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Growth and Distribution of Bacteria in Contaminated Whole Blood and Derived Blood Components

Ute Gravemann[a] Wiebke Handke[b] Torsten J. Schulze[a] Axel Seltsam[b]

[a]German Red Cross Blood Service NSTOB, Institute Springe, Springe, Germany; [b]Bavarian Red Cross Blood Service, Institute Nuremberg, Nuremberg, Germany

Keywords
Bacterial contamination · Whole blood · Buffy coat platelets

Abstract

Introduction: Bacterial contamination of blood products presumably occurs mainly during blood collection, starting from low initial concentrations of 10–100 colony-forming units (CFUs) per bag. As little is known about bacterial growth behavior and distribution in stored whole blood (WB) and WB-derived blood products, this study aims to provide data on this subject. ***Methods:*** WB units were inoculated with transfusion-relevant bacterial species (*Acinetobacter baumannii, Bacillus cereus, Escherichia coli, Klebsiella pneumoniae, Listeria monocytogenes, Pseudomonas fluorescens, Serratia marcescens, Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus dysgalactiae, Streptococcus pyogenes, Yersinia enterocolitica*; $n = 12$ for each species), stored for 22–24 h at room temperature, and then centrifuged for separation into plasma, red blood cells (RBCs), and buffy coats (BCs). The latter were pooled with 3 random donor BCs and one unit of PAS-E each to yield plasma-reduced platelet concentrates (PCs). Samples for bacterial colony counting were collected after WB storage and immediately after blood component production. Sterility testing in PCs ($n = 12$ for each species) was performed by bacterial culture after 7 days of storage. ***Results:*** Bacterial growth in WB varied remarkably between donations and species. Streptococcus species produced the highest titers in WB, whereas *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli,* and *Pseudomonas fluorescens* did not multiply. Centrifugation resulted in preferential accumulation of bacteria in BCs, with titers of up to 3.5×10^3 CFU/mL in BCs and up to $\leq 0.9 \times 10^3$ CFU/mL in BC-derived PCs. Overall, 72/144 PCs (50%) tested positive for bacteria after storage. Sterility test results were species-dependent, ranging from 12 of 12 PCs tested positive for *Streptococcus pyogenes* to 1 of 12 PCs positive for *Escherichia coli*. Bacterial contamination of RBC and plasma units was much less common and was associated with higher initial bacterial counts in the parent WB units. ***Conclusions:*** Bacterial growth in WB is species-dependent and varies greatly between donations. Preferential accumulation of bacteria in BCs during manufacturing is a critical determinant of the contamination risk of BC-derived pooled PCs.

Introduction

While rare, transfusion-transmitted bacterial infections (TTBIs) are regularly reported to national hemovigilance authorities [1, 2]. Although improvements in donor screening, testing and donation practices have significantly reduced the risk of TTBI, bacterial contamination of blood products still contributes to the most frequently reported serious adverse events. Bacterial contamination is presumed to occur mainly during the donation process. The initial number of bacterial contaminants, though too small to measure, is estimated to be around 10–100 colony-forming units (CFUs) per bag [3].

karger@karger.com
www.karger.com/tmh

Correspondence to:
Ute Gravemann, ute.gravemann@bsd-nstob.de

Due to the rarity of TTBIs and detection limit issues, little is known about the growth behavior and distribution of bacteria in stored whole-blood (WB) units and WB-derived blood products. Spiking experiments are needed to generate data on bacterial contamination of blood products. Most previous studies of bacterial growth in WB units are limited to single bacterial strains [4–6] or high spiking concentrations of 10^2 CFU/mL or higher [7–10], neither of which reflects the true strain diversity and low initial numbers of bacteria present at the time of blood donation.

Several factors influence the growth of bacteria in blood and blood components. Blood has antimicrobial properties because it contains different immune system components, such as WB cells (WBCs), platelets (PLTs), complement components, and antimicrobial peptides [10, 11]. Consequently, the proliferation of bacterial contaminants in WB may be not only strain-dependent but also donor-dependent [8]. Storage conditions such as storage time also influence bacterial growth. The risk for bacterial contamination is highest for platelet concentrates (PCs), the blood products with the most favorable storage conditions for bacterial growth.

Various mitigation strategies are used to minimize the risk of bacterial contamination, including diversion of the first millimeters of collected blood, bacterial testing, and pathogen inactivation (PI). Skin and donation site preparation/disinfection also plays a role in bacterial contamination of donated blood, as does technique of the phlebotomist.

PI systems are available for plasma and PLTs but not for red blood cells (RBCs) and WB. The latter systems are under development and will hopefully become available soon [12–14]. Knowledge about the growth and distribution of bacteria in WB and WB-derived blood components is important for risk assessments, bacterial selection for validation experiments, and adequate inactivation study design. Two different panels of transfusion-relevant bacteria reference strains (TRBRS) have been tested in international studies, shown to reproducibly grow in PCs or RBC units, and recommended by the World Health Organization (WHO) for use in studies of the microbial safety of blood products [15–17]. The aim of the present study was to determine the growth and distribution characteristics of twelve transfusion-relevant bacterial strains in WB units and WB-derived blood components with a special focus on PCs.

Materials and Methods

Blood Donors

Blood donors were selected based on our local donor selection standards. Only regular blood donors who met the requirements for blood donation and gave their informed consent approved by the Local Ethics Committee were included in the study.

WB Processing

WB (450–500 mL) was collected in bags containing 70 mL citrate phosphate dextrose anticoagulant solution (day 0) and stored overnight for 14–19 h at room temperature (22 ± 2°C) until inoculation and further processing. Using standard blood bank equipment under conditions equivalent to routine production, each WB unit was separated into components by centrifugation (4,000 *g* for 10 min at room temperature) using a Cryofuge 6,000i centrifuge (Heraeus). RBCs, buffy coat (BC), and plasma were prepared using a MacoSmart bag press (Macopharma). RBCs were manufactured with 110 mL of PAGGS-M additive solution and leukodepleted by filtration.

Plasma-reduced PCs were prepared from pools of four BCs according to the method of Eriksson and colleagues [18]. The pools were mixed with 280 mL of SSP+ PLT storage solution (MacoPharma), which is identical to PAS-E [19]. After soft spin centrifugation, PCs were isolated from suspension, leukoreduced by inline filtration, and transferred to standard storage bags (Haemonetics).

Spiking and Sampling

The study design is shown in Figure 1. We investigated the impact of WB processing on bacterial distribution and growth in blood components with a special focus on PCs. In PCs, we studied the contamination rate and bacterial load in fresh and stored products as well as the bacterial load at a time point when PI treatment may be performed. Each WB was tested for sterility before spiking using the BACT/ALERT system (bioMérieux, Nuertingen, Germany). In total, twelve different bacteria strains were used (Table 1). On the day after donation, each strain was inoculated in twelve WB units (inoculum size: approximately 10 CFU/bag). WB units were stored for additional 22–24 h at room temperature and then separated into blood components as described above. Study donor BCs were pooled with 3 random donor BCs and one unit of PAS-E to yield plasma-reduced PCs. Samples for bacterial testing were taken after 22–24 h of storage at room temperature (WB), immediately after preparation (RBCs, BCs, PCs, and plasma), and after 4–6 h of storage at 22°C (PCs), respectively, mimicking the time period after preparation that may be required to carry out PI treatment of PCs. After 7 days of storage, PC was sampled for sterility testing using the BACT/ALERT system (BioMérieux) and for determination of the bacterial concentration by plating.

Bacterial Strains

The bacteria used in this study are gram-negative and gram-positive species known to contaminate blood products. Most belong to the WHO International Repository of Platelet Transfusion-Relevant Reference Strains for PLTs and RBCs and were obtained from the Paul-Ehrlich-Institute [PEI] in Langen, Germany. *Acinetobacter baumannii* was obtained from the ATCC (American Type Culture Collection) (LGC Standards, Wesel, Germany) (Table 1). Each WHO reference strain has been shown to grow reproducibly in PCs or RBCs after minimal inoculation (10–100 CFU/unit) in multiple international laboratories [15–17].

Bacteria were cultured in tryptic soy broth and aliquots of 1 mL were frozen after the addition of 50% human serum albumin. The suspensions were stored at temperatures below −70°C, and the bacterial concentration of the stock was determined by plating assay.

Aliquots thawed at 37°C without further cultivation were used for spiking. The inoculum was prepared by serial dilutions of the stock in sodium chloride solution. Dilution factor and volume were calculated based on the known concentration of the stock. The dilution factor chosen resulted in an inoculum volume in the range of 100–1,000 µL. The spiking concentration was confirmed by plating the spiked volume of the inoculum.

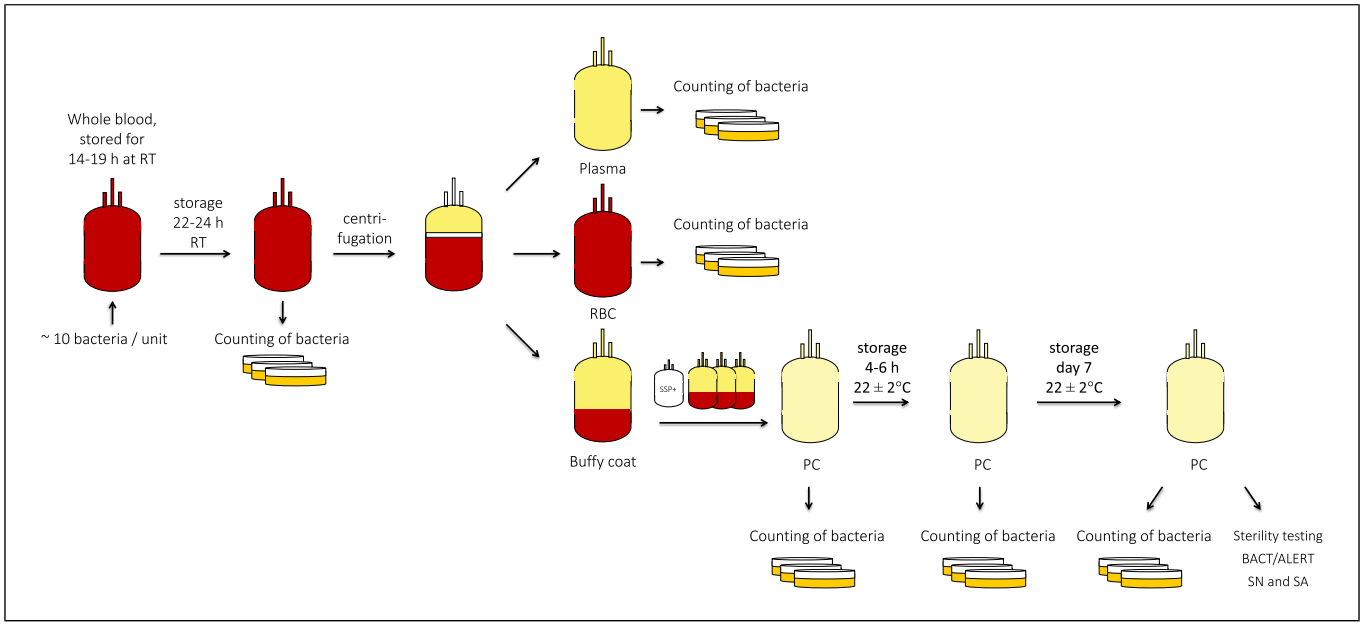

Fig. 1. Design of spiking experiments using 12 different bacterial species to spike 12 WB units per species.

Table 1. List and origin of bacterial species used in the study

Bacteria	Gram	Strain	Reference
Acinetobacter baumannii	Negative	ATCC-17961	Isolated from blood
Bacillus cereus	Positive	PEI-B-P-57	WHO PLT 2
Escherichia coli	Negative	PEI-B-P-19	WHO PLT 1
Klebsiella pneumoniae	Negative	PEI-B-P-08	WHO PLT 1
Listeria monocytogenes	Positive	PEI-A-199	WHO RBC
Pseudomonas fluorescens	Negative	PEI-B-P-77	WHO PLT 2, WHO RBC
Serratia marcescens	Negative	PEI-B-P-56	WHO PLT 2
Staphylococcus aureus	Positive	PEI-B-P-63	WHO PLT 2
Staphylococcus epidermidis	Positive	PEI-B-P-06	WHO PLT 1
Streptococcus dysgalactiae	Positive	PEI-B-P-71	WHO PLT 2
Streptococcus pyogenes	Positive	PEI-B-P-20	WHO PLT 1
Yersinia enterocolitica	Negative	PEI-A-105	WHO RBC

Bacteria belong to the First WHO International Repository of Platelet Strains (WHO PLT 1) [15], to the enlargement panel (WHO PLT 2) [16] or to the RBC reference strains (WHO RBC) [17].

Enumeration of Bacterial Cells and Automated Blood Culture

Bacterial concentrations were determined using standard plating methods and expressed as the number of viable CFUs per milliliter (CFU/mL). *Streptococcus dysgalactiae* was cultured on Columbia blood agar (Merck, Darmstadt, Germany). All other bacterial species were plated on CASO agar (Merck, Darmstadt, Germany). Samples with an expected count of >100 CFU/mL were inoculated using a spiral plater. Serial ten-fold dilutions of each sample were transferred to agar plates (2 plates/dilution, 50 µL/plate). Samples with an expected count of <100 CFU/mL were cultured using the pour plate method (2 plates/sample, 0.5–1 mL/plate). All agar plates were incubated at 37°C (except for *Serratia marcescens*, which was incubated at 30°C) for 1–2 days until colonies could be counted.

PC samples collected on day 7 of storage were tested for sterility with the BACT/ALERT 3D 60 Microbial Detection System (bioMérieux, Nuertingen, Germany). Briefly, after the collection of PC samples (20 mL each) under sterile conditions on day 7 of storage, half (10 mL) was injected into a BACT/ALERT SA (aerobic) and SN (anaerobic) culture bottle, respectively, and cultivated under automated blood culture conditions at 36°C in the BACT/ALERT incubator (bioMérieux) for 7 days.

Results

WB was spiked with 3–15 CFU per bag (corresponding to approximately 0.006–0.03 CFU/mL) and stored for 22–24 h before sampling (Table 2). Bacterial growth in WB varied remarkably between donations and bacterial species. *Streptococcus pyogenes* and *Serratia marcescens* exhibited the most rapid growth in WB, whereas

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Table 2. Bacterial growth and distribution in blood products derived from spiked WB (n = 12 per species)

Species	Spike, CFU/bag	Titer, CFU/mL, mean, min–max							PC BacT/Alert day 7[a]
		WB 22–24 h	RBC	Plasma	BC	PC after separation	PC 4–6 h	PC day 7*	
Klebsiella pneumoniae	9–15	13.3 0–70	0.0 0	0.2 0–1.5	34.6 0–278	4.6 0–43	77.8 0–823	1.5×10^9 $0.8–1.8 \times 10^9$	8/12 pos
Streptococcus pyogenes	7–9	252.9 25–539	48.1 0–202	162.4 0–695	1,085 163–3,506	225 18–906	3.9×10^3 2.5×10^2–2.6×10^4	1.3×10^6 $0.2–3.6 \times 10^6$	12/12 pos
Staphylococcus aureus	6–7.5	3.5 0–20	0.1 0–1	0.0 0	0.9 0–4	0.0 0	0.0 0	8.6×10^7 $3.6 \times 10^6 – 1.6 \times 10^8$	6/12 pos
Streptococcus dysgalactiae	3–9	6.3 0–31	0.0 0	0.0 0–0.5	32.7 0–161	0.3 0–1	2.0 0–7	1.0×10^8 $0.8–1.5 \times 10^8$	11/12 pos
Escherichia coli	8–14	2.2 0–21	0.0 0	0.2 0–2.5	0.6 0–6	0.0 0	0.0 0	3.8×10^8 n.a	1/12 pos
Staphylococcus epidermidis	7–10	0.0 0	0.0 0	0.0 0	0.0 0	0.0 0	0.0 0	3.7×10^7 n.a	1/12 pos
Pseudomonas fluorescens	3–8	10.8 0–42	1.5 0–13	0.0 0	71.7 2–284	2.8 0–11	79.7 0–396	1.6×10^9 $0.4–3.3 \times 10^9$	12/12 pos
Yersinia enterocolitica	8–12	0.1 0	0.1 0–2	0.1 0–1	0.0 0	0.0 0	0.0 0	n.a	0/12 pos
Serratia marcescens	15	40.1 0–360	0.2 0–2	3.2 0–38	95.0 0–864	2.3 0–10	63.1 $0–2.5 \times 10^2$	0.9×10^{10} $0.2–3.0 \times 10^{10}$	9/12 pos
Acinetobacter baumannii	7–11	0.0 0	0.0 0	0.0 0	0.4 0–2.5	0.1 0–1	0.0 0	Titer could not be determined[b]	2/12 pos
Listeria monocytogenes	9–15	0.0 0–0.5	0.0 0	0.0 0	0.0 0	0.0 0–1	0.0 0	1.3×10^7 $0.1–2.8 \times 10^7$	4/12 pos
Bacillus cereus	2–8	23.2 0–124	0.0 0	0.3 0–1	53.4 0–392	0.4 0–3	23.2 0–44	4.1×10^6 $2.7–7.1 \times 10^6$	6/12 pos

*Titers determined in PCs tested positive in BACT/ALERT. [a]pos = Samples of PCs tested positive in BACT/ALERT 3D 60 Microbial Detection System (Biomerieux). [b]In single PCs, the titer could not be determined accurately because the dilution factors were inappropriate and the number of colonies was too low or too high to be counted.

Staphylococcus aureus, *Escherichia coli,* and *Pseudomonas fluorescens* did not multiply but remained detectable in some spiked units. In WB units spiked with *Staphylococcus epidermidis*, *Yersinia enterocolitica*, or *Acinetobacter baumannii* no bacteria were detected after storage prior to component preparation.

Bacteria preferentially accumulated in BCs during separation, reaching titers of up to 3.5×10^3 CFU/mL in BCs and up to 0.9×10^3 CFU/mL in BC-derived PCs immediately after preparation. Bacterial contamination of RBC and plasma units was much less frequently detected and was associated with higher initial bacterial counts in the parent WB units. Maximum bacterial counts were 202 CFU/mL for RBCs and 695 CFU/mL for plasma.

Bacterial counts in PCs directly after preparation were lower than those in the contaminated BCs. Count reduction is probably due to a combination of different effects like leukodepletion by filtration and the dilution effect obtained by BC pooling and addition of storage solution. However, bacteria started to proliferate during storage for 4–6 h at room temperature. The highest bacterial counts of up to 2.6×10^4 CFU/mL were obtained in PCs for *Streptococcus pyogenes*. Titers above 1×10^2 CFU/mL were detected for *Klebsiella pneumoniae* (up to 8.2×10^2 CFU/mL), *Pseudomonas fluorescens* (up to 4×10^2 CFU/mL), and *Serratia marcescens* (up to 2.5×10^2 CFU/mL), while the titers in PCs spiked with the other bacteria species were lower or zero at this point of time.

Overall, 72 out of 144 PCs tested positive for bacteria after 7 days of storage. Bacterial counts on day 7 in PCs that tested positive in BACT/ALERT were variable and ranged from 10^6 – 10^{10} CFU/mL (Table 2). PC contamination rates varied depending on the type of species used for WB spiking: 12/12 PCs tested positive for *Streptococcus pyogenes* and *Pseudomonas fluorescens*, while only 1/12 tested positive for *Escherichia coli* and *Staphylococcus epidermidis*, and none tested positive for *Yersinia enterocolitica*.

Discussion

This systematic study of bacterial growth behavior and distribution in WB and WB-derived blood components demonstrated that very low-level inoculation with various transfusion-relevant bacterial species can cause bacterial contamination in all three blood products studied. Bacterial growth differed between bacterial species and blood donors. Contaminating bacteria preferentially accumulated in BCs during WB centrifugation and were transferred to the PC during further preparation, while accumulation of bacteria into RBC and plasma units was associated with higher initial bacterial counts in the parent WB units. Even if risk reduction measures such as skin disinfection and diversion of the first 10–50 mL of donated blood are taken, transient or commensal bacteria on the donor's skin may enter the blood collection bag via venipuncture during the WB donation process. During storage, bacteria in blood components may grow to levels capable of inducing acute sepsis and life-threatening or fatal reactions in the recipient [20]. Bacterial contamination can be missed in early testing due to low bacterial concentrations. By interpolating from counts determined at a later time point of storage, Benjamin and Wagner estimated the number of contaminating bacteria to be less than 62 CFU/unit 12–36 h after donation [21]. In the current study, we mimicked routine transfusion conditions by contaminating WB units with low levels of transfusion-relevant bacteria thought to be capable of growing in blood products like PCs and RBCs [15–17]. Our findings show that these bacteria were also able to survive and grow in the WB units, suggesting that they may also be suited as reference strains for WB spiking studies.

Several bacterial spiking studies in WB have been performed in the past to shed light on different aspects of transfusion safety. Some investigated the effects of leukocyte depletion [22] or different storage conditions [4, 7] on bacterial growth. Leukocyte depletion by filtration was shown to significantly reduce bacterial counts by partially removing bacterial contaminants [8, 22–24]. Prolonged storage of WB in the presence of donor WBCs was shown to delay the growth of some bacteria [4]. Taha et al. [9] demonstrated that overnight holding of bacterially inoculated WB eliminated some bacterial strains, while others were able to survive and proliferate. Similar results were achieved by Siblini et al. [24] who also investigated the bactericidal properties of WB. Our results are consistent with data from other studies demonstrating differences in the growth of different bacterial strains or even isolates from the same species [22]. The donor-dependent differences in bacterial growth observed in our experiments have also been described previously and may be de due to differences in the presence and activity of immune factors between donors.

In our study, we observed no or limited growth of *Yersinia enterocolitica and Staphylococcus epidermidis*, respectively, in WB. This is in accordance with the findings of Siblini and Taha who both showed a reduced viability of *Yersinia enterocolitica* in WB during room temperature storage [9, 25]. We cannot conclude from our investigations whether bactericidal or other factors were responsible for the limited ability to grow. However, it was reported for *Staphylococcus epidermidis* that the clearance of bacteria from WB is dependent on the inoculum size [11]. While small inocula of *Staphylococcus epidermidis* were cleared rapidly from WB, it took up to 24 h of storage in WB to eliminate larger inocula. This might explain why we could not detect growth of

Staphylococcus epidermidis in our study using low titer spiking concentrations, whereas others reported growth of this species. The fact that one of the PCs spiked with *Staphylococcus epidermidis* tested positive at the end of storage, although bacteria could not be detected in the underlying WB unit, is most probably based on a sampling error due to a low bacterial count in the WB. However, accidental contamination during inoculation of culture bottles cannot be excluded in this case, as identification methods were not applied.

Consistent with our findings, Taha et al. [9] and Mohr et al. [8] also described the segregation of bacteria into specific blood components. Because the density ranges of many bacteria overlap with those of RBCs and PLTs, centrifugation of WB results in the preferential accumulation of bacteria in BCs under routine blood banking conditions [25]. Taha et al. [9] investigated the distribution of bacteria through BC-based PC production and subsequent PC storage after spiking and overnight storage of WB. In this study, bacteria segregated preferably toward the cellular fractions (RBCs and BC) compared to plasma. Mohr et al. looked at the bacteria concentration in WB after storage for 8 h at 22°C and at different process steps during the production and storage of pooled PCs. While some of the investigated strains did not grow or were even killed during storage, the concentrations of bacteria in WB after 8 h and in the freshly prepared BC were in a similar order of magnitude. The main differences between these studies and our investigation are the higher spiking concentration (10^2–10^4 CFU/mL) used and the correspondingly higher titers achieved after WB storage. However, several other factors including the centrifugation time and speed, the anticoagulant chemistry, the bacterial load, and the geometry of the blood container also influence the separation of blood constituents and contaminants [26]. The less rigid centrifugation conditions usually used to recover PLTs from pooled BCs not only support the separation of the PLTs from RBCs but also seem to cause bacteria to accumulate with the PLTs in the supernatant used for BC-derived PC preparation. However, clinical data and our spiking experiments show that this separation effect is not perfect. Hemovigilance reports indicate that TTBI rates are highest for PCs and that TTBIs are less frequently caused by RBC and plasma transfusions [2, 27]. In the present study, bacterial contamination was also detected in RBCs and plasma and was associated with bacterial strains able to grow rapidly in WB. Clinically relevant concentrations of the fast-growing strain *Streptococcus pyogenes* were detected in all blood components examined here.

Bacterial growth in blood components is mainly determined by storage conditions. While RBCs are stored at 1–6°C and plasma is usually kept frozen during storage, PCs are stored at 20–24°C under continuous agitation for gas exchange to preserve function and survival. These storage conditions make PCs an excellent growth medium for many bacteria. Rapid-growing strains, such as *S. aureus*, *E. coli,* and *S. pyogenes* can reach clinically relevant counts in PCs within a few hours of storage [28]. Nevertheless, psychrophilic bacteria like *Y. enterocolitica* or *S. marcescens* [17, 29] can grow in RBCs at 4°C or in refrigerated plasma, stored for several days to ensure rapid availability for emergency purposes [30]. The high rates of PCs testing positive for bacteria after 7 days of storage in the present study confirm that PCs are particularly susceptible to bacterial contamination. With our experimental approach, we tried to define factors that influence the bacterial contamination of blood components during WB manufacturing. Our findings and those from other studies suggest that the distribution of bacteria in WB critically determines the contamination rate of blood components [4].

Different strategies to control bacterial risk exist, and most focus special attention on PCs. Bacterial testing poses significant logistical challenges, so many countries have decided to implement PI for PLTs. In the future, universal PI may be introduced as soon as PI technologies for RBCs or WB are approved and become commercially available. Production processes must be adapted for the implementation of PI systems, and time frames for PI procedures must be defined to ensure that processing does not exceed the inactivation capacity of the system. Whereas viruses or parasites do not replicate in blood products, bacteria are able to grow to high titers [31]. Inactivation of bacteria is a challenging task, and incomplete inactivation allows residual bacteria to recover and grow again. Several studies of different PI systems have revealed that fast-growing bacterial strains are not completely inactivated if the bacterial count at the time of inactivation exceeds the inactivation capacity of the PI system [3, 32–34]. Our study provides information on relevant bacterial strains and conditions that can be used to challenge and validate PI systems. In addition, the results of this study on the occurrence of bacterial contamination in blood components in the early post-preparation period may be helpful for planning infection risk assessments and defining process parameters to optimize the efficacy of PI processes. Our results show that bacterial contamination may reach titers of up to 0.5×10^3 CFU/mL in WB after 24 h of storage, even though initial contamination was very low. Therefore, a bacteria inactivation capacity of more than three $\log_{10}$ reduction steps may be required for a PI system for WB to ensure sterility of the WB and the corresponding blood products.

There are some limitations to this study. First, WB units were spiked on the day after donation and not immediately for logistic reasons. Some immunologic factors like the complement system [35] and WBC

activity may have been diminished during storage. Second, the design of our study does not cover the scenario of secondary contamination with environmental bacteria recently reported in the USA for PCs contaminated with *Acinetobacter baumannii* by storage container defects [36]. Third, production parameters like storage time and temperature, centrifugation conditions, or the timing of leukocyte depletion may have had an additional impact on bacterial distribution and growth [4, 8, 11, 22]. Thus, the bacterial concentrations observed in this study may differ slightly from those detected under routine conditions. Methods of manufacturing blood components can differ between production sites. Automated processes like apheresis were not investigated in this study. Therefore, the present study is only representative of bacterial growth and distribution under the manufacturing conditions used at our blood center. However, these production processes are similar to those used at blood services in many other European countries. Finally, differences in the growth of different bacterial strains or even isolates from the same species were shown in several studies [10, 11, 22, 37]. The results of our study therefore are limited to the strains and species described herein and cannot be used to extrapolate the growth of other isolates.

In conclusion, TTBIs still pose a serious risk for transfusion recipients. Although there are several risk mitigation strategies, like bacterial testing or PI, none of them completely eliminates the risk for bacterial contamination. Reasons for this include sampling errors in bacterial testing [38–40] or secondary contamination during storage [41, 42]. However, PI technologies have been shown to significantly improve the bacterial safety of blood transfusion, particularly in countries using 100% pathogen-reduced PCs [43]. The present study adds to the knowledge about risk factors for bacterial contamination of blood components and provides information on critical factors to consider when validating and implementing bacterial risk reduction strategies.

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Statement of Ethics

Samples used in this study were obtained as part of routine blood donation. Written informed consent was obtained from participants prior to the study. Use of these samples for research purposes did not require ethical approval in accordance with local/national guidelines.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

Ute Gravemann, Wiebke Handke, and Axel Seltsam designed the study. Ute Gravemann, Torsten Schulze, and Axel Seltsam analyzed the data and wrote the manuscript. All authors read and approved the final manuscript.

Data Availability Statement

All data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author.

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