Effect of Safety Measures on Bacterial Contamination Rates of Blood Components in Germany

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Keywords

Bacterial contamination · Blood components ·
Contamination rate · Quality control

Summary

Requirements for bacterial testing of blood components on a defined quantity as part of routine quality control were introduced in Germany by the National Advisory Committee Blood of the German Federal Ministry of Health in 1997. The philosophy was to establish standardized methods for bacterial testing. Numerous measures to reduce the risk of bacterial contamination were implemented into the blood donation and manufacturing processes between 1999 and 2002. German Blood establishments performed culture-based bacterial testing on random samples of platelet concentrates (PCs), red blood cells (RBCs) and fresh frozen plasma (FFP) and reported data out of the production periods 1998, 2001 and 2005/2006. While the bacterial contamination rate of apheresis PCs remained nearly unchanged, it decreased by 70% for pooled PCs to a rate of 0.158% in the last observation period. Leukocyte-depleted RBCs with diversion of the initial blood volume showed a contamination rate of 0.029% which is significantly lower than that of RBCs without leukocyte depletion and diversion (0.157%). The contamination rate of plasma decreased by 80%. Preventive measures resulted in a significant reduction of bacterial contamination of blood components. Long-term monitoring with standardized methods for bacteria testing supports evaluation of the cumulative effect of contamination reducing measures.

Schlüsselwörter

Bakterielle Kontamination · Blutkomponenten ·
Kontaminationsrate · Qualitätskontrolle

Zusammenfassung

In Deutschland wurde 1997 mit einem Votum des Arbeitskreises Blut die Prüfung von Blutkomponenten auf bakterielle Kontamination an einer definierten Stichprobe als Bestandteil der Qualitätskontrolle eingeführt. Ziel war die Etablierung standardisierter Methoden zur Testung auf Bakterien. Zwischen 1999 und 2002 wurde eine Reihe von Maßnahmen in die Prozesse bei der Blutspende und Komponentenherstellung integriert, um das Risiko der bakteriellen Kontamination zu reduzieren. Die Blutspendedienste führten an einer Zufallsstichprobe von Thrombozytenkonzentraten, Erythrozytenkonzentraten und Plasmen eine Testung auf Bakterien mittels Kulturverfahren durch und berichteten ihre Ergebnisse aus den Produktionszeiträumen 1998, 2001 und 2005/2006. Während die bakterielle Kontaminationsrate bei Apherese-Thrombozytenkonzentraten nahezu unverändert blieb, sank die Rate bei Pool-Thrombozytenkonzentraten um 70% auf 0,158% im letzten Beobachtungszeitraum. Leukozytendepletierte Erythrozytenkonzentrate mit Abtrennung des initialen Blutvolumens bei der Blutspende zeigten mit einer Kontaminationsrate von 0,029% eine signifikante Abnahme des Bakterieneintrags gegenüber Konzentraten ohne Leukozytendepletion und Diversion mit einer Rate von 0,157%. Die Kontaminationsrate von Plasma nahm um 80% ab. Präventive Maßnahmen führten zu einer signifikanten Reduktion des Bakterieneintrags in Blutkomponenten. Die Langzeitbeobachtung mit standardisierten Testmethoden ermöglicht die Bewertung des kumulativen Effekts kontaminationsreduzierender Maßnahmen.

Introduction

Transfusion-associated bacterial infection still is a serious risk of morbidity and mortality related to blood transfusion. In the past decades various measures have been implemented in blood establishments to reduce this hazard and to improve bacterial safety of blood components.

For the purpose of donor screening, separately obtained blood samples can be tested by serological and molecular biological techniques. The resulting progress in terms of reduced risk of viral transmission, in particular, contrasts with a still relatively high risk of transfusion reactions due to bacteria in blood components.

In a first retrospective survey performed in 1997, data on bacterial contamination of blood components in Germany during the production period 1995/1996 were obtained [1]. The participating blood establishments employed highly variable methods in terms of time of sampling, sample volume, incubation temperature and incubation time. Both conventional culture methods with liquid and solid media and automated methods were used. In 1997 the working party on bacteria safety in transfusion medicine of the National Advisory Committee Blood of the German Federal Ministry of Health (Arbeitskreis Blut) introduced national guidelines to monitor bacterial contamination of blood components as part of routine quality control in all blood establishments [2].

The intention of these guidelines was to establish standardized methods for bacterial testing to gain data on a broad basis for observation and analysis of contamination rates over long time periods. In this article, we present an overview on the results of bacterial testing of blood components in German blood establishments in the production periods 1998, 2001 and 2005/2006, and focus on the influence of precautionary measures implemented to reduce bacterial contamination.

Contamination Reduction Measures

General and specific measures to prevent bacterial contamination were introduced in Germany between 1999 and 2002. Integrity testing of sterile tubing welds to identify leakages is mandatory since 1999 [3]. All tube connections have to be checked by a tube stripping device test procedure as part of in-process control. Details on careful donor selection and on skin cleansing procedures were introduced by revised guidelines on the collection of blood and blood components in 2000 [4]. The donor questionnaire was amended by questions on diarrhea, dental treatments, abscesses and osteomyelitis as risk factors for asymptomatic bacteremia. Skin disinfection has to be performed by double application of a licensed and certified antiseptic and a defined exposure time. Starting from October 2001 leukocyte depletion of cel-

Table 1. Contamination rates of PCs by bacterial testing as part of routine quality control

Type of PC	Production period	Number tested	Contamination rate (confirmed positive) in %
APCs	1998	4,523	0.155
APCs	2001	8,302	0.157
APCs	2005/2006	11,452	0.114
PPCs	2001	3,947	0.507
PPCs	2006/2006	8,850	0.158
Data published in: Vox Sang 2006;90:177–182 and Vox Sang 2011;100:356–366.			

lular blood components was introduced as a general requirement [5].

Diversion of the initial blood volume prior to blood collection was implemented in 2002 [6].

Bacterial Contamination of Platelet Concentrates

Results of bacterial testing of platelet concentrates (PCs) at the end of shelf life derived from single platelet concentrates (SPCs) prepared from whole blood, pooled platelet concentrates (PPCs) prepared from four buffy coats and from apheresis platelet concentrates (APCs). The authors analyzed whether the contamination rates of PCs in the 3 surveys were influenced by production methods such as apheresis, pooling, leukocyte depletion and diversion [7, 8].

APCs showed a nearly constant contamination rate over all study periods (table 1). The authors concluded that diversion and leukocyte depletion seem to have no significant impact on the frequency of bacterial contamination of APCs.

Leukocyte depletion of cellular blood components as a general requirement induced a change in manufacturing processes of PCs from whole blood-derived single-unit PCs to buffy coat-derived and leukocyte-depleted PCs as one therapeutic unit. While 0.507% out of 3,947 PPCs tested in 2001 turned out to be contaminated, 8,850 PPCs tested in 2005/2006 showed a contamination rate of 0.158% (table 1).

Diversion was not yet implemented in 2001 but in 2005/2006. Comparison of contamination rates of PPCs without and with diversion showed a 70% reduction in bacterial contamination and confirms results of a series of studies on the effect of diversion on PCs [9–11].

Since PCs are thought to be at considerably higher risk of contamination than RBC concentrates, in many countries bacterial testing of PCs was implemented. In most cases, automated culture methods such as BacT/ALERT (bioMérieux, Nürtingen, Germany) are used [12].

Comparability of data is rendered difficult by differences in the test methods used: time of sampling at the beginning or end of shelf life, sample volume, aerobic and anaerobic cul-

Table 2. Contamination rates of RBC concentrates by bacterial testing as part of routine quality control

Type of RBC concentrate	Production period	Number tested	Contamination rate (confirmed positive) in %
RBC concentrates not leukocyte-depleted	1998, 2001	28,218	0.167
RBC concentrates leukocyte-depleted, without diversion	2001	27,336	0.087
RBC concentrates leukocyte-depleted, with diversion	2005/2006	54,845	0.029
Data published in: Vox Sang 2011;100:356–366.			

ture versus aerobic culture only, incubation period and last but not least statistical sample size.

In several countries pre-release screening of PCs with automated culture systems was implemented to prevent issue and transfusion of contaminated platelets [9, 12–18]. Sampling for platelet surveillance cultures is performed soon after production to dispatch only PCs with negative cultures.

In Germany one study was conducted that compared contamination rates of APCs and PPCs derived from four buffy coats with sampling at the beginning of shelf life [19]. Manufacturing and testing conditions were similar to those for PCs in the 2005/2006 survey. Consistent with the survey data, contamination rates of APCs and PPCs did not differ significantly. The rate of confirmed positive PPCs with an early postproduction sampling was significantly lower than that of the 2005/2006 survey (0.06% vs. 0.157%). Sampling at the end of shelf life probably detects bacteria that were missed at the beginning of the shelf life period because of their low number in the PCs. Results are consistent with data from studies performed with re-testing of outdated PCs to determine the effectiveness of the screening for bacteria. Murphy et al. [20] reported confirmed positive results with a BacT/ALERT screening on early sampling on 0.08% of PCs. At a follow-up test on day 4, 0.12% of PCs were positive, and on testing after expiry date 0.22% of PCs showed to be contaminated. Pearce et al. [21] published comparable data, with contamination rates of 0.06% detected during initial testing of PCs and 0.09% detected by testing of outdated PCs. The authors concluded that the sensitivity of bacterial screening by early sampling is $\leq 40\%$ because of the initially low bacterial load. As a consequence, septic transfusion reactions have been reported from PCs with initially negative screening results [13–16, 18, 19].

As described before, the purpose of sampling shortly after the date of manufacture is to detect contamination prior to transfusion. Random bacterial testing at the end of the storage period in Germany serves as a quality control method for the collection and production processes. Production methods for PCs vary from country to country and include apheresis, production of single-donor concentrates from whole blood, production of pooled concentrates from 4 to 6 single-donor concentrates in a functionally closed system with sterile tubing, bedside pooling using plug-in connections, and production from 4–6 pooled buffy coats with plasma or storage solu-

tion [22]. Thus, comparability of contamination rates on an international basis is limited, not only because of different test methods but also because of different manufacturing methods for PCs. The same applies to shelf life of PCs, which varies between 72 h to 7 days [12].

Bacterial Contamination of Red Blood Cell Concentrates

German blood establishments performed culture-based testing on a random sample of 110,399 RBC concentrates out of a total production of 16,338,293 RBC concentrates in the periods 1998, 2001 and 2005/2006 [7, 8]. To investigate the potential influence of leukocyte depletion, authors combined test results of RBC concentrates with and without leukocyte depletion (table 2). Compared to unfiltered RBC concentrates with a contamination rate of 0.167%, leukocyte depletion of RBC concentrates resulted in a significant decrease to 0.087%. Implementation of diversion of the initial blood flow away from the blood collection bag resulted in a further decrease of the contamination rate of RBC concentrates to 0.029% in the 2005/2006 survey. Data indicate a favorable effect of leukocyte removal. Studies have shown that bacteria are phagocytosed by leukocytes in the whole blood storage phase and can be eliminated by leukocyte filtration [23–26].

Similar to other studies, German data showed that diversion of the initial blood flow contributes to reducing bacterial contamination of whole blood donations by skin surface bacterial [27–30]. Data on results of bacteria monitoring of time-expired RBCs as performed in Germany were only available from the National Blood Service in England. Six confirmed positives out of 8,585 tested RBC concentrates were reported in the years 2002–2005 after implementation of leukocyte depletion and diversion, resulting in a contamination rate of 0.065% [12].

Bacterial Contamination of Plasma

Progressive reduction of the contamination rate of fresh frozen plasma (FFP) could be achieved from 1998 to 2005/2006. The combination of efforts resulted in a rate of 0.019% con-

firmed contaminated FFP units which means a reduction of 80% when compared with the rate of 0.100% in 1998. In contrast to cellular blood components, data from studies on frequency of bacteria in FFP are not available. Storage below freezing point and a short interval between thawing and transfusion are unfavorable conditions for survival and proliferation of bacterial [31]. Case reports of septic reactions associated with transfusion of plasma indicate that water baths can cause microbial entry during thawing process [32, 33].

Bacterial Species

Determination of bacterial species detected in the 3 surveys showed that organisms predominantly belong to the transient and resident skin flora. Gram-positive bacteria from the common skin flora also presented the majority of species in published studies. Fast growing Gram-negative bacteria known to include some of the most clinically significant organisms were not isolated from blood component in the three surveys.

The authors contribute this to the fact that these bacteria rarely occur as contaminants of blood components and therefore were not detected in the sample.

Gram-negative organisms tend to cause more severe reactions, due to presence of endotoxins often produced by such organisms [34, 35]. However, all detected bacterial species may induce transfusion-associated complications depending on the bacterial load and on the patient's condition.

Conclusions

Data on routine quality control testing on a random sample of blood components according to specified guidelines provide a basis for monitoring bacterial contamination over long time periods. As previously described, numerous measures were introduced in the collection and manufacturing of blood components between 1997 and 2002 to prevent bacterial contamination. As no head-to-head comparison was possible, contamination rates of the different production periods and of different products were used to estimate the effect of leukocyte depletion for RBC concentrates or PCs and of diversion for RBC concentrates, PCs or FFP. Preventive measures resulted in a significant reduction of bacterial contamination of blood components. Nevertheless, transfusion of bacterially contaminated components, especially platelets, is an ongoing risk. The procedures currently used in obtaining and manufacturing blood components seem to offer no further potential for improvement.

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Disclosure Statement

The authors declared no conflict of interest.

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