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Microbial safety of cellular therapeutics—lessons from over ten years' experience in microbial safety of platelet concentrates

Melanie Störmer,[1] Erica M. Wood[2] & Birgit Gathof[1]

[1]*Transfusion Medicine, University Hospital Cologne, Cologne, Germany*

[2]*Transfusion Research Unit, Department of Epidemiology and Preventive Medicine, Monash University, Melbourne, VIC, Australia*

Bacterial safety of cellular preparations, including blood products and cellular therapeutics, presents ongoing challenges for physicians, manufacturers and regulators. Although there have been many new approaches to enhance the microbial safety of cellular products during the last decade, established methods for microbiological control still need to be fully adapted to the special circumstances of cellular preparations. The experience from transfusion medicine regarding microbial safety of blood components has demonstrated the variety of problems and risk factors for the development of new strategies for microbial safety. Special attention has been given to the prevention and detection of bacterial contamination of platelet concentrates. But so far, none of the targeted strategies for rapid detection or pathogen reduction have become routinely implemented worldwide, in part at least because development and requirements of new technologies and their implementation into the routine setting are a whole different problem. But factors including the short shelf life and nontraditional lot sizes for cellular and gene therapy products are driving the need for rapid microbiological methods. In conclusion, lessons from the microbial safety of platelet concentrates enable us to understand that the detection or reduction in bacteria represents a more difficult challenge in comparison with viruses. Recent regulatory changes demonstrate that we are getting closer to the goal of a shift from the traditional view of sterility evaluation (identify and inactivate anything and everything) to a new thinking about microbiological control.

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Introduction

Cellular therapy, in which a cellular material is injected into a patient, will be expected to continue to grow rapidly in the near future [1]. Cellular therapies, including cellular immunotherapies, cancer vaccines and other types of both autologous and allogeneic cells for certain therapeutic indications (e.g. hematopoietic stem cells), are a class of products that present multiple challenges to ensure safety, purity and potency [2].

Correspondence: Melanie Störmer, Institute for Transfusion Medicine, University Hospital Cologne, Kerpener Str. 62, 50937 Cologne, Germany
E-mails: melanie.stoermer@uk-koeln.de; melanie_stoermer@gmx.de

Over the past century, conventional microbiological culture methods described in the European, Japanese and US Pharmacopoeias have helped manufacturers to ensure the production of microbiologically safe noncellular products [3]. But with the development and production of cellular-based products, the conventional technologies reached their limits. Significant changes in regulatory requirements have been made recently by revising chapters of the European Pharmacopeia or by the US Food and Drug Administration (FDA) making changes to the requirements to address the needs of next-generation therapies. The other side of the coin is that standardized conditions will require extensive validation based on the approach of the corresponding authorities.

Due to many similarities between cell therapeutics and cellular blood components, experiences of transfusion medicine in bacterial contamination can be applied to the improvement of microbial safety of other cell-based products [4–6]. In the last decade, special attention has been given to strategies and recommendations for the prevention, detection or reduction in bacterial contamination of platelet concentrates (PCs). In general, the strategies for microbiological control can be divided into as follows: (1) primary testing using a culture-based or adequate direct bacterial detection method early in storage, (2) secondary testing late in storage using a detection method in addition to the primary testing, (3) bacterial testing late in storage to extend the shelf life of the PC, (4) pathogen reduction after production of PC. Depending on the shelf life of PCs (4–7 days) and individual legal regulations (mandatory bacterial testing, no bacterial testing and bacterial testing to extend PC shelf life), different countries applied varying strategies. But the most common strategy represents the primary testing strategy by cultivation early in storage using the negative-to-date concept, where all PCs are screened within 24 h after blood donation before release and incubation are continued for up to 7 days [7]. This applies also for other blood products, cell-based products including hematopoietic progenitor and other regenerative cells [8–11]. Nevertheless, factors including the short shelf life and nontraditional lot sizes for advanced cellular products are driving the need for the development of novel and rapid microbiological methods. But the expectation and requirement of these new technologies, to achieve the same sensitivity as it is stated for the conventional method, limited the authority's approval and implementation into the routine setting up to now.

This review, while addressing the current microbial safety concept of platelet concentrates, will focus on the challenges of cellular products in general with respect to a strong microbial safety concept applicable broadly to different types of products and their corresponding regulations.

Concepts of microbial safety

The achievements in improving the viral safety of blood components led to major expectations of microbial safety across the board, but viruses are not able to grow during component preparation or storage and are detected by using specific detection methods for each virus in the donor's blood. In contrast, for microbiological (bacterial) control, the ability to detect a broad range of different bacterial species in a dynamic environment and often at very low levels in the final product is required.

Microbial safety of noncellular products

As shown in Fig. 1, bacterially contaminated noncellular products are extremely rare, owing to the efficiency of processes according to the Good Manufacturing Practice (GMP) in the pharmaceutical industry [12]. Elements of these processes include the use of standardized starting materials as well as strict adherence to use of aseptic equipment, materials and handling techniques during all manufacturing steps. In general, sterilization of source materials, intermediates and finished products can be performed using various methods such as sterile filtration at the end of the procedure (e.g. for vaccines). Each batch must undergo the sterility testing following procedures established and proven over decades according to the Pharmacopoeias worldwide. The principle of classical sterility testing is a parallel incubation of the sample under aerobic conditions at 20–25°C in a representative microbiological aerobic medium and a second sample under anaerobic conditions at 30–35°C in an anaerobic medium for up to 14 days. The turbidity of the medium is used as parameter for microbial growth. Sample preparation can be performed by direct inoculation or filtration [4, 12–15]. Moreover, automated culture systems (e.g. BacT/Alert, bioMérieux, France; Bactec, BD, USA; VersaTREK, TREK Diagnostic Systems, USA) have been used successfully in quality control of plasma fractionated from whole blood donations or apheresis for bacterial

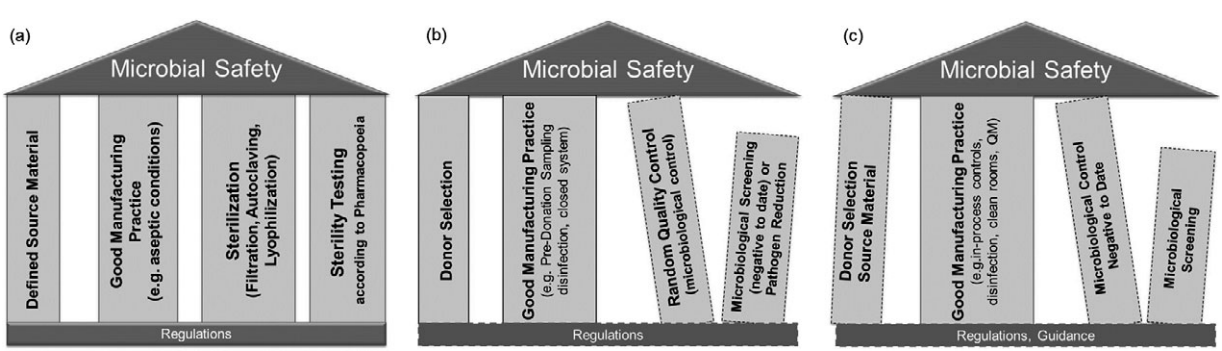

Fig. 1 Concepts of microbial safety of (a) Noncellular products, (b) Platelet concentrates and (c) Cellular therapeutics.

contamination for more than a decade and provide the advantage to be applicable to turbid products [16, 17].

Microbial safety of platelet concentrates

In contrast, the microbial, particularly bacterial, safety concept of PCs has a less secure foundation (Fig. 1b). The main keystones include careful donor selection and procedures such as the selection of the venepuncture site, effective skin disinfection, separation of the first volume from the blood donation (predonation sampling, also called diversion) and the consistent monitoring of the bag systems. Collectively, these have proven very effective in reducing the rates of bacterial contamination and associated septic transfusions reactions by 50 to 75% [17–23]. Nonetheless, the residual contamination risk is approximately 1 in 10 000 transfused PCs with nonfatal reactions occurring in 1 in 100 000 and fatal reactions in 1 in 500 000 transfused PCs, respectively, further action is needed [11, 24, 25].

Due to the fact that blood components are not sterilizable and the specification of the final product can never be 'sterile', the keystone 'sterilization' has to be replaced by the less certain support of microbiological control [26]. The introduction of the 'Minimum Requirements for sterility control of blood components in 1997' by the working party on bacteria safety in transfusion medicine of the National Advisory Committee Blood of the German Federal Ministry of Health (Arbeitskreis Blut, AKB) could be seen as one milestone in bacterial safety in Germany. These national guidelines were introduced to monitor bacterial contamination of blood components as part of routine quality control to establish standardized methods for bacterial testing [16, 27]. The standardized protocols included the time of sampling, sample quantity, sampling procedure and microbiological control according to the European Pharmacopoeia 2·6·1 or 2·6·27 using aerobic and anaerobic cultivation for 14 days if conventional liquid culture is performed [13] and 7 days if an automated system is used [28], identification of contaminating bacteria and performing a second culture as a confirmatory test using material from the same blood bag [12, 18, 29]. Following microbiological monitoring has been mandatory in Belgium in 1998 and in the Netherlands in November 2001 [18, 30, 31]. However, random quality control testing is less strong as a base for microbial safety than 100% screening.

Since the AABB Standard 5·1·5·1 required implementation of methods to limit or detect bacterial contamination in 2004, a variety of growth-based and non-growth-based methods for platelet bacteria screening including NAT, flow cytometry, colorimetric assays and immunoassays have been evaluated in many studies worldwide using the first established repository of platelet transfusion bacterial reference strains [18, 32, 33]. To meet this standard, platelet bacteria screening by automated culture was widely implemented worldwide using the 'negative-to-date' concept for early culturing of PCs. This strategy includes an early sampling, typically within 24–48 h of collection, followed by a 6- to 12-h holding period and PCs released with a negative diagnostic status while cultivation continues [7, 11, 31, 34–36]. If the result status changes from negative to reactive, physicians have to be informed immediately and products need to be recalled. Since the implementation of the standard, the incidence of transfusion-transmitted bacterial infections has fallen but the risk has not been eliminated due to rare false-negative results. The rate of bacterial contamination escaping detection by routine early culturing is approximately 1 in 1500 units [25, 37]. Nevertheless, the implementation of bacterial screening showed to be an effective risk reduction measure and increased the safety of the blood supply [11, 35].

Moreover, different pathogen reduction technologies were developed and optimized in parallel and implemented in some countries to improve the microbial and viral safety of PCs [34, 38–40]. Over the intervening years, the shelf life of PCs was reduced in some countries based on suspicion of increased transfusion-transmitted bacterial infections by longer storage of PCs until a bacterial platelet screening or reduction strategy was implemented [18, 31, 41, 42]. While in some countries, pathogen reduction became mandatory, many countries have implemented automated culture systems.

It has been difficult for other rapid screening technologies to find their place in the implementation into routine testing up to now, due to lower sensitivity and specificity [43, 44]. In 2009, a commercially available flow cytometric technology originally established for the food industry was introduced for bacterial screening of PCs in Germany [45]. Using this technology, in combination with a sampling on day 3 or 4 of platelet shelf life is accepted by the German national authorities (Paul-Ehrlich-Institute) to extend the storage period of PCs from 4 back to 5 days [7, 18, 46]. In contrast, an immunoassay was cleared by the FDA in 2007 as an adjunct quality control test for pooled platelets within 4 h of transfusion [42]. This test system has been implemented for routine testing of PCs in some US centres as a secondary test and is performed at the day of issue [47, 48].

In March 2016, the US FDA issued the second draft guidance entitled 'Bacterial Risk Control Strategies for Blood Collection Establishments and Transfusion Services' to enhance the safety and availability of platelets for transfusion. This guidance recommends controlling the risk of bacterial contamination through PRT or bacterial

testing. PRT has to be performed shortly after platelet collection by blood collection establishments. Bacterial testing encompasses initial testing (primary testing) of platelets by blood collection establishments, and subsequent retesting (secondary testing) prior to transfusion, principally by transfusion services, but also by blood collection establishments [42, 49].

As shown, the strategies for bacterial safety measures of PCs vary substantially among different countries depending on national regulations and are still under development. Therefore, the keystone 'Microbial Screening/Pathogen Reduction' which developed during the last years is not as stable as it was expected to be a decade later.

Microbial safety of advanced cellular therapeutics

Microbial contamination of cellular products used in transplantation and regenerative medicine can occur during collection or after *in vitro* manipulation [10, 50, 51]. Reported rates in various types of progenitor cell products range from 0·2 to more than 25%, averaging about 3% [10, 51, 52]. The clinical significance of microbial contamination on HBC engraftment and transplant outcome is unclear. It is the general experience of the specific transplant community [52]. Comparable to cellular blood products, the most common micro-organisms are those associated with skin, including coagulase-negative staphylococci and *Propionibacterium acnes* [10]. Source materials for some cellular products are obtained from patients in intensive care units, clinically dead patients or cadavers. Moreover, the source materials cannot be sterilized due to the effect on viability and potency [4, 26, 53]. Therefore, even the selection of source material represents a risk to microbial safety (Fig. 1c).

In contrast, the high standards of manufacturing processes in cellular therapy are significantly responsible for the current state of microbial safety. Any procedure requires a strict control according to GMP including quality control and quality assurance programmes to control collection, processing, storage and release of cell therapy products. Moreover, elements such as (1) the design, access and maintenance of the facility, (2) the purchase, use and maintenance of the equipment, (3) the specifications, purchase, storage and use of materials, and (4) the quality assurance including quality control, validation, qualification and document control needs to be addressed [54–57].

Moreover, many cell therapeutics have short shelf lives, which often necessitate administration to the patient before results from currently available sterility tests are available [26, 55].

As already shown for cellular blood products, most of the approaches for microbiological control used in the pharmaceutical industry for filterable products are not applicable in case of products containing living cells. As shown in Table 1, the present limitations related to cellular products include low production volumes, limited manufacturing time, short shelf lives, the introduction of an additional product or process variables which may affect the test outcome, the requirement for large sample volumes and the cellular (nonfilterable and nonsterilizable) matrix [53, 55]. In general, the addition of cell suspensions to culture media inevitably causes them to become opaque so that these methods are as a rule not recommendable for microbiological control of cellular products. Taking into account these limitations of classical methods, the European Pharmacopoeia Commission and others introduced the use of automatic culturing for microbiological control of cellular products [28] which is currently widely used for the detection of micro-organisms in cellular therapeutics [8, 10, 50, 55, 58–60]. The advantage of the automated culture system is the shortened incubation period of 7 days. But considering the extremely short shelf life of much cell therapeutics, novel rapid and effective principles are needed. Moreover, specific components have specific requirements regarding microbiological control. Regarding cord blood, the small final product volume represents a big challenge that culture bottles for small inocula should be used [8, 61]. In particular, the presence of antibiotics or other supplements such as DMSO used in autologous stem cell products or tissues may interfere with the results of microbiological testing why culture bottles with antimicrobial removal systems are needed [50, 62]. Finally, the small final product volume or product itself may lead to the impossibility of the final product testing. Therefore, there may be no alternative to test the culture or storage media of these products as performed for corneal transplants [62, 63]. Since blood culture bottles are only validated by the manufacturer for blood and blood products, an additional validation for the use of fluids from tissue preparations is necessary [63].

In 2008, the US FDA published guidance for industry about the validation of growth-based rapid microbiological methods for testing of cellular and gene therapy products. It was stated that there is a significant need to develop, validate and implement test methods that are more rapid than the classical ones [2, 6]. But due to the lack of existing regulations, there was a slow progress in the validation and implementation of alternative methods. To address the ongoing challenges, the chapter 2·6·27 of the European Pharmacopoeia was revised and was published in 2017 [28]. This revision represents a milestone in microbial safety of cellular products and may lead to

Table 1 Challenges for microbial safety concepts of cellular therapeutics

Challenge	Reasons
Source material	Sterility cannot be guaranteed due to the biological nature of the material (source and method of collection). Most products are highly individualized and patient-specific.
Manufacturing processes	The manufacturing process often represents a complex and multistep process during which bacteria can enter the product.
Bacterial contamination	The initial bacterial count is very low (high risk of sampling error). Bacteria may grow during storage and after testing. Bacterial testing needs to detect a broad range of bacterial species. This may lead to lower sensitivity and lower specificity.
Shelf life	If not cryopreserved, product shelf life ranges from hours to few days. Conventional sterility testing is not applicable (too slow). Products currently have to be administered to the patient before microbiological testing is complete.
Sample size	Batch sizes for autologous or direct use are variable and often very small (e.g. one bag or vial). Final products cannot undergo sterility testing due to volume limitations to guarantee potency. High sampling error occurs due to small sample sizes.
Sample matrix	In some cases, the cell-based preparation itself can inactivate contaminating micro-organisms resulting in a false-negative. The sample matrix can interfere with some detection technologies and may lead to false-positive results, low sensitivity and low specificity. No sterilization process is possible due to the resulting damage and loss of activity and potency. Sterile filtration is not possible due to the cellular matrix. Micro-organisms may adhere to or penetrate the cells.
Additives	The presence of antibiotics or other supplements like DMSO may interfere with the results of microbiological testing.
Expectations and change management	Viral safety success for blood components leads to high expectations of microbial safety. Regulatory, medical, cost and environmental challenges. New technologies are not comparable to conventional sterility testing. They may need major validation efforts and may not be applicable for routine use. Limited availability of microbiological expertise in this specialized area.

the acceptance and the use of alternative and adequate methods for microbial detection. This step will contribute to a stronger concept of microbial safety.

Challenges for alternative methods—reluctance to change from the classical culture method

As shown in Table 1, there are many difficulties and reasons for reluctance to change from the classical culture method to alternative methods. Product characteristics, applicability and ease of incorporation into routine processes, obsolete regulations and high expectations challange manufacturers and regulators to develop and approve alternative methods. The expected perfect test for rapid microbiological control should have an extremely high diagnostic sensitivity for a range of different bacteria and other organisms, a low false-positive rate, is cheap, reliable and fast. Up to now, none of the evaluated methods could fulfil all these requirements [64]. For example, the cellular matrix can affect both sensitivity and specificity because the presence of cells may influence the detection technology due to cell debris causing false positive results.

Further challenges include the effort in validation and the applicability into routine operational processes. In general, sampling should be performed as late as possible, ideally immediately before administration to the patient, but this is logistically and clinically challenging. In regard to PCs, point-of-release testing is often impractical for the transfusion service due to unpredictable and often urgent demands for PCs. Moreover, the implementation of a rapid test is associated with increased workload and costs [42]. Notably, the shelf life of several days makes the screening strategy more difficult. Detection or PRT of PCs during storage enables blood centres to prolong the shelf life, but due to the growth ability of bacteria, these cells can still grow up to high numbers after testing. The time between testing or treatment and the administration of the product to the patient needs to be as short as possible. Therefore, cellular therapeutics with a very short shelf life may benefit from this characteristic in comparison with products with a shelf life of several days

regarding the implementation of rapid methods with a lower sensitivity. A new understanding is required.

Step forward—revision of regulations

In 2017, the revised chapter 2·6·27 of the European Pharmacopoeia 'Microbiological examination of cell based preparations' was published. This chapter takes into account the special characteristics and limitations of cell-based preparations, in particular the short shelf life. The main changes to the previous version include the following:

- Description of specific characteristics of why the test for sterility described in chapter 2·6·1 Sterility is not suitable
- Recommendations of more flexible incubation temperature
- Replacement of *Yersina enterocolitica* with *Micrococcus sp*. for method suitability assessment
- Information about the sensitivity to be achieved during validation (capacity to detect 100 CFU or less)
- Opportunity to refer to general chapter 2·6·1. Sterility, which may be applied
- Opportunity to use alternative rapid test methods with or without a preincubation step by referring to general chapter 5·1·6. 'Alternative methods for control of microbiological quality' although at an expense of lower sensitivity in comparison with growth-based methods. This chapter was also revised and published in 2017 [65].

These revisions will lead to a greater awareness of the issues, as well as acceptance and willingness to develop and implement alternative methods, and will in time lead to a more stable microbiological safety concept for these life-saving products.

Conclusion

For the release of traditional biologicals that are produced in large lots with longer stability profiles such as vaccines or allergens, the turnaround time on conventional microbiological tests which are sensitive and robust is not a concern with respect to the ability to obtain reliable results. Next-generation products including the advanced therapy medicinal products (gene therapy medicinal, somatic cell therapy medicinal and tissue-engineered products) have short shelf life and require novel technologies to detect micro-organisms that are more rapid than traditional methods [3, 66, 67]. Nowadays, the implementation of a rapid method requires an initial risk assessment and method validation. Up to now, one main criterion was the level of sensitivity where the method must be able to detect down to one colony-forming unit as it is stated for the conventional methods. But significant revisions of the chapters 2·6·27 and 5·6·1 of the European Pharmacopeia now address the needs of next-generation therapies although at the expense of lower sensitivity and therefore smooth the way for a better acceptance for implementation by authorities. Now, we should focus on the advantage of alternative methods to obtain the result before product release and adapt already developed methods for microbiological control of blood products to the specific requirements of cellular therapeutics. For this reason, specifications including the bacterial load of clinical significance and the time between testing and administration to the patient need to be defined for each single product type depending on the shelf life, processing and storage conditions.

In conclusion, now is the time for the development of novel principles in microbial safety of cell therapeutics. The paradigm shift in thinking from regarding sterility is 'we have to find everything' to 'we have to find as much as possible within the time frame available' is just getting started.

Conflict of interest

The authors have no conflict of interests. Reference to technologies is for informational purposes only.

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