

Bacterial Contamination in Random Donor Platelet Concentrates Using the BacT/Alert 3D Microbial Detection System and VITEK 2 Bacterial Identification System

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ABSTRACT:

BACKGROUND:

Platelet transfusions are associated with an increased risk of infection and sepsis due to their susceptibility to bacterial contamination. Stored at 20–24°C for up to five days, platelet concentrates (PC) provide an optimal environment for bacterial growth. Bacterial contamination remains a significant cause of transfusion-related morbidity and mortality. Accurate estimates of contamination rates are essential to guide safety practices and testing protocols.

OBJECTIVE:

Determine the frequency of bacterial contamination in random blood donor PC and to identify the most common bacterial species implicated in contamination.

MATERIALS, AND METHODS:

Samples from 130 random donor PC taken 18–24 hours' post-donation and before pooling were processed using the Reveos® automated whole blood processing system. Each sample was tested using the BacT/Alert® 3D microbial detection system over five days and simultaneously cultured on solid agar media (blood agar, MacConkey agar, and Sabouraud agar) for 48 hours at 32–34°C. Any microbial growth is supposed to be identified using the VITEK® 2 compact system.

RESULTS:

All 130 samples tested negative for bacterial and fungal contamination across both testing methods.

CONCLUSION:

This study found no evidence of bacterial contamination in the tested PC, unlike prior Iraqi data suggesting greater rates. The results highlight the efficiency of present collecting and storing methods, therefore underlining the need for rigorous aseptic practices and quality control in guaranteeing transfusion safety.

KEYWORDS: Random Donors, Platelet Concentrates, Reveos, BacT/Alert, VITEK 2.

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INTRODUCTION:

Platelet concentrates (PC) are the second most commonly used blood component after red blood cells, playing a critical role in the prevention and treatment of bleeding disorders⁽¹⁾. In pooled preparations, PC from 4–6 random donors are combined, typically yielding 240–360 × 10⁹ platelets suspended in 200–350 mL of plasma or plasma substitutes. Apheresis-derived PC, on the other hand, provide 200–400 × 10⁹ platelets in 200–300 mL of plasma from a single donor, thus reducing donor exposure and potentially lowering the risk of transfusion-related complications⁽²⁾. PC are still especially susceptible to bacterial contamination even with major developments in donor screening and collecting techniques. Their storage conditions at room temperature (20–24°C) with continuous

agitation for up to five days can enable bacterial development to clinically hazardous levels⁽³⁾. Before transfusion all platelet products have to be checked for bacterial contamination. Although conventional techniques are still the gold standard for identifying bacterial contamination, they are not without limits. These include the high cost, generally poor application of culture methods in blood bank environments, and the delay in getting findings compared to the limited shelf life of platelets⁽⁴⁾. The estimated rate of bacterial contamination in PC whether whole blood or apheresis-derived, ranges from 1 in 2,000 to 1 in 3,000 units. Alarmingly, one in every six transfused contaminated units may result in severe sepsis. This makes bacterial contamination up to 50–250 times more likely

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than the combined risk of transmitting viruses such as human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV), and human T-lymphotropic viruses I and II through transfusion. Therefore, robust bacterial surveillance systems are essential⁽⁴⁾. While passive surveillance, relying on clinician reports of transfusion-transmitted bacterial infections, is commonly used, its accuracy is limited. Clinical signs of bacterial sepsis can easily be misinterpreted as other types of transfusion reactions, leading to underreporting. Hence, active and reliable testing methods are needed to ensure transfusion safety. The BacT/Alert® 3D system is a widely adopted, automated colorimetric culture method that detects microbial growth based on carbon dioxide (CO₂) production, which alters the color of a pH-sensitive sensor at the base of the culture bottles. It effectively identifies aerobic, anaerobic, fungal, and yeast contaminants⁽⁵⁾. Complementing this, the VITEK® 2 compact system, developed by BioMérieux, offers rapid and precise bacterial identification and antibiotic susceptibility profiling using fluorescence-based technology. It has been validated in numerous studies for its reliability and efficiency with pure bacterial cultures⁽⁶⁾. This study aims to detect the frequency of bacterial contamination frequently implicated in the contamination of random blood donor PC and to identify the common types of bacterial contamination in PC.

METHOD:

This cross-sectional study was conducted at the National Blood Transfusion Center (NBTC) in Baghdad, Iraq. Research approvals were obtained from the Iraqi Board for Medical Specializations (IRB no.: Path53 on 28 Apr 2024). Whole blood donations were collected from healthy donors who met standard eligibility criteria. After collection, the whole blood was stored in a Fiocchetti Scientific Refrigerator at 20–24°C for 18–24 hours. Platelet concentrates were then prepared using the REVEOS®

automated whole blood processing system. Donor units testing positive for HIV, HBV, HCV, and syphilis were excluded from the study. A total of 130 random donor PC units were included. To ensure aseptic collection, sterile gloves were used and frequently changed. Inside a Class II biological safety cabinet, a platelet segment was disinfected with 70% alcohol, allowed to dry, and 10 mL of sample was aspirated and transferred to BacT/Alert bottles and solid agar media (blood agar, MacConkey agar, and Sabouraud agar). Blood agar was prepared using nutrient agar with 5–10% sterile defibrinated blood⁽⁷⁾, MacConkey agar for isolating enteric bacteria⁽⁸⁾, and Sabouraud dextrose agar for fungi⁽⁹⁾. Each medium was autoclaved, cooled, poured into sterile Petri dishes, and stored at 2–8°C. For BacT/Alert® 3D (BioMérieux, France), 10 mL of each sample was inoculated into FA plus bottles and incubated for 5 days. Simultaneously, a drop of each sample was cultured onto agar media and incubated for 48 hours at 32–34°C. Growth detection was followed by microbial identification using the VITEK® 2 compact system⁽⁶⁾. Platelet content was estimated using the platelet yield index (PYI) on the REVEOS system⁽¹⁰⁾. Quality control was verified using five known positive controls for BacT/Alert to ensure the accuracy of the system. **Statistical Analysis:** Data were analyzed using SPSS version 26. Results were shown in Tables. Description of numerical values expressed as mean ± standard deviation (SD), and qualitative data was expressed as frequency and percentages.

RESULTS:

The interim platelet units (IPUs) of 130 random donor prepared by the Reveos® automated whole blood processing system showed no aerobic bacterial or fungal contamination detected by both the BacT/Alert 3D microbial detection system and in solid agar media (blood agar, MacConkey agar, and Sabouraud agar), as shown in Table 1.

Table 1: Culture results of this study.

Number of PC samples	Culture in BacT/Alert FA Plus bottle	Culture in Blood agar plate	Culture in MacConkey agar plate	Culture in Sabouraud agar plate
130	No growth	No growth	No growth	No growth

The IPUs were analyzed, with a mean PYI of 7.73×10^{10} and a standard deviation of 1.88. The PYI ranged from 4.1×10^{10} to 13×10^{10} . Among

the samples, 90% (117 out of 130) had a PYI of $\geq 5.5 \times 10^{10}$, while 10% (13 out of 130) had a PYI of $< 5.5 \times 10^{10}$. These results are shown in Table 2.

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Table 2: Platelet Yield Index of Interim Platelet Units Obtained from the Reveos Automated Whole Blood Processing System.

	Total number of samples	mean	SD	Minimum-Maximum PYI	Number of PYI $<5.5 \times 10^{10}$	Number of PYI $\geq 5.5 \times 10^{10}$
IPUs ($\times 10^{10}$)	130	7.73	1.88	4.1 – 13	13/130 (10%)	117/130 (90%)

IPU, interim platelet unit; SD, standard deviation; PYI, platelets yield index

DISCUSSION:

Although transfusion-transmitted infections have significantly declined with improved screening and processing methods, bacterial contamination of platelet products remains a persistent concern. This is particularly alarming given that platelets are stored at room temperature (20–24°C), which supports bacterial proliferation during their 5-day shelf life. Bacterial contamination is currently the second leading cause of transfusion-related fatalities in the United States, with 2,000 to 4,000 contaminated platelet units causing hundreds of sepsis cases and deaths annually^(5,11). These statistics emphasize the critical importance of strengthening safety protocols in platelet transfusion. In this study, no bacterial contamination was detected in 130 random donor PC, corresponding to a 0% contamination rate. This contrasts sharply with a previous NBTC study, which reported a 38% contamination rate in 100 PC, most commonly

with *Bacillus subtilis* (63%) and *Staphylococcus epidermidis* (16%)—organisms commonly found on human skin^(12,13). An analysis conducted at the NBTC in Baghdad, Iraq, examined 1,542 manually prepared PC collected between 2015 and 2023, revealing an overall contamination rate of 5% (78 / 1542). The annual contamination rates were 9.4% in 2015, 6.2% in 2019, 6% in 2020, 2.4% in 2022, and 3.8% in 2023, as shown in Figure 1. The findings highlighted the superior sterility of the Reveos Automated Whole Blood Processing System compared to manual methods. The automated system's closed, controlled environment reduces human error and exposure to environmental contaminants, whereas manual processing remains more susceptible to contamination. These results emphasize the advantages of adopting automation to improve the safety, consistency, and quality of PC preparation.

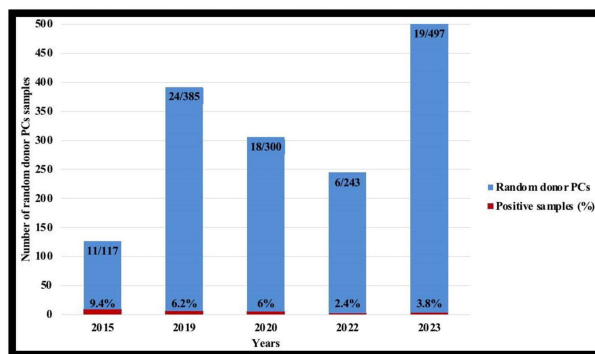


Figure 1: Contamination rates in a previous analysis of manually prepared PC at the NBTC, Baghdad, Iraq, during the years 2015, 2019, 2020, 2022, and 2023, using aerobic BacT/Alert culture bottles.

Globally, contamination rates of PC vary, as shown in Table 3, with significantly higher rates reported in developing countries—for instance, a rate of 20% has been documented in Nigeria⁽¹⁴⁾, Kenya (12.1%)⁽¹⁵⁾, and Mexico (9%)⁽³⁾, while developed countries report substantially lower rates—e.g., Belgium (0.74%)⁽²⁾, China (0.22%)⁽¹⁶⁾, and Canada (0.01%)⁽¹⁷⁾. This study's zero contamination rate aligns more closely with the latter, suggesting that the integration of

automation, such as the Reveos system, can elevate safety standards in developing countries to match those of advanced healthcare systems. Additionally, the study evaluated platelet content using the Platelet Yield Index (PYI) in the Reveos system⁽¹⁸⁾. Results revealed a mean yield of 7.73×10^{10} , exceeding the minimum clinical standard of 5.5×10^{10} ⁽¹⁹⁾. Though a few units showed yields below this threshold (as low as 4.1×10^{10}), overall performance was consistent with

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other findings, highlighting the efficiency of the units suitable for transfusion. Reveos system in producing high-quality platelet

Table 3: Bacterial contamination rates in different blood banks worldwide.

Country, year (reference)	Sample size	Frequency of Contaminated PC	Time of sample taking, methods of testing
Nigeria, 2024 ⁽¹⁴⁾	10	20 %	Brain-heart infusion broth and thioglycolate broth
Kenya, 2018 ⁽¹⁵⁾	91	12.1 %	Tryptic Soy Broth
Zimbabwe, 2015 ⁽²⁰⁾	39	10.3 %	Bottles of trypton soy broth
Ghana, 2009 ⁽²¹⁾	22	9.0 %	Brain-heart infusion broth
Mexico, 2017 ⁽³⁾	100	9.0 %	6-hour, BacT/Alert aerobic & anaerobic bottles
Pakistan, 2018 ⁽²²⁾	200	1.0 %	48-hour, Oxoid's Signal blood culture bottles
Belgium, 2005 ⁽²⁾	107,827	0.74% ^a	Day 0 (2-22 hours), BacT/Alert aerobic & anaerobic bottles
Netherlands, 2005 ⁽²⁾	38,664	0.67% ^b	Day 1 (16-24 hours), BacT/Alert aerobic & anaerobic bottles
Morocco, 2020 ⁽²³⁾	3,898	0.44 %	<2 days, Bacterial cultures broths in an aerobic atmosphere
Taiwan, 2009 ⁽²⁴⁾	2,338	0.34% ^c	Day 1-5, BacT/Alert aerobic & anaerobic bottles
Denmark, 2004 ⁽²⁵⁾	22,057	0.32% ^a	Day 0-1 (<30 hours), BacT/Alert aerobic bottles
Sub-Saharan Africa, 2016 ⁽²⁶⁾	337	0.3 %	Immediately prior to transfusion, Becton Dickinson Bactec™ Peds Plus™ Aerobic/F culture vial and Gram staining
China, 2018 ⁽¹⁶⁾	28,711	0.22 %	12 hours, BacT/Alert aerobic & anaerobic bottles
USA, 2008 ⁽²⁷⁾	5,211	0.21% ^b	Day 1 or later, BacT/Alert aerobic bottles
	20,725	0.10% ^d	
Iran, 2016 ⁽²⁸⁾	2000	0.20 %	Thioglycolate sodium broth
Australia, 2011 ⁽²⁹⁾	302,386	0.18% ^e	Day 1 (24-hour), BacT/Alert aerobic & anaerobic bottles
Japan, 2009 ⁽³⁰⁾	21,786	0.17 % ^b	After at least 4 days, BacT/Alert aerobic and anaerobic culture bottles
	21,783	0.05% ^d	
Denmark, 2005 ⁽²⁾	22,165	0.15% ^c	Immediately post-production, BacT/Alert aerobic bottles
Germany, 2007 ⁽³¹⁾	52,243	0.07%	Day 0 (18-hour, BacT/Alert aerobic & anaerobic bottles
Wales, 2011 ⁽³²⁾	54,828	0.06%	Day 1, BacT/Alert aerobic & anaerobic bottles
China, 2009 ⁽³³⁾	8,000	0.06%	Day 0 (18-24 hours), BacT/Alert aerobic & anaerobic bottles
Germany, 2008 ⁽³⁴⁾	4,355	0.05%	Day 1, BacT/Alert aerobic & anaerobic bottles
New Zealand, 2013 ⁽³⁵⁾	59,461	0.04%	Day 2, BacT/Alert aerobic bottles
Ireland, 2008 ⁽³⁶⁾	43,230	0.03%	Day 2 (36-hour), BacT/Alert aerobic & anaerobic bottles
Norway, 2005 ⁽³⁷⁾	36,896	0.03% ^c	Day 1, BacT/Alert aerobic bottles only
USA, 2010 ⁽³⁸⁾	388,903	0.02%	Day 1 (24-36 hours), BacT/Alert aerobic & anaerobic bottles
USA, 2009 ⁽³⁹⁾	1,004,206	0.02%	BacT/Alert aerobic bottles
	781,936	0.02%	BacT/Alert aerobic bottles
Canada, 2011 ⁽¹⁷⁾	489,847	0.01%	Day 1-2 (24-48 hours), BacT/Alert aerobic bottles

a, did not use diversion pouch; **b**, prior to implementation of diversion pouch; **c**, not stated whether diversion was used; **d**, after sample diversion implementation; **e**, includes indeterminate results.

CONCLUSION:

No bacterial contamination was found in PC, contradicting a previous Iraqi report. The Reveos automated whole blood processing system improves bacterial control through enhanced collection and processing. Effective contamination control requires meticulous cleaning, antiseptic techniques, and tight infection control standards.

- **Conflict of Interest Statement: The authors declare no conflict of interest.**

- **Authors' Contributions: Zaid Abbas Kareem** contributed to the study design and data collection;

Haithem Ahmed A-Rubaie performed the statistical analysis; **Yaqoob Abdulwahed Saleh** drafted the manuscript. All authors reviewed and approved the final version.

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