Diagnostic Methods for Platelet Bacteria Screening: Current Status and Developments

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Summary

Bacterial contamination of blood components and the prevention of transfusion-associated bacterial infection still remains a major challenge in transfusion medicine. Over the past few decades, a significant reduction in the transmission of viral infections has been achieved due to the introduction of mandatory virus screening. Platelet concentrates (PCs) represent one of the highest risks for bacterial infection. This is due to the required storage conditions for PCs in gas-permeable containers at room temperature with constant agitation, which support bacterial proliferation from low contamination levels to high titers. In contrast to virus screening, since 1997 in Germany bacterial testing of PCs is only performed as a routine quality control or, since 2008, to prolong the shelf life to 5 days. In general, bacterial screening of PCs by cultivation methods is implemented by the various blood services. Although these culturing systems will remain the gold standard, the significance of rapid methods for screening for bacterial contamination has increased over the last few years. These new methods provide powerful tools for increasing the bacterial safety of blood components. This article summarizes the course of policies and provisions introduced to increase bacterial safety of blood components in Germany. Furthermore, we give an overview of the different diagnostic methods for bacterial screening of PCs and their current applicability in routine screening processes.

Introduction

Bacterial contamination of platelet concentrates (PCs) represents an ongoing risk in transfusion medicine even though a variety of strategies have been developed for minimizing the risk of transfusion-transmitted sepsis. These essential strategies for preventing bacterial contamination of blood components include careful donor selection, selection of the puncture site, effective skin disinfection, separation of the first volume from the blood donation (pre-donation sampling, also called diversion) and the consistent monitoring of the bag systems, including monitoring of additional connections to the bag by the so-called sterile connecting device. Policies regarding these precautionary measures have already been implemented in Germany [1–7].

The introduction of the ‘Minimum requirements for sterility control of blood components (1997)’ by the working party on bacteria safety in transfusion medicine of the National Advisory Committee Blood of the German Federal Ministry of Health (Arbeitskreis Blut, AKB) was a milestone in bacteria safety in Germany. These national guidelines were introduced to monitor bacterial contamination of blood components as part of routine quality control to establish standardized methods for bacterial testing [6]. The standardized protocols included the time of sampling (expiry date + 3 days), the sample quantity ($0.4 \times$ root of monthly produced blood component per production facility), sampling procedure, microbiological control (aerobic and anaerobic cultivation using 4–10 ml sample each with incubation at 30–37 °C for 14 days if conventional liquid culture is performed and 7 days if an automated system is used), identification of contaminating microorganisms, and performing a second culture as a confirmatory test using material from the same blood bag [1, 5, 8]. As a result of the obtained and analyzed data; statement V16 was revised and adopted as statement V43 in 2012 by the AKB. Since

Fig. 1. Overview of the essential German strategies for the prevention of bacterial contamination of blood components and their influence on the rate of transfusion-transmitted bacterial infection (TTBI). Different strategies have been described and published in terms of different votes by of the National Advisory Committee Blood of the German Federal Ministry of Health (Arbeitskreis Blut, AKB). The rate of TTBI was assumed from the German hemovigilance report published by Funk et al. [10].

blood components are not sterilizable and the specification of the final product can never be 'sterile', the term sterility testing was replaced by microbiological control in this statement. Moreover, the main revision focuses on the sample quantity needed for quality control. Up to now the sample reference parameter for the calculation of the sample quantity of each blood component was defined as the number of each individual product produced in the institute. Now the definition is the number of performed principles (Vote 43 [9]). Altogether, due to all these improvements in the acquisition of blood, the bacterial contamination rate was reduced over the years. Nonetheless, hemovigilance data showed that severe transfusion reactions still occurred in Germany [10]. As a consequence, the shelf life of platelets was reduced to 4 days beginning at midnight on the day the blood was drawn, but may be prolonged back to 5 days if pathogen reduction or bacterial detection is implemented (Vote 38, [11]). The suspicion that increased transfusion-transmitted bacterial infections (TTBIs) may be due to longer storage of PCs, led to the shelf life also being reduced in the USA to 5 days and in Japan to 3 days. However, after implementing a bacterial platelet screening strategy several countries, including Denmark, Ireland, The Netherlands, and UK, extended the storage time up to 6.5 or 7 days [12, 13]. Since the American Association of Blood Banks (AABB) standard 5.15.1 in 2004 recommended the implementation of methods to limit or detect bacterial contamination, a variety of methods for platelet bacteria screening have been evaluated in many studies worldwide. Moreover, microbiological monitoring has been mandatory in Belgium since 1998 and in The Netherlands since November 2001 [14].

Figure 1 displays an overview of these essential strategies and their influence on the frequency of TTBIs [10]. Although these policies represent important steps for improving the safety of blood products, the total rate of reported TTBIs has remained almost stable over the last 16 years. This review focuses on the different diagnostic methods and strategies for detecting bacterial contamination.

Bacterial Growth – Bacterial Reference Strains

A fundamental difference between contamination by viruses or bacteria is that the latter can simply continue to replicate in a PC during its shelf life. Viruses need target cells for replication, which are normally reduced in packed red cells, PCs or plasma products. Under the usual storage conditions at 22–24 °C, even an extremely small number of bacteria can multiply to vast and clinically dangerous levels during their storage period [15]. However, bacterial contamination of blood components does not always result in bacterial multiplication. Organisms may die due to autosterilization, others may survive in the unit in low numbers but not multiply, and some may grow rapidly to high transfusion relevant numbers [16]. The behavior of bacteria in the setting of blood component production, storage and use must be taken into account, as replication of microorganisms in transfusable components may behave differently than in other microbiological culture media [5]. To validate and assess methods for bacterial screening in a consistent manner, bacterial strains that are able to proliferate in blood components are needed. Therefore, an international validation study was or-

ganized by the ‘Subgroup on Bacteria’ of the ‘Working Party on Transfusion-Transmitted Infectious Diseases of the International Society Blood Transfusion’ of the International Society of Blood Transfusion in 2008 to establish a WHO Repository of Platelet Transfusion Relevant Bacteria Reference Strains [17]. These deep-frozen bacterial suspensions are defined in bacterial species, bacterial cell count, and proven for the ability to grow in PCs under routine storage conditions. The repository currently includes 4 bacterial strains but will be enlarged in the near future. To validate new bacterial detection technologies, the bacterial solutions only need to be thawed and diluted down to the recommended final count for spiking experiments to simulate real-life conditions. This standard can now be ordered over the homepage of the Paul-Ehrlich institute.

Diagnostic Methods – Bacterial Screening Strategies

The perfect test should have an extremely high diagnostic sensitivity and specificity, and be inexpensive, reliable, and fast. Up to now, none of the evaluated methods has been able to fulfill all these requirements [18]. For a long time measurements of glucose and pH as surrogate indicators and microscopic examination were the usual methods for detecting bacteria in PCs, but have been shown to be ineffective in clinical use and have led to many units being discarded due to false-positive results [15, 19]. Besides conventional microbiological approaches or surrogate markers, several efficient methods able to detect bacterial contamination in platelets were developed over the past decade (for review see [12, 15]).

In general, diagnostic methods for the detection of bacteria in PCs can be divided into: i) methods using cultures in combination with an early sampling strategy; ii) methods using cultures in combination with a late sampling strategy; iii) methods using rapid direct detection in combination with a late sampling strategy; and iv) methods using rapid indirect detection of bacteria in combination with a late sampling strategy. An overview of the currently commercially available bacterial screening methods that are presented in the following sections is provided in table 1.

Culture-Based Methods in Combination with Early Sampling Following the ‘Negative-to-Date’ Concept

Automated Culture Systems

Many countries have implemented automated culture systems using the negative-to-date concept to screen PCs for bacterial contamination [13, 20–23]. Cultivation or incubation methods require time for signal production as they depend on microbial growth. Therefore, samples are collected from the PCs early in the shelf life, in general within 24 h after donation. 4–10 ml samples are then inoculated into aerobic (in some countries into aerobic and anaerobic) culture bottles and incubated in the au-

tomated culture system up to 7 days at 35–37 °C [24]. PCs with a negative diagnostic status are released but cultivation is continued. If the result status changes from negative to reactive, physicians have to be informed immediately and products need to be recalled. If PCs were already transfused a look-back procedure has to be initiated [25]. The sample collection at an early stage of shelf life exhibits a high risk of sampling error in the range 1:14,000–1:50,000 [5], due to the low number of contaminating bacteria at the beginning of PC shelf life, particularly when slow-growing bacteria are involved in the contamination. The risk of sampling errors continually decreases during PC storage; the detection rate of bacterial contamination changed from 14.9% for sampling on day 1 to 100% for sampling on day 7 [15]. In general, cultural methods still represent the method with the highest analytical sensitivity of less than 10 colony-forming unit (CFU) per ml, but use of the ‘Negative-to-Date’ concept considerably reduces the diagnostic sensitivity as a relevant percentage of platelets had already been transfused by the time a positive signal occurred. Further disadvantages of culture methods include the detection of bacterial contaminations with non-transfusion-relevant bacterial species or titers. For example, *Propionibacterium acnes*, which is often detected in PCs with cultural methods, lacks the capability to proliferate in PCs due to the storage conditions in the gas-permeable bags [26, 27]. Furthermore, bacteria that usually die due to autosterilization during PC storage were also detected using the early sampling procedure. These 2 aspects result in a higher rate of positive PCs accompanied by high diagnostic efforts and a discard of products.

Three automated culture methods have been in routine use or have been validated for platelet quality control: the BacT/ALERT system (BioMérieux, France), the Bactec (BD Diagnostics, USA), and the VersaTrek system (Trek Diagnostics, USA). The BacT/Alert is by far the most common in reports and has been cleared for this application by the Food and Drug Administration (FDA) [18, 25, 28]. It monitors CO₂ production due to bacterial growth, which changes the color of a gas-permeable sensor at the bottom of the culture bottle from grey to yellow [25]. The Bactec system is very similar to the BacT/Alert system but uses a fluorimetric instead of the colorimetric detection principle [18]. The VersaTREK monitors bacterial growth by detecting pressure changes in the headspace of the blood culture bottle secondary to gas consumption/production [29]. All of these systems are available on the market and were validated in various studies for the use in quality control of PCs with comparable results, but as yet the Bactec and the VersaTREK have not been cleared by the FDA for the quality control of PCs [28–31].

Haemonetics eBDS (Formerly Pall eBDS)

Another principle that was introduced into routine practice and cleared by the FDA is the Pall eBDS system (Pall Corporation, USA), now Haemonetics eBDS (Haemonetics Corporation, USA). This monitors the concentration of oxygen in the headspace of a satellite bag that is incubated at 37 °C [5]. The eBDS system comprises: a disposable sample set, with a

Method (Manufacturer)	FDA/ CE-IVD	Principle of detection	Analytical sensitivity, CFU/ml	False-positive rate, %	Sample volume	Hands-on-time/ time-to-result	References	Advantages	Disadvantages
Culture methods combined with early sampling									
BacTUAlert (BioMérieux)	Yes	Colorimetric detection of CO ₂ production during automated cultivation	1–10	0.03–0.36	4–10 ml	5 min/depending on bacterial load	[13, 21–23, 31, 55, 66, 67]	High sensitivity Easy performance Automation	Risk of sampling error Time-to-results strongly depending on bacterial load Limited clinical value (negative-to-date)
Bactec (BD Biosciences)	No	Fluorimetric detection of CO ₂ production during automated cultivation	1–10	0.10	4–10 ml		[28, 30, 31]		
VersaTrek (Trek Diagnostics)	No	Detection of pressure changes in culture bottle due to gas consumption/production	10–20	n.s.	4 ml		[29]		
Haemonetics eBDS (Haemonetics)	Yes	Electrochemical detection of oxygen consumption at the end of cultivation	1	0.008–3.5	2–3 ml	n.s./24–30 h	[28, 32, 33, 55, 66]		
Rapid methods combined with late sampling									
BactiFlow (BioMérieux)	No ¹	Fluorescence-assorted cell sorting based on esterase activity of viable cells	300–500	0.05–0.57	1 ml	5 min/1 h	[35–37]	Short hands-on-time, time-to-result	Decreased sensitivity
16S rDNA real-time PCR	Yes	Amplification of nucleic acids	GP: 10 ³ to 10 ⁴	n.s.	1.0	30–60 min/4 h	[35]	time, time-to-result	PC release without testing
PDG (Verax Biomedical Inc.)	Yes	Lateral-flow immunoprecipitation of bacterial lipopolysaccharide or lipoteichoic acid	GN: 10 ³ to 10 ⁵ , some > 10 ⁶	0.5–1.15	0.5 ml	5 min/1.5 h	[23, 42–45]	Reduced risk for sampling errors	
BacTx ² (Immunetics)		Colorimetric detection of bacterial peptidoglycan	10 ³ to 10 ⁴	n.s.	1.0 ml	1 h	[51]	High clinical efficiency	
GN = Gram-negative, GP = Gram-positive, n.s. = not specified. ¹ Currently in progress. ² No published comprehensive validation studies.									

²²No published comprehensive validation studies.

pouch containing a readily dissolvable tablet of sodium polyanethol sulfonate to avoid platelet aggregation; trypticase soy broth as a source of nutrition for microorganisms; a flatbed agitator; an incubator and an oxygen analyzer. This system is based on the principle that the growing aerobic and facultative anaerobic bacteria consume oxygen in the plasma and that the oxygen in the air of the sample pouch will equilibrate with the plasma. After collection of a 3-ml sample, the automated culture system should be run for a minimum of 24 h before the pouch is heat-sealed and incubated at 35 °C with constant agitation and subsequent measurement of oxygen concentration. Bacterial contamination is assumed if the O₂ reading is less than 12.5%, which is the cut-off value between a positive and negative reading [28, 32]. Using this technology only bacteria that are able to grow under aerobic conditions will be detected, which confers a risk for false-negative screening results [25]. The sensitivity of the Pall eBDS system is similar to the BacT/ALERT system in the order of 1 CFU/ml [33].

Microcalorimetry

Measuring heat due to replicating microorganisms in culture has been used for detection of bacteria in PCs. A microcalorimetry thermostat is used to measure the heat flow curves. Any heat generated or absorbed by the sample is measured continuously over time. Trampuz et al. [34] artificially contaminated PC samples with different bacterial species at cell counts of 1–10⁵ CFU/ml. Heat changes in PCs contaminated with 1 CFU/ml were mostly not detectable. The time needed for detection increased with decreasing initial concentration of microorganisms. Fast-growing organisms were detected within 8 h of incubation. The method is easy to perform and needs only a minimum of manual effort and technical expertise. However, due to the cultivation step this method needs to be combined with an early sampling strategy and, therefore, the risk of sampling error seems to be comparatively high, as mentioned for the automated culture methods.

Culture-Based Methods in Combination with a Late Sampling

The implementation of culture methods in combination with late sampling is currently under discussion. The utilization of the BacT/Alert as a possible rapid screening method was proposed by Sireis et al. [35], but basically all other culture-based methods are also applicable. In this study, a bacterial panel including bacteria with fast and stable growth characteristics [17] was used for spiking experiments, and sampling after 3 days of storage resulted in an incubation time of <12 h. Nevertheless, spiking experiments with other bacteria demonstrating lower growth kinetics [36, 37] are necessary for further evaluation of this strategy. Alternatively, the application of the negative-to-date concept is also an option.

Conclusion for Culture Methods

Concluding the introduction on platelet bacterial screening, automated cultivation should be considered as an important milestone in improving bacterial safety of blood components [5], but so far culture methods have been always combined with early sampling and, therefore, exhibit a high risk of sampling error. The predictive value of culture methods and the negative-to-date release concept limit their clinical value for decreasing the infection risks of platelets at the time of issue [15]. The potential for sampling error, delayed time to result, and release of platelets cannot be solved with culture approaches combined with an early screening strategy [36]; only the combination with a late sampling could potentially overcome this limitation. Nevertheless, rapid detection methods are the focus of interest for bacterial screening because they are able to produce a diagnosis within 2 h (e.g. BactiFlow, Pan Genera Detection (PGD)) or at least within a working day (e.g. nucleic acid testing, NAT). Therefore, rapid methods allow postponing of sample withdrawal and minimize the sampling error [5].

Direct Detection of Bacteria in PCs Using Rapid Detection Methods in Combination with Late Sampling

Real-Time PCR

Numerous investigators have focused on the detection of bacterial contamination in PCs by real-time PCR [35, 38–40]. Conserved nucleic acid sequences in the 2 target regions 16S rDNA and 23S rDNA have been used to develop real-time PCR assays. The analytical sensitivities vary from 5 to 50 CFU/ml. However, the main drawback of these assays is amplification of background signals originating from the utilized PCR reagents, in particular from polymerases since these were produced in bacteria and are, therefore, not free of contaminating bacterial genomic DNA originating from the host [25]. Numerous attempts have been made to reduce background amplification, e.g. decontamination of PCR reagents by enzymatic digestion or the production of ‘bacterial DNA-free polymerases’, often lowering the assay sensitivity and influencing assay efficiency [41]. Furthermore, the design of primer and probes for NAT testing is optimized for the detection of commonly contaminating bacteria, so that detection of some species may be reduced or may completely fail. Therefore, primers and probes must be constantly verified either by testing of appropriated reference strains or in silico. Taken together, NAT testing is more laborious and expensive (equipment, reagents) compared to other methods (table 1). To our knowledge, only the assay developed by Sireis et al. [35] received CE-IVD (in vitro diagnostics) certification and is commercially available.

PGD

The test principle of the FDA-licensed, qualitative PGD (Verax Biomedical Inc., Worcester, MA, USA) assay is based on a lateral-flow immunoprecipitation of bacterial cell wall

antigens (lipopolysaccharide or lipoteichoic acid), which are present at high copy numbers (>200,000 copies/cell) [25]. The short hands-on time and the minimum requirement for laboratory instrumentation provide the opportunity for a point-of-issue bacterial detection test immediately before transfusion of PCs [42]. The analytical sensitivity of this assay is specified as 10^3 – 10^4 CFU/ml for Gram-positive bacteria and 10^3 – 10^5 CFU/ml for Gram-negative bacteria. However, for some Gram-negative strains the detection limit is greater than 10^6 CFU/ml [42]. Further disadvantages of the PGD assay are the costs, a high rate of false-positive results [23] and, currently, the subjective result interpretation [42]. This assay has been evaluated in various studies for use in quality control of PCs [43–45]. In January 2011, the AABB approved a new standard (5.1.5.1.1) in which the PGD test is recommended as an option for meeting the intent of this standard [46]. However, currently the PGD assay is the only system that might be feasible as a bedside detection system. Nevertheless, since centrifuges are required for testing, it is not clear if the system can be implemented as a bedside test immediately before transfusion. Furthermore, this setting might pose a challenge of responsibilities because blood donor services would no longer be responsible for testing, and staff on the board should do the bacterial screening. Flow Cytometry

Labeling of bacterial components by fluorescent dyes presents another approach for detecting and quantifying bacteria in PCs by flow cytometry. A method based on reagents from BD Biosciences (Becton Dickinson GmbH, Heidelberg, Germany) has been evaluated for investigating PCs [47]. The membrane-permeable fluorescent dye thiazole orange binds specifically to bacterial nucleic acids and emits a bright green-orange fluorescence. In a two-step procedure platelets are lysed and bacteria are labeled. The result is available in less than 15 min. The low sensitivity of 10^4 CFU/ml could be increased by an additional short pre-incubation of the platelet sample at 37 °C [5, 47, 48]. Unfortunately, reagents are no longer commercially available. Furthermore, the FDA-cleared solid-phase flow cytometric method called Scansystem is also no longer being marketed [15]. This method was based on filtration of PC samples followed by picogreen staining of bacteria and detection using a laser-based, solid-phase scanning cytometry [49].

Sireis et al. [35] evaluated the use of flow cytometry for prolonging the shelf life of PCs using an in-house-validated procedure. Platelets are incubated for 20 min at room temperature with CD61-phycoerythrin (PE) (BD Bioscience). Bacterial cells are then stained with thiazole orange and incubated for additional 10 min. A fluorescence filter set for FL1 (BP 515–545 nm) is used to collect the fluorescence emission from thiazole orange complexed with nucleic acids to detect bacteria. Using the FL2 filter set (BP 572–595 nm), the CD61–PE-stained platelets, which do not contain DNA, are detected [35]. This procedure shows a reduced sensitivity due to the fact that bacteria are clotted together with platelets removed by out gating CD61-positive cells to reduce the background noise.

In 2009, a commercially available flow cytometric technology originally established for the food industry was introduced for bacterial screening of PCs. The BactiFlow assay detects and enumerates bacteria by fluorescent labeling of viable cells. A non-fluorescent fluorochrome passes the cell membrane of cells with intact membrane integrity and enzymatic activity, and is cleaved by intracellular esterases. To reduce the background noise, the esterase activity of platelets is selectively eliminated by enzymatic digestion [36]. The analytical sensitivity of this assay is validated to be 300–500 CFU/ml [35, 36]. This technology is used for routine bacterial screening of PCs and is accepted by the German national authorities (Paul-Ehrlich institute) to extend the storage period of PCs from 4 back to 5 days [35–37, 50].

BacTx Peptidoglycan Assay

The BacTx assay is a rapid, qualitative, colorimetric assay for detecting the presence of the peptidoglycan present in bacteria cell walls. The PC sample (0.5 ml) is added to a microfuge tube containing a lysis reagent and is centrifuged to pellet insoluble platelet debris and bacterial cell wall fragments. The pellet is then homogenized by adding an extraction reagent to release peptidoglycan from the bacterial cell walls for optimal detection. After neutralization, the mixture is added to a tube containing the lyophilized detection reagents placed in the BacTx photometer reader, which monitors the detection reaction and interprets the result using software installed on the laptop provided. The test has a sensitivity of approximately 10^4 CFU/ml, and provides results within 1 h [15]. The sensitivity varies between different bacterial strains, and, according to the manufacturer, ranges from a minimum of 1.3×10^3 CFU/ml (*Staphylococcus epidermidis* ATCC49134) to 7.6×10^4 CFU/ml (*Escherichia coli* ATCC 25922) in apheresis-derived PC and 1.7×10^3 CFU/ml (*Bacillus cereus* ATCC11778) to 5.8×10^4 CFU/ml (*Serratia marcescens* ATCC43862) for whole blood-derived platelets. The BacTx received FDA clearance for bacterial contamination of PCs in 2012. First results by Jacobs et al. [51] demonstrated the suitability for use near the time of issue of PCs.

Other Methods under Development

Wang et al. [52] developed a strategy for detecting specific nucleic acid fragments by combining universal PCR with lateral-flow dipstick (LFD) technology. This assay utilizes the amplification of genes followed by hybridization by a set of probes that are specific for the conserved region of the target. Colloidal gold-labeled avidin and specific anti-FITC (fluorescein isothiocyanate) antibodies immobilized on the LFD nitrocellulose membrane then capture a probe-amplicon complex, producing a visible red line on the membrane. According to the results of the group, the total analysis time of LFD technology is only about 2 h, but the sensitivity varies depending on the bacterial species from 5 to 10^4 CFU/ml.

A spore-based biosensor for detecting bacteria in real time has been recently developed by Rotman and Cote [53]. The

system, termed LEXSAS, exploits the ability of spores to produce fluorescence when sensing neighboring bacterial cells. The LEXSAS contains microbial spores suspended in an enzymatic substrate that produces a germinant upon enzymatic catalysis combined with diacetate fluorescein, a component that fluoresces when hydrolyzed by esterases. Signal to noise ratio values are determined by comparing signals from contaminated units to signals from the negative control [15]. Bacterial detection using a bioimaging system based on a fluorescent indicator for esterase activity of bacteria was also described by Motoyama et al. [54].

To our knowledge no other reports evaluating these 2 procedures have been published. Therefore, it is difficult to assess the utility of these methods for platelet bacteria screening.

Conclusion for Direct Detection Methods

Currently, the 3 rapid methods, the BactiFlow, NAT and PGD systems, have been routinely evaluated and have been shown to be feasible for bacterial screening of PCs [35, 36, 43, 44, 50, 55–57]. The performance of the PGD assay comes closest to a bedside test; however, the comparably low sensitivity of $>10^3$ CFU/ml indicates that this method is acceptable only if the screened PCs are transfused immediately after testing. The high sensitivity of the BactiFlow and NAT assays means that transfusion could be postponed after testing, but implementation is more laborious. The other rapid methods described in this review also present promising approaches, but there is a need for further studies regarding their applicability in a routine screening setting to evaluate more fully their relevance and acceptance for bacterial screening of PCs.

Indirect Detection of Bacteria in PCs Using Rapid Detection Methods in Combination with Continuous Sampling during Storage or Late Sampling

Platelet Aggregation

Platelet function is one of the most discussed topics within the field of hemostasis. Although there are various platelet function tests available, light transmittance aggregometry (LTA) invented by Born [58] is still considered the gold standard for the evaluation of platelet function. Moreover, it has been shown that bacteria interact with platelets, and may trigger platelet activation and subsequent aggregation depending on the bacterial strain properties and bacterial concentration. Therefore, Störmer et al. [59] evaluated the suitability of platelet aggregation as a parameter for bacterial contamination of apheresis PCs in comparison to bacterial count calculation. They demonstrated that platelet aggregation was reduced in bacterially contaminated products in comparison to sterile products. The level of reduction was dependent on donors and the growth properties of the bacterial strain in the product [60]. Furthermore, a time shift between reaching high bacterial counts in the product and the influence on platelet

quality was observed. Therefore, the principle of indirect detection represents only the situation at a specific time before measurement due to the time between reaching high bacterial cell counts and inducing the detectable effect. Measuring platelet aggregation immediately prior to transfusion would not only improve microbiological safety but also the quality and efficiency of platelet transfusion.

Non-Invasive pH Measurement

A decrease in pH can be the consequence of bacterial contamination with acid producing bacteria. An approach has been developed allowing in-line measuring of pH in platelets through the use of the BCSI pH SAFE reader without opening the bag system (Blood Cell Storage Inc., Seattle, WA, USA). PCs are stored in a bag containing the integrated pH sensor for non-invasive pH monitoring. This sensor comprises a pH-sensitive fluorescent membrane fixed to a clear window inside a small sensor tube, which is welded in the rim of the PC container. Fluorescence of the sensor membrane is measured through the window using a fiber optic probe and the BCSI pH SAFE reader calculates pH based on the ratio of yellow and red fluorescence measured [61, 62]. Preliminary studies by Montag et al. [63] demonstrated a decrease of pH (6.7 to 6.5) in bacterially contaminated units after reaching high bacterial counts. Thereafter, an increase in pH values was observed in all contaminated PCs. In general, pH measurement in PCs as a solitary approach for platelet bacteria screening is not acceptable because of its low sensitivity, but on the other hand this system allows pH measurement immediately before transfusion without opening the bag, which gives a clear advantage. Due to the fact that there are only explicit time points at which the pH provides a warning signal, and due to the recovering effect of pH during storage, a single measurement before transfusion may not prevent transfusion of contaminated products (false-negative-result). There is a need for further studies and development to provide a real-time monitoring system during PC storage.

Oxygen Measurements

A new continuous non-invasive bacterial detection method has been developed using O_2 measurements in the platelet fluids. The principle of the sensor operation is based on the quenching of luminescence caused by collision between molecular oxygen and luminescent dye molecules in the excited state. Oxygen concentration in platelet fluids is continuously detected by oxygen sensing probes and registered every 20 s. Probes can be labeled by barcodes and integrated into the platelet storage bag. First studies by Mueller et al. [64] were performed by placing disinfected probes directly in the platelet fluid of sterile and artificially contaminated PCs. On the basis of the generated baseline data for sterile PCs, a bacterial-positive blood product was defined as showing an O_2 saturation of $< 10\%$ because all negative samples had oxygen saturation higher than 30% over a time period of 7 days. A

major benefit of the described PreSense O₂ system is a continuous measurement in a closed system. Using a radio frequency identification technology bacterial screening might become feasible up to the time of transfusion.

Conclusion for Indirect Detection Methods

The indirect detection methods presented here also provide adaptable methodological concepts, but further studies are needed to evaluate the details with respect to their applicability in a routine screening and their performance regarding different bacterial strains. For example, the pH drop observed for some strains demand continuous monitoring and extensive examination of a large number of different strains. Nevertheless, the approach of a continuous non-invasive monitoring does avoid the risk for sampling errors.

Conclusions

Beside conventional microbiological approaches or surrogate markers, several efficient methods able to detect bacterial contamination in platelets have been investigated since the AABB standard 5.15.1 insisted on the implementation of methods to limit, or at least detect, bacterial contamination. The introduction of automated cultures for quality control of PCs was seen as a milestone in improving microbial safety. However, cultivation or incubation methods require some time for signal production as they depend on growth of microbes. Thus, they have to be combined with early sampling, which can imply a high sampling error risk. Their advantage is the uncomplicated introduction into the logistics of blood banks. Rapid methods are able to produce the diagnosis within a short time, so that sample drawing can be postponed, minimizing the sampling error risk. However, it was thought that these strategies might sometimes cause logistical complications. We know now that the implantation of rapid methods in the routine screening process is not that complicated. The implementation of the BactiFlow instrument or real-time

PCR in German blood centers, and their adoption by the German competent authority PEI (Paul-Ehrlich institute) for prolonging the shelf life to 5 days demonstrate their suitability and robustness for routine platelet bacteria screening [25, 35–37, 50, 57]. The statement included in vote 38 [65], i.e. ‘It is presently not possible to recommend any of the rapid testing procedures due to the absence of sufficient routine experiences’, should be revised on the basis of the current state of studies. The late sampling in combination with an effective and reliable method seems to represent a good strategy for bacterial screening of PCs, obtaining explicit results even for slow-growing organisms. However, the standardization of rapid methods is still an important aspect to allow a comparison of assay sensitivities, specificities and performances. To solve this challenge, the establishment of the WHO Repository of Platelet Transfusion Relevant Bacteria Reference Strains [17] is an essential step forward. Additionally, the introduction of internal and external quality controls by collaborative trials and further national or international multicenter studies are opportunities for the near future. The extension of the published data from the current German surveillance system could possibly provide additional important data for the reduction of TTBI. Information about the age of PC, the contaminating bacterial species and titer, and specification of the intolerable secondary effects, as well as about the recipient (age, immune status, antibiosis) will aid in assessing TTBI, resulting in a potential modification of current diagnostic methods and screening strategies.

In conclusion, platelet bacterial screening in general improves microbial safety of PCs. Furthermore, considering the supply for patients, the prolongation of the shelf life due to the introduction of a screening strategy will improve the availability of PCs especially on holidays and weekends.

Disclosure Statement

Both authors state that they have no potential conflict of interest.

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