Austin Hematology

Open Access

Austin Publishing Group

Review Article

Bacterial Screening of Blood Components: Past, Present and Possibly Future Methodologies for Improving Transfusion Safety

Debrincat A[1], Gialanze JP[2], Spiteri N[2] and Zammit V[1,2]*

[1]Faculty of Health Sciences, Department of Applied Biomedical Science, University of Malta, Malta
[2]National Blood Transfusion Service, Malta

***Corresponding author:** Zammit Vanessa, National Blood Transfusion Service, Telghet Gwardamangia, Pieta, PTA010, Malta

Received: January 26, 2021; **Accepted:** February 20, 2021; **Published:** February 27, 2021

Abstract

Major developments have been made in the past two decades to ensure that products are free from viral pathogens. Such improvements were achieved, mainly by the introduction of advanced screening methodologies such as Nucleic Acid Testing. Even though this has vastly improved transfusion safety, bacterial contamination remains a persistent threat. To mitigate this, improved donor arm disinfection, blood diversion pouches and proactive screening of blood components have been implemented by transfusion services all over the globe with great success. This review will focus on how current bacterial screening methodologies have improved over the years and how newer developments within this sector may further reduce the undesired possibility of transfusing contaminated blood products.

Keywords: Blood transfusion; Bacterial detection; Contamination; Platelet concentrates

Abbreviations

TTBI: Transfusion Transmitted Bacterial Infections; SAR: Serious Adverse Reactions; EU: European Union; PCs: Platelet Concentrates; AABB: American Association of Blood Banks; eBDS: enhanced Bacterial Detection System; FDA: US Food and Drug Administration; NAT: Nucleic Acid Testing; PCR: Polymerase Chain Reaction

Introduction

The need for bacterial surveillance is a must in today's transfusion settings. Passive vigilance relates to the reporting of Transfusion Transmitted Bacterial Infections (TTBI) or septic events by clinicians followed by identification of the causative agent. However this practice is not always accurate since the signs and symptoms of bacterial infection are hard to differentiate from other adverse transfusion reactions [1]. As a result of misinterpreting such symptoms, instances of sepsis can be easily overlooked and may not be reported within the respective national hemovigilance network. The prevalence of reactions caused by TTBIs was estimated being around 1 in 10,000 to 1 in 100,000 of platelet units transfused [2]. Active screening by means of blood culturing systems is a major measure that can prevent TTBIs from occurring. Members of the European Union (EU) are required to submit a yearly report detailing all the Serious Adverse Reactions (SARs) as a result of transfusion (EU Article 8 of Directive 2005/61/EC). In 2017, a total of 25, 093, 906 blood product units were transfused within the EU member states and Norway, with more than three fifths consisting of red cell concentrates. A total of 3, 114 SARs were reported by most participating countries, which include 16 episodes that were a result of bacterial infection [3]. Although this signifies a small proportion of SAR's, two deaths out of the 28 deaths reported due to SARs within the same report indicated their cause as being transfusion associated bacterial infection. Although the risk of contracting a bacterial infection in this regard is small, the mortality rate can be quite high. This risk is disproportionately higher in immunocompromised patients. Apart from infection, the infusion of endotoxins produced by Gram-negative bacteria within the blood product also poses a risk of causing shock or death [4].

Blood product contamination may arise due to improper donor skin disinfection [5], during phlebotomy [6], contaminated donation equipment [7], or donor bacteremia [8]. Unlike viruses, contaminating bacteria and fungi can proliferate within blood products during storage, especially within Platelet Concentrates (PCs) [9]. It is estimated that between 1:1000 to 1:2000 platelet units are contaminated. Recipients of such contaminated units therefore have a high risk of developing sepsis [10]. Due to the elevated risk presented by contaminated platelets, monitoring of said blood component is a major focus point for the prevention of septic transfusions. The majority of contaminating bacteria originate from the skin, most notably those of the *Staphylococcus, Corynebacterium* and *Propionibacterium* species. Other bacteria such as gram-negative organisms are also encountered and are responsible for many of the reported TTBI related fatalities [11]. Pooled random donor platelets require buffy coats from different donors. As a result, single donor apheresis platelets are much less likely to result in transfusion associated bacterial infection since the likelihood of contamination is reduced, as there is less chance of failure of disinfection during collection [12]. According to the 5.1.5.1 standard set by the American Association of Blood Banks (AABB) in 2004, all accredited blood banks must implement some form of bacterial testing for platelet units. This measure was formulated in response to adverse reactions being observed caused by the transfusion of contaminated blood products [13]. As a result, this standard lead to the widespread adoption of automated blood culture systems for maintaining the sterility of PCs [14]. In the EU, blood establishments are required to have in place a system that limits bacterial contamination through collection and

Citation: Debrincat A, Gialanze JP, Spiteri N and Zammit V. Bacterial Screening of Blood Components: Past, Present and Possibly Future Methodologies for Improving Transfusion Safety. Austin Hematol. 2021; 6(1): 1033.

processing of blood components [15]. Various different approaches have been implemented since there is no universal standard or law that dictates the requirements for bacterial screening within the EU [16].

Blood Culture Systems

The use of automated blood culture systems has been credited in the reduction of 69.7% in septic platelet transfusion reactions [17]. Automated blood culture systems allow for the release of platelet concentrates on the basis of a negative-to-date result. Although this helps to maintain PCs stockpiles, it does not give a 100% guarantee of sterility since negative results may be caused by under-inoculation or the presence of organisms with a prolonged lag phase and slow growth [11]. The volume of PCs used for testing must also be considered with regards to the PC volume available upon transfusion. When sampling 15ml of PC for inoculation of both aerobic and anaerobic blood culture bottles, Macauley et al., [18], noted a reduction of 6% to the final platelet count within the concentrate. To reduce this, they suggested to utilize only aerobic cultures since the majority isolates identified were aerobes. Anaerobic culture bottles have also been shown to produce a higher rate of false positive results when cultured with PCs [19]. Yet it was proven that even for PCs the use of both bottles resulted in increased detection of positive cultures, simply due to the fact that one is screening a larger inoculum volume, increasing the likelihood of detection as a result [17]. Anaerobic blood culture bottles will also detect the presence of any facultative anaerobes such as *Propionibacterium acnes*, which would not be possible within aerobic bottles [17,20]. Brecher and Hay [21] concluded that the time of detection for certain organisms was noted to be equivalent or faster when using anaerobic bottles, justifying the need to use both for a platelet sample. In the case of delayed sampling for bacterial screening, extending the platelet shelf-life should be taken in consideration since a longer waiting time is required [21].

Many different automated systems are currently in use for facilitating the use of blood cultures for diagnostic purposes. In the early nineties investigations were carried out to determine the validity of using such systems within the context of ensuring transfusion safety [22]. Different countries in the EU which perform blood cultures on PCs have different sampling timing as required by their set guidelines [16]. This lack of consensus surrounding sampling timing eventually results in varying sensitivities. Fuller et al., [14], have suggested culturing PCs after 24 hours which allows the proliferation of contaminating bacteria, thus increasing the overall concentration within the initial inoculum, subsequently reducing the instances of false negative results [23]. Slow growing bacteria may require further incubation of up to 48 hours to achieve detection. For certain, slow growing organisms such as *Propionibacterium acnes* detection prior to release of PCs may not be possible since the time-to-detection by the system can take longer than the actual shelf life of the platelets themselves [24].

The Different Blood Culture Systems in Use for Detection of Bacterial Contamination

BacT/ALERT

One of the most prominent systems in use is the BacT/ALERT Microbial Detection System (BioMérieux SA, France). This system was granted clearance by the US Food and Drug Administration (FDA) for the monitoring of bacterial contamination within PCs [10]. The system utilizes a colorimetric sensor embedded at the bottom of proprietary blood culture bottles, which is read every ten minutes within the system by a light emitting diode. When carbon dioxide is produced by bacteria as a by-product of metabolism of the culture medium, the sensor changes colour. This increases the amount of red light reflected by the sensor, which alters the voltage registered by a photodiode. This is then interpreted by the system's on-board computer as an indication of positive microbial growth [25,26]. In a study conducted by Lui et al., [27], the efficacy of BacT/ALERT blood cultures as a prospective screening tool was determined by a spiking experiment using PCs spiked with bacteria commonly responsible for contamination of blood products. Positive results were obtained within an incubation time of 28 hours post inoculation. This established that short-term routine blood cultures using this system is suitable for detection of bacterial contamination of blood products. The newer BacT/ALERT Virtuoso system (BioMerieux SA, France) was found to have reduced further the time to detection of bacteria in sepsis patients [28]. This system also has the advantage of automated loading and unloading of culture bottles, further increasing turnaround time [29].

BacTec

The BACTEC line of systems developed by BD provides similar results in terms of detection time within platelet concentrates when compared to the BacT/ALERT systems [30]. This system works utilizing a sensor bound to the blood culture bottles that emits fluorescence upon production of CO_2 and consumption of O_2 by a cultured organism. This is monitored every 10 minutes within the system for detection [31]. This is an evolution of the previously used radiolabeled carbon and infrared detection methods [32]. The blood culture bottles contain the proprietary sensor and can hold a maximum of ten milliliters of sample. The BACTEC 9240 system was shown to be capable of detecting bacterial contamination within PCs containing a concentration 10CFU/mL, with a time-to-detection of between 6.5 to 17.6 hours when tested using aerobic blood culture bottles [33].

VersaTREK

The VersaTREK manometric blood culture systems tracks either the production or consumption of gases by analyzing the pressure within the inoculated blood culture bottles [34]. The blood culture bottles can be inoculated with a volume of up to 10ml of sample and contain stir rods that homogenise the sample. Unlike the previous systems mentioned which rely on the production of CO_2 by bacteria for detection, VersaTREK system measures changes in the overall pressure with the culture bottles. This increases sensitivity for bacteria that produce little CO_2 or due to the sample having a high white blood cell count [35] which could result in a linear increase of the gas during incubation, resulting in false positives [36]. This system was compared against BACTEC FX by Chetouane et al. [37] using spiked PCs. The results from their study indicated that the VersaTREK system was capable of detecting bacteria under aerobic conditions, with results comparable to BACTEC FX system, utilizing small 5ml PC samples [37].

Pall Enhanced Bacterial Detection System

The Pall enhanced Bacterial Detection System (eBDS) oxygen analyzer (Pall corporation, USA) utilizes a pouch that houses 3ml of platelet sample. This incubated at a temperature of 35°C for 24 or 30 hours under constant agitation, which further enhances the proliferation of contaminants. A filter attached to the sample pouch removes the majority of the platelets present [10]. A specifically formulated nutrient tablet is present within the pouch containing sodium polyanethol sulfonate which prevents the aggregation of platelets and, trypticase soy broth, which allows for the proliferation of contaminants [38]. An oxygen sensor measures the changes in the electrical potential caused by the consumption of oxygen within the solution during the incubation period. A positive result for bacterial contamination is obtained when the percentage of oxygen falls below 12.5% resulting in the time-to-detection reading [10,38]. McDonald et al., [39] suggested lowering the cut-off for detection down to 9.4%, to reduce the rate of false positives. They also noted that in tests performed with platelets spiked with *Pseudomonas aeruginosa*, false negative results were obtained due to the oxygen concentration being very close to the reduced cut-off level. In two separate runs, they also noted that this particular bacterium did not proliferate during the incubation period and did not result in a detectable oxygen decrease. This was possibly due to bacteriocidic or antibacterial properties of proteins present within the PCs. A big shortcoming of this system is that it cannot perform anaerobic cultures since it relies on oxygen measurement. This can be an issue since anaerobic organisms such as *Propionibacterium acnes* may not proliferate and its detection is therefore hindered [9].

Shortcomings of Blood Culture Systems

False negative blood cultures are a worrying consequence of the limitations of this technique. Such results may be caused when the inoculum cultured within an automated system has a very low initial bacterial count, resulting in failure for detection during the apparatus's pre-determined incubation period. To prevent this, delayed sampling after collection and separation of PCs can ensure a larger initial bacterial count upon inoculation [9,40]. With regards to screening of PCs, biofilm forming bacteria such as *Staphylococcus epidermidis*, can evade detection by assimilating cells spherically within PCs, reducing the number of free bacterial cells that can be cultured after sampling. This issue is compounded by the bacteria's slow growth rate [41]. Biofilm producing bacteria can infect patients *via* intravenous infusion of a PCs concentrate, after being dislodged from the bag due to agitation after evading detection [42]. Other slow growing organisms such as *Propionibacterium acnes* [23,42] have also been repeatedly been shown to produce false negative results. On the other hand, false positives may also occur when using culture-based systems. These are usually the result of contamination from a source other than from donation. In a study carried out by Macauley et al. [18], 0.31% of the total positive platelet cultures produced a negative result upon re-culturing, leading to the conclusion that contamination could have occurred during inoculation.

Non-Culture-Based Methods for Detection

Blood culture methods remain the most popular solution for detecting bacterial contamination due to their reliability and widespread use within the transfusion setting. Nonetheless, non-culture-based techniques have been devised to achieve rapid detection for use as pre-release point-of-care testing solutions [43]. This allows for a secondary line of testing to detect the presence of bacteria after failure to do so by blood cultures and also for allowing transfusion services to prolong the shelf-life of platelets beyond five days of storage [44].

Scansystem

The Scansystem (Hemosystem, France) was a bacterial detection method which utilises a solid-phase scanning cytometry technique which was discontinued in 2011 [45] In this method, 3 different 3ml PCs samples are pooled together and 3ml of the resultant mixture is then stained using a mixture containing picogreen, a DNA binding dye. The resulting mixture is agitated for 40 minutes to allow for the aggregation of platelets and then filtered and stained. The solution is then analyzed using an epifluorescence microscope and 50 random fields are analyzed for fluorescent fragments. This technique allows for rapid detection of bacteria but has a sensitivity of around 100CFU/ml and like all microscopy-based techniques it success depends heavily on the initial concentration of the inoculum used [46,47].

Staining Methods for Microscopy

Gram stain and acridine orange stains are two stains that can be used for the microscopic identification of bacteria in smears derived from blood components. Microscopic procedures utilizing both stains have been declared a suitable method for the detection of contamination within PCs by the US FDA and subsequently recommended by the AABB [11]. Staining methods are cheap and easy to perform, but such methodologies are of limited use within the modern transfusion practice due to them being laborious, making them incompatible for high workload scenarios [9]. Sensitivity of staining procedures relies heavily on the bacterial count within the PCs being investigated as well as the skill level of the observer performing counts. The sensitivity of the Gram stain method can be increased when a platelet sample is centrifuged prior to staining. This was demonstrated by Steen et al., [48] were they compared centrifuged versus non centrifuged aliquots obtained from previously inoculated platelet concentrates. All of the spun samples were positive for the presence of bacteria, whilst a few of the unspun samples produced false negative results [48]. Steen et al. also recommended the use of the Gram stains for detecting contamination at the end of the PCs shelf life. Stains are now mainly used as part of the identification and confirmation procedures for the presence of bacteria within positive blood cultures [49].

pH Testing

An easy way of determining the presence of bacterial contaminants is by taking a pH reading from PCs. This form of rapid point of care test relies on a pH drop within PCs as a result of acid producing bacteria. In the past, such readings were achieved using blood gas analyzers designed for use of whole blood specimens tested at temperatures of 37°C. This produced erroneous readings since platelets are present in a totally different fluid matrix than whole blood and are stored at a temperature of 22°C [50]. Therefore, pH readers need to be specifically intended for this use. A pH of 7.0 was determined as suitable cut-off reading by performing studies

on spiked PCs [51]. When the pH fell below this value, the PCS unit was said to be most likely contaminated and removed from storage. Unfortunately this technique does not take into account the possibility of a pH drop resulting from the platelets and white blood cells within the concentrate as a result of their metabolic processes, leading to a potential for high rates of false positives [51].

Flow Cytometry

Flow cytometry has been postulated as a rapid, point-of-issue test for identifying contamination within blood products. The technique is based on the principle of passing individual cells from a sample trough an interrogation point *via* laminar flow using sheath fluid. The individual cells are hit by light emitted from a laser diode, which can then be absorbed, scattered or emitted as fluorescence. This light is directed towards a photomultiplier tube that generates a voltage. This is then converted to an analogue signal and interpreted digitally [52]. A fluorescent dye such as thiazole orange is used, which binds to the nucleic acid of bacterial cells. The characteristic side scatter and fluorescence emitted by bacteria differs from that of platelets and allows for their quantification within a sample [38,53]. In the case of said concentrate, the sample must be purified by first removing platelets by means of enzymatic digestion. The flow cytometer provides counts for the bacterial cells and the sample is determined to be contaminated using a pre-determined cut-off value [54]. The rapid turn-around time of around one minute per sample after processing, allows for supplementary testing for the extension of PCs shelf life [54]. Another benefit is that a small sample volume is needed for detection [38,53]. To reduce the possibility of background fluorescence from undigested platelets, a non-fluorescent fluorochrome such as in the case of the BactiFlow (BioMerieux, France) assay can be used. Fluorescence is only achieved after the fluorochrome has been cleaved by esterase enzymes found within bacterial cells [55]. A downside for this type of testing is that detection is dependent on the growth kinetics of bacteria during platelet storage. Therefore some slow growing bacteria may only be detected after a late period of storage, reducing its effectiveness when testing newer PCs [53].

Verax Platelet Pan Genera Detection

The Verax Platelet Pan Genera Detection test (Verax Biomedical, USA) is an immunoassay-based technique that allows for rapid checking of contamination within PCs prior to transfusion. The test targets lipopolysaccharide and lipoteichoic acid antigens for Gram-negative and Gram-positive organisms respectively, using antibodies embedded on a test strip. The test utilizes a few drops from a PCs sample and only requires a few minutes for a positive result to appear as bands on the strip, making it easy to perform and to interpret the result visually. Interpretation may be hindered when very faint bands appear, producing an ambiguous result. Sensitivity varies from 103 to 105 CFU/ml depending on the bacteria present [56]. This method is much less sensitive than the other tests discussed so far, making it suitable only for testing PCs units prior to transfusion. When compared to a pH based detection method, this technique has been shown to yield a higher rate of false positive results [57].

Realtime Polymerase Chain Reaction/ Nucleic Acid Testing

Nucleic Acid Testing (NAT) using the Real Time Polymerase Chain Reaction (PCR) technique allows for the replication and quantification of bacterial genetic material in real time for the purpose of diagnosing the presence of a potential pathogen. Certain steps of the technique such as extraction can be automated for use in sterility testing of blood components [58]. Real time PCR involves the use of fluorescent probes, which are attached to targeted bacterial DNA sequences to allow for their quantification whilst amplification is taking place. For this reason, probes have to be specifically engineered to bind target sequences of specific bacterial species, allowing for the identification of a contaminating bacterium [59]. Another approach to this technique is the targeting of specific genes and 16S ribosomal RNA, which are commonly expressed by bacteria, allowing for more broad detection [60]. One of the issues with using NAT testing for bacterial detection is that the polymerase enzymes used in the PCR process originate from bacteria themselves and thus may contain contaminating bacterial DNA. This process requires specialized equipment as well as trained personnel, limiting its availability due to its high initial cost requirement for procurement and training [38]. Future implementations of NAT include being used as bedside test prior to the transfusion of a PCs or to check for sterility after pathogen reduction [61].

Conclusion

Culture based bacterial detection systems remain the gold standard for the prevention of TTBIs during the production process of blood products due to their proven track record in many different transfusion services worldwide. As it currently stands, non-culture-based methods are mainly indicated for use as point-of-care and pre-transfusion tests to be used in conjunction with blood cultures, either to enhance detection or to prolong PCs shelf life. Due to the possibility of failure when using current bacterial detection technology, pathogen reduction is an attractive solution to mitigate this problem. Pathogen reduction eliminates pathogens with minimal effect on the blood product or added risk to the recipient [62]. Different methodologies utilize chemical compounds, some requiring a photosensitisation step with ultraviolet light such as with the use of amotosalen or riboflavin. Other methodologies such as with the use of amustaline do not require photosensitisation [62,63]. Although this can pave way for a contaminant free blood supply, the high cost of pathogen reduction remains a major barrier for wider adoption by transfusion services [64].

References

1. Hong H, Xiao W, Lazarus HM, Good CE, Maitta RW, Jacobs MR. Detection of septic transfusion reactions to platelet transfusions by active and passive surveillance. Blood. 2016; 127: 496-502.

2. Wagner SJ. Transfusion-transmitted bacterial infection: risks, sources and interventions. Vox Sang. 2004; 86: 157-163.

3. European Commission directorate General for Health and Food Safety. Summary of the 2018 annual Reporting of Serious Adverse Reactions and events for Blood and Blood Components. 2018.

4. Hillyer CD, Josephson CD, Blajchman MA, Vostal JG, Epstein JS, Goodman JL. Bacterial contamination of blood components: risks, strategies, and regulation: joint ASH and AABB educational session in transfusion medicine. Hematology Am Soc Hematol Educ Program. 2003; 575-589.

5. Debrincat A, Gialanze J, Spiteri N, Zammit V. Blood Donor Arm Disinfection-Preventing the Contamination of Blood Components. Emed Res. 2020.

6. Buchta C, Nedorost N, Regele H, Egerbacher M, Kormoczi G, Hocker P, et al.

Skin plugs in phlebotomy puncture for blood donation. Wien Klin Wochenschr. 2005; 117: 141-144.

7. Heltberg O, Skov F, Gerner-Smidt P, Kolmos HJ, Dybkjaer E, Gutschik E, et al. Nosocomial epidemic of Serratia marcescens septicemia ascribed to contaminated blood transfusion bags. Transfusion. 1993; 33: 221-227.

8. Tipple MA, Bland LA, Murphy JJ, Arduino MJ, Panlilio AL, Farmer JJ, et al. Sepsis associated with transfusion of red cells contaminated with Yersinia enterocolitica. Transfusion. 1990; 30: 207-213.

9. Montag T. Strategies of bacteria screening in cellular blood components. Clin Chem Lab Med. 2008; 46: 926-932.

10. Savini V, Balbinot A, Giancola R, Quaglietta A, Accorsi P, D'Antonio D, et al. Comparison between the BACTEC 9240 and the Pall eBDS system for detection of bacterial platelet concentrate contamination. Transfusion. 2009; 49: 1217-1223.

11. Palavecino EL, Yomtovian RA, Jacobs MR. Detecting bacterial contamination in platelet products. Clin Lab. 2006; 52: 443-456.

12. Illert WE, Sanger W, Weise W. Bacterial contamination of single-donor blood components. Transfus Med. 1995; 5: 57-61.

13. Badlou B, Farhad R, Dabirmoghadam A. Measurement of BacT/ALERT sensitivity after inoculation certain amount of *E.coli* and *S.epidermis*. Journal of Blood Disorders and Transfusion. 2015.

14. Fuller AK, Uglik KM, Savage WJ, Ness PM, King KE. Bacterial culture reduces but does not eliminate the risk of septic transfusion reactions to single-donor platelets. Transfusion. 2009; 49: 2588-2593.

15. European Directorate for the Quality of Medicines & Healthcare. EDQM-European Directorate for the Quality of Medicines. 2021.

16. Prax M, Bekeredjian-Ding I, Krut O. Microbiological Screening of Platelet Concentrates in Europe. Transfus Med Hemother. 2019; 46: 76-86.

17. Benjamin R, McDonald C. The international experience of bacterial screen testing of platelet components with an automated microbial detection system: a need for consensus testing and reporting guidelines. Transfusion medicine reviews. 2014; 28: 61-71.

18. Macauley A, Chandrasekar A, Geddis G, Morris KG, McClelland WM. Operational feasibility of routine bacterial monitoring of platelets. Transfus Med. 2003; 13: 189-195.

19. de Korte D. 10 Year's Experience with Bacterial Screening of Platelet Concentrates in the Netherlands. Transfus Med Hemother. 2011; 38: 251-254.

20. Lee CK, Ho PL, Lee KY, Tsui GTF, Chua E, Tsoi WC, et al. Value of anaerobic culture in bacterial surveillance program for platelet concentrates. Transfusion. 2008; 48: 2606-2611.

21. Brecher ME, Hay SN. Bacterial contamination of blood components. Clin Microbiol Rev. 2005; 18: 195-204.

22. Gong J, Hogman CF, Lundholm M, Gustafsson I. Novel automated microbial screening of platelet concentrates. APMIS. 1994; 102: 72-78.

23. Abela MA, Fenning S, Maguire KA, Morris KG. Bacterial contamination of platelet components not detected by BacT/ALERT®. Transfus Med. 2018; 28: 65-70.

24. McDonald CP, Rogers A, Cox M, Smith R, Roy A, Robbins S, et al. Evaluation of the 3D BacT/ALERT automated culture system for the detection of microbial contamination of platelet concentrates. Transfus Med. 2002; 12: 303-309.

25. Thorpe TC, Wilson ML, Turner JE, DiGuiseppi JL, Willert M, Mirrett S, et al. BacT/Alert: an automated colorimetric microbial detection system. J Clin Microbiol. 1990; 28: 1608-1612.

26. Hall RA, De Sordi L, Maccallum DM, Topal H, Eaton R, Bloor JW, et al. CO_2 acts as a signalling molecule in populations of the fungal pathogen Candida albicans. PLoS Pathog. 2010; 6: e1001193.

27. Liu HW, Yuen KY, Cheng TS, Lee KB, Chua EK, Ho PL, et al. Reduction of platelet transfusion- associated sepsis by short-term bacterial culture. Vox Sang. 1999; 77: 1-5.

28. Congestrì F, Pedna MF, Fantini M, Samuelli M, Schiavone P, Torri A, et al. Comparison of 'time to detection' values between BacT/ALERT VIRTUO and BacT/ALERT 3D instruments for clinical blood culture samples. Int J Infect Dis. 2017; 62: 1-5.

29. Jacobs MR, Mazzulli T, Hazen KC, Good CE, Abdelhamed AM, Lo P, et al. Multicenter Clinical Evaluation of BacT/Alert Virtuo Blood Culture System. J Clin Microbiol. 2017; 55: 2413-2421.

30. Riedel S, Siwek G, Beekmann SE, Richter SS, Raife T, Doern GV. Comparison of the BACTEC 9240 and BacT/Alert blood culture systems for detection of bacterial contamination in platelet concentrates. J Clin Microbiol. 2006; 44: 2262-2274.

31. BMS Diagnostics. Fluorescent Technology in the BD BACTECTM Automated Blood Culture System. 2014.

32. Dunne M, Burnham C-AD. The Dark Art of Blood Cultures. The Dark Art of Blood Cultures. American Society of Microbiology. 2017.

33. Dunne WM, Case LK, Isgriggs L, Lublin DM. In-house validation of the BACTEC 9240 blood culture system for detection of bacterial contamination in platelet concentrates. Transfusion. 2005; 45: 1138–1142.

34. Yue P, Zhou M, Kudinha T, Xie X, Du J, Song H, et al. Clinical Performance Evaluation of VersaTrek 528 Blood Culture System in a Chinese Tertiary Hospital. Front Microbiol. 2018; 9: 2027.

35. Dreyer A, Ismail N, Nkosi D, Lindeque K, Matthews M, Van Zyl D, et al. Comparison of the VersaTREK blood culture system against the Bactec9240 system in patients with suspected bloodstream infections. Annals of clinical microbiology and antimicrobials. 2011; 10: 4.

36. Karakonstantis S, Manika I, Vakonaki M, Boula A. False-Positive Blood Cultures in Acute Leukemia: An Underrecognized Finding. Case Reports in Medicine. Hindawi. 2018: 2018; e7090931.

37. Chetouane Y, Gallian P, Chetouane K, Dubourg G, Chiaroni J, Raoult D, et al. Comparing two blood culture systems for the detection of bacterial contamination in platelet concentrates. Transfusion. 2018; 58: 2604-2610.

38. Störmer M, Vollmer T. Diagnostic methods for platelet bacteria screening: current status and developments. Transfus Med Hemother. 2014; 41: 19-27.

39. McDonald CP, Pearce S, Wilkins K, Colvin J, Robbins S, Colley L, et al. Pall eBDS: an enhanced bacterial detection system for screening platelet concentrates. Transfus Med. 2005; 15: 259-268.

40. Wagner SJ, Eder AF. A model to predict the improvement of automated blood culture bacterial detection by doubling platelet sample volume. Transfusion. 2007; 47: 430-433.

41. Ali H, Greco-Stewart VS, Jacobs MR, Yomtovian RA, Rood IGH, de Korte D, et al. Characterization of the growth dynamics and biofilm formation of Staphylococcus epidermidis strains isolated from contaminated platelet units. Journal of Medical Microbiology. 2014; 63: 884-891.

42. Greco C, Martincic I, Gusinjac A, Kalab M, Yang A-F, Ramírez-Arcos S. Staphylococcus epidermidis forms biofilms under simulated platelet storage conditions. Transfusion. 2007; 47: 1143-1153.

43. Schmidt M. Comparison of different methods of bacterial detection in blood components. ISBT Science Series. 2009; 4: 80-86.

44. US Food and Drug Administration. Bacterial Risk Control Strategies for Blood Collection Establishments and Transfusion Services to Enhance the Safety and Availability of Platelets for Transfusion [Internet]. U.S. Food and Drug Administration. FDA; 2020.

45. Schmidt M, Sireis W, Seifried E. Implementation of Bacterial Detection Methods into Blood Donor Screening - Overview of Different Technologies. Transfus Med Hemother. 2011; 38: 259-265.

46. Jacobs MR, Bajaksouzian S, Windau A, Palavecino EL, Yomtovian R. Evaluation of the Scansystem method for detection of bacterially contaminated platelets. Transfusion. 2005; 45: 265-269.

47. Schmidt M, Hourfar MK, Heck J, Weis C, Montag T, Nicol S-B, et al. Scansystem™ Enables Rapid and Sensitive Bacterial Detection in Platelets Stored in Additive Solution with Implementation of Standard Positive Control

Capsules'. TMH. 2006; 33: 274-278.

48. Steen RM, Hanson-Steen AS, John CSt, Indrikovs AJ. The Use of the Gram Stain to Screen Platelet Concentrates for Bacterial Contamination. Laboratory Medicine. 2003; 34: 609-612.

49. Neeraja M, Lakshmi V, Padmasri C, Padmaja K. Utility of Acridine Orange staining for detection of bacteria from positive blood cultures. J Microbiol Methods. 2017; 139: 215-217.

50. Ringwald J, Luther R, Zimmermann R, Strobel J, Weiss D, Eckstein R. Precise pH measuring of platelet concentrates containing additive solution--the impact of the temperature. Vox Sang. 2012; 103: 49-54.

51. Yazer MH, Triulzi DJ. Use of a pH meter for bacterial screening of whole blood platelets. Transfusion. 2005; 45: 1133-1137.

52. Ambriz-Avina V, Contreras-Garduno JA, Pedraza-Reyes M. Applications of flow cytometry to characterize bacterial physiological responses. Biomed Res Int. 2014; 2014: 461941.

53. Mohr H, Lambrecht B, Bayer A, Spengler H-P, Nicol S-B, Montag T, et al. Basics of flow cytometry–based sterility testing of platelet concentrates. Transfusion. 2006; 46: 41-49.

54. Vollmer T, Hinse D, Schottstedt V, Bux J, Tapernon K, Sibrowski W, et al. Inter-laboratory comparison of different rapid methods for the detection of bacterial contamination in platelet concentrates. Vox sanguinis. 2011; 103: 1-9.

55. Vollmer T, Kleesiek K, Dreier J. Detection of bacterial contamination in platelet concentrates using flow cytometry and real-time PCR methods. Methods Mol Biol. 2013; 943: 91-103.

56. Vollmer T, Hinse D, Kleesiek K, Dreier J. The Pan Genera Detection immunoassay: a novel point-of-issue method for detection of bacterial contamination in platelet concentrates. J Clin Microbiol. 2010; 48: 3475-3481.

57. Harm SK, Delaney M, Charapata M, Aubuchon JP, Triulzi DJ, Yazer MH. Routine use of a rapid test to detect bacteria at the time of issue for nonleukoreduced, whole blood-derived platelets. Transfusion. 2013; 53: 843-850.

58. Schmidt M, Seifried E. Improving blood donor screening by nucleic acid technology (NAT). ISBT Science Series. 2010; 5: 219-229.

59. Viana JD, Ferreira SC, Matana SR, Rossi F, Patel P, Garson JA, et al. Detection of bacterial contamination in platelet concentrates from Brazilian donors by molecular amplification of the ribosomal 16S gene. Transfusion Medicine. 2018; 28: 420-426.

60. Dreier J, Stormer M, Kleesiek K. Real-time polymerase chain reaction in transfusion medicine: applications for detection of bacterial contamination in blood products. Transfus Med Rev. 2007; 21: 237-254.

61. Roth WK. History and Future of Nucleic Acid Amplification Technology Blood Donor Testing. TMH. 2019; 46: 67-75.

62. Solheim BG. Pathogen reduction of blood components. Transfus Apher Sci. 2008; 39: 75-82.

63. Mufti NA, Erickson AC, North AK, Hanson D, Sawyer L, Corash LM, et al. Treatment of Whole Blood (WB) and red Blood Cells (RBC) with S-303 inactivates pathogens and retains *in vitro* quality of stored RBC. Biologicals. 2010; 38: 14-19.

64. Kacker S, Bloch EM, Ness PM, Gehrie EA, Marshall CE, Lokhandwala PM, et al. Financial impact of alternative approaches to reduce bacterial contamination of platelet transfusions. Transfusion. 2019; 59: 1291-1299.