Evaluation of the Sensitivity and Specificity of Use of Glucose and pH for Bacterial Screening of Platelet Concentrates Compared to the Bact/Alert

Farhad Razjou¹ · Hossein Timori Naghadeh¹ · Shirin Ferdowsi¹ · Abolfazl Dabirmoghadam¹

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Abstract Bacterial contamination of blood components is the major infectious risk in transfusion medicine. Since platelets should be stored at room temperature that makes them an excellent growth medium for bacteria; it is mentioned as a major problem in transfusion medicine. Transfusion risk of a bacterial contaminated platelet concentrate is higher than viral pathogen such as HIV, HBV, HCV and HTLV. The objective of this study was to evaluation of the sensitivity and specificity of use of glucose and pH for bacterial screening of platelet concentrates compared to the Bact/Alert. 1332 platelet concentrates were screened by the Bact/Alert system for aerobic and anaerobic bacterial contamination. Bacterial contamination was also evaluated by using urine reagent strips (Multistix10 SG Bayer) and culture methods. Moreover PH screening with a pH meter (Metrohm 744 Swiss) and glucose was also used for detection of bacterial contamination. The rate of bacterial contamination detected by the Bact/Alert system in platelet concentrates was 25 in 1332 (1.9 %). It contained 15 (1.1 %) for aerobic bacteria and 10 (.8 %) for anaerobic bacteria. 226 of 1332 were considered as containing bacteria by using urine reagent strips. Six of the 226 units were also positive by the Bact/Alert system. Three of those units were culture positive for aerobic bacteria and three for anaerobic. The result of platelet concentrates that underwent pH screening by use of pH meter and a pH portion of urine reagent strips was the same. The sensitivity and specificity of considering glucose

alone for detection of bacterial contamination were 12 and 98 % respectively. For pH alone, these were 24 and 83 %. For glucose and/or pH, these were 24 and 83 %; and for combination of glucose and pH, these were 12 and 98 %. Our results showed use of glucose/pH strips would improve the safety of blood products and should be encouraged.

Keywords Platelet concentrate · Bacterial contamination · Reagent strips

Background and Objectives

Bacterial contamination evaluation of blood products is regarded as an important point of blood safety. Mortality due to bacterial contamination of blood has been estimated to occur as many as 500–750 deaths annually in the United States [1]. Since platelets should be stored at room temperature that makes them an excellent growth medium for bacteria, it is declared as a major difficulty in transfusion medicine [2]. The transfusion risk of a bacterial contaminated platelet concentrates (PC) is higher than viral pathogen such as HIV, HBV, HCV and HTLV [3]. Bacterial contamination is considered the third most common cause of death overall from transfusion reported to the US Food and Drug Association (FDA), following transfusion—related acute lung injury and hemolytic reactions. The sepsis related mortality risk of platelet transfusion is ranging from 1/20,000 to 1/85,000 donor exposure [4]. The exact incidence of bacterial contamination of blood products reported by different studies is unknown because of using variable methods of bacterial detection [5]. Donor skin flora, donor asymptomatic bacteria and contamination during product processing are three primary sources of bacterial contamination [6]. Beside donor arm derived is

✉ Abolfazl Dabirmoghadam
dabirmoghadam@yahoo.com

¹ Blood Transfusion Research Center, High Institute for Research and Education in Transfusion Medicine, IBTO, Tehran, Iran

the major source [7]. For reducing this risk attempt on improvement of the skin disinfection procedure and detection of contaminating agents affecting platelets during preparation procedure have an important role. Surrogate (nonculture) bacterial detection methods are proposed for this purpose as being rapid and less expensive. They are based on changes in platelet unit strip pH and glucose that occurs following increasing levels of microbial contamination [6]. Overall sensitivity reported for the test was 95 % with a specificity of 98–100 % at 10^7 colony-forming units per milliliter (CFU/mL) [8]. The Bact/Alert system is cleared by FDA for quality control of bacterial culture in platelets. The Bact/Alert system monitors CO_2 produced by bacterial proliferation and detect contamination of platelets inoculated to 1 CFU/mL in 12–26 h [9]. The objective of this study was to evaluation of the sensitivity and specificity of use of glucose and pH for bacterial screening of PC compared to the Bact/Alert.

Materials and Methods

During the 1 year study period, 1332 unselected random platelet concentrates units were processed from whole blood and collected in platelet containers (JMS, Mo Kio, Singapore). The CPDA-1 solution was used as an anticoagulant. All these PC units were screened by the Bact/Alert system for bacterial contamination in Tehran blood transfusion center. Bacterial contamination was also evaluated by using urine reagent strips (for pH and Glucose). Sampling was performed 48–72 h after collection of whole blood. 20 ml of the PLT component was collected by syringe from the port site of bag after the site was disinfected with a swap with 70 % isopropyl alcohol. Thereafter the sample was used for following purposes:

1. Bacterial contamination was evaluated by using Bact/Alert standard bottles named Bacterial Platelet Aerobic (BPA) (bioMerieux, Inc., Durham, NC) for detection of aerobic bacteria and Bacterial Platelet Anaerobic (BPN) (bioMerieux, Inc., Durham, NC) for detection of anaerobic bacteria. The bottles were incubated for 7 days at 36 °C. When a bottle was detected positive it was confirmed by culture based bacterial detection method. Culture was prepared from BPN bottle was incubated in anaerobic jar for confirmation and identification of anaerobic bacteria.
2. Bacterial contamination was also evaluated by using culture methods for detection of aerobic bacteria. These included Thioglycolate, Trypticase Soy Broth & Agar, Blood Agar (Merck, 64271 Darmstadt, Germany).
3. Drops of the sample were placed on the glucose and pH part of urine reagent strip (Multistix 10 SG, Bayer

Corporation, Elkhart, IN) for detection of aerobic and anaerobic bacteria; Results were interpreted at 30 s for glucose and 60 s for pH. A glucose level less than 250 mg per dL and/or a pH level value of less than 7.0 was considered positive for bacterial contamination. In this study glucose and pH level in bags containing CPD were used as the cut-off for predicting of bacterial contamination.

4. Drop of sample underwent pH screening with a pH meter (Metrohm 744, Swiss) for detection of bacterial contamination. pH level value of less than 7.0 was considered positive.
5. The organism involved was identified by using Mini Api (bioMerieux, Inc., Durham, NC) device and its standard kits named Rapid ID 32 A.E, Strep (bioMerieux, Inc., Durham, NC).

Results

The rate of bacterial contamination in platelet concentrates detected by the Bact/Alert system was 25 in 1332 platelet concentrates (1.9 %). It contained 15 (1.1 %) for aerobic bacteria and 10 (.8 %) for anaerobic bacteria.

The confirmatory aerobic manual culture prepared from platelet bags was positive in 13 of the total amount of samples resulted in a positive signal (Bact/Alert BPA bottles) whereas 2 did not reveal any microorganisms. All of the confirmatory aerobic and anaerobic manual cultures prepared from BPA and BPN bottles resulted in a positive signal were positive. The isolated bacteria included, 10 anaerobic bacteria (*Propionibacterium acnes*), 2 *Streptococci*, 1 *Serratia marcescens*, 2 *Enterobacter cloacae*, 4 *Corynebacterium diphtheria*, 2 *Staphylococcus aureus* and 4 *Staphylococci epidermidis* (Table 1).

Twenty-seven of 1332 platelet concentrates were classified as containing bacteria by measurement of glucose alone in urine reagent strips. Only three of the 27 platelet concentrates were also positive by the Bact/Alert system. Aerobic bacteria were isolated from all units. The sensitivity of this method was 12 % and the specificity was 98 %.

The result of platelet concentrates that underwent pH screening by use of pH meters and urine reagent strips (pH portion) was the same. Of the 1332 platelet concentrates that screened by measurement of pH, 224 had a pH value of less than 7.0. Six of those were confirmed by the Bact/Alert system. Three of them were culture positive for aerobic bacteria and the rest were positive for anaerobic bacteria. The sensitivity of this method was 24 % and the specificity was 83 %.

226 of 1332 were considered as containing bacteria by using urine reagent strips. Six of the 226 units were also

Table 1 Rate of bacterial contamination in platelet concentrates

Bacterial type	Bacteria	No. of cases	Percentage (%)
(A) Anaerobic bacteria	<i>Propionibacterium acnes</i>	10	40
(B) Aerobic bacteria	<i>Streptococci</i> spp	2	8
	<i>Serratia marcescens</i>	1	4
	<i>Enterobacter cloacae</i>	2	8
	<i>Corynebacterium diptherioied</i>	4	16
	<i>Staphylococci aureous</i>	2	8
	<i>Staphylococci epidermidis</i>	4	16

positive by the Bact/Alert system. Three of those units were culture positive for aerobic bacteria and three for anaerobic bacteria. Sensitivity for the study was 24 % with a specificity of 83 %. If the combination of glucose and pH level values were considered positive for bacterial contamination, then 25 Unit would be detected by urine reagent strips. Only three of these units were confirmed by the Bact/Alert system as aerobic bacteria. The sensitivity of this study was 12 % and the specificity was 98 %. Test accuracy for detecting bacterial contamination by glucose and pH screening test is presented in Table 2.

Discussion

Bacterial contamination of blood component is the major infectious risk in transfusion medicine [10]. The rate of contamination of PC is cited at between .02 and 1.2 %, depending on the production and bacterial culture techniques used [2]. After using bacterial culture systems for detection of bacterial contamination in platelet units prepared by aphaeresis method, the rate of sepsis and mortality reduced to 1:75,000–1:500,000 [11]. In Europe culturing of each PC presents logistical problems, so many centers have chosen surrogate methods like measurement of pH and glucose as indicators of contamination [9]; although they are not sufficiently sensitive to meet current AABB standards [12]. Only in the USA most of the whole blood derived platelets are screened by measuring the pH or glucose or other rapid methods. These tests had 4–6 folds lower detection rate than the culture based methods [13].

In our study, all PC units were screened by the Bact/Alert system for bacterial contamination in the Tehran blood transfusion center. Sampling was performed 48–72 h after collection of whole blood. Holding PLT_s for 48–72 h before sampling improves the detection sensitivity for PLT_s contaminated with low concentration of bacteria [14]. The rate of bacterial contamination was 25 in 1332 platelet concentrates (1.9 %). It contained 15 (1.1 %) for aerobic bacteria and 10 (.8 %) for anaerobic bacteria (This rate reduced to .1 % after implementation of Good

Manufacturing Practice (GMP) for skin preparation, removal of skin plug with diversion of the first 10 ml of collection and platelet production) (Table 1). The results obtained from a pH portion of reagent strips were the same as a pH meter. Reagent strips results are semi quantitative and are more subjective. On the other hand we found that 224 (16.8 %) units of the 1332 platelets had a pH value of less than 7. 226 of 1332 were considered as containing bacteria by using urine reagent strips.

In this study screening of PC units by considering glucose and/or pH part of the reagent strip method resulted in 165 units per 1000 being wasted, but prevented 6 patients from receiving a contaminated platelet unit. Meanwhile screening by the combination of glucose and pH resulted only 17 units per 1000 being wasted, but prevented just 3 patients from receiving a contaminated platelet unit. Although the sensitivity of pH screening of platelet concentrates individually is 2 times more than of the glucose screening alone, but pH screening method results in significant platelet wastage.

In one study, [15] 450 (1.2 %) units of the 37,060 whole blood derived platelets had a pH value of less than 7. Four of those units were confirmed by culture method. Higher WBC and also PLT content likely explains pH failure not due to bacterial contamination. In another study [8], thirty of the 3093 PC units was classified as positive by the reagent strips. Only two of 30 reagent strip screen positive units were also positive by the standard bacterial culture method. *Bacillus cereus* was isolated from both units. It should be noted that the assessment of pH is not without problems. Certain bacterial species like *Klebsiella* sp. *Bacillus subtilis* and *Proteus mirabilis* which produce acetone and other neutral metabolites rather than acid metabolites from glucose may prevent the expected decline of pH in the presence of bacterial growth [16]. None of these bacteria have been isolated in the present study. Regarding the role of bacteria to risk the blood safety (particularly PC) it is necessary to diminish the risky agents as low as possible by improving the quality assurance and developing platelet safety by using appropriate detecting procedures. Although measurement of pH and glucose are not sufficiently sensitive to meet current AABB standards,

Table 2 Test accuracy for detecting bacterial contamination by glucose and pH screening test

Screening test	True positive	True negative	False positive	False negative	Accuracy, % (95 % CI)	Sensitivity, % (95 % CI)	Specificity, % (95 % CI)	PPV, % (95 % CI)	NPV, % (95 % CI)	PLR (95 % CI)	NLR (95 % CI)
Glucose alone	3	1282	25	22	96 (95–97)	12 (4–30)	98 (97–99)	11 (4–27)	98 (97–99)	6.27 (.05–817.2)	.90 (.82–.98)
pH screening	6	1089	218	19	82 (80–84)	24 (12–43)	83 (81–85)	3 (1–6)	98 (97–99)	1.44 (.51–4.09)	.91 (.82–1.01)
Both glucose and pH	3	1284	22	22	97 (96–98)	12 (4–30)	98 (97–99)	12 (4–30)	98 (97–99)	7.12 (.05–937.9)	.90 (.82–.98)
Glucose and or pH (at least one of them)	6	1086	220	19	82 (80–84)	24 (12–43)	83 (81–85)	3 (1–6)	98 (97–99)	1.43 (.50–4.04)	.91 (.82–1.01)

PPV indicated positive predictive value, NPV negative predictive value, PLR positive likelihood ratio, NLR negative likelihood ratio

* For one of the platelet concentrates, bacterial contamination base on Bact/Alert system was missing

however, it can be used at the time of transfusion beside. It is less expensive and should not require specialized equipment or specially trained personnel. No existing screening test meets all of the criteria for the ideal test. The precautionary principle which is called foresight principle may be applied to this dilemma. According to this principle when human health is threatened for example by bacterial contamination of blood products, the threat warrants the implementation of available methods as interim measures to reduce the risk of bacterial contamination [16]. According to limitation of current screening tests, it is necessary to look for new methods in future.

Conclusion

In spite of the fact that no perfect solution exists for bacterial screening of platelet concentrates, in the interim, widespread use of glucose/pH strips or any available methods would improve the safety of blood products and should be encouraged.

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