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Three different bacterial detection systems for platelet concentrates under inter-laboratory conditions

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ABSTRACT

Background: A variety of screening methods are currently used worldwide in order to decrease the risk of transfusion-transmitted sepsis and improve the safety of PCs.
Methods/materials: PCs inoculated with five different transfusion-relevant species of bacteria at concentrations of 1, 10, and 100 colony-forming units (CFU) ml^{-1} were stored at 22 °C for 7 days. Flow cytometry (FACS), BacT/Alert automated culturing, and a quantitative real-time PCR (Q-PCR) were then used to detect the presence of bacteria in samples prepared from these PCs.
Results: At the initial spiking concentrations of 1, 10, and 100 CFU ml^{-1}, Q-PCR detected all five bacterial species tested. Screening with the BacT/Alert culture-based system allowed bacterial detection (inoculated on day 0) within a mean time of 15.13 h for all three spiking concentrations. Using FACS, positive signals were obtained for all three concentrations of *Escherichia coli* and *Bacillus cereus* on day 1 and for initial spiking concentrations of *Pseudomonas aeruginosa* and *Staphylococcus aureus* of 1 CFU ml^{-1} on day 2. For *Staphylococcus epidermidis*, detection of an initial inoculum of 1 CFU ml^{-1} was possible only beginning on day 6.
Conclusion: This study shows that under standard laboratory conditions the sensitivity of FACS in the detection of bacterial contamination of PCs was lower than that of either the BacT/Alert automated culturing method or Q-PCR.

1. Introduction

Currently, the risk of transfusion-transmitted viral infection has been dramatically reduced, while the bacterial contamination of blood and blood products for transfusion remains a serious problem. The prevalence of bacterial contamination of cellular blood products has been reported approximately 1 in 3000 units. Significant clinical events following platelet and RBC transfusions occur with a prevalence of approximately 1:25,000 and 1:250,000, respectively [1]. Sepsis in patients receiving platelet concentrates (PCs) is of particular concern as these preparations are stored at room temperature rather than frozen. As a result, even an initial contamination with very small numbers of bacteria can causes vast and clinically dangerous levels of bacteria following a 5–7-day storage period [2]. As shown in a previous report, the remain risk of obtaining bacterial infection by transfusion is 100–1000 times higher than that of viral infection, and approximately one out of every 2000–3000 units of PCs is probably to have some type of bacterial contamination from the donor's skin or bloodstream[2,3]. Consequently, the detection or reduction of bacterial contamination is an urgent goal of

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blood banking and transfusion medicine. In fact, in several countries, including the United States, Hong Kong, and most of European countries, all PCs are routinely screened for bacterial contamination while, in Northern Ireland, this is the case for the majority of PCs [4].

Based on recent studies, methods to investigate the prevalence of bacterial contamination could be divided into two major groups: (a) incubation or culture methods; (b) fast detection methods. The latter include nucleic acid amplification methods, fluorescence-activated cell sorting (FACS), and immunological detection methods (e.g., the Pan Genera Detection system) [5,6]. The limit of detection of these methods varies dramatically depending on their sensitivity and the level of contamination [7]. For the detection of most of the bacterial species that typically contaminate PCs, culture techniques are the most sensitive [8]. Among these, BacT/Alert 3D is the most widely used commercial quality-control assay, with the ability to detect 1–10 CFU (ml PC)$^{-1}$ [9]. By comparison, the analytical sensitivity of methods based on nucleic acid screening is 10–100 CFU (ml PC)$^{-1}$, depending on the bacterial species as well as the DNA extraction and amplification procedures, while FACS analysis, which is based on fluorescence staining of bacteria DNA, has a sensitivity of only 10^3–10^4 CFU (ml PC)$^{-1}$ [5,10]

Over the past decade, numerous studies have focused on the prevalence of bacterial contamination of PCs as well as the source and the spectrum of the bacterial species implicated in contamination [2,7,11]. These studies identified the skin flora of the donor (i.e., at the phlebotomy site) as the most common source of bacterial PC contamination, with asymptomatic donor bacterial infection or during processing of the units as less frequent causes. Each of these sources may lead to contamination with either gram-positive or gram-negative bacteria by skin contamination is more commonly related with gram-positive bacteria and asymptomatic donor bacteremia with gram-negative pathogens [12]. A small number of enteric or environmental species have also been identified in PCs and were shown to result in the most serious reactions and the majority of the sepsis-related deaths in these patients [7].

In the Chinese blood banking system, PCs are not routinely screened for the presence of bacteria, to the extent that very few laboratories in China have assessed the various bacterial detection methods. However, with the growing recognition in our country of the importance of PC screening, we examined three bacterial detection methods (BacT/Alert 3D culture method, an in-house developed nucleic acid amplification technique, and FACS analysis) and compared their sensitivity in our lab by screening for the presence of five microorganisms that are common sources of transfusion-transmitted bacterial infection in PCs. In addition, the manageability and efficiency of these systems were also being evaluated.

2. Materials and methods

2.1. Bacterial strains and culture conditions

This study focused on five microorganisms frequently related to PC contamination: *Escherichia coli, Bacillus cereus, Staphylococcus epidermidis, Pseudomonas aeruginosa,* and *Staphylococcus aureus.* The bacterial strains were grown in beef cream culture medium under oxic conditions at 37 °C. To investigate the actual number of CFU ml$^{-1,}$ the bacteria were cultured until the cultures attained an optical density (OD) at 600 nm of 1.0. Serial 10-fold dilutions of these suspensions in phosphate-buffered saline (PBS) were prepared and then plated in triplicate on agar plates. Aliquots were also taken from the appropriate dilutions and used to inoculate PCs. The recovered concentrations were determined by plating on agar plates.

2.2. PC inoculation

For each of the tested bacterial species, the PCs were pooled and then separate into 12 smaller sub-pools of 40 ml. Three of these served as negative controls (without spiking) and the other nine were inoculated with the appropriate bacterial suspensions to acquire triplicate final concentrations of 1, 10, and 100 CFU ml^{-1}. To detect the existence of bacteria in these samples, two aliquots of 10 ml each obtained from each spiked PC bag were used immediately after spiking (day 0) to inoculate BacT/Alert standard aerobic and standard anaerobic culture bottles (bioMérieux, Boxtel, the Netherlands). These bottles were incubated in the BacT/Alert automated broth system until either a positive signal developed or for a maximum of 7 days in the absence of signal. As a negative control, the BacT/Alert culture bottles were inoculated with aliquots from unspiked PCs. The existence of bacteria in the remaining PCs was surveyed through quantitative real-time PCR (Q-PCR) and FACS analysis. For these experiments, 1 ml aliquots were sampled from each of the units at different time-points during their storage. Thus, each PC bag was taken on day 0 (preparation of PCs and inoculation) and on days 1, 2, 3, 6, and 7 after inoculation. The samples were investigated in parallel with the BacT/Alert samples (Fig. 1).

3. Bacterial detection methods

3.1. BacT/Alert automated culturing method

BacT/ALERT (bioMerieux, Durham, NC) is an FDA-approved system for the detection of bacterial contamination in PC units. It relies on the detection of CO_2 production resulting from the growth of the bacteria. In this study, two 10 ml aliquots of PCs were, respectively, inoculated into BacT/ALERT aerobic (BPA) and anaerobic (BPN) culture bottles using aseptic technique in a laminar airflow cabinet. The bottles were then incubated in the BacT/ALERT 3D system at 35–35.5 °C, for up to 7 days in the case of no reaction, or until a positive reaction was detected by the monitoring unit of the BacT/ALERT system.

4. 16S rDNA quantitative real-time PCR

4.1. DNA extraction

Nucleic acids were extracted from 1 ml of PCs at different time-points during their storage using the TIANamp