Measurement of BacT/ALERT Sensitivity after Inoculation Certain Amount of E. coli and S. epidermis

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Abstract

Background: Transfusion of contaminated human platelets concentrates (PCs) causes septic reaction and death of recipients. Current rate of detection of bacterial-contaminated of the PCs are very important tools to warranty patients'safety. BacT/ALERT system was introduced as one of the most sensitive, specific, and rapid screening test, which could be implemented in Blood Transfusion Centers.

The aim of this study is to investigate whether the BacT/ALERT system as introduced, can function as rapid detection system.

Material and Methods: In this study we used the whole blood from 24 healthy human subjects with informed consent, The PCs were collected from these whole blood samples by differential centrifugation and Buffy coat system. All PCs (50-70 mL) stored under standard blood banking conditions. The PCs were randomly selected and divided in two groups of 12, and 10 CFU/mL was of either E. coli (gram negative) added into first group (n=12 PCs), or S. epidermidis (gram positive) bacteria into another group (n=12 PCs). Then samples were inoculated in BPA culture medium of BacT/ALERT system and studied after 0, 6, 24, 48 hrs.

Results: The BacT/ALERT system showed rapidly positive results with E. coli samples (T0) with all introduced volumes (0.5, 1, 2 ml) but with S. epidermidis samples showed 83% with low volumes (0.5 and 1 ml), and 91.6% positive alert results with high volume (2 ml) after 48 hrs incubations.

Conclusion: This indicates that the sensitivity and specificity of BacT/ALERT system is disputable and not universal, which remained to be investigated in details.

Keywords: Blood transfusion; Bacterial screening; E. coli; Transfusion transmitted diseases, S. epidermidis; Microbiology

Introduction

Transfusion of contaminated human platelets concentrates (PCs) results in transfusion transmitted diseases (TTD), septic reaction and death of recipients, however [1-3]. Detection methods of bacterial contamination of PCs are very important tools to warranty patients'safety [2,4]. The TTD and septic reactions steadily are reported with culture-tested PCs, possibly due to false-negative outcome associated with inadequate sampling of low-concentration of certain bacteria [3]. Current detection technologies to screen bacterial contamination of blood products are rather aspecific, not 100% sensitive, and relatively lethargic. Hence, these failures should question the usefulness of systematic bacterial detection of the PCs, when the lethal accidents are occurring after routine controls [5,6].

Recently developed automated techniques for detection of bacteria are much more rapid than direct plating techniques, and the blood culture [4,7]. One assumes that such rapid techniques can be useful to monitor the sterility of cellular blood products with greater sensitivity, using a small aliquot of the blood product taken soon after collection [8].

Veniuncture during blood collection is probably the major cause of bacterial contamination of blood components since the majority of contaminant organisms are part of the normal or transient skin flora [9]. To reduce hazardous of bacterial contamination and proliferation it is important to implement a rapid and highly specific detecting test in every Blood Bank. Globally, microbial infection of blood products is a determined but often ignored problem in the Blood Transfusion Centers [10]. The possible sources of microbial contamination of blood products consist of different origin i.e. donor bacteremia,5 during and after blood collection procedure, from the collection pack, during the blood manufacturing process and storage temperature (20-24°C) [11].

Unfortunately still the most frequent TTDs ’complication occurs with PCs transfusion with an incidence between 1:1000 and 1:3000 [10,12]. Obviously, dissimilarities in transfusion reactions against bacterial inocula transfused, lack of standard procedure, and at least but not last lack of 100% sensitive/specific machines affected ‘rate of detection’ [13]. Timely detection of contaminated PCs play pivotal role.
in the fundamentals of cascade of failures, which might unnecessarily expose hemato-oncologic patients to unknown dosis of microorganisms posttransfusion [14]. After recognition of lethal effects of contaminated PCs prompted the College of American Pathologists (CAP) and the American Association of Blood Banks (AABB) to set new standards requiring the screening of PCs for bacterial contamination [13,15].

The aim of this study is to investigate whether BacT/ALERT system as introduced, can function as reliable detection system under our circumstances. Furthermore, to evaluate ‘rate of detection’ by introducing different volume of contaminated PCs inoculated with known endconcentration of certain bacteria i.e. E. coli and S. epidermidis.

Here we report that after introducing different PCs inoculated with known concentration of certain bacteria BacT/ALERT system showed positive results immediately after adding 10 CFU/mL but it obviously works sort-dependent.

Materials and Study Design

This descriptive research study was done with 1% of daily processed PCs in the IBTO from 24 healthy donors whole blood sample with informed consent, which were separated by differential centrifugation into PCs. All processed PCs (n=24) containing 50-70 ml volume stored under standard blood banking’s conditions at 22-24°C with agitation, prior to detection tests (Figure 1).

In order to improve the diagnosis of bacterial contamination, we used the BacT/ALERT system to screen bacterial infections of PCs (6). The BacT/ALERT system is alarming when minimal endconcentration of certain microorganism (inoculum) reaches 10 CFU/mL in blood products, and its alert signal is based on CO$_2$ emissions in the culture medium (colorimetric technology/ sensor culture bottles) [16]. The system with the use of specific media that is embeded in the device, allows continuous monitoring of cultivation via the display of the BacT/ALERT system. Two relevant bacteria i.e. E. coli and S. epidermidis strains were isolated and purified for this study, and were confirmed by biochemical reactions as described [17]. Both contaminants were prepared for PCs specifically. Then, fresh cultures of bacteria isolated from the colonies and inoculated into the Trypticase broth environment (Construction company, Merck, Germany). Subsequently, the new colonies of the bacteria were prepared in a standard fashion i.e. 0.5 McFarland, 10 CFU/mL aliquote dilutions (Figure 2). Bacteria populations were counted and 10 CFU/mL was added to each randomly selected PC, which supposedly high enough to be detected by BacT/ALERT system as CO$_2$ release causes sensor bottle to turn yellow, Instrument measures and detects color change, analyzes data to determine positivity, alerts when culture is positive.(5) Next different harvested volumes (0.5, 1, 2 mL) (Figure 3A-3C) were used for each inoculation, under sterile conditions. The PCs were inoculated with the certain bacteria, which commonly contaminate PCs pretransfusion i.e. E. coli and S. epidermidis. Furthermore, randomly selected PCs were incubated with BPA medium under defined condition, and then daily the samples were checked by employing BacT/ALERT standard bottles BPA (BioMerieux, Inc., Durham, NC) for detection of aerobic bacteria. The BacT/ALERT’s bacterial display after the first alarm automatically records all positive results.

Figure 1: The human platelets concentrates (PCs) contamination and multiplication processes after 48 hours storage.

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Figure 2: Bacterial contamination tests with known artificially made contaminants.

Figure 3: Rate of detection of positive results of BacT/ALERT system of contaminated PCs with A) 0,5, B) 1, and C) 2 mL volume of certain known bacteria i.e. E. coli and S. epidermidis (10 CFU/mL)

Acquired data were analyzed by ANOVA test and p<0.05 was considered significant (n=288).
Results

Because E. coli (fast) and S. epidermidis (slow) have different growth rates, the results of our studies gathered, and compared with each others. Due to the multifactorial aspects of detection tests i.e. rate of detection, sensitively, and specificity the capability of the BacT/ALERT system was investigated in the IBTO. We set priorities and focused on detection alarm rate after addition of three different volumes 0.5, 1, and 2 mL. (Figure 3A-3C) contaminants containing at least a begin concentration of 10 CFU/mL of E. coli and/or S. epidermidis at time point zero (T0).

Comparison of different measurements showed that the BacT/ALERT system displayed with different rate a positive results when the PCs were inoculated with 0.5 mL. Immediately after incubation (T0) (Control groups 1, C1), BacT/ALERT system significantly (p<0.05) displayed the E. coli samples much quiker as positive than S. epidermidis (rate of detection >10 hrs after 10 CFU/mL). Moreover, compared to C1 after 6, 24, 48 hrs inoculation we observed again E. coli was quicker detected than S. epidermidis, respectively.

In the case of S. epidermidis immediately after incubation (T0) (control groups 1, C2), BacT/ALERT system displayed positive with 0.5 and 1 mL, only 10 cases, while with 2 mL volume 11 of 12 cases. However, The BacT/ALERT system displayed contaminated samples for 100 % positive (12 of 12) after 6, 24, and 48 hrs incubation with all volumes.

As it is shown in the Figure 3B the BacT/ALERT system displayed with different rates a positive results when PCs were inoculated with 1 mL. Inoculation time from 6 up to 48 hrs showed inverse relation versus rate of detection displayed by the BacT/ALERT system. Obviously, multiplication of bacteria increases when more adjacent bacteria is present irrespective of bacteria sort.

Compared to control group, the mean rate of detection reduced to 5 hrs when 2 mL of E. coli-contaminated PCs 48 hrs was inoculated (Figure 3C). With other words, the amount of bacteria when passes a detection alarm delayed considerably, as such that using of the BacT/ALERT system gave positive alert signal. When PCs were inoculated with highst volume (2 mL) the BacT/ALERT system displayed with different rates a positive results (Figure 3).

Nonetheless BacT/ALERT system failed to detect minimum of S. epidermidis (10 CFU/mL) even after addition of 2mL to solutions, earlier than 48 hrs (at T0).

Discussion

In this study random human PCs inoculated with known final concentration (10 CFU/mL) of known bacteria (E. coli and S. epidermidis) to investigate specificity and sensitivity of BacT/ALERT system. Compared to control group after 24 inoculation of E. coli the BacT/ALERT system displayed a 100% sensitivity but 83.3% (n=28; false negative).

These data confirm other laboratory findings. Why in our study 2 samples of 12 at T0 were not displayed as positive might be caused by either failure of the BacT/ALERT system or something else, which needs more investigations.

Data acquired were from 24 samples shows that BacT/ALERT system has not 100% sensitivity tested by known final concentration of S. epidermidis (10 CFU/mL). Unfortunately this percentage is too high when patients safety and costeffectiveness concern play pivotal role to use this system.

In fact every Blood bank is expecting to have a rapid bacterial detection test that warranty any blood products/stability and safty. In one hand the BacT/ALERT system was partially able to alert us in less than 10 hrs. In the other hand rate of detection time efficacy, and costeffectiveness of the BacT/ALERT system studied were not favorable. One may speculate what would be the reason to buy such expensive equipement with so specificity and sensitivity.

Taken together, our results at least have shown that the BacT/ALERT system under different circumstances should be tested again. Despite the consequences on patients safety the BacT/ALERT displayed a dose- and sort dependent results at its best condition. The rate of detection was not so impressive and missed positive samples, which might not a justifiable issue. In our opinion, based on this scores of the BacT/ALERT system, the Blood Banks and Transfusion Centers take high responsibilities for patients safety.

References


