

## Can dipstick method help in bacterial detection in platelet bags? A tertiary care hospital's blood bank review

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### Abstract

**Objective:** To find the bacterial and biochemical details of bags used in platelet transfusion.

**Methods:** This cross-sectional study was carried out at a tertiary care hospital of Saudi Arabia (King Khalid Hospital, Najran) from January to June 2012, and comprised platelet bags. Samples for bacterial detection and biochemical testing of platelet bags were taken from blood bags on Day 6 of donation. Bacterial detection was done by using aerobic culture bottle, different gram stain, cultures and analytical profile index strips. Glucose, pH and protein measurements were done by Multistix dipstick method. SPSS 16 was used for data analysis.

**Results:** Of the 352 platelet bags, 1(0.28%) showed bacterial growth on Day 6 of collection. That bacterium was *Staphylococcus epidermidis*. Glucose content and pH of that platelet bag was 144.14mg/dl and 5, respectively. The overall mean pH of platelet bags was  $6.69 \pm 0.55$  (range: 3-7). Moreover, 255(72.4%) bags showed pH of 7, 90(25.5%) of 6, 5(1.4%) of 5 and 2(0.57%) showed pH of 3 on Day 6. The overall mean protein level was  $6.162 \pm 0.204$ g/dl (range: 5.8-6.6). Pearson bivariate correlation between platelet bag's pH and glucose content was 0.707 ( $p=0.001$ ).

**Conclusion:** Positive correlation was found between platelet bag's glucose and pH levels.

**Keywords:** Platelets; Glucose; Bacteria. (JPMA 66: 1258; 2016)

### Introduction

Blood transfusion is one of the important aspects in patient care. Viral screening and nucleic acid testing remained the most common methods for detection of Human Immunodeficiency Virus (HIV), Hepatitis B and C.<sup>1</sup> But still, blood-transmitted infections like viruses, protozoans, helminths, prions and bacteria are some of the biggest problems of transfusion medicine.<sup>2</sup> This concern becomes even more serious in the case of platelets as they are the particular component of the blood which is ideal for the growth of bacteria.<sup>3</sup> Since 1990, several methods have been used to minimise the risk of bacterial transfusion reaction. Among these measures, donor screening and skin decontamination by iodine or chlorhexidine were important steps, as majority of bacteria isolated from blood bags had been skin flora.<sup>4</sup> That is why pathogens are mostly gram-positive like *Staphylococcus aureus*, coagulase negative *Staphylococci*, *Corynebacteria*, *Bacillus* spp. *viridans* group *Streptococci* and anaerobic diptheroid gram-positive bacilli, for example *Propionibacterium acnes*.<sup>3</sup>

Some platelet storage solutions are being introduced which reduce the plasma and prevent bacteria to grow.<sup>5</sup> Chances of bacteria avoidance in platelet bag is not

reduced to zero, so detection of bacteria in blood bags has great importance. Culture-based methods have gained the great significance to detect bacteria.<sup>4</sup> Gram stain, accrine orange and Wright's stain are used to detect bacteria in blood bags, but with high false positive results. Inexpensive but insensitive and non-specific measures like pH, glucose level, oxygen level and other metabolic changes are also used as an indication of growth of bacteria.<sup>3</sup> Fluorescent cytometric techniques and nucleic acid polymerase chain reaction (PCR) methods are future consideration to detect bacteria in blood bags.<sup>3,6</sup>

Platelet bag quality assurance is also a vital issue in the field of blood transfusion. There are several factors which affect the platelet quality in platelet concentrates, including method of forming units, temperature at which it is stored, platelet count of platelet bags and sufficient oxygen to carry on metabolism. Platelet concentrates need constant agitation to optimise the transport of oxygen and carbon dioxide through the container. Many morphological, biochemical and functional changes occur during this storage period of platelets, which are termed as platelet storage lesions. These lesions are important because they can affect post-transfusion survival of cells.<sup>7</sup>

The significance and applicability of this study can be assessed by findings of Eder et al., 2007,<sup>8</sup> who estimated the ratio of septic reaction to transfusion at 1:25,000 and the number of platelet units found to cause bacterial contamination after applying detection techniques at

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1:2,000 to 1:3,000. Also, lots of blood component recipients are neutropenic and febrile patients, who are already taking antibiotics. So, whole clinical scenario can be changed after bacterial detection techniques implication.<sup>8</sup> Bacterial detection has not been applied as a policy in many hospitals of Saudi Arabia. The current study was planned to determine microbiological and biochemical changes in platelet bags after storage for 5 days, so that the quality of platelet bags and bacterial growth could be assessed.

## Materials and Methods

This cross-sectional study was carried out at a tertiary care hospital (King Khalid Hospital, Najran) of Saudi Arabia, from January to June 2012, and comprised platelet bags. Approval was obtained from the ethics committee of the hospital. Blood was drawn from the donors as mentioned in the guidelines of the formerly American Association of Blood Banks (AABB).<sup>9</sup> Then 450ml of whole blood was collected in a 450-ml quadruple bag containing 63ml of citrate phosphate dextrose (CPD) anticoagulant, with additive solution (SAGM) (TERUMO PENPOL Limited). The whole bloods were separated into platelets, fresh frozen plasma and packed red blood cells by hard spin and then light spin method, as described in AABB guidelines.<sup>10</sup> Platelet bags were kept in a Helmer agitator for 5 days.

Samples were selected by simple random sampling technique. Samples for bacterial detection and biochemical testing of platelet bags were taken from expired blood bags i.e. on Day 6 of donation (as the platelet bag were considered expired on Day 5). Under aseptic measures, 10ml sample was taken from platelet bag. Four ml sample was inoculated in aerobic culture bottle. Three ml sample was taken in sterile plastic tubes for pH, glucose and protein measurement by Multistix dipstick method. Three ml sample was taken in sterile red top tube for glucose and protein measurement by Roche kit using COBAS instrument. Bacteria detection was carried out by inoculation of platelets from platelet bag in aerobic bottle on Day 6 of blood component preparation as recommended by BACTEC.<sup>11</sup> Bacteria were then further characterised by different gram stain, cultures and analytical profile index (API) strips.

Statistical analysis was done using SPSS 16. Descriptive statistics were obtained and bivariate Pearson correlation

was found for different parameters.

## Results

Of the 352 platelet bags, 1(0.28%) showed bacterial growth on Day 6 of collection. That bacterium was *Staphylococcus epidermidis*. Glucose content and pH of that platelet bag was 144.14 mg/dl and 5, respectively. The overall mean pH of platelet bags was  $6.69 \pm 0.55$  (range: 3-7) (Table-1). Moreover, 255(72.4%) bags showed pH of 7, 90(25.5%) showed pH of 6, 5(1.4%) showed pH of 5 and 2(0.57%) showed pH of 3 on Day 6. The mean glucose content of the bags was  $256.93 \pm 136.21$  (8.46-484.86) (Table-1). The glucose content of 211(60%) bags was between 180.18-360.36 mg/dl. Moreover, 46(13%) platelet bags showed glucose value less than 54.05 mg/dl. Moreover, 137(39%) and 167(47.7%) of platelet concentrates had 4+ and 3+ of glucose (Table-2). The overall mean protein level was  $6.162 \pm 0.204$ g/dl (range: 5.8-6.6)

Pearson bivariate correlation between platelet bag's pH

**Table-1:** Means and ranges of pH, glucose and protein.

	Mean±Standard Deviation	Range
pH [N=352 (100%)]	6.6971±0.55	3-7
Glucose determined by Roche kit (mg/dl) [N=352 (100%)]	256.93±136.21	8.46-484.86
Protein (g/dl) [N=352 (100%)]	6.1619±2.04	5.8-6.6

**Table-2:** Glucose and protein (by dipsticks) percentages.

Scores	1+N(%)	2+ N(%)	3+ N(%)	4+ N(%)
Glucose by dipstick [N=352 (100%)]	12 (3.2%)	36 (10.1%)	167 (47.7%)	137 (39%)
Protein by dipstick [N=352 (100%)]	0 (0%)	2 (0.5%)	276 (78.2%)	74 (21.3%)

**Table-3:** Pearson correlation between pH and glucose content of platelet bags (determined by Roche Kit).

	Pearson bivariate Correlation	p-value (significant if <0.05)
pH and Glucose content of platelet bags (mg/dl)	0.707	< .001

**Table-4:** Dipstick glucose relationship to other parameters.

Glucose by dipstick	1+	2+	3+	4+
Mean Glucose (determined by Roche Kit) ± Standard Deviation(mg/dl) [N=352 (100%)]	16.93±11.89	141.08±116.03	268.29±108.64	276.57±121.98
Mean pH ± Standard Deviation [N=352 (100%)]	6.17±0.41	6.35±0.67	6.72±0.58	6.79±0.41

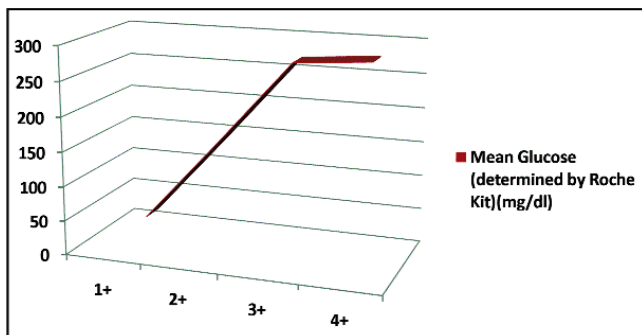


Figure-1: Dipstick glucose relationship to glucose by Roche Kit.

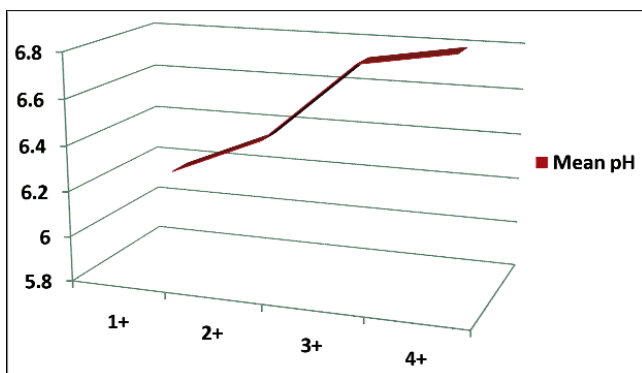


Figure-2: Dipstick pH relationship to pH.

and glucose content was 0.707 (Table-3) ( $p=0.001$ ).

## Discussion

Platelets are an excellent growth medium for bacterial growth. Bacteria can grow in platelet concentrates and can cause transfusion reactions. In some studies it is mentioned that there are chances of 1:2,000 to 1:5,000 of bacterial contamination in platelet concentrates. Among them some can cause sepsis i.e. 1:20,000 to 1:50,000, out of which 10% can become fatal.<sup>12</sup> It is reported that 1 platelet bag is usually infected among 1000-2000 platelet bags.<sup>13</sup> In our study, only one bag showed bacterial growth among 352 bags on Day 6 of collection, which is equal to 0.28%. In a Taiwan study, Hsueh JC et al. found that the infection rate of platelet bags was 0.31% (7 in 2,256).<sup>5</sup> Lee CK et al. established detection rate of 0.15% (3 in 10,035 platelet units) in Hong Kong.<sup>14</sup> Another study carried out by American Red Cross showed the results of 0.019% (226 of 350,658 platelet bags). In that study, 47% of bacteria isolates were of staphylococcal species.<sup>15</sup> Similarly, other studies showed prevalence of bacterial contamination from 0.14%-1.41%, with the mean value being 0.43%. These studies also mentioned that most common bacteria isolated from platelet concentrates was staphylococcus, as in our study.<sup>16</sup>

Wagner SJ concluded that 56% of times bacteria isolated from transfusion-related sepsis was aerobic gram-positive.<sup>17</sup> But gram-negative organisms are also found in blood bag contamination and they are more morbid than gram-positive. Yomtavian RA et al. showed division of the cases in sepsis as Staphylococcus spp. in 42% of cases, Escherichia coli in 9%, Bacillus spp. in 9%, Salmonella spp. in 9%, Streptococcus spp. in 12%, Serratia spp. in 8%, Enterobacter spp. in 7%, and other organisms in 4%.<sup>18</sup> Donor bacteraemia is one of the less frequent causes of bacterial transmission through blood components. Ness P et al. concluded that 62.5% and 34.8% of cases of septic reaction by transfusion were shown to be related to skin flora bacteria and transient donor bacteraemia, respectively.<sup>19</sup>

Moreover, pH has been used as a quality control of platelet concentrates. Its value below 6.8 has been declared as a bad indicator of in-vivo platelets survival. Platelets release different chemicals including lactate which lowers the pH.<sup>20</sup> Similarly, growth of bacteria can affect pH of platelet bags due to production of acids. It has been mentioned in study that dipstick accuracy for pH detection is good at pH around 7.<sup>21</sup> Therefore, we used dipsticks to judge the pH of platelet bags.

In our study, the majority (72.6%) of bags showed pH of 7 on Day 6. Besides, 25.5% of bags showed pH of 6 and only 1.4% and 0.5% showed pH of 5 and 3, respectively. In one study, pH of 7.125 and pH of 7.140 was detected on Days 5 and 7 of platelet collection, respectively.<sup>7</sup> Doescher A et al. detected pH of 7 on Day 6.<sup>20</sup> In another study, pH of 7.73 was the result on Day 5 of collection.<sup>22</sup> This shows our study results were mostly in concordance with other studies.

Furthermore, 27.4% of platelet concentrates showed pH of 6 and less. This is an indicator of non-viable platelets in platelet bags. Platelets become spherical at pH of 6.8 and metabolism of platelets is captured at pH of 6 or less.<sup>22</sup> The pH of the platelet bag which was found infected with bacterium was 5. This might be due to acid released by bacterium.

To maintain the good quality of platelets, glucose should be maintained during storage period. In our study, the mean glucose content of 352 platelet bags was 256.93mg/dl, with a range of 8.46-484.86. Besides, 60% of platelet concentrates showed glucose content between 180.18-360.36 mg/dl. Chandra T et al. found that glucose concentration decreased from 225.58mg/dl to 197.47mg/dl from the day of collection to Day 7.<sup>23</sup> We came to know that 39% and 47.7% of platelet concentrates had 4+ and 3+ of glucose with dipstick method. A significant positive correlation was found

between pH and glucose content of platelet bags. The same correlation was also found for glucose measured by dipstick method and pH of platelet bags. As the pH of platelet bags decreased, glucose content also decreased. This explains the fact that as platelet bags get older, more glucose is converted into acids, which results in reduction of pH.<sup>11,20</sup>

In our study, protein content (range: 5.8-6.6g/dl) in all of the platelet bags was near the lower normal range of plasma protein (6.4-8.2 g/dl) (Table-1). This indicates that protein content of the healthy donor was diluted in plasma and citrate of blood bags. Easy and cheap determination of platelet bag quality is an area of interest in field of transfusion medicine.<sup>20</sup> In our study, we found a positive correlation between glucose determined by dipstick and glucose determined by Roche Kit and, pH (Figure-1 and 2, Table-4). Glucose determination with dipstick is an easy technique. It gives an idea about the quality of platelet bag contents according to our analysis in this study. It is a known fact that bacterial growth in platelet bags causes decrease in pH and glucose content.<sup>20</sup> Therefore, we can suggest that dipstick technique can help in detection of bacterial growth. Sample can be taken from platelet bag tubing and can be tested for glucose content by dipstick during storage period. Further studies are needed to establish the relationship between glucose content in tubing and platelet bags during the storage period of platelet bags.

## Conclusion

The dipstick technique was found to be helpful in detection of pH and glucose content of platelet bags. Glucose and pH content of platelet bags were found to be good indicators of bacterial growth.

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**Conflict of Interest:** None.

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