

# Microbial contamination risk in hematopoietic stem cell products: retrospective analysis of 1996–2016 data

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**Abstract**

Quality assurance and safety of hematopoietic stem cells (HSC) with special emphasis on bacterial and fungal contamination is the prerequisite for any transplantation procedure. The aim was to determine the incidence rate of such contamination during processing of transplantation material with regard to HSC source: peripheral blood stem cell (PBSC), bone marrow (BM), or cord blood (CB). Analysis involved autologous and allogenic products dedicated for patients and comprised in all 4135 donations, including 112 BM (2.70%), 3787 PBSC (91.60%), and 236 CB (5.70%) processed in cell bank over the period 1996–2016. Aerobic and anaerobic contamination was determined.

Analysis of the 20-year data revealed 42 contaminated products: 25 PBSC (0.66% of tested units) and 17 CB (7.20% of tested units). No microbial contamination of BM products was detected. Overall percentage of contaminated products was 1.01%, mostly with *Staphylococcus epidermidis* (61.36%). Bacterial contamination rate at cell bank is relatively low and processing in a closed system does not seem as crucial as might be expected. This is particularly true for BM components. Equally important are evaluation of donor's medical status and condition of the puncture site for collection of source material. Implementation of appropriate sample collection procedures should help minimize the risk of false-positive results due to environmental contamination.

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**Keywords:**

microbial contamination, microbiological control, hematopoietic stem cell, cell transplantation

## Introduction

Quality and safety of hematopoietic stem cells (HSC) is the prerequisite of any successful transplantation. Ready-for-use HSC transplantation material is the outcome of several preparation steps including the following: source material collection, sample collection, centrifugation, removal of unwanted plasma and cells, cryopreservation, storage, thawing, etc. Each step of stem cells collection and processing is burdened with a risk of product contamination – especially bacterial – and the overall risk rises with introduction of each additional activity [1, 2].

Discussions on the safety of substances of human origin are typically focused on viral infections, although bacterial contamination is of significance as it occurs more frequently and bacteria are observed to have developed resistance [3].

Safeguard against microbial contamination of HSC products is the subject of numerous studies which focus attention on bacterial detection methods, immediate access to test results, sensitivity of tests, sample collection techniques, and the effect of cryoprotectants [4].

It is therefore important for any stem cell bank involved in collection and preparation of transplantation material to follow good practice guidelines and regulations [5, 6, 7] already in force for several years both in European countries and the United States [8]. In many cases, HSC processing requires open system which increases the risk of bacterial contamination. The risk can be reduced by extending the closed system over the entire preparation process. Special kits were

therefore developed for all activities to be performed within a closed system and cryoprotectant pre-filled syringes were used to secure sterile connections.

Identification of bacterial contamination especially in autologous transplantation material leads to product discard and either shortage of such material or higher risk of unsuccessful clinical outcome. For most allogeneic transplantations, the infusion of stem cells is performed immediately after collection so the risk of bacterial contamination is higher because at that moment the microbial status of the HSC product is yet unknown (test results become available after several days).

Infections in immunocompromised patients are a particular challenge. Apart from HSC donor, infection from another possible source is transmission of viral infection from one cryopreserved component to another. Certain procedures have been launched to eliminate such infection routes which – apart from HSC donor testing – include implementation of storage methods that contribute to reduction of infection risk [9, 10]. Appropriate procedures of HSC collection and processing as well as diagnostic testing are crucial for prevention of bacterial contamination.

Up to date observations and literature reports reveal a variety of infections that can be transmitted with transplantation material. Diagnosis is complicated by factors such as type of bacteria (aerobic/anaerobic), low abundance of specimen, or slow bacterial growth. It is therefore essential for quality control to provide adequate culture media [11, 12]. Prevention of bacterial and fungal contamination is the fundamental task of any cell bank processing transplantation material.

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The study aim was to estimate the rate of bacterial and fungal contamination during processing of transplantation material in our cell bank over a period of 20 years with regard to type of HSC source, that is, peripheral blood stem cell (PBSC), bone marrow (BM), or umbilical cord blood (CB).

## Materials and Methods

We performed an analysis of HSC products dedicated for clinical use and processed from PBSC, BM, and CB donations collected in the period 1996–2016. PBSC and BM materials were collected at the Institute of Hematology and Transfusion Medicine (IHTM), whereas CB was obtained from gynecological-obstetrics hospitals. All products were processed at the IHTM cell bank. The analysis also included allogenic HSC products received from other centers, subjected to preparation at IHTM and dedicated for the IHTM patients (491 products in the years 2012–2016).

A total of 4135 HSC products were analyzed, including 112 BM (2.70%), 3787 PBSC (91.60%), and 236 CB (5.70%), all of which were prepared at the IHTM cell bank over the 1996–2016 period. Autologous products accounted for 79% of all studied PBSC grafts and 66% of BM products.

BM was collected in operating room conditions following puncture-site disinfection according to surgical procedures. It was then subjected to filtration and further processing with Cobe Spectra (Gambro) and Optia (Terumo BCT) cell separators.

PBSCs were collected using CS-3000 (Baxter), Cobe Spectra (Gambro), and Optia (Terumo BCT) cell separators with a two-step disinfection method of venipuncture site or using vascular catheter.

CB was collected following disinfection with standard agents routinely used at gynecological and obstetric wards.

PBSC and BM collected at IHTM were subjected to preparation within an hour of collection. Allogenic products from other centers were processed within 48 hours of collection.

Once the preparation process was completed and the cryoprotectant added, samples were collected for bacteriological testing of BM and PBSC destined for long-term storage. Until the end of 2014, samples from PBSC and BM components were collected only after processing was completed. Since 2015, samples were collected both after collection (post-collection samples) and after processing. CB samples were collected both before and after processing. The final DMSO concentration in the product was 10%.

Each sample was collected approximately 1 hour of collection directly from the product. Samples of 1 ml for each culture bottle (aerobic and anaerobic) were collected from drain segments and directly inoculated in a laminar air flow chamber. Aerobic and anaerobic bacteria were tested at room temperature and 35°C using various media. Two (2) trypticase-soybean (TSB-T) and one (1) Schaedler + Vit K3 (BioMerieux) media were used for PBSC and BM; Bactec Peds Plus/F and Bactec Lytic/10/Anaerobic/F (Becton-Dickinson) were used for CB. Samples were incubated for 7 days. In the years 1996–2002, PBSC and BM samples were inoculated on home-made Trypticase-soybean and on Schaedler media in the Laboratory of Microbiology IHTM. Until the end of 2014, samples from PBSC and BM components were collected only after completion of processing. In 2015, we decided to collect samples both after collection (post-collection samples) and after processing.

Until 2012, cell processing was performed mostly within a closed system with the exception of BM filtration and preparation of a cryoprotectant mixture which took place in a laminar air flow chamber. In 2012, a decision was made to replace 5% albumin used for preparation of cryoprotectant mixture with plasma and since then only the closed system was in use. BM was subjected to filtration immediately after collection in operating room conditions.

The laminar air flow chamber was subjected to systematic weekly microbiological control; no bacterial contamination was reported.

## Results

A total of 42 contaminated products were detected including 25 PBSC (0.66% of all tested PBSC units) and 17 CB (7.20% of all tested CB units). No contamination was reported for BM components (Tab. I). The overall percentage of contaminated components was 1.01%. Until the end of 2014, when all samples were collected only in the post-processing period, 22 contaminations were found. In 2015, two (2) contaminated products were determined; 1 sample tested in aerobic environment was contaminated with *Staphylococcus epidermidis* and 1 sample from the other product was tested in anaerobic environment and found contaminated with *Priopionobacterium acnes*. In 2016, we found one product contaminated with *Staphylococcus epidermidis* – one sample tested in aerobic environment. Eight (8) CB samples were found contaminated in the post-collection period and 15 in the post-processing period. In two samples identified as contaminated before processing no bacteria were identified after the processing

**Table I. Number of processed products in consecutive years including contaminated products**

Year	PBSC (contaminated)	BM (contaminated)	CB (contaminated)
1996	7 (1)	0	0
1997	27 (1)	0	17
1998	21	2	18 (1)
1999	26	9	55 (6)
2000	54	8	60 (4)
2001	60	4	10
2002	44	10	3
2003	55	6	8
2004	53	5	3
2005	98	3	0
2006	107 (2)	3	0
2007	108	1	0
2008	255 (1)	3	0
2009	288 (2)	10	0
2010	211 (6)	12	0
2011	278 (1)	7	30 (3)
2012	282 (3)	12	32 (3)
2013	348 (1)	12	0
2014	425 (4)	2	0
2015	468 (2)	2	0
2016	572 (1)	1	0
<b>Overall</b>	<b>3787 (25)</b>	<b>112</b>	<b>236 (17)</b>

was completed. *Staphylococcus epidermidis* species predominated (61.36%). No fungal contamination was observed. Table II presents the detected contaminations with regard to the type of component. The contamination rate varied during the period under analysis (Fig. 1). In 2010, three (3) products were detected as contaminated with *Staphylococcus epidermidis*; all three came from the same patient with a vascular catheter and were collected on consecutive days. In another patient, two different bacteria species (*Staphylococcus epidermidis* and *Staphylococcus aureus*) were identified on consecutive days. In 2012, two (2) different bacterial species *Enterococcus faecalis* and *Staphylococcus epidermidis* were identified in one product. Products collected in other centers were tested on site immediately after collection and no contamination was found.

## Discussion

Bacteriological control of HSC products dedicated for clinical use is an extremely significant pre-condition for successful transplantation. Some centers perform transplantations of bacteria-contaminated HSC products but patients are supported by antibiotic therapy [1, 2, 13, 14, 15]. In others, contaminated products are tested for bacterial presence also after thawing, which facilitates the patient's follow-up. On the other hand, it has also been determined that cryopreservation might reduce the growth capacity of bacteria [16].

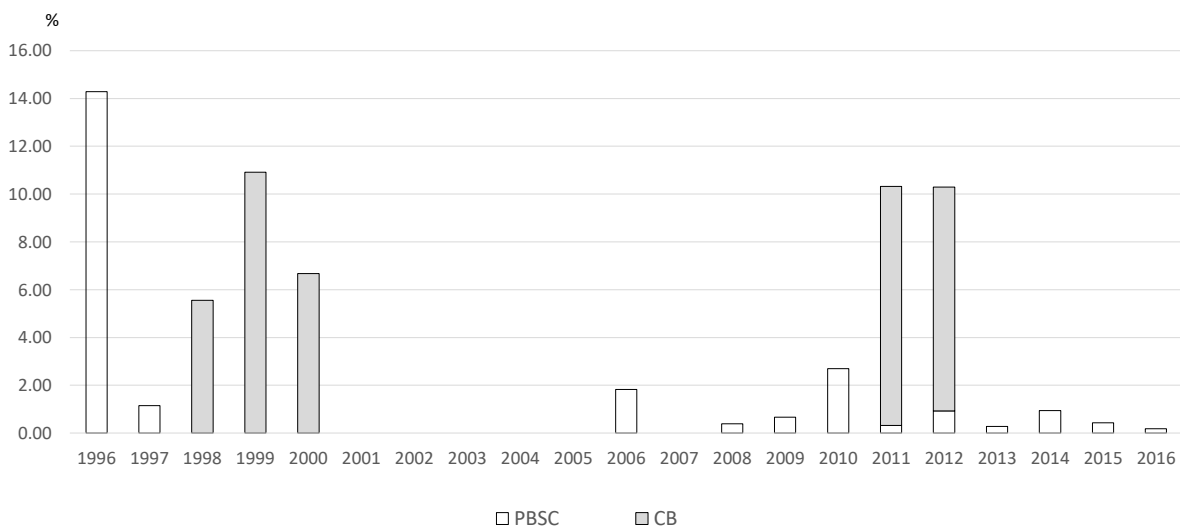
IHTM regulations state that any bacteria-contaminated component – either autologous or allogenic – is deferred from clinical use. It is therefore crucial to make sure that the number of discarded components is as low as possible. The testing of samples both after collection and after processing is the crucial stage of proper cell banking. Such protocol helps to evaluate the quality of performance both during collection and processing and may also improve the probability of detection of small number of bacteria in product.

Our study results demonstrate that the contamination rate for HSC products at IHTM is low as compared to several other centers. Literature data report that the contamination rate for all products varies from 0.2% to as much as 26.3% [1, 5, 8, 16, 17]. It is even

higher for CB products; the percentage amounts up to 48% [18]. In our study, we also observed that CB contaminations occur more frequently than for PBSC and BM. Moreover, in more than 50% of infected samples, bacteria were directly detected in the post-collection period. The very conditions of CB collection increase the risk of bacterial contamination either directly or through kit contamination. Processing of CB for clinical use is another challenge due to small specimen volume and special sterility requirements. Ongoing studies on optimization of CB sampling are focused on dilution of source material, determination of the best timing for sample collection, collection of test samples from CB remnants, that is, plasma or red blood cells [19]. To a lesser degree, this refers to PBSC and BM testing mostly because of larger volume of product. It should be kept in mind, however, that each sample collection reduces the amount of stem cells for transplantation.

**Table II. Microorganisms identified in PBSC and CB products in years 1996–2016**

Microorganism	No. of contaminated products	
	PBSC	CB
<i>Staphylococcus epidermidis</i>	19	8
<i>Priopionobacterium acnes</i>	2	-
<i>Streptococcus agalactiae</i>	-	2
<i>Escherichia coli</i>	-	2
<i>Staphylococcus aureus</i>	1	-
<i>Enterococcus faecalis</i>	1	1
<i>Staphylococcus cohnii</i>	1	-
<i>Staphylococcus vitulinus</i>	1	-
<i>Brevibacterium</i> spp.	1	-
<i>Dermabacter hominis</i>	-	1
<i>Leuconostoc</i> spp.	-	1
<i>Staphylococcus capitis</i>	-	1
<i>Micrococcus</i> spp.	-	1
<i>Streptococcus sanguinis</i>	-	1



**Fig. 1. Frequency of bacterial contamination in HSC products in relations to HSC source in years 1996–2016**

Surprisingly, no bacterial contaminations were found in BM components, although collection and processing procedures were performed in open system. It would seem that BM components are more exposed to bacterial contamination because of the type and conditions of activities performed as well as the large specimen volume. In other centers, BM was typically contaminated directly after collection as well as during processing [11, 13, 20, 21].

The most common contamination reported for HSC products is with human skin bacteria. This is also confirmed by our study where *Staphylococcus epidermidis* was detected in 61.36% of all collected samples [17, 21, 22]. Microorganisms found in BM products differed from those in PBSC products. In his study, Vanneaux et al. [20] also identified *Priopionobacterium acnes* as the most common microorganism beside coagulase-negative *Staphylococcus*. In the study of Kozłowska-Skrzypczak et al. [23], however, the most common microbial contamination detected in both PBSC and in BM was with *Bacillus species* although in this cell bank the preparation procedure was performed in a closed system exclusively. The same bacterial species was detected in laminar flow chamber where test samples were collected. In our cell bank where test samples are collected once a week, no bacteria were found in laminar flow.

The case of one patient (all products contaminated) revealed the source of contamination to be neither the processing nor sampling technique but the tip of the catheter used for cell collection. It is therefore crucial to make sure the collection procedure does not affect component sterility even before the HSC collection begins. Cell collection should be undertaken after a thorough check of the vascular catheter; patients at risk of asymptomatic bacteremia should be deferred.

CB was tested after collection as well as after processing which allowed to detect contamination related to inadequate disinfection of the umbilical cord or failure to observe microbiological purity standards during collection. No bacterial growth was found in three samples collected after processing; the same component was found contaminated just after collection. This might be due to the bactericidal effect of DMSO or small amount of bacteria in product. Similar results were reported by Padley et al. for components repeatedly tested after thawing [1].

In one of our study samples, two different bacterial species were identified before (*Leuconostoc* spp.) and after processing (*Staphylococcus capitis*). This may be the result of the small number of bacteria in the sample or their uneven concentration and distribution in the product.

Our analysis demonstrates the need for implementation of bacteriological monitoring of components immediately after collection as well as after processing; in 2015, this requirement was implemented for all HSC products. This procedure revealed that bacterial contamination found in all three components had most probably occurred in the processing period. At the same time, it is likely that a small number of bacteria in the product immediately after collection may give false-negative results in the test sample.

Moreover, bacteriological analysis demonstrated that this may have occurred as superinfection during sampling, the more so that the whole processing procedure was performed in a closed system. Since sampling is not performed within a closed system, it may be identified as the critical step of activity. It is therefore reasonable to consider a change of sampling technique, for example, transfer of drain segments

to a microbiological laboratory for direct media inoculation to ensure adequate quality of the sample.

The results of our study demonstrate that the bacterial contamination rate at IHTM is relatively low. Of crucial importance here are internal audits as well as constant monitoring of all activities. At the same time, we may see that processing in a closed system is not as crucial as might be expected. This is particularly true for BM components. Similar results are reported by Cassens et al. who found no significant impact of the processing environment on bacterial contamination rate. The authors compared the bacterial contamination rate during cell processing in a laminar air flow chamber subjected to systematic microbiological control located in a B class clean room with that for laminar air flow chamber inspected only once a year and located in a no-class room [8].

Cell processing is most commonly performed in clean rooms according to Good Manufacturing Practice (GMP). Alternatively, the processing may take place in a closed system which reduces high maintenance costs of such clean rooms [24, 25].

Bacterial contamination implies the loss of transplantation material. As mentioned earlier, the IHTM policy permits no bacteria-contaminated components for clinical use. Extremely dangerous for the recipient is transplantation of components bacteria-contaminated directly after collection when results of culture tests are still unavailable. However, cryopreservation and cryoprotectants may also contribute to bacterial viability; the bacteria remaining in the contaminated product may grow after the product is thawed and infused to the recipient [18].

Our study results identify staff experience as crucial for prevention of HSC contamination. After the initial 3-year period (1996–1998) when HSC components were found bacteria-contaminated, no contaminations were reported for several consecutive years. Increase in contamination rate was reported during the next several years most likely as outcome of liberal approach of staff to the routinely performed activities. In 2010, an increase in the number of contaminated autologous components was reported most likely due to liberalization of the approach to the health of autologous donors and the use of vascular catheters. More attention was therefore directed to donor health and processing procedures in the years that followed.

Staff overload is another risk factor; the same number of people has to prepare a growing number of HSC components. Until 2012, no significant changes have been introduced as regards performance at each step of the preparation process.

Implementation of a closed system should have completely eliminated contamination during the preparation process. It follows therefore that the sample collecting procedure requires change to avoid superinfection and acquisition of appropriate information on bacteriological safety of the component. At stake here is prevention of unwarranted deferral of components from clinical use.

We conclude from our study that careful attention should be paid to the puncture site from which source material is collected. Apart from using the closed system during preparation, components should be investigated for contamination directly after HSC collection as well as after processing. The use of an appropriate method and technique for sample collection is of utmost priority.

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**Authors' contributions**

JA-P – conceived the idea for the study and was responsible for research design, interpretation of results and preparing of the manuscript. EL, AR – were responsible for data collection. MŁ – was responsible for the final review of the manuscript.

**Conflict of interest**

The authors have no conflicts of interest to declare.

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**Ethics**

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; EU Directive 2010/63/EU for animal experiments; Uniform requirements for manuscripts submitted to biomedical journals.

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