Scansystem™ Enables Rapid and Sensitive Bacterial Detection in Platelets Stored in Additive Solution with Implementation of Standard Positive Control Capsules*

Michael Schmidt^a Michael K. Hourfar^a Julia Heck^a Christina Weis^a Thomas Montag^b
Sven-Boris Nicol^b E. Seifried^a

^aInstitute of Transfusion Medicine and Immunohematology, German Red Cross, Johann Wolfgang Goethe University, Frankfurt/M.,

^bPaul Ehrlich Institute, Langen, Germany

Key Words

Rapid bacterial screening method · T-Sol® ·
Scansystem™

Summary

It has been estimated that as many as 1 in 2,000 to 1 in 3,000 units of platelet concentrates (PCs) are bacterially contaminated. In this study we evaluated the new Scansystem bacterial detection method for pool PCs stored in additive solution (T-Sol®). At specific times during incubation, PCs spiked with a range of bacteria were analyzed by the Scansystem. *Staphylococcus aureus*, *Bacillus cereus*, *Klebsiella pneumoniae*, *Escherichia coli*, *Staphylococcus epidermidis*, and *Streptococcus pyogenes* were reliably detected when sample volumes were taken 24 h post inoculation. Of the bacteria used for the spiking study, only *Propionibacterium acnes* was not detected by the Scansystem method during the whole incubation period of 36 h. In summary, Scansystem was able to detect 6 transfusion-relevant bacteria species in PCs stored in additive solution. Two major benefits of Scansystem were found: First, the results are obtained within 70 min, and second, standardized and lyophilised positive control capsules are provided for daily validation of the testing procedure. However, analytical sensitivity of the method might be slightly reduced when applied on platelets stored in additive solution T-Sol as compared to platelets stored in 100% plasma.

Schlüsselwörter

Schnellmethode zum Bakterien-Screening · T-Sol® ·
Scansystem™

Zusammenfassung

Aktuell wird das bakterielle Kontaminationsrisiko für Thrombozytenkonzentrate mit 1:2000 bis 1:3000 angegeben. In der vorliegenden Studie wurde das neue Scansystem-Detektionsverfahren für Thrombozytenkonzentrate (TKs), hergestellt in Additivlösung (T-Sol®), untersucht. Die «beimpften» TKs wurden an definierten Zeitpunkten mit Scansystem untersucht. *Staphylococcus aureus*, *Bacillus cereus*, *Klebsiella pneumoniae*, *Escherichia coli*, *Staphylococcus epidermidis* und *Streptococcus pyogenes* wurden zuverlässig nach einer Inkubationszeit von 24 h nachgewiesen. Lediglich *Propionibacterium acnes* konnte nicht im vorgegebenen Untersuchungszeitraum von 36 h nachgewiesen werden. Insgesamt war es möglich, mit Hilfe von Scansystem 6 transfusionsmedizinisch relevante Bakterien in Thrombozyten mit Additivlösung nachzuweisen. Scansystem bietet somit zwei wesentliche Vorteile: Erstens, benötigt eine einzelne Untersuchung nur 70 min, und zweitens wurde standardisiertes lyophilisiertes Kontrollmaterial entwickelt, welches eine tägliche Validierung des Testverfahrens ermöglicht. Jedoch war die analytische Sensitivität dieser Methode bei der Untersuchung von TKs in Additivlösung verglichen mit TKs in 100% Plasma, leicht reduziert.

*Michael Schmidt and Michael K. Hourfar contributed equally to the manuscript.

Introduction

Since the beginning of the last century it has been known that infections by microorganisms could be transmitted by blood transfusion. In 1951 Borden et al. [1] published a case of transfusion-related death caused by massive bacterial contamination. Bacterial contamination of blood products remains a persistent problem in transfusion medicine. Currently, the risk of receiving bacterially contaminated platelets is 10–1,000 times higher than the combined risk of transfusion-related infection with HIV, HCV, HBV, and HTLV [2, 3]. The risk of bacterial infection through platelet transfusion is about 1 in 2,000 to 1 in 3,000, and the risk of a severe septic reaction after platelet transfusion has been estimated at approximately 1 in 50,000 [4–7]. Mortality following transfusion of a bacterially contaminated platelet unit is estimated to be in the range from 1:7,500 to 1:100,000 [6, 8]. Since 2004 blood screening for bacterial contamination is mandatory in the USA for all platelets [9]. Many countries who have already implemented bacterial screening used culture methods such as BacT/ALERT® (bioMérieux, Nürtingen, Germany). In a multicenter study of eight German transfusion centers [10] the German Red Cross study group found 0.9% of false-positive results when analyzing the first 4,000 samples tested with BacT/ALERT (35 of 4,000 samples). In this study, we evaluated the new Scansystem detection method for its ability to detect bacteria in pool platelets stored in additive solution (T-Sol®, Baxter, Vienna, Austria).

Materials and Methods

Study Design

In this study pool platelet concentrates (PCs), stored in additive solution T-Sol, were individually contaminated with seven different bacterial strains associated with transfusion-transmitted bacterial infection. Each bacterial strain was tested in nine replicates. PCs were spiked with approximately 10 CFU/ml and were incubated at 20–24 °C on a flat bed agitator. Scansystem analysis was performed 24 h post inoculation (p.i.). The test was repeated at 30 h p.i. and 36 h p.i. if at least one test result was negative at the previous point of time. The number of living bacteria in the PCs was quantified by plating on blood agar at 0 h for sterile control, immediately after spiking (spiking control) as well as at 24 h p.i., 30 h p.i. and 36 h p.i. A sample volume of 1 ml was cultured on blood agar plates for at least 24 h at 37 °C with exception for *Propionibacterium acnes*. Blood agar plates for *P. acnes* were cultured for at least 72 h at 37 °C under anaerobic conditions.

Scansystem

The Scansystem™ (Hemosystem, Marseilles, France) was developed for detection of bacteria in leukoreduced platelet-rich plasma (PRP)-derived platelets and apheresis platelets 30 h after blood collection. In this study bacterial detection was performed according to the manufacturer's instructions [11, 12]. Scansystem is performed as a pool testing method of three individual platelet bags. The test method relies on staining of the bacterial genome by the DNA-intercalating dye picogreen. The system consists of Scansystem platelet kits and the Scansystem solid-phase cytometry system. 3 ml of three PCs (one spiked with bacteria and two ster-

Fig. 1. Scansystem platelet kit. Scansystem is designed as a pooling test of three platelet bags. In case of a positive result all bag were tested individually.

ile samples to imitate pool testing procedure) are each inoculated into a plastic chamber. Thereafter Luer locks were broken, and all three samples were mixed (fig. 1). 3 ml of this mixture (dilution 1:3) are incubated with 1 ml of BLS1 (polyethylenimine, 60 mg/l, monoclonal antibody CD9 6B1, 30 mg/l; 1:2,000 dilution of picogreen nucleic acid-binding dye) and placed onto a flat bed agitator for 40 min to aggregate platelets. After filtering through a 5 µm pore-size filter, bacterial cell membranes were permeabilized and stained with BLS2 (EDTA, 1.86 g/l; nisin, 8 mg/l; N-octyl-β-D-glucopyranoside, 2.5 g/l; chlorhexidine diacetate, 150 mg/l, all in distilled water). Thereafter, all fluid was filtered onto a 0.4 µm pore-size black membrane, scanned with an argon laser (488 nm excitation light) and analyzed with an epifluorescence microscope. At least 50 microscopic fields were visually analyzed. If more than 10 bacteria-specific fluorescence spots were detected, the ratio between detected bacteria and analyzed fields was ≥ 0.2 . These samples were consequently taken to be positive. Under routine conditions, in case of a positive pool result individual tests of the three PCs were necessary. In the current spiking study all sterile samples were controlled for cross-contamination at each time by plating on blood agar plates. The measurement is simple and takes only 70 min. Lyophilised positive control capsules are provided for daily verification of the performance of the Scansystem method.

Bacterial Strains

In this study, five bacteria species – *Klebsiella pneumoniae* (PEI-B-08-07), *Streptococcus pyogenes* (PEI-B-20-03), *Staphylococcus epidermidis* (PEI-

Table 1. Scansystem screening method^a

Bacterium	24 h				30 h				36 h			
	N positive / N tested (%)	mean ratio	SD	% CV	N positive / N tested (%)	mean ratio	SD	% CV	N positive / N tested (%)	mean ratio	SD	% CV
Gram-positive												
<i>S. epidermidis</i> (PEI-B-06-05)	5/9 (55.6)	0.26	0.19	73.1	9/9 (100)	1.00	0.0	0.0				
<i>S. aureus</i> (PEI-B-23-03)	9/9 (100)	0.98	0.03	3.1								
<i>S. pyogenes</i> (PEI-B-20-3)	8/9 (88.9)	0.89	0.33	37.1	9/9 (100)	1.00	0.0	0.0				
<i>B. cereus</i> (JWG)	9/9 (100)	0.94	0.05	5.32								
<i>P. acnes</i> (PEI-B-22-03)	0/9 (0)	0.01	0.01	100.0 0	0/9 (0)	0.02	0.03	150.0	0/9 (0)	0.03	0.03	100.0
Gram-negative												
<i>K. pneumoniae</i> (PEI-B-08-07)	9/9 (100)	0.96	0.08	8.3								
<i>E. coli</i> (ATCC 25922)	9/9 (100)	1.00	0.00	0.0								

SD = standard deviation; % CV = percentage coefficient of variance; Mean ratio = mean value of 9 tested bags (ratio = bacteria-positive spots / counted spots).

^aPool PCs were spiked with approximately 10 CFU/ml of one of the bacterial strains listed in the table. Samples were analyzed at 24, 30 and 36 h if at least one Scansystem test was negative at the previous timepoint. Each bacterial strain was spiked into 9 PCs stored in T-Sol. At least 50 microscopic fields were visually analyzed. If 10 or more fields showed bacteria specific fluorescence signals, the ratio of detected bacteria to analyzed fields was 0.2 or above. These samples were taken to be positive. SD and % CV refer to the mean ratio.

B-06-05), *Staphylococcus aureus* (PEI-B-23-03), and *P. acnes* (PEI-B-22-03) – were provided by the Paul Ehrlich Institute (Langen, Germany). Two bacteria species, *Escherichia coli* (ATCC 25922) and *Bacillus cereus* (clinical isolate), were obtained from the Institute for Medical Microbiology of the Johann Wolfgang Goethe University (Frankfurt/M., Germany). Lyophilised capsules containing *Lactobacillus casei* subspecies *rhamnosus* were used as positive controls.

Platelet concentrates

Pool PCs were prepared according to the procedures routinely used at the Frankfurt German Red Cross Institute. In short, whole blood units were centrifuged and separated into plasma, red cell concentrate and buffy coat (70 ± 10 ml) using an automated device (Compomat, Fresenius Hemocare, Bad Homburg, Germany). Four buffy coats were pooled by sterile docking and mixed with 200 ml T-Sol® (Baxter, Frankfurt/M., Germany). Following soft spin centrifugation at 548 × g for 10 min, the PRP was transferred to a storage container using the Compomat and then filtered. After preparation, the pool PCs contained at least 2 × 10¹¹ platelets/bag.

Statistical Analysis

The standard deviation (SD) and coefficient of variation (CV) of Scansystem analysis and blood agar plates were calculated by using MS Excel 2000 software.

Results

In total seven different bacterial species were tested, each in nine replicates. Aliquots of three PCs (one spiked PC and two sterile negative controls) are pooled for analysis with Scansys-

tem, to imitate routine testing. In table 1 the results for the seven tested bacterial strains are shown. *S. aureus*, *B. cereus*, *K. pneumoniae* and *E. coli* were detected in all 9 PCs when sampling 24 h after spiking. *S. epidermidis* was detected in 5 out of 9 PCs after 24 h and in all PCs after 30 h. *S. pyogenes* was detected in 8 out of 9 PCs after 24 h and in all PCs after 30 h. In contrast *P. acnes* was not detected with the Scansystem method in this study even after 36 h of platelet storage. In all experiments, the bacterial number after spiking (0 h) was between 5 and 50 CFU/ml (table 2). Depending on the growth kinetics, bacterial counts at 24 h p.i. increased to >4 log CFU/ml for *S. epidermidis*, >5 log CFU/ml for *S. aureus* and *K. pneumoniae*, >6 log CFU/ml for *S. pyogenes* and *B. cereus*, and >7 log CFU/ml for *E. coli*. Only *P. acnes* did not show any growth for the first 24 h and a just small increase up to a mean value of 830 CFU/ml 36 h p.i.

To exclude secondary contamination, bacteria in the inoculum and in all spiked PCs were compared by Gram staining and DNA fingerprints at the Paul Ehrlich Institute. Cross-contamination could be ruled out in all experiments as Gram staining as well as DNA fingerprints showed 100% congruence between the spiked bacteria and the bacteria in the platelet bags (data not shown).

Twenty lyophilised capsules containing *L. casei* subspecies *rhamnosus* were solved in PBS and tested with the Scansystem over a period of 2 weeks twice a day. By culturing on

Table 2. Number of bacteria colonies on blood agar plates^a

Bacterium	0 h		24 h		30 h		36 h	
	mean no. (range), CFU/ml	SD	mean no. (range), CFU/ml	SD	mean no. (range), CFU/ml	SD	mean no. (range), CFU/ml	SD
Gram-positive								
<i>S. epidermidis</i> (PEI-B-06-05)	15.1 (12–21)	3.14	1.3×10^4 (4.7×10^2 to 4.0×10^4)	1.3×10^4	3.2×10^5 (3.5×10^4 to 1.6×10^6)	5.1×10^5		
<i>S. aureus</i> (PEI-B-23-03)	7.7 (6–10)	1.22	6.8×10^5 (1.6×10^5 to 1.6×10^6)	5.6×10^5	7.38×10^6 (2.0×10^6 to 1.4×10^7)	4.3×10^6		
<i>S. pyogenes</i> (PEI-B-20-3)	6.8 (5–9)	1.20	7.7×10 (1.9×10^4 to 2.5×10^7)	8.3×10^6	1.6×10^7 (1.5×10^5 to 3.4×10^7)	1.2×10^7		
<i>B. cereus</i> (JWG)	9.1 (6–14)	2.67	3.3×10^6 (2.1×10^5 to 1.4×10^7)	4.1×10^6				
<i>P. acnes</i> (PEI-B-22-03)	13.8 (7–19)	4.15	18.3 (12–25)	4.36	326 (212–427)	83.5	830 (466–1,120)	226.6
Gram-negative								
<i>K. pneumoniae</i> (PEI-B-08-07)	25.9 (24–30)	2.15	$>5.0 \times 10^5$ (N.D.)	N.D.				
<i>E. coli</i> (ATCC 25922)	24.2 (10–32)	7.74	4.4×10^6 (1.2×10^5 to 3.4×10^7)	1.1×10^7				

^aBacteria concentration was determined immediately after spiking and after each time point (24, 30 and 36h). Bacteria number was analyzed by culturing 1 ml on blood agar plates for at least 24 h. For *P. acnes* blood agar plates were cultured for 72 h under anaerobic conditions. Nine PCs were tested per bacterial strain.

blood agar plates, the detection limit was shown to be between 2×10^3 and 4×10^3 CFU/ml. Positive controls were detected in 100% of the experiments (data not shown).

Discussion

The whole process from blood donation to transfusion of blood products offers various opportunities to reduce the risk of bacterial contamination [8]. Improved puncture site skin disinfection of the collection site [13], pre-donation sampling, checking of integrity of sterile connection sites, use of apheresis PCs from a single donor instead of pooled PCs prepared from several donors [14], and reduction of platelet shelf life from 7 to 5 days [15] are just some examples. However, none of these methods is able to ensure a 100% prevention of bacterial contamination. Therefore, the implementation of screening measures for detection of bacterial contamination in PCs was suggested (AABB Standards, 2003) [9]. A desirable screening method should be highly sensitive and specific. Additionally, due to the limited shelf life of platelets, it should not require an incubation for several days. The rapid detection method described in this article was developed for detection of bacterial contamination within 30 h after blood collection. All bacteria were detected within 30 h p.i., except for *P. acnes*. *P. acnes*, however, is known as a very slowly growing bacterium. Accordingly BacT/Alert, an established automated culture system, usually showed positive signals for *P. acnes* after

90–110 h culture [10] (data not shown). Although the clinical importance of an infection with *P. acnes* is discussed controversially, one has to keep in mind that many recipients of blood products are immunosuppressed. In those patients, even harmless skin bacteria may cause severe sepsis [16]. Wilson et al. [17] analyzed risk factors for mortality in patients with anaerobic bacteremia. Clinical significance depended on the bacteria strain and ranged from 0% (0/53) for *Propionibacterium* spp. to 96% (43/45) for *Bacteroides* spp. On the other hand, there are reports on severe transfusion-transmitted infections caused by *Propionibacteria* [16].

Each bacterium was tested in nine independent pool PCs. Considerable variations in bacterial counts were observed between the different contaminated PCs. The variation between the minimum and maximum number of bacteria in each PC at 24 h p.i. was between 1 and 2 log units. An exception was *S. pyogenes* with a range of variation of 3 log units. One pool platelet bag spiked with *S. pyogenes* showed a lower growth kinetic for this bacteria, corresponding to the negative Scan-system test at 24 h. A spiking error could be excluded as the number of bacteria after inoculation in this bag was comparable to the other 8 bags. These heterogeneous results may be explained by antibacterial antibodies of at least one of the donors of this specific PC.

Due to the highest bacterial counts reached at 24 h p.i., the highest sensitivity was observed for *S. epidermidis*. In all experiments, no negative results were found if the bacterial count was 3.5×10^4 CFU/ml or higher (table 2). These results

differ slightly from those reported by Ribault et al. [12] or Jacobs et al. [18] and from those of our own experiments analyzing platelets stored in 100% plasma [19]. Against the manufacturer's instructions, Ribault et al. [12] analyzed the whole membrane instead of 50 random microscopic fields. When one bacterium was found on the whole membrane, the sample was considered to be positive. This approach, however, is extremely laborious and is not applicable for routine use. Jacobs et al. [18] analyzed 200 leukoreduced single donor apheresis platelets spiked with ten different bacterial strains. They found a sensitivity of 163 CFU/ml for Scansystem whereas sensitivity of our previous study was about 100 CFU/ml [19]. The apparently lower sensitivity in the current study may be explained by different products (platelet stored in T-Sol in the present study versus apheresis platelets stored in 100% plasma in the previous study) or by different bacterial strains used. Nevertheless, we could demonstrate for the first time that Scansystem is suitable to detect bacteria in pool platelets prepared with the buffy coat method as well as in PRP platelets [11].

Scansystem is the first rapid detection system which provides a standardized positive control. In view of the comparably low incidence of bacterially contaminated platelet products (approximately 1 in 2,000), a daily validation of the system should improve blood safety by preventing false-negative results. The

positive control capsules are containing a defined number of lyophilised bacteria and allow to prepare adjusted bacterial solutions, e.g. with a concentration equal to twice the detection limit of the system. Therefore the sensitivity of Scansystem can be verified on a daily basis by use of the positive control capsules.

In summary, the Scansystem was able to detect six strains of transfusion-relevant bacteria species in PCs stored in additive solution within 30 h after blood collection. However, the sensitivity might be reduced when compared to platelets stored in 100% plasma. One major benefit of the Scansystem might be the rapidity of the measurement. PCs that have been stored for at least 30 h p.i. can be examined for bacterial contamination within 70 min. This feature could be of interest for emergency cases. Additionally, lyophilised positive control capsules are provided for daily monitoring of the performance of the Scansystem bacterial detection method.

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