

Review Article

Evaluations of Detection Methods of Bacterial Contamination in Platelet Components

Mohammad Ali Esmaili¹Ph.D., Farhad Razjou²M.D., Behzad Nazel Khosroshahi²Ph.D., Ali Amini¹Ph.D., Soodeh Namjoo¹Ph.D.
Peyman Beigi³B.Sc., Mohammad Reza Rezvani^{4*}Ph.D.

¹Department of Hematology, School of Allied Medical Sciences, Iran University of Medical Sciences, Tehran, Iran.

²Blood Transfusion Research Center, High Institute for Research and Education in Transfusion Medicine, Tehran, Iran.

³Faculty of Allied Medicine, Alborz University of Medical Sciences, Alborz, Iran.

⁴Department of Hematology, Faculty of Allied Medicine, Iran University of Medical Science, Tehran, Iran.

ABSTRACT

Article history

Received 5 Sep 2017

Accepted 13 Nov 2017

Available online 31 Dec 2017

Key words

Bacterial contamination

BacT/ALERT

Culture based methods

Platelet components

Platelet components (PCs) have widespread applications in clinical cases. Since PCs store in room temperature (between 20-24°C), they are susceptible to bacterial contamination. There are varied approaches for identifying bacterial contamination in PCs. These methods categorized into two groups: Firstly, culture based methods and secondly, non-culture based methods. Both of them have a couple of merits and demerits. BacT/ALERT is a culture-based technique, which has been approved by the food and drug administration. Although sensitivity and specificity of this method could be debatable and is not universal. This method is considered as gold standard contemporary method and it is far more dependable and superb in comparison with the contamination detection methods. It is assumed that, application of rapid methods play an important role in detection of bacterial contamination in the future. Accordingly, this study aimed to represent a summary of each method, which was used for bacterial contamination detection in PCs with detailed assessment of culture-based methods, specifically BacT/ALERT.

Introduction

Platelet is enucleated cellular fragments produced by megakaryocytes. They play an important role in hemostasis as well as thrombosis and bleeding [1, 2]. Hence, platelet components (PCs) transfusion is a vital procedure in a variety of patients especially those who have hematological and oncological diseases [3]. PCs have vast clinical utilization. Even though researchers have done diverse endeavors to eliminate bacterial contamination of PCs, but it still remains a health concern throughout the world. PCs storage in room temperature (between 20-24°C) is one of important reasons, which makes these blood products vulnerable to bacterial contamination. Consequently, blood centers have to carry out quality control on PCs before use so that they make sure these products do not have any bacterial contamination. [4, 5]. There are a wide range of methods for detecting bacterial contaminations like measuring pH, glucose concentration as well as culture base methodology, molecular evaluation and flow cytometry [6-8]. Contaminating of PCs with bacteria occur far higher (100-1000 times) in comparison with viral contamination. In America after transfusion mistakes, bacterial contamination is the second most prevalent cause of death related to blood transfusion. In general, it is asserted that, approximately one out of every 2000–3000 PCs units is probably have some kinds of bacterial contamination (Table 1) owing to the donor's skin or bloodstream. [9-12]. Therefore, with regard to

high prevalence of bacterial contamination in PCs compare to other infectious agents, this study aimed to evaluate different methods for detecting bacterial contamination in PCs. Moreover, in this investigation the advantages and disadvantages of each assessment method have been studied with an emphasis on culture-based methods, specifically BacT/ALERT.

Platelet Components

Platelets components are produced by two main procedures: single donor apheresis platelets (SDP) method and whole blood derived platelets (WBP). One SDP unit from a single donor can yield one to three adult therapeutic doses of platelets whereas pooling of 4 to 6 WBP units produce one therapeutic dose. In the United States, the WBP is manufactured using the platelet rich plasma (PRP) technique while in the majority of European societies it is produced by the buffy coat (BC) technique. The BC technique has developed in the 1970s by European scientists to reduce residual donor leucocytes.

Producing the PCs by PRP technique commence by doing a light spin centrifugation in 2000 g followed by a heavy spin centrifugation in 5000 g. BC approaches begin with 'high speed' centrifugation (2800 g), which separate the platelet-poor plasma and the packed red blood cells. The residual BC is re-suspended and pooled with three to five other donor BCs and after that the BC pool is centrifuged at 'low speed' (700 g) (Fig.1).

Table 1. Comparison of the bacterial contamination prevalence in PCs in Iran, America and French

Country name	bacterial contamination prevalence in PCs
United States	one out of every 3000–4000 PCs units
French	one out of every 2500 PCs units
Iran	four out of every 2000– PCs units

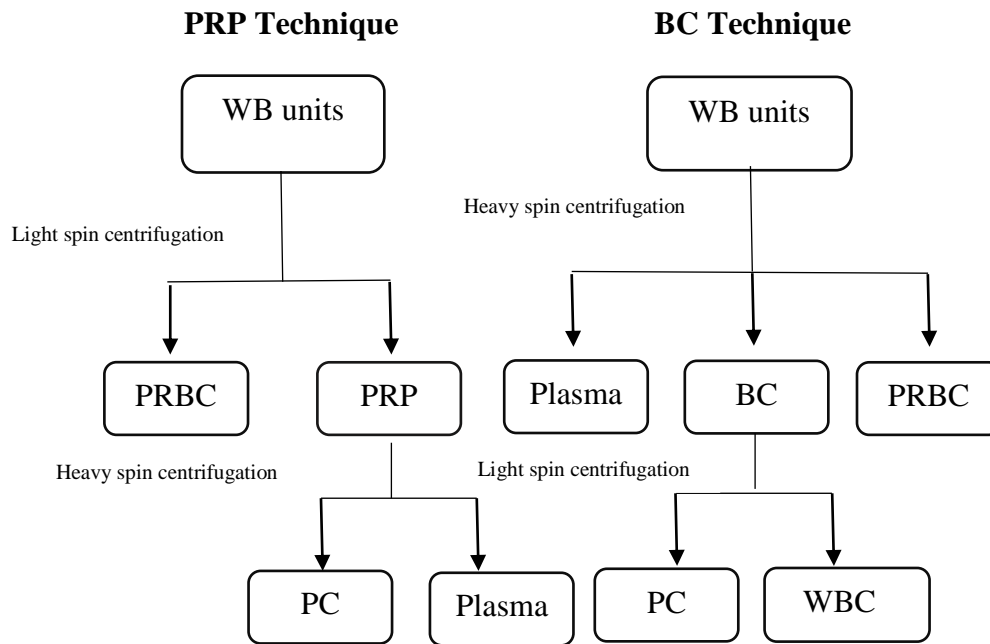


Fig. 1. Methods of PCs preparation from anticoagulated whole blood. Whole blood (WB), packed red blood cells (PRBC), platelet rich plasma (PRP), buffy coat (BC), white blood cells (WBC) and platelet concentrate (PC).

To sum up, BC approaches is fabulous in comparison with PRP method because it provides much higher yield even though PRP method is simple and inexpensive [13-18].

Bacterial contamination in PCs

Bacterial contamination of cellular blood components is defined as contaminating these blood products by bacteria, which is create transfusion reaction or any adverse event following either whole blood or its other derived product transfusion [19]. PCs storage in room temperature (between 20-24°C) makes these blood products susceptible to the

bacterial contamination [20, 21]. Storage in this temperature is necessary because it boosts post-transfusion platelet function and prevents platelet aggregation [18]. Gram-positive cocci are the most prevalent contaminants of platelet, consist of *staphylococci* and *streptococci* [22]. By way of illustration, *Staphylococcus epidermidis* is most common type of bacteria, which isolated in contaminated PCs [4, 23]. These bacteria encompass part of skin normal flora [24]. Statistics demonstrate that the WBP products create more transfusions-associated septic reactions in patients compare with the SDP

products due to higher amount different donor for collecting WBP than SDP [25-27]. Most cases of PCs bacterial contamination occur during venipuncture. Moreover, less frequently, it occurs owing to asymptomatic donor bacteremia or during preparation of PCs [28]. Another point of significance is bacterial contamination of the blood products that is not unique to PCs and may occur in red blood cells products similar to PCs. Indeed, major difference attributed to storage condition [29]. Plasma products such as fresh frozen plasma and cryoprecipitate are frozen, that is why they are rarely associated with bacterial contaminations [4]. Platelet concentrates have a 5-day shelf life in the room temperature. Storage length was reduced from 7 to 5 days by food and drug administration (FDA) ought to bacterial contamination in PCs. In a couple of cases, their useful shelf life is limited to 3 days. Recently, the key requirement for the changing of platelet shelf life from 5 days to 6 and 7 days is provided. These requirements and activities seems be effective and beneficial. However, it needs the registration and confirmation by FDA. It is asserted that, we should eradicate PCs bacterial contamination, it will feasible that we store them for a longer time. Bacterial contamination of PCs has severe and adverse consequences for patients and it is one of the crucial causes of fatality following transfusion [30-33]. Despite employing all of proposed solutions for eliminating bacterial contamination, we cannot eradicate it from PCs.

There are significant challenges in detection of the bacterial contamination in PCs following as:

1. Contaminant bacteria diversity (heterogeneity of the target organisms): For this reason, it is necessary to apply a method with high sensitivity and specificity so that be able to detect almost all types of contaminant bacteria.
2. Bacterial contamination identifications during sample collection. (Most cases of PCs bacterial contamination occur at the time of venipuncture due to skin normal flora mentioned).
3. Maintaining platelet products at room temperature (between 20-24°C).
4. Short shelf-life of PCs, therefore, our detection method should be rapid [34].
5. It is impossible to disinfect the depth of skin, which enhance the risk of PCs contamination detection [29].

A couple of strategies and precautions, which reduce bacterial contamination, listed below:

1. Skin where phlebotomy tended to perform should not be touched before puncture.
2. The skin of phlebotomy area should be ideally disinfected [35, 36].
3. Initial sample diversion (technique of diverting the first 30 ml of blood after venipuncture into a sample pouch) [37]. These first three approaches can reduce bacterial load in the initial blood collection [29] and prevent bacterial contamination resulting from skin commensal organisms [36, 38-40]. Previous investigations indicate that, diversion methodology can reduce 71% of bacterial contamination compared to the control group [40].
4. History to identify asymptomatic bacteremia in donors.

5. Consistent implementation of superb manufacturing goods with defined hygiene standards [37, 41].

6. Pathogen inactivation or pathogen reduction. (eradication or elimination process of pathogens, including viruses, bacteria, and fungi in blood components). Pathogen inactivation increases shelf life of platelet unit. Additionally, this technology has the capability to makes the blood product safer and it broadly eliminates infectious organisms without either need to screen for detecting specific pathogens or irradiate products. [42, 43]. Technologies, which are developed for pathogen inactivation of PCs, are including Amotosalen plus ultraviolet A light and riboflavin plus ultraviolet light. Both of them are based on damaging to pathogens nucleic acids. Amotosalen plus ultraviolet A light has approved by the US FDA in 2014 [44]. However, this technique has a couple of limitations, including possible toxicity, possible reduction in component quality, cost, incomplete inactivation, as well as inability to inactivate prions and new or unrecognized pathogens [45, 46]. All of the items listed above impact the rate of bacterial contamination and eventually occurrence rate of septic reactions in the patients who receive blood products. Hence, bacterial contamination detection in PCs is consequential, due to fierce consequences (even fatal), which these contaminated components may create in patients [47-49].

Bacterial contamination detection methods in PCs

There are wide ranges of methods to detect bacterial contamination in PCs, which some of

them have been approved and the others are under development. Each of these ways has relative sensitivity and specificity. Testing methods of bacterial contamination can be either active or inactive (passive). In active type, PCs are tested prior to administration, but in passive type, only clinically important transfusion reactions are tested retrospectively. However, it seems that passive method leads to underestimate the incidence of bacterial contamination [34, 50, 51]. In an investigation, reported data by national septic transfusion reactions duo to bacterial contaminated platelets based on the passive surveillance evaluated and they found the limitation of passive surveillance. [52]. Active type scheme for detecting bacterial contamination categorized into two groups: first, culture based methods and second, non-culture based methods [29] (Table 2). In the current investigation, both of them will be discussed in detail.

Culture based methods

Culture method is done either during the product shelf life (for instance, between 12 to 36 hours of holding time) or at or near the time of product transfusion. In the first type, since the bacterial load is too low, it is demanding to be detectable. Nonetheless, at the time of product transfusion bacterial contamination is more detectable owing to high bacterial load. However, the detection at this time is too late in order to prevent from transfusion. It is recommended that, it is better to perform culture on the third or fourth day of platelet shelf-time [7].

Table 2. Comparison of culture based methods and non-culture based methods

Methods	Specifications
Culture based (1-15 CFU/ml)	Advantages: They can be very sensitive, which this feature relies on the sample size and volume of the PCs. Disadvantages: They are prone to false positive and false negative. Usually, they are used in the start of the PCs shelf life.
Non-culture based	Advantages: To do in the shortest possible time (rapidity). Are used at or before of PCs transfusion Disadvantages: They are demanding and expensive. Their implementation is difficult.
Highly sensitive (10³- 10⁵ CFU/ml)	
Lowly sensitive (10³- 10⁶ CFU/ml)	Low sensitivity and low true positive rates

CFU= colony-forming unit; PCs= Platelet components

Culture based methods require that bacteria grow in the culture conditions of the test. These tests are capable to detect as few as one colony-forming unit (CFU) of bacteria in the inoculated sample, and can reliably detect 10-100 CFU in the sample. The sensitivity of them relies on the sample size and volume of the PCs. Indeed, bacterial contaminations in the samples with greater amount are more likely detected in this method [7, 53]. In a review study, it determined that 8 ml sample volume yields higher true-positive rates than 4 ml, which cause significant increase in the detection rate of the bacterial contamination in PCs [54]. To conclude, culture base systems are the most sensitive method for the detection of bacterial contamination of PCs, however, demanding large sample volume and pretty long incubation times (more than 24 hours) are disadvantage of its application [4, 55]. Procedures approved by the american association of blood banks (AABB) are included BacT/ALERT monitoring, CO₂

production as well as enhanced bacterial detection system (EBDS) monitoring oxygen consumption and pan genera detection (PGD), and a point-of-release bacterial detection assay [56-58]. PGD testing is not a culture-based method. Therefore, it is not discussed here and will be explained in non-culture based methods section.

BacT/ALERT

BacT/ALERT is a colorimetric culture system with an FDA-approved indication for testing platelet contamination. Samples should be taken at least 24 hours after collection process and using one aerobic blood product aerobic, and one anaerobic blood product anaerobic, bottles. Each bottle should be poured with 4 ml (5-10 ml) of platelet sample [39]. This procedure optimizes bacterial detection and allows bacterial growth in PCs (boost bacterial concentrations) the results in bacterial concentration will become more likely greater than detection range of culture-based system (1-10 CFU/ml) [59-61].

Moreover, platelets is inoculated with known transfusion relevant bacterial strains in order to carry out the quality control of so-called system and find out its ability to reliably detect a wide range of transfusion relevant bacterial strains at a sensitivity of 1 to 10 CFU/ml in a 7-days culture and 12-hour incubation period, respectively [47, 62-65].

In addition, increasing the sample volume has a noteworthy impression on culture sensitivity since it will boom probability of existence at least one viable organism in contaminated sample [37, 39, 66, 67]. In BacT/ALERT method, it should be considered that a minimum of 10 hours incubation (depending on the bacterial load) requires to obtain the result [68].

There are two techniques almost similar to BacT/ALERT: The Bactec system, which use a fluorometric system rather than colorimetric detection principle and the Versa TREK monitors bacterial growth by detecting pressure changes in the headspace of the blood culture bottle secondary to gas consumption/production [29, 69]. BacT/Alert system is most common type of system, which is applied and seen in reports in comparison with other systems of detection [29, 70, 71]. The BacT/ALERT system is a quick approaches in comparison with other culture systems (nearly 2 times faster than the manual culture method) with high accuracy in PCs bacterial contamination detection and it enhances platelet health [72]. However, culturing systems have a couple of disadvantages. By way of illustration, culturing is time consuming that is why PCs may have been

transfused before test (for example BacT/ALERT) become positive [37]. Moreover, BacT/ALERT system under different circumstances should be tested again. Indeed, despite the consequences on patient's safety, the BacT/ALERT displayed a dose and volume dependent results at its best condition. It is asserted that, based on these scores of the BacT/ALERT system, blood banks and transfusion centers should take high responsibilities for the patients' safety [73, 74].

Enhanced Bacterial Detection System (eBDS)

eBDS is an FDA-approved system, which works according to the measurement of reduction in oxygen concentration due to the bacterial growth at 37°C. This system consists of a disposable sample pouch, an incubator, and an oxygen analyzer. Platelet sample (2-3 ml) is transferred into a satellite bag and incubated for 18- 24 hours at 35°C with agitation.

A filter in the system allows bacteria to enter the sample while preventing platelets and other cellular components from contaminating the sample. Following incubation for 24 hours, the oxygen level is measured in the headspace. Bacterial contamination in PCs will consider should the O₂ amount founded to be less than 19%, which is the cut-off value between a positive and negative reading [20, 34]. In fact, this system uses the oxygen consumption by bacteria as a marker for detection. It has capability to detect aerobic and facultative anaerobic bacteria, which consider a risk for false-negative screening results [71]. *In vitro* sensitivity of this method is between 1-15

CFU/ml and sensitivity of the Pall eBDS and BacT/ALERT is similar [58, 75].

Non-culture based methods

Non-culture based methods are rapid and this attribute is major merits of this kind of methods. These methods allow us to detect toxic levels of bacteria at the time of testing or shortly before PCs components transfusion. These methods divided into two types: Highly sensitive and lowly sensitive.

Highly sensitive: Polymerase chain reaction (PCR) and flow cytometry

These methods are very sensitive, but utilization of samples with low volume is too insensitive for early testing. Compared to culture-based methods, there are combination of high sensitivity and specificity, low contamination risk, ease of performance, and speed [76-81].

PCR

Analytical sensitivity of nucleic acid screening based methods is 10-100 CFU (ml PC), depending on contaminant bacterial species as well as DNA extraction and amplification procedures. On the contrary, flow cytometry analysis, which is based on fluorescence staining of bacterial DNA, has a sensitivity of only 10^3 - 10^4 CFU (ml PC) [9, 76]. Conserved nucleic acid sequences in the two- target regions 16S rDNA and 23S rDNA have been used to develop real-time PCR assays. However, there is the possibility of DNA contamination of PCR reagents, which can produce false-positive results [71, 78]. Applying decontamination of PCR reagents by enzymatic digestion often reduced the assay

sensitivity and influencing assay efficiency [79]. Furthermore, primers and probes must be constantly verified [80]. Overall, PCR testing is demanding and expensive.

Flow cytometry

Application of fluorescence-activated cell sorting to screen PCs for bacterial contamination is a rapid and feasible approach. It requires only about 30 min to yield results. On the contrary, results of nucleic acid extension are obtained within approximately 4 h (Extraction 90 min. for 20 samples, amplification 90 min., analysis 5 min., sampling and pipetting 30 min.), which is a disadvantage of this method, especially for slow-growing species such as *Staphylococcus epidermidis* [68]. Flow cytometry assay is typically carried out either as a rapid test or in combination with a pre-incubation step aimed at increasing the analytical sensitivity [9]. However, without a pre-incubation step, the analytical sensitivity of fluorescence-activated cell sorting method is very low (10^3 to 10^5 CFU/ml) [9, 68, 81]. However, the sensitivity is high, but implementation is more difficult [20, 82].

Lowly sensitive: Such as gram stain, acridine orange, glucose consumption, PH measurement, pan genera detection and enzymatic method of bio responsive polymers.

Gram stain and acridine orange

It is clear that gram stain and acridine orange have similar sensitivity (10^5 - 10^6 CFU/ml) and low cost, particularly in older units, but they are very time consuming and requires specialized training. They can be done at the time of issue, providing a real time assay for

bacteria at concentrations most likely to cause serious adverse events [34, 82]. However, these resulted in low true positive rates.

Glucose consumption and PH measurement

Both of glucose consumption and PH measurement in the platelet components are thought to change in the presence of increasing bacteria. As the growth of bacteria leads to glucose consumption and acid production, glucose and PH decrease in the platelet components. However, these both have been shown to be inapplicable in the clinical use and have led to wasting many platelet units because of false positive results. For instance, in PH testing, it was found that all of positive results were false and nearly 2% of all platelet units were discarded [27, 34]. Consequently, it can be said, determination of PH and glucose lead to very high false positive rates. Hence, PH and glucose measurement have low true positive rates.

Pan genera detection

The pan genera detection assay is a rapid and qualitative immunoassay. This method has cleared by the FDA for the detection of aerobic and anaerobic Gram-positive and -negative bacteria directly prior to transfusion.

The test principle is based on the immunological detection of lipopolysaccharide for Gram-negative bacteria or lipo-teichoic acid for Gram-positive bacteria [34, 83]. The analytical sensitivity of this assay is specified as 10^3 – 10^4 CFU/ml and 10^3 – 10^5 CFU/ml for Gram-positive and negative bacteria, respectively [83]. For quality control testing of PCs, this system should be used as an adjunct quality control test following testing with an FDA-

cleared bacterial detection device. The pan genera detection test comes as a commercial kit containing lateral flow test device (Including two simultaneously run test strips specific for detection of Gram-positive and Gram-negative bacteria) reagents, controls, and other disposable materials [34]. The major disadvantages of the pan genera detection assay are high costs and high rate of false-positive results [84]. Since this method has low sensitivity, it seems that it is merely acceptable when the screened PCs are transfused immediately after testing [20].

Bio responsive polymers

Researches have shown that technology of bio responsive polymers based on enzyme operate reactions is an appropriate method to detect both contaminating organisms and wound infection [85-89]. This technology can be used to find contaminations at an early stage and it can be used for the detection of pathogens in PCs. A visible color reaction indicates a contamination and gives a clear yes/no signal. However, it seems that this procedure has a low sensitivity and maybe fluorescent dyes can be used to improve the sensitivity of this detection system [90].

Conclusions

To conclude, the method used for testing of the bacterial contamination of PCs should be sensitive, specific and inexpensive. It should have a rapid turn-around time and demand a small sample volume [91]. The efficiency of bacterial detection in PCs depends on several factors such as sampling time, sample volume as well as sensitivity of the detection method

and bacterial growth kinetics [47]. Each of the methods described in this study has a couple of advantages and disadvantages. It seems that culture-based methods, especially BacT/Alert, which has been approved by the FDA, are the most sensitive methods among them. Consequently, wide varieties of countries are using BacT/Alert method as a routine bacterial screening method [11, 13, 53, 59, 62, 92-94]. Additionally, it asserted that BacT/Alert technique has high sensitivity and specificity; however, it can be controversial and is not universal. It is believed that this claim still needs further study. In general, this method is far more dependable and fabulous in comparison with other PCs contamination identification method and considers as gold standard method. Rapid methods are able to diagnose and give us test results within a short time period, which it minimizes the sampling error risk like low volume or sampling time errors. Additionally, a couple of these methods not in use or no longer fulfill requirements for bacterial contamination detection, most cases are still clinically important. Furthermore, a couple of methods like molecular methods are important in research centers. Application of

rapid methods more likely will play an crucial role in transfusion medicine in near future. This requires more and more investigations, especially in clinical indications.

Eventually, it clears that consideration of a comprehensive assessment is consequential, which all of following items must be included:

1. Consistent implementation of the principles of good manufacturing practice.
2. Accurate identification asymptomatic bacteremia and the considering permissible protocol for it.
3. Disinfection of phlebotomy area in proper manner.
4. Technique of diverting first 30 ml of blood sample after venipuncture.
5. Verification of PCs storage containers and storage temperature to increase PCs shelf life.
6. Utilization of a superb method for testing PCs bacterial contamination (Sensitive, specific, inexpensive and rapid).
7. Pathogen inactivation or pathogen reduction for increasing platelet units' shelf life.

Conflict of Interest

The authors report no declarations of interest.

Acknowledgment

There is no acknowledgment to declare.

References

- [1].Esmaili MA, Sharifi Z, Yari F. Conjugates of B cells and platelet microparticles in the human peripheral blood. *Archives of Medical Laboratory Sciences* 2016; 2(3): 79-83.
- [2].Esmaili MA, Yari F, Sharifi Z, Nikougoftar M, Fadaei R. Effects of platelet microparticles on the activation of B cells. *Modares Journal of Medical Sciences: Pathobiology* 2013; 15(4): 1-10.
- [3].Niu MT, Knippen M, Simmons L, Holness LG. Transfusion-transmitted *Klebsiella pneumoniae* fatalities, 1995 to 2004. *Transfus Med Rev.* 2006; 20(2): 149-57.
- [4].Brecher ME, Hay SN. Bacterial contamination of blood components. *Clin Microbiol Rev.* 2005; 18(1): 195-204.
- [5].Jacobs MR, Palavecino E, Yomtovian R. Don't bug me: the problem of bacterial contamination of

- blood components--challenges and solutions. *Transfusion* 2001; 41(11): 1331-334.
- [6]. Dreier J, Vollmer T, Kleesiek K. Novel flow cytometry-based screening for bacterial contamination of donor platelet preparations compared with other rapid screening methods. *Clin Chem*. 2009; 55(8): 1492-502.
- [7]. Murphy WG, Foley M, Doherty C, Tierney G, Kinsella A, Salami A, et al. Screening platelet concentrates for bacterial contamination: low numbers of bacteria and slow growth in contaminated units mandate an alternative approach to product safety. *Vox Sang*. 2008; 95(1): 13-19.
- [8]. Rotman B, Cote MA. Application of a real-time biosensor to detect bacteria in platelet concentrates. *Biochem Biophys Res Commun*. 2003; 300(1): 197-200.
- [9]. Schmidt M, Hourfar MK, Nicol SB, Wahl A, Heck J, Weis C, et al. A comparison of three rapid bacterial detection methods under simulated real-life conditions. *Transfusion* 2006; 46(8): 1367-373.
- [10]. Blajchman MA, Beckers EA, Dickmeiss E, Lin L, Moore G, Muylle L. Bacterial detection of platelets: current problems and possible resolutions. *Transfus Med Rev*. 2005; 19(4): 259-72.
- [11]. Fang CT, Chambers LA, Kennedy J, Strupp A, Fucci MC, Janas JA, et al. Detection of bacterial contamination in apheresis platelet products: American Red Cross experience, 2004. *Transfusion* 2005; 45(12): 1845-852.
- [12]. Farzad BB, Farshad B, Zahra B, Nahid A, Mahsa KB. Bacterial contamination of platelet products in the Blood Transfusion Center of Isfahan, Iran. *GMS hygiene and infection control*. 2016; 11.
- [13]. Benjamin RJ, Kline L, Dy BA, Kennedy J, Pisciotto P, Sapatnekar S, et al. Bacterial contamination of whole-blood-derived platelets: the introduction of sample diversion and prestorage pooling with culture testing in the American Red Cross. *Transfusion* 2008; 48(11): 2348-355.
- [14]. Borosak M, Wood E. Bacterial Pre-Release Testing of Platelets - the Australian Red Cross Blood Service Clinical Experience. *Transfus Med Hemother*. 2011; 38(4): 239-41.
- [15]. Jenkins C, Ramirez-Arcos S, Goldman M, Devine DV. Bacterial contamination in platelets: incremental improvements drive down but do not eliminate risk. *Transfusion* 2011; 51(12): 2555-565.
- [16]. Lee CK, Wong HK, Ho PL, Tsoi WC, Lee KY, Tsui GT, et al. Significant bacterial contamination risk reduction with the use of diversion pouch. *Transfus Med*. 2012; 22(6): 404-408.
- [17]. Murphy S, Heaton WA, Rebullia P. Platelet production in the Old World--and the New. *Transfusion* 1996; 36(8): 751-54.
- [18]. Katus MC, Szczepiorkowski ZM, Dumont LJ, Dunbar NM. Safety of platelet transfusion: past, present and future. *Vox Sang*. 2014; 107(2): 103-13.
- [19]. Goldman M, Blajchman MA. Blood product-associated bacterial sepsis. *Transfus Med Rev*. 1991; 5(1): 73-83.
- [20]. Stormer M, Vollmer T. Diagnostic methods for platelet bacteria screening: current status and developments. *Transfus Med Hemother*. 2014; 41(1): 19-27.
- [21]. Esmaeili MA, Yari F, Amini A, Rezvani MR. The effect of cell derived microparticles in transfusion medicine and adaptive immune system. *Archives of Medical Laboratory Sciences* 2016; 2(1): 29-35.
- [22]. Greco C, Mastronardi C, Pagotto F, Mack D, Ramirez-Arcos S. Assessment of biofilm-forming ability of coagulase-negative staphylococci isolated from contaminated platelet preparations in Canada. *Transfusion* 2008; 48(5): 969-77.
- [23]. Larsen CP, Ezligini F, Hermansen NO, Kjeldsen-Kragh J. Six years' experience of using the BacT/ALERT system to screen all platelet concentrates, and additional testing of outdated platelet concentrates to estimate the frequency of false-negative results. *Vox Sang*. 2005; 88(2): 93-7.
- [24]. von Eiff C, Peters G, Heilmann C. Pathogenesis of infections due to coagulase-negative staphylococci. *Lancet Infect Dis*. 2002; 2(11): 677-85.
- [25]. Vamvakas EC. Relative safety of pooled whole blood-derived versus single-donor (apheresis) platelets in the United States: a systematic review of disparate risks. *Transfusion* 2009; 49(12): 2743-758.
- [26]. Ness P, Braine H, King K, Barrasso C, Kickler T, Fuller A, et al. Single-donor platelets reduce the risk of septic platelet transfusion reactions. *Transfusion* 2001; 41(7): 857-61.
- [27]. Yomtovian RA, Palavecino EL, Dysktra AH, Downes KA, Morrissey AM, Bajaksouzian S, et al. Evolution of surveillance methods for detection of bacterial contamination of platelets in a university hospital, 1991 through 2004. *Transfusion* 2006; 46(5): 719-30.
- [28]. Buchholz DH, Young VM, Friedman NR, Reilly JA, Mardiney MR. Detection and quantitation of bacteria in platelet products stored at ambient temperature. *Transfusion* 1973; 13(5): 268-75.
- [29]. Murphy WG, Coakley P. Testing platelet components for bacterial contamination. *Transfus Apher Sci*. 2011; 45(1): 69-74.
- [30]. Te Boekhorst PA, Beckers EA, Vos MC, Vermeij H, Van Rhenen DJ. Clinical significance

- of bacteriologic screening in platelet concentrates. *Transfusion* 2005; 45(4): 514-19.
- [31]. Braine HG, Kickler TS, Charache P, Ness PM, Davis J, Reichart C, et al. Bacterial sepsis secondary to platelet transfusion: an adverse effect of extended storage at room temperature. *Transfusion* 1986; 26(4): 391-93.
- [32]. Pietersz RN, Engelfriet CP, Reesink HW, Wood EM, Winzar S, Keller AJ, et al. Detection of bacterial contamination of platelet concentrates. *Vox Sang.* 2007; 93(3): 260-77.
- [33]. de Korte D, Marcelis JH, Verhoeven AJ, Soeterboek AM. Diversion of first blood volume results in a reduction of bacterial contamination for whole-blood collections. *Vox Sang.* 2002; 83(1): 13-16.
- [34]. Bessos H, Seghatchian J. What's happening? The expanding role of apheresis platelet support in neonatal alloimmune thrombocytopenia: current status and future trends. *Transfus Apher Sci.* 2005; 33(2): 191-97.
- [35]. Goldman M, Roy G, Frechette N, Decary F, Massicotte L, Delage G. Evaluation of donor skin disinfection methods. *Transfusion* 1997; 37(3): 309-12.
- [36]. McDonald CP, Lowe P, Roy A, Robbins S, Hartley S, Harrison JF, et al. Evaluation of donor arm disinfection techniques. *Vox Sang.* 2001; 80(3): 135-41.
- [37]. de Korte D, Curvers J, de Kort WL, Hoekstra T, van der Poel CL, Beckers EA, et al. Effects of skin disinfection method, deviation bag, and bacterial screening on clinical safety of platelet transfusions in the Netherlands. *Transfusion* 2006; 46(3): 476-85.
- [38]. Eder AF, Kennedy JM, Dy BA, Notari EP, Weiss JW, Fang CT, et al. Bacterial screening of apheresis platelets and the residual risk of septic transfusion reactions: the American Red Cross experience (2004-2006). *Transfusion* 2007; 47(7): 1134-142.
- [39]. Eder AF, Kennedy JM, Dy BA, Notari EP, Skeate R, Bachowski G, et al. Limiting and detecting bacterial contamination of apheresis platelets: inlet-line diversion and increased culture volume improve component safety. *Transfusion* 2009; 49(8): 1554-563.
- [40]. Satake M, Mitani T, Oikawa S, Nagumo H, Sugiura S, Tateyama H, et al. Frequency of bacterial contamination of platelet concentrates before and after introduction of diversion method in Japan. *Transfusion* 2009; 49(10): 2152-157.
- [41]. McDonald CP, Roy A, Mahajan P, Smith R, Charlett A, Barbara JA. Relative values of the interventions of diversion and improved donor-arm disinfection to reduce the bacterial risk from blood transfusion. *Vox Sang.* 2004; 86(3): 178-82.
- [42]. Lozano M, Cid J, Prowse C, McCullough J, Klein HG, Aubuchon JP. Pathogen inactivation or pathogen reduction: proposal for standardization of nomenclature. *Transfusion* 2015; 55(3): 690.
- [43]. Allain JP, Owusu-Ofori AK, Assennato SM, Marschner S, Goodrich RP, Owusu-Ofori S. Effect of Plasmodium inactivation in whole blood on the incidence of blood transfusion-transmitted malaria in endemic regions: the African Investigation of the Mirasol System (AIMS) randomised controlled trial. *Lancet* 2016; 387(10029): 1753-761.
- [44]. Cazenave JP, Folléa G, Bardiaux L, Boiron JM, Lafeuillade B, Debost M, et al. A randomized controlled clinical trial evaluating the performance and safety of platelets treated with MIRASOL pathogen reduction technology. *Transfusion* 2010; 50(11): 2362-375.
- [45]. Klein HG, Dodd R, Dzik W, Luban N, Ness PM, Pisciotto P, et al. Current status of solvent/detergent-treated frozen plasma. *Transfusion* 1998; 38(1): 102-107.
- [46]. Ludlam CA, Powderly WG, Bozzette S, Diamond M, Koerper MA, Kulkarni R, et al. Clinical perspectives of emerging pathogens in bleeding disorders. *Lancet* 2006; 367(9506): 252-61.
- [47]. Brecher ME, Holland PV, Pineda AA, Tegtmeier GE, Yomtovian R. Growth of bacteria in inoculated platelets: implications for bacteria detection and the extension of platelet storage. *Transfusion* 2000; 40(11): 1308-312.
- [48]. Mohr H, Bayer A, Gravemann U, Muller TH. Elimination and multiplication of bacteria during preparation and storage of buffy coat-derived platelet concentrates. *Transfusion* 2006; 46(6): 949-55.
- [49]. Wagner SJ, Friedman LI, Dodd RY. Transfusion-associated bacterial sepsis. *Clin Microbiol Rev.* 1994; 7(3): 290-302.
- [50]. Roth VR, Kuehnert MJ, Haley NR, Gregory KR, Schreiber GB, Arduino MJ, et al. Evaluation of a reporting system for bacterial contamination of blood components in the United States. *Transfusion* 2001; 41(12): 1486-492.
- [51]. Jacobs MR, Good CE, Lazarus HM, Yomtovian RA. Relationship between bacterial load, species virulence, and transfusion reaction with transfusion of bacterially contaminated platelets. *Clin Infect Dis.* 2008; 46(8): 1214-220.
- [52]. 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(4): 496-502.
- [53]. Benjamin RJ, Wagner SJ. The residual risk of sepsis: modeling the effect of concentration on bacterial detection in two-bottle culture systems and an estimation of false-negative culture rates. *Transfusion* 2007; 47(8): 1381-389.
- [54]. Bruhn R, Custer B, Vanderpool S, Townsend M, Kamel H, Tomasulo P. Impact of increasing

- sample volume from 4 ml to 8 ml on bacterial detection rates in apheresis platelets: a meta-analysis. *Vox Sang.* 2015; 108(3): 318-20.
- [55].Blajchman MA, Goldman M, Baeza F. Improving the bacteriological safety of platelet transfusions. *Transfus Med Rev.* 2004; 18(1): 11-24.
- [56].Brecher ME, Hay SN, Rothenberg SJ. Evaluation of a new generation of plastic culture bottles with an automated microbial detection system for nine common contaminating organisms found in PLT components. *Transfusion* 2004; 44(3): 359-63.
- [57].Brecher ME, Hay SN, Rothenberg SJ. Validation of BacT/ALERT plastic culture bottles for use in testing of whole-blood-derived leukoreduced platelet-rich-plasma-derived platelets. *Transfusion* 2004; 44(8): 1174-178.
- [58].Holme S, McAlister MB, Ortolano GA, Chong C, Cortus MA, Jacobs MR, et al. Enhancement of a culture-based bacterial detection system (eBDS) for platelet products based on measurement of oxygen consumption. *Transfusion* 2005; 45(6): 984-93.
- [59].Ezaki S, Kawabata K, Kanno T, Ohto H. Culture-based bacterial detection systems for platelets: the effect of time prior to sampling and duration of incubation required for detection with aerobic culture. *Transfusion* 2007; 47(11): 2044-2049.
- [60].Wagner SJ, Moroff G, Katz AJ, Friedman LI. Comparison of bacteria growth in single and pooled platelet concentrates after deliberate inoculation and storage. *Transfusion* 1995; 35(4): 298-302.
- [61].Wagner SJ, Robinette D. Evaluation of an automated microbiologic blood culture device for detection of bacteria in platelet components. *Transfusion* 1998; 38(7): 674-79.
- [62].Brecher ME, Hay SN, Rose AD, Rothenberg SJ. Evaluation of BacT/ALERT plastic culture bottles for use in testing pooled whole blood-derived leukoreduced platelet-rich plasma platelets with a single contaminated unit. *Transfusion* 2005; 45(9): 1512-517.
- [63].Brecher ME, Means N, Jere CS, Heath D, Rothenberg S, Stutzman LC. Evaluation of an automated culture system for detecting bacterial contamination of platelets: an analysis with 15 contaminating organisms. *Transfusion* 2001; 41(4): 477-82.
- [64].Muller TH, Mohr H, Montag T. Methods for the detection of bacterial contamination in blood products. *Clin Chem Lab Med.* 2008; 46(7): 933-46.
- [65].Brecher ME, Hay SN, Rothenberg SJ. Monitoring of apheresis platelet bacterial contamination with an automated liquid culture system: a university experience. *Transfusion* 2003; 43(7): 974-78.
- [66].Tomasulo PA, Wagner SJ. Predicting improvement in detection of bacteria in apheresis platelets by maintaining constant component sampling proportion. *Transfusion* 2013; 53(4): 835-42.
- [67].Kim SC, Kim S, Lee DH, Choi SR, Kim JS. Effect of blood volume in standard anaerobic blood culture bottles of the BacT/ALERT 3D system used for the detection of pathogens and time to detection. *PLoS One* 2015; 10(2): e0116728.
- [68].GuoHui B, Chunhui Y, Miao H, Hong W, Jiaxin L, Ye C, et al. Three different bacterial detection systems for platelet concentrates under inter-laboratory conditions. *Transfus Apher Sci.* 2013; 49(3): 600-607.
- [69].Nanua S, Weber C, Isgriggs L, Dunne WM. Performance evaluation of the VersaTREK blood culture system for quality control testing of platelet units. *J Clin Microbiol.* 2009; 47(3): 817-18.
- [70].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(6): 1217-223.
- [71].Schmidt M, Sireis W, Seifried E. Implementation of Bacterial Detection Methods into Blood Donor Screening-Overview of Different Technologies. *Transfus Med Hemother.* 2011; 38(4): 259-65.
- [72].Razjou F, Dabirmoghadam A. Comparison of the Bact/Alert blood culture system and manual culture method for detection of aerobic and facultative anaerobic bacterial contamination in platelet concentrates. *Scientific Journal of Iranian Blood Transfusion Organization* 2012; 8(4): 265-71.
- [73].Dabirmoghadam A, Razjou F, Kokab Sayar E. Evaluation of an automated microbiological blood culture for detection of bacteria in platelet units. *Scientific Journal of Iranian Blood Transfusion Organization* 2013; 9(4): 399-405.
- [74].Dabirmoghadam A, Razjou F, Badlou BA. Measurement of BacT/ALERT Sensitivity after Inoculation Certain Amount of *E. coli* and *S. epidermis*. *J Blood Disorders Transf.* 2015; 2015.
- [75].Fournier-Wirth C, Deschaseaux M, Defer C, Godreuil S, Carriere C, Bertrand X, et al. Evaluation of the enhanced bacterial detection system for screening of contaminated platelets. *Transfusion* 2006; 46(2): 220-24.
- [76].Dreier J, Stormer M, Kleesiek K. Two novel real-time reverse transcriptase PCR assays for rapid detection of bacterial contamination in platelet concentrates. *J Clin Microbiol.* 2004; 42(10): 4759-764.
- [77].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.
- [78]. Mohammadi T, Reesink HW, Vandenbroucke-Grauls CM, Savelkoul PH. Optimization of real-time PCR assay for rapid and sensitive detection of eubacterial 16S ribosomal DNA in platelet concentrates. *J Clin Microbiol.* 2003; 41(10): 4796-798.
- [79]. 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(3): 237-54.
- [80]. Sireis W, Ruster B, Daiss C, Hourfar MK, Capalbo G, Pfeiffer HU, et al. Extension of platelet shelf life from 4 to 5 days by implementation of a new screening strategy in Germany. *Vox Sang.* 2011; 101(3): 191-99.
- [81]. Mohr H, Lambrecht B, Bayer A, Spengler HP, Nicol SB, Montag T, et al. Sterility testing of platelet concentrates prepared from deliberately infected blood donations. *Transfusion* 2006; 46(3): 486-91.
- [82]. Yomtavian R, Lazarus HM, Goodnough LT, Hirschler NV, Morrissey AM, Jacobs MR. A prospective microbiologic surveillance program to detect and prevent the transfusion of bacterially contaminated platelets. *Transfusion* 1993; 33(11): 902-909.
- [83]. 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(10): 3475-481.
- [84]. Ramirez-Arcos S, Kou Y, Mastronardi C, Perkins H, Goldman M. Bacterial screening of outdated buffy coat platelet pools using a culture system and a rapid immunoassay. *Transfusion* 2011; 51(12): 2566-572.
- [85]. Hasmann A, Gewessler U, Hulla E, Schneider KP, Binder B, Francesko A, et al. Sensor materials for the detection of human neutrophil elastase and cathepsin G activity in wound fluid. *Exp Dermatol.* 2011; 20(6): 508-13.
- [86]. Hasmann A, Wehrsuetz-Sigl E, Kanzler G, Gewessler U, Hulla E, Schneider KP, et al. Novel peptidoglycan-based diagnostic devices for detection of wound infection. *Diagn Microbiol Infect Dis.* 2011; 71(1): 12-23.
- [87]. Hasmann A, Wehrsuetz-Sigl E, Marold A, Wiesbauer H, Schoeftner R, Gewessler U, et al. Analysis of myeloperoxidase activity in wound fluids as a marker of infection. *Ann Clin Biochem.* 2013; 50(Pt 3): 245-54.
- [88]. Schneider KP, Gewessler U, Flock T, Heinzle A, Schenk V, Kaufmann F, et al. Signal enhancement in polysaccharide based sensors for infections by incorporation of chemically modified laccase. *N Biotechnol.* 2012; 29(4): 502-509.
- [89]. Schneider KP, Rollett A, Wehrsuetz-Sigl E, Hasmann A, Zankel A, Muehlebach A, et al. Bioresponsive systems based on polygalacturonate containing hydrogels. *Enzyme Microb Technol.* 2011; 48(4-5): 312-18.
- [90]. Gamerith C, Heinzle A, Schneider KP, Hulla-Gumbsch E, Gewessler U, Ducoroy L, et al. Bioresponsive polymers for the detection of bacterial contaminations in platelet concentrates. *N Biotechnol.* 2014; 31(2): 150-55.
- [91]. Hsueh JC, Ho CF, Chang SH, Pan FZ, Chen SC, Shi MD, et al. Blood surveillance and detection on platelet bacterial contamination associated with septic events. *Transfus Med.* 2009; 19(6): 350-56.
- [92]. 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(7): 1138-142.
- [93]. Nussbaumer W, Allerstorfer D, Grabmer C, Rheinschmidt M, Lin L, Schonitzer D, et al. Prevention of transfusion of platelet components contaminated with low levels of bacteria: a comparison of bacteria culture and pathogen inactivation methods. *Transfusion* 2007; 47(7): 1125-133.
- [94]. Su LL, Kamel H, Custer B, Vanderpool S, Harpool D, Busch M, et al. Bacterial detection in apheresis platelets: blood systems experience with a two-bottle and one-bottle culture system. *Transfusion* 2008; 48(9): 1842-852.