are very useful in spiking experiments, where defined inocula are needed (Schmidt *et al.*, 2005; Mohr *et al.*, 2006; Stormer *et al.*, 2007). On the basis of control samples and the analytical validation of the assay, a result was considered positive when the Ct value was  $\leq 30.4$ . The Ct values above this cycle number indicate a negative result, which is due to the intercept of the magnitude of the fluorescence signal ( $\Delta R_n$ ), with the horizontal threshold line in bold representing the Ct value for a given sample. The fluorescence signal at 30.5 corresponds to the NTC representing the negative control. This interpretation of the results was valid only when DNA extraction was efficient and no PCR inhibition was observed. This implied that the IPC was consistently amplified (Ct value of 27–28). If the IPC was not amplified or the PCR seemed to be inhibited, a new aliquot of the same sample of PC was re-extracted and reamplified. The serial dilutions of a gram-positive



**Fig. 2.** Standard curve generated with DNA extracted from serial dilutions of a pure culture of *St. epidermidis* and *St. aureus* (a), and *E. coli* and *Se. marcescens* (b). The assay was linear over a wide range varying between  $1 \times 10^1$  and  $1 \times 10^5$  CFU equivalents per PCR procedure. For *St. epidermidis*, slope,  $-3.433$ ; intercept,  $25.947$  and  $R^2$ ,  $0.975$  were found. For *St. aureus*, slope,  $-3.475$ ; intercept  $26.249$  and  $R^2$ ,  $0.968$  were found. For *E. coli*, slope,  $-3.513$ ; intercept,  $22.785$  and  $R^2$ ,  $0.984$  were found. For *Se. marcescens*, slope,  $-3.587$ ; intercept,  $23.239$  and  $R^2$ ,  $0.971$  were found. The x-axis represents the starting copy number expressed as log CFU equivalents per PCR procedure. The Ct values are plotted on the y-axis.

(Fig. 2a) or gram-negative (Fig. 2b) culture in PCs were determined. The sensitivity of the real-time assay for detecting *St. epidermidis* and *St. aureus* in PC was 100 CFU mL at 24 h, and that for *E. coli* and *Se. marcescens* in PC was 100 CFU mL at 48 h when DNA was used as a template. The sensitivity of the NAT system was evaluated by probit analysis, which showed a 95% level of detection and confidence interval. Table 1 summarises the results of the test. The 95% limit of detection for the bacteria, as indicated in the respective package inserts, is shown together with the concentration

**Table 1.** Spiking of PCs with bacterial titres

Bacterial strain (n = 24)	BacT/ALERT			PCR			PCR			PCR		
	Pos	Time (h)	Plate	Day 1 (24 h)			Day 2 (48 h)			Day 5 (120 h)		
				DNA	Ct	Plate	DNA	Ct	Plate	DNA	Ct	Plate
<i>St. epidermidis</i> (1 CFU mL)	24	12.9	0	—	0	24	26.52 ± 0.22	24	24	27.25 ± 0.15	24	24
<i>St. epidermidis</i> (10 CFU mL)	24	12.7	0	—	0	24	25.33 ± 0.15	24	24	26.15 ± 0.23	24	24
<i>St. epidermidis</i> (100 CFU mL)	24	10.3	24	30.40 ± 0.02	24	24	24.15 ± 0.37	24	24	24.22 ± 0.47	24	24
<i>St. aureus</i> (1 CFU mL)	24	13.3	0	—	0	25	25.99 ± 0.14	24	24	25.57 ± 0.15	24	24
<i>St. aureus</i> (10 CFU mL)	24	12.5	0	—	0	24	24.12 ± 0.47	24	24	19.20 ± 0.40	24	24
<i>St. aureus</i> (100 CFU mL)	24	11.3	24	26.32 ± 0.13	24	24	23.87 ± 0.77	24	24	19.00 ± 0.73	24	24
<i>E. Coli</i> (1 CFU mL)	24	14.0	0	—	0	0	—	24	24	28.74 ± 0.06	24	24
<i>E. Coli</i> (10 CFU mL)	24	13.2	0	—	0	24	23.87 ± 0.07	24	24	24.44 ± 0.03	24	24
<i>E. Coli</i> (100 CFU mL)	24	11.0	0	—	0	24	23.43 ± 0.65	24	24	22.83 ± 0.29	24	24
<i>Se. marcescens</i> (1 CFU mL)	24	13.5	0	—	0	0	—	24	24	20.69 ± 0.28	24	24
<i>Se. marcescens</i> (10 CFU mL)	24	12.7	0	—	0	24	24.69 ± 0.12	24	24	19.14 ± 0.26	24	24
<i>Se. marcescens</i> (100 CFU mL)	24	11.5	0	—	0	24	23.14 ± 0.41	24	24	19.55 ± 0.29	24	24
NTC	24	—	0	30.50 ± 0.12	0	0	30.50 ± 0.09	0	0	30.50 ± 0.05	0	0

Bacteria were detected by culturing in the BacT/ALERT, PCR (DNA assay) and growth on PCA plates. For BacT/ALERT, time until detection is given in hours. For PCR, all samples were negative on day 0 (0 h), day 3 and day 4 gave the same results as day 2.

of the bacteria tested by both systems. The number of reactive samples of the total number of samples tested is also shown, with more than 95% of the replicates showing positive results.

*Linear range and limit of detection: estimation of bacterial load in contaminated PCs*

A combination of gram-positive and gram-negative bacterial DNA was used as the standard in the determination of bacterial number by real-time PCR. After the standard curve was calculated, the software automatically quantified the number of bacterial DNA, comparing the Ct of the sample to the Ct of the standard curve. To determine the validity of using the universal probe and primer sets to estimate the total number of bacteria in culture, four bacteria, *E. coli*, *Se. marcescens*, *St. epidermidis* and *St. aureus*, were grown separately *in vitro* to late-exponential, early stationary phase, and equal volumes of the cultures were mixed together. Inocula were prepared using a 0.5 McFarland standard (approximately  $10^8$  CFU mL) in sterile saline with 10-fold serial dilutions to achieve the requisite inoculum level. The titres of the bacterial suspensions were determined by 'spread plate' assays using PCA (Probac). Twenty-four PC samples were spiked for each bacterium. The resulting curve was used to estimate the bacterial load (reflected as CFU equivalents per PCR procedure) in the PCs, representing a quantitative test. An example of representative standard curves in triplicate is shown in Fig. 2. The curve was linear between  $1 \times 10^5$  and  $1 \times 10^1$  CFU per PCR reaction. In this study, a standard control curve was made for each PCR run.

In our analyses, the correlation coefficient ( $R^2$ ) for *St. epidermidis* was above 0.975, corresponding to 95%; for *St. aureus* was above 0.967, corresponding to 94% efficiency of the reaction (Fig. 2a); for *E. coli* was above 0.983, corresponding to 92% and for *Se. marcescens* was above 0.970, corresponding to 91% efficiency of the reaction (Fig. 2b) for all experiments. The possible advantages of real-time PCR were investigated by performing a similar experiment with the four different bacterial strains by using the BacT/ALERT method. All PCs spiked with gram-positive and gram-negative bacteria gave a positive result with the BacT/ALERT culture system (Table 1). Positive results with the BacT/ALERT culture system were always found within 14 h after spiking. In contrast, at day 0, none of the spiked PC samples were found to be positive for growth on PCA plates or with real-time PCR analysis. Only the *St. epidermidis* and *St. aureus* samples with an initial concentration of 100 CFU mL were found to be positive within 24 h (day 1). After 24 h, all samples were found to be positive until the last day of the experiment, except for the

*E. coli* and *Se. marcescens* samples with an initial concentration of 1 CFU mL, which were detected by PCR only after 120 h (Table 1).

*Number of viable cells during growth*

To monitor the level of bacterial growth, gram-negative and gram-positive mixtures were cultured in BHI broth at 37 °C. Samples were taken every hour by measuring the optical density at 600 nm.

*PCR real-time vs BacT/ALERT*

All PCs spiked with gram-positive and gram-negative bacteria gave a positive result with the BacT/ALERT culture system (Table 1), with the positive results always found within 14 h after spiking. On other hand, for real-time PCR, only the gram-positive samples with a concentration of 100 CFU mL tested positive immediately after spiking (day 1). After 24 h, all samples were found positive until the last day of the experiment, except for the gram-negative samples with a concentration of 1 CFU mL, which was detected by PCR only after 120 h (Table 1).

## DISCUSSION

Currently, culture-based methods, including the BacT/ALERT system, are the most common tools used in blood banks for bacterial screening of PCs (Pietersz *et al.*, 2007). Indeed, the BacT/ALERT system is considered to be the gold standard in most of the blood centres for the detection of bacterial contamination of PCs. Although BacT/ALERT has reduced the risk of transfusion of bacterially contaminated PCs, the method is not ideal because of false-negative results (Benjamin & Wagner, 2007; Lessa *et al.*, 2008; Eder *et al.*, 2009). Studies evaluating the performance of the BacT/ALERT have shown the ability of this automated culture system to reliably detect as few as 1–10 CFU mL (Wagner & Robinette, 1998; Brecher *et al.*, 2001; De Korte *et al.*, 2001; McDonald *et al.*, 2001). However, McDonald *et al.* (2001) showed that the BacT/ALERT system detected inoculums only at a concentration of 10 CFU mL or greater. Theoretically, the system will detect one viable organism. On the other hand, in our study, both BacT/ALERT and real-time PCR were able to detect 1 CFU mL regardless of time. In view of the aforementioned disadvantages, a faster method to test PCs for bacterial contamination is desirable. Several rapid methods have been developed for sterile testing of blood products (Wagner, 2004; Muller *et al.*, 2006). In comparison with our previously described results with real-time

PCR assays (Rood *et al.*, 2008, 2010) using DNA as a template, the results of this study showed lower background Ct values for PCs (30.4 vs 30.6 and 33.7 of previous results) (Nadkarni *et al.*, 2002; Mohammadi *et al.*, 2003). This difference could be explained by batch-to-batch variation of exogenous bacterial DNA present in isolation and amplification reagents (Corless *et al.*, 2000). The presence of exogenous bacterial 16S rDNA in reagents affects the sensitivity of nucleic acid-based assays. Various studies have reported that these bacteria are the common contaminants found in PCs (Wagner & Robinette, 1998; De Korte *et al.*, 2001; McDonald *et al.*, 2001; Brecher *et al.*, 2003). In this validation study, all bacteria were detected in cultures within 5 days. In the case of PC contamination, the initial bacterial number in the PC preparation is usually very low (Eder *et al.*, 2009). The DNA assay is not sensitive enough to detect bacterial contamination of platelets directly after production. However, the BacT/ALERT system may also not always detect bacterial contamination of PCs directly after production (Brecher *et al.*, 2000). A mathematical model showed that an 8 mL sample taken from a 300 mL PC with a bacterial concentration of 0.15 CFU/mL that is inoculated into two culture bottles only has a 60% chance of detection (Benjamin & Wagner, 2007). The introduction of CFU standards will help to accurately determine the sensitivity of different DNA-based methods. In addition to their use in molecular genetics methods, bacterial CFU standards are very useful in spiking experiments in which defined inocula are needed (Schmidt *et al.*, 2005; Mohr *et al.*, 2006; Stormer *et al.*, 2007). In our study, bacterial contamination was simulated by spiking the PCs with low numbers of bacteria (e.g. 1 CFU/mL) that are known to proliferate in PCs. It should be noted that the bacterial standards we used consisted of bacteria that were in their logarithmic growth phase and adapted to growth in plasma. These bacteria were expected to start growing in PCs almost immediately after spiking. In the real event of bacterial contamination of PCs, there could be a delay before the bacteria start growing in PCs (Stormer *et al.*, 2008a,b). The BacT/ALERT did not give a false-negative signal. According to a mathematical model (Benjamin & Wagner, 2007), there would be five false negatives expected for the BacT/ALERT. Twenty-four PCs spiked with *E. coli* and *Se. marcescens* (1 CFU/mL) remained negative in the PCR, on the fourth day, but were positive in the BacT/ALERT culture and plating assays. This could be because the PCs were spiked with a very low bacterial concentration. This experiment shows that testing the platelets for bacterial contamination at a later time point with the DNA assay could be better than screening immediately after production as done with the BacT/ALERT method. Because of the short turnaround

time of the real-time PCR assay, it is possible to postpone testing of the PCs from immediately after production to shortly before use in transfusion when the amount of bacteria might be higher than 10 CFU/mL. This could also help to reduce the incidence of false-positive results (i.e. positive cultures that are not confirmed) found with the BacT/ALERT method (Pietersz *et al.*, 2007), as the process of autosterilisation takes more time to occur in PCs (Ezuki *et al.*, 2007). This means that low amounts of bacteria can be killed during PC storage as a result of pre-formed antibodies, complement proteins, lysozymes or the presence of lipoproteins in the plasma (Ezuki *et al.*, 2007). The DNA of these small amounts of bacteria will not be detected by real-time PCR assay.

In conclusion, the real-time PCR assay can be an alternative for, or can be used in addition to, culture-based methods when PCs are tested at a later time point, thus allowing an extended shelf life of the platelets. Further investigation may determine whether the real-time PCR assay is applicable for routine screening of PCs in blood banks. To date, the BacT/ALERT system remains the gold standard. Finally, the cost of the testing should be evaluated because, thus far, the implementation of NAT is not feasible for routine tests in all blood banks because of its high cost compared with the currently used methods. Considering that the BacT/ALERT system is presently the only practical method of routine screening for the detection of bacterial contamination of PCs, real-time PCR merits serious consideration as an alternative approach to detect bacterial contamination and reduce bacterial transmission by transfusion. The Research Ethics Committee of University Federal of São Paulo/São Paulo Hospital examined and approved this research project (no. 0516/10).

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#### CONFLICT OF INTEREST

All authors declare that they have no competing interests.

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