

Detection of Bacterial Contamination in Single-Donor Platelets Using BACT/ALERT and VITEK Systems

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ABSTRACT:

BACKGROUND:

Bacterial contamination of platelet units is a serious concern in transfusion medicine, potentially leading to septic transfusion reactions, which can be life-threatening. Due to their storage at room temperature (20–24°C) with constant agitation, platelet concentrates are particularly susceptible to bacterial growth, especially from skin flora introduced during blood collection or processing. Common contaminants include *Staphylococcus aureus*, coagulase-negative staphylococci, and Gram-negative organisms such as *Escherichia coli*. Contamination may result from inadequate aseptic techniques, faulty collection equipment, or undetected donor bacteremia. Early detection and the application of pathogen reduction strategies are critical for ensuring transfusion safety.

OBJECTIVE:

This study aimed to determine the frequency and types of bacterial contamination in platelet apheresis units.

METHODS:

A cross-sectional study was conducted at the National Blood Transfusion Center between May 20 and July 22, 2024. A total of 130 platelet apheresis units, collected from voluntary and replacement donors and screened negative for HIV, HBV, HCV, and syphilis, were evaluated. Samples were taken on the second day post-collection and cultured using standard media and the BacT/ALERT microbial detection system.

RESULTS:

All 130 platelet units showed no evidence of bacterial or fungal contamination in either the conventional culture media or BacT/ALERT system.

CONCLUSION:

The absence of detectable contamination may reflect effective donor skin disinfection and sterile collection practices. However, the limited sample size, single-point testing, and potential for false negatives suggest the need for further studies. Future work should incorporate larger sample sizes, advanced rapid detection methods, and delayed sampling for improved accuracy.

KEYWORDS: Platelet transfusion, bacterial contamination, apheresis, BacT/ALERT, transfusion safety.

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INTRODUCTION:

Blood transfusion is one of the most frequently performed procedures in modern healthcare, with over 100 million units transfused annually worldwide ⁽¹⁾. Among the various components, platelet concentrates (PCs) play a crucial role in the management of patients with hematological malignancies, bone marrow failure syndromes, and those undergoing intensive chemotherapy or surgery ⁽²⁾. Despite significant improvements in donor screening, blood collection, and storage protocols, bacterial contamination of PCs remains a persistent and serious challenge in transfusion medicine. Unlike red blood cells or

plasma, platelets must be stored at room temperature (20–24°C) with constant agitation to maintain their viability and function. Unfortunately, these storage conditions are ideal for bacterial growth, which increases the risk of transfusion-transmitted infections ⁽²⁾. The estimated prevalence of bacterial contamination in platelet units' ranges from 1 in 2,000 to 1 in 3,000, and clinical sepsis occurs in approximately one out of every six contaminated transfusions. In fact, in the United States, bacterial contamination ranks as the second leading cause of transfusion-related fatalities,

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surpassed only by transfusion errors⁽³⁾. The most common route of bacterial entry into platelet products is during the collection process. Bacteria from the donor's skin flora—despite rigorous disinfection protocols—can enter the unit during venipuncture. Common skin-derived contaminants include *Staphylococcus aureus*, *Staphylococcus epidermidis*, and other coagulase-negative staphylococci⁽⁴⁾. In addition, transient bacteremia, which may go unnoticed in asymptomatic donors, can introduce bacteria such as *Enterococcus faecalis* and viridans group streptococci into the bloodstream. Contamination can also arise from environmental sources or flaws in blood collection equipment⁽⁵⁾. Transfusion of contaminated platelets can result in a spectrum of adverse reactions, from mild febrile non-hemolytic transfusion reactions to severe, life-threatening septicemia. Clinical symptoms typically manifest shortly after transfusion and include fever, chills, tachycardia, and hypotension. Gram-negative bacteria, in particular, pose a high risk due to rapid endotoxin release, which can precipitate septic shock⁽²⁾. To address these risks, multiple safety strategies have been developed. These include improved skin disinfection, diversion of the initial blood draw, and the implementation of bacterial detection systems such as culture-based screening. Among these, the BacT/ALERT automated culture system is widely used and regarded as a gold standard. However, it requires an incubation period of 24–48 hours, and its sensitivity may be limited when bacterial load is initially low, increasing the risk of false-negative results and the inadvertent transfusion of contaminated units⁽³⁾. Therefore, the need for more rapid and reliable detection methods, alongside pathogen reduction technologies, is critical to improving the overall safety of platelet transfusion. This study aimed to determine the frequency and potential causes of bacterial contamination in single-donor platelet units using BacT/ALERT and conventional culture methods.

METHOD:

This cross-sectional study was conducted at the National Blood Transfusion Centre between May 20 and July 22, 2024. A total of 130 platelet apheresis units, collected from both voluntary and replacement donors, were evaluated for bacterial contamination. Ethical approval for the study was obtained from the Iraqi Board for Medical Specialisations, Scientific Council of Pathology (Issue Path55, dated 6/5/2024).

Inclusion and Exclusion Criteria: All included platelet units were collected from single-donor

apheresis and met the standard eligibility criteria for donation. Donors had normal platelet counts ($150\text{--}450 \times 10^9/\text{L}$) and white blood cell counts ($4.0\text{--}11.0 \times 10^9/\text{L}$). Units testing positive for HIV, HBV, HCV, or syphilis were excluded from the study to minimise the confounding risks of coexisting transfusion-transmitted infections.

Platelet Collection and Storage: Platelets were collected using the *Trima® Accel* automated blood collection system (Terumo BCT, Colorado, USA). The donor's arm was disinfected using a concentric circular technique with povidone-iodine followed by 70% isopropyl alcohol to ensure asepsis. The initial 20–30 mL of drawn blood was diverted to minimise skin flora contamination. During the procedure, anticoagulant acid citrate dextrose (ACD) was used in a controlled ratio, and donors were monitored for signs of hypocalcaemia. Each apheresis unit targeted a platelet yield of approximately $3 \times 10^{11}/\text{L}$. After collection, the units were stored at room temperature (20–24°C) with continuous agitation for 24 hours before bacterial testing, as recommended in prior studies for optimal platelet preservation.

Sample Collection and Testing Protocol: All laboratory steps were conducted under aseptic conditions inside a biosafety cabinet. From each unit, approximately 5 mL of platelet-rich plasma was collected from the sample diversion pouch, using sterile techniques and equipment. About 4 mL of the sample was placed into BacT/ALERT aerobic culture bottles (BioMérieux, Marcy-l'Étoile, France), mixed gently, and marked with unique labels. These were incubated at 37°C using the BacT/ALERT 3D system for five days to detect microbial growth, aligning with protocols described in established microbiological screening literature. Additionally, 0.5 mL of each sample was cultured on three media types: blood agar and MacConkey agar for bacterial detection (Gram-positive and Gram-negative, respectively) and Sabouraud agar for fungal identification. Plates were incubated at 32–34°C for 48 hours using a refrigerated incubator (Froilabo). If growth was detected, isolates were identified using the VITEK® 2 Compact System, ensuring precise organism classification. Ethical considerations: the study was approved by the Iraqi Postgraduate Medical Journal. Delayed bacterial testing, typically conducted ≥ 24 hours post-collection, is a well-established practice aimed at improving the reliability of culture-based detection methods. Bacteria introduced during collection, such as skin flora from donors, are often present in low quantities initially. Allowing a 24-hour

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incubation period provides sufficient time for these microorganisms to proliferate to detectable levels, thereby minimizing the risk of false-negative results when testing is performed too early (e.g., on Day 0 or 1). The sensitivity of commonly used aerobic and anaerobic culture systems, such as BacT/ALERT, is significantly enhanced when samples are tested after this initial incubation period. Empirical evidence supports that the likelihood of detecting bacterial contamination increases when cultures are initiated at least 24 hours following collection. Furthermore, adherence to delayed testing is reinforced by regulatory guidelines and best practice recommendations. Institutions such as the AABB and the U.S. Food and Drug Administration (FDA) advocate for bacterial testing at or beyond 24 hours post-collection, a standard that has been widely adopted in transfusion medicine and blood bank protocols internationally. Using a small sample volume (e.g., 4 mL) may not reliably detect low-level bacterial contamination in platelet units, particularly during early stages. Increasing the sample volume to 8 mL or more significantly

improves detection sensitivity. Additionally, employing both aerobic and anaerobic culture bottles enhances the identification of a broader range of bacterial species. A quality control test was performed to assess the performance of the BacT/ALERT® system using five pre-confirmed positive samples containing Gram-positive and Gram-negative bacteria. Each sample was inoculated into separate BacT/ALERT culture bottles. The system reliably detected bacterial growth, with all samples testing positive within 12 hours of incubation.

RESULTS:

Out of the 130 apheresis platelet samples analyzed, all units demonstrated no microbial growth in both testing methods. Specifically, no aerobic bacterial or fungal growth was detected after five days of incubation using BacT/ALERT bottle culture, and no growth was observed after 48 hours on conventional culture media including MacConkey agar, blood agar, and Sabouraud agar. These findings indicate zero incidence of detectable bacterial contamination among the tested apheresis platelet units. As shown in Table 1.

Table 1: Apheresis Culture Result of 130 Units.

BacT/ALERT bottle culture	Culture media (MacConkey agar, blood agar, and Sabouraud Agar)
No growth	No growth

DISCUSSION:

The detection of bacterial contamination in platelet concentrates (PCs) is influenced by multiple factors, including the method used, sample volume, and timing of testing. In this study, none of the 130 apheresis platelet units demonstrated bacterial growth using the BacT/ALERT® 3D system, a finding consistent with similar studies conducted in low- and middle-income countries. For example, Kumari et al. (2018–2022) at Aga Khan University in Pakistan reported a 0% contamination rate among 476 apheresis units tested using the same system, despite screening over 84,000 total platelet units⁽³⁾. Similarly, Anna Maria Leo detected only one contaminated unit among 481 tested (0.2%), with the contaminant identified as *Staphylococcus epidermidis*, a skin flora organism, detected on day five post-collection⁽⁴⁾. These findings suggest a low contamination rate for apheresis units, especially when optimal collection and handling practices are implemented. However, studies also caution against overreliance on negative culture results, particularly when testing small volumes or using only aerobic bottles. The BacT/ALERT®

system, while widely utilized, has documented limitations. Abela et al. reported false-negative results involving *Staphylococcus aureus* in three cases, later confirmed through molecular identification⁽⁵⁾. The formation of bacterial biofilms and platelet aggregation may contribute to uneven bacterial distribution and sampling errors, reducing detection sensitivity⁽⁹⁻¹¹⁾. Additionally, culture sensitivity depends heavily on the initial bacterial load, with concentrations below 1–10 CFU/mL often escaping detection^(12,13). Our study used a 4 mL sample volume, which, while standard in many protocols, is lower than the 8–10 mL volumes shown to increase detection sensitivity significantly. Kamel et al. demonstrated that increasing sample volume using a Proportional Sample Volume (PSV) strategy doubled the true-positive detection rate in PCs⁽¹⁴⁾. Similarly, Benjamin reported false-negative rates between 10–30% when low bacterial loads or slow-growing organisms were present⁽¹⁵⁾. Testing exclusively with aerobic bottles may also compromise sensitivity. Data from NHS Blood and Transplant revealed that 66% of positive cultures

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were detected only in anaerobic bottles, emphasizing the benefit of using both aerobic and anaerobic media for comprehensive screening⁽¹⁰⁾. Australian data supported this two-bottle strategy, enhancing detection across diverse bacterial profiles⁽¹⁵⁾. Preventive strategies remain critical. Diverting the first 20–30 mL of donor blood has significantly reduced contamination rates, as demonstrated in studies by de Korte and others, lowering risks from skin flora such as coagulase-negative staphylococci^(16,17). Moreover, rigorous skin disinfection protocols, as shown by Arghittu et al., reduced the microbial load on the donor's skin by 84.4%, further contributing to sterility⁽¹⁸⁾. While our findings confirm the efficacy of current protocols, limitations such as small sample size, low culture volume, and single-mode detection may underestimate true contamination rates. Enhancing sample volume, employing dual-bottle cultures, and adopting Large Volume Delayed Sampling (LVDS) can further reduce residual risks in platelet transfusions^(19,20).

CONCLUSION:

This study found no bacterial contamination in apheresis platelet units, reflecting effective donor skin disinfection, initial blood diversion, and sterile collection protocols. However, factors such as small sample volume, exclusive use of aerobic media, and early sampling may contribute to false negatives. Future research should involve larger sample sizes and implement advanced detection methods, such as rapid immunoassays (e.g., PGD tests). Adoption of large-volume delayed sampling (LVDS) using both aerobic and anaerobic bottles at 36–48 hours post-collection is recommended. Secondary testing on days 3–4 may further enhance safety. As recommendations further studies should explore biofilm-associated bacterial resistance and optimize cost-effective detection strategies. Implement Large Volume Delayed Sampling (LVDS) to enhance pathogen yield and reduce false negatives in bloodstream infection detection. Utilize dual-bottle blood culture systems to improve microbial recovery rates and diagnostic accuracy.

Conflict of Interest: The authors declare no conflict of interest.

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Authors' Contributions

Authors' Contributions: The 1st author contributed to the study design and data collection; The 2nd author performed the statistical analysis; The 3rd author drafted the

manuscript. All authors reviewed and approved the final version.

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