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Analysis

Prospective Evaluation of a Practical Guideline for Managing Positive Sterility Test Results in Cell Therapy Products

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A B S T R A C T

Product safety assurance is crucial for the clinical use of manufactured cellular therapies. A rational approach for delivering products that fail release criteria (because of potentially false-positive sterility results) is important to avoid unwarranted wastage of highly personalized and costly therapies in critically ill patients where benefits may outweigh risk. Accurate and timely interpretation of microbial sterility assays represents a major challenge in cell therapies. We developed a systematic protocol for the assessment of positive microbial sterility test results using retrospective data from 2007 to 2016. This protocol was validated and applied prospectively between October 2016 and September 2017 to 13 products from which positive sterility results had been reported. Viable and nonviable environmental monitoring (EM) data were collected concurrently as part of a facility control assessment. Three of 13 (23%) positive sterility results were attributable to bone marrow collections that had been contaminated with skin flora during harvest; all were infused without pertinent infectious sequelae. Of the remaining 10, 1 was deemed a true positive and was discarded before infusion, whereas 9 were classified as false positives attributed to laboratory sampling and/or culturing processes. Three products deemed false positive were infused and 6 were withheld because of patient issues unrelated to microbial sterility results. No postinfusion-associated infectious complications were documented. Almost half of the positive EM findings were skin flora. Paired detection of an organism in both product and associated EM was identified in 1 case. Application of our validated protocol to positive product sterility test results allowed for systematic data compilation for regulatory evaluation and provided comprehensive information to clinical investigators to ensure timely and strategic management for product recipients.

INTRODUCTION

Mitigation of infectious risks associated with transfusion of human blood and/or its subcomponents is well recognized. After the delayed recognition of widespread HIV transmission through infected donor blood in the 1980s, enhanced regulatory oversight and stringent blood product screening were implemented [1]. Meticulous donor selection and education combined with highly sensitive laboratory testing and postdonation tracking has resulted in almost zero risk of HIV, hepatitis B virus, and hepatitis C virus transfusion-related transmissions in the developed world. However, microbial contamination of manufactured blood and cell therapy products are recognized as significant residual safety problems [2]. Furthermore, laboratory testing methods and performance evaluations for blood products and cell therapies are still not well defined.

Blood Component Sterility

RBC and platelet product-related bloodstream infections are mitigated by optimization of collection, processing, and storage techniques. These include skin disinfection [3] and diversion of the first collected aliquot [4], leukocyte reduction postcollection, use of single-donor apheresis platelets [5], storage time optimization, and closed-system processing. Pretransfusion sterility testing by visual inspection and direct cultures by manual or automated methods further reduce infectious risks in recipients [6]. Enclosed system pathogen inactivation technologies have been successfully applied to clinical platelet products, bolstering protection against known and emerging pathogens [7]. Fractionated plasma derivatives,

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which are regulated like pharmaceuticals, are collected in closed systems, pooled, and processed in highly controlled manufacturing facilities. Sterile filtration, lyophilization, followed by pathogen inactivation, and/or final sterility testing are also performed to minimize and monitor for microbial contamination. Pathogen inactivation methods within enclosed systems offer the advantage of removing the requirement for product sampling, thus reducing the risk of introducing microbial contaminants.

Safety Practices for Cellular Therapies

Product safety assurance in cellular therapeutics is significantly more challenging. Source material collection procedures, such as bone marrow harvests or surgical graft extractions, are more prone to contamination compared with a relatively quick, aseptic venipuncture [8]. Product-donor ineligibility, as determined by the blood donor history questionnaire, is often inapplicable to cellular therapies given the limited donor pool and an urgent medical need among recipients. Products manufactured in longer-term, open-system cultures with research-grade reagents are at increased risk of sterility breaches. Antimicrobial agents may not always be used during product manufacturing because of the risk of hypersensitivity reactions in recipients. Also, unlike most pharmaceuticals and medical devices, cellular therapies are "living drugs" that cannot undergo postmanufacture sterilization.

Cellular therapies also present challenges with regard to the assessment of microbial contamination. Culture turbidity as an indicator of microbial growth may be confounded by the turbidity of the cell suspension itself. Also, because of the need to maximize cell suspension for infusion, only a small volume (~1 mL; 1% to 10% of the cellular product) may be available for sterility testing, and this may compromise culture sensitivity. Automated systems, such as blood culture instruments, have demonstrated faster time detection compared with the manual compendial United States Pharmacopeia (USP)<71> method in some studies [9-11]. Hence, aseptic processing in a controlled, clean facility (per International Standards Organization (ISO) standards) and rigorous in-process sterility sampling paired with an active environmental monitoring (EM) program are critical in reducing risk of product contamination and can provide investigative data in the event of a positive sterility result that requires antimicrobial intervention.

METHODS

Using retrospective data collected between 2007 and 2016 we developed a systematic approach for the risk management of cellular therapy products that had positive sterility testing results. We applied this validated protocol in a prospective analysis for products manufactured between October 2016 and September 2017. EM data trends are also presented.

Aseptic Processing to Prevent Product Contamination

At our facility cell and gene therapy products, including minimally manipulated marrow or peripheral blood stem cells used for hematopoietic stem cell transplantation and those that required extensive cell culture manipulations, were processed aseptically following current Good Manufacturing Practices. Aseptic processing involved the optimization of operating conditions and the use of processing controls to prevent and actively monitor for microbial contamination risk. These included close monitoring and validation of the production facility, equipment, processes, personnel, and documentation practices. The facility was controlled for temperature, airflow, humidity, differential pressure, particle counts, and microbial bioburden. Equipment, reagents, and supplies were sterilized and/or filtered where applicable. Facilities and equipment were routinely cleaned and sanitized as part of a robust quality control and assurance program and in response to any evidence of contamination. All raw materials were qualified for use on the basis of source, purity, potency, safety, and suitability. Operating personnel were trained to high standards of hygiene and cleanliness and were required to don protective gowns, shoes, masks, and gloves. Personnel were retrained periodically and underwent proficiency testing and annual competency assessment. Where possible, cells were cultured and/or manipulated in closed systems, with engineering controls. All aspects of aseptic processing were ultimately verified by a "media fill" procedure, before product manufacture. This served as a simulation experiment in which cell product and reagents were replaced with culture media that supported microbial growth to monitor for contamination during the aseptic manufacturing process. Starting cellular raw material, intermediates, and final products were tested for microbial contamination at 1 or more time points during manufacture in compliance with regulatory guidelines [12,13].

Sterility Testing of Manufactured Cell Therapy Products

Federal regulations (21 CFR 1271: current Good Tissue Practices) require the collection, processing, packaging, and distribution of all cellular therapeutics by methods that prevent the introduction, transmission, or spread of communicable diseases, including viral, bacterial, fungal, and/or parasitic infections. For products including gene therapies and more than minimally manipulated cell therapies in prolonged cultures, regulations further specify sterility testing for bacteria and fungi using microbial testing methods equivalent to or better than those described in 21 CFR 610.12 [9]. In our institution traditional USP<71> testing for cell therapy products was replaced in 2004 with direct inoculation of aerobic and anaerobic plus bottles incubated at 30° to 35°C for 14 days on the Bactec FX automated blood culture system (Becton Dickinson, Sparks, MD) [10,11]. In 2015 direct inoculation of a Sabouraud dextrose agar (SDA; incubated at 20° to 25°C for 14 days) was added to improve the detection of molds after observation of fungal balls in Bactec bottles that failed to flag positive on the Bactec instrument. The Bactec bottles are inoculated in the processing facility, whereas the SDA fungal culture is inoculated in the laboratory. For products requiring rapid release, direct product Gram stain and Endotoxin assay (LAL Pryogent-5000; Lonza Biotech, Walkersville, MD) are performed, with turn-around times of 2 to 3 hours. Products are sampled multiple times during in-process production and upon final product release. Surface and air viable EM is performed as indicated in USP<797>. Matrix Assisted Laser Desorption Ionization - Time of Flight, Mass Spectrometry (MALDI-TOF MS) (Bruker Daltonics, Billerica, MA) was used to identify bacterial and fungal isolates from positive product and EM cultures [14]. Bacterial and fungal isolates were reflexed to 16S rRNA and ITS sequencing [15,16], respectively, if no identification was available by MALDI-TOF MS.

RESULTS

Systematic Investigation of Positive Sterilities in the Cell Therapy Lab

Between 2007 and 2017 an average of 325 cellular therapy products (range, 253 to 513) were manufactured annually in our facility (Supplementary Table S1). These included minimally manipulated hematopoietic stem cell grafts and more highly processed products. Manufactured products decreased in number but increased in complexity over the 10 years. Each year an average of 1202 (range, 629 to 1792) sterility tests were performed. Sterility tests included direct product Gram stain, Bactec bottle culture, SDA fungal culture (2015 onward), and endotoxin testing. On average, 12 (1%) positive product microbial tests (over an average of 8 [2.5%] products) were reported each year. Of these, an average of 4 were associated with allogeneic bone marrow collections performed at the National Institutes of Health or at outside facilities.

In October 2015 after failure of the Bactec system to automatically detect growth of *Aspergillus* spp. (even though fungal balls were observed upon manual inspection), fungal testing was supplemented by the addition of the SDA plate in an effort to improve mold detection. Consequently, the total number of sterility tests on products increased to 1792 in 2016. Of these, 22 (1.2%) were reported positive; 5 were related to bone marrow collections. To our knowledge no false negatives were uncovered by clinical follow-up since the introduction of SDA; however, false-positive rates increased 4-fold since introducing the additional fungal test. Importantly, laboratory processing was performed in the routine Bio Safety Level (BSL2) hospital clinical microbiology laboratory up until February 2017 when product sterility testing and culture handling was moved into a dedicated ISO classified space that was separated from the clinical laboratory. In addition, specially trained Good

Manufacturing Practices staff were employed to handle only product sterility testing and environmental cultures. As of May 2018, no positive sterility results were reported from >700 tests performed.

As a way to address the increase in false-positive rates, after a retrospective analysis of data collected between 2007 and 2016 we developed a systematic approach to assess positive sterility test results on a case-by-case basis. In this prospective analysis (October 2016 and September 2017) our goal was to attain the highest levels of patient safety while minimizing product wastage. The root cause evaluation protocol allowed for data-driven decision-making on infusions that had associated positive sterility testing results. For products that had positive sterility results, the assessment protocol (Figure 1A) assisted with determining true- from false-positive results, enabling timely notification of the US Food and Drug Administration and the Institutional Review Board as well as appropriate patient observation and treatment. Upon receiving a positive sterility report, immediate verification was performed using 2 or more patient or donor identifiers. Subsequently, a product investigation was initiated to gather information covering 3 main areas: donor history, cell collection details, and other available sterility testing results. Information collected for donor history includes recent travel or illness, signs/symptoms of ongoing infections, and clinical lab results, if available. If the product was obtained from an autologous donor, primary disease, treatment history, medications/antibiotic prophylaxis, and clinical lab data at baseline were collected. Details of cell collection included apheresis versus bone marrow biopsy (National Marrow Donor Program site versus onsite operating suite). If the collection was via apheresis, gathered data included the type; location; date and time of central venous catheter placement; details of site cleaning performance and confirmation; vital signs before, during, and after the procedure; duration of the procedure; and any abnormal occurrences during collection. Data from other sterility testing results were collected from parent product or split product, including in-process and final samples. If the product was

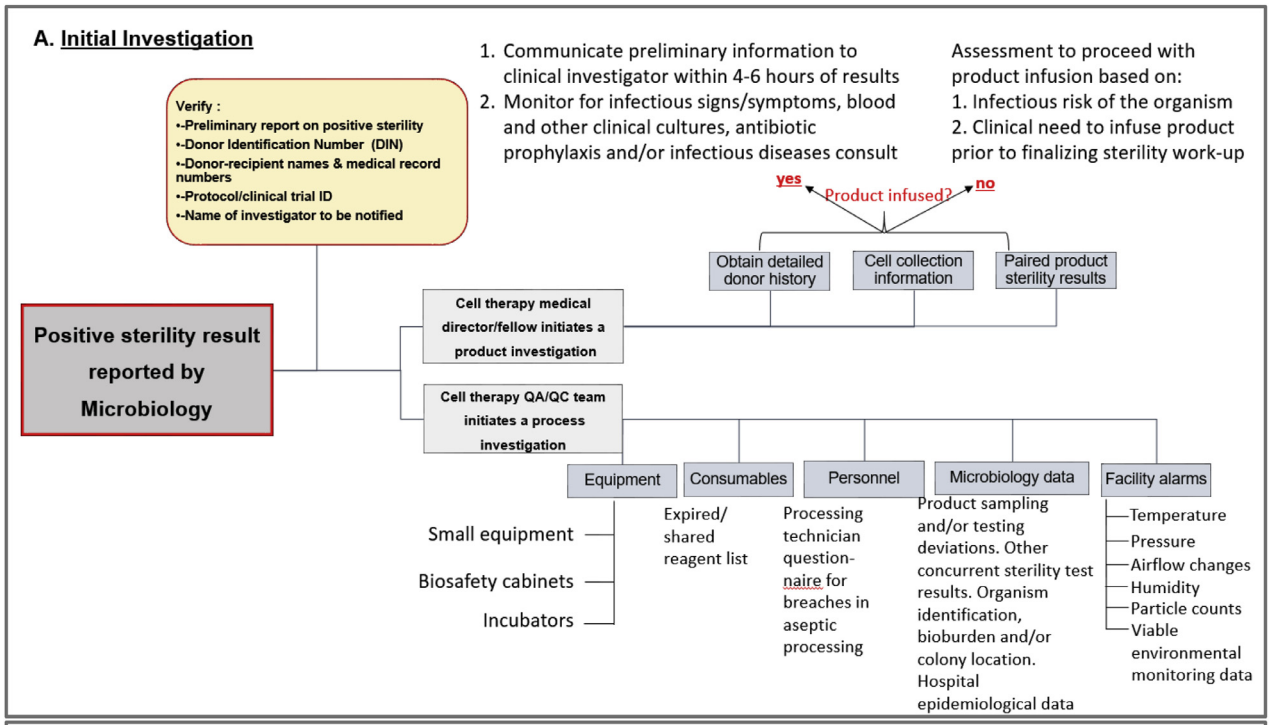

Figure 1. Systematic assessment of positive product microbial sterilities. (A) Initial investigation. Systematic data collection on product and process-related deviations/unusual occurrences in the pericollection/processing period. (B) Final report. Timely compilation and distribution of the final root cause analysis (RCA) report to the appropriate clinical and quality units.

already infused, preliminary sterility results were communicated to the clinical investigator within 4 to 6 hours of result availability and the patient was monitored for infectious signs/symptoms. Blood cultures were drawn before antibiotic prophylaxis (if needed) after an infectious disease consult. If the product was pending infusion, the decision to proceed was based on the infectious risk of the organism and/or the clinical urgency before completing sterility evaluation.

Concomitant with the product investigation, the cell therapy quality unit initiated a process investigation. Records concerning equipment, consumables, and reagents used in manufacturing were reviewed. Personnel involved with the manufacturing were interviewed to identify breaches in aseptic techniques. Communications with the microbiology laboratory regarding potential processing and/or environmental deviations and other concurrent sterility data were documented. Automated reports on facility controls (temperature, room pressure, airflow changes, humidity, particulate counts) were evaluated for anomalies during the time of processing. Product-related microbiologic data along with environmental bioburden data from around the time of manufacture were surveyed. All information was consolidated into a root cause analysis report. This was circulated within the department and provided to the primary team for further regulatory review (Figure 1B). Supplementary Table S2 provides an example of detailed investigational data (using the designed protocol) on 2 cases with positive sterility results from 2017 and a final assessment. The final decision to label products as false positive was based clinical judgement, that is, on the absence of other parameters suggestive of a possible infectious source (concomitant positive blood or repeat product cultures or patient illness at the time of cell collection) or evidence of sterility breaches during processing.

Positive Product Sterility Results and Concomitant EM Data

From January 2016 to September 2016 the protocol was validated by the absence of clinical adverse events with infusions associated with positive sterility results that were classified as false positive by the root cause analysis (Supplementary Table S3). Prospectively, from October 2016 to September 2017, 13 products were reported with positive sterility results (Table 1). Assessments were performed in each case to determine the validity of the positive result and the potential risks associated with infusion. Figure 2 shows the EM data trends collected over the same time period. Environmental data were used primarily to institute remedial measures for breaches in facility sanitization. However, EM data were also useful in defining facility flora and linking the environment as the potential source for product contamination if organism identification, collection locations, and times matched.

In this 1-year prospective evaluation 3 of 13 (23%) positive sterility results were related to allogeneic bone marrow harvests contaminated with skin flora (Table 1; cases 11,12, and 13). This was consistent with historical data. Also, although these cases were determined to be truly contaminated, all bone marrow products were infused as planned because prior clinical studies have demonstrated an insignificant impact of positive bone marrow sterility results on long-term engraftment and survival [17-20]. Of the 10 remaining cellular products with positive sterility results, only 1 case (case 3) was determined to be a true positive and was not infused. To our knowledge, based on clinical chart review and discussion with the primary investigator and clinical teams, none of the infused products classified as false positive by our assessment protocol resulted in transfusion-related infectious complications in the recipients.

EM data collected during the study period were unremarkable (Figure 2). On average, positive environmental cultures were identified in 1%, 10%, and 40% of samples sent from ISO 5, 7, and 8 zones, respectively. Between 56% and 69% of these were categorized as skin or oral flora, 13% to 23% were environmental organisms, and 8% to 29% were reported as objectionable (defined as yeasts, mold, gram-negative rods, and coagulase-positive *Staphylococcus*). Among the bacteria, *Micrococcus luteus* was most commonly identified (19%), followed by *Kocuria* spp. (10%), *Staphylococcus hominis* (7%), *Staphylococcus epidermidis* (5%), and *Streptococcus mitis* (4%). Common fungi included *Aspergillus sydowii* (20%), *Aspergillus fumigatus* (13%), *Cladosporium* spp. (12%), *Penicillium* spp. (10%), and *Aspergillus niger* (9%). In 1 case EM identified *M. luteus* that had also been cultured from a manufactured product at around the same time. This product was not infused for reasons unrelated to sterility testing results.

DISCUSSION

Confidence in product safety is critical to the success of early-phase clinical cell therapy studies. However, the biologic diversity of the products and the complexity of the cell production often necessitate an individualized assessment within a predetermined framework of safety enforcement statutes and guidance documents. The US Food and Drug Administration assesses manufacturing requisites and product testing on a case-by-case basis, depending on the current scientific knowledge, regulatory precedents and experience with similar products and/or indications, the phase of product development, and the risk-to-benefit profile of the target patient population [21]. In addition to developing strategies to avoid product microbial contamination, the cell processing facility must maintain accurate and systematic records of cases that deviate from prescribed safety norms. It is also incumbent upon the facility to promptly inform clinical investigators of a safety deviation to initiate appropriate prophylactic measures.

Our clinical cell processing laboratory manufactures relatively low volumes of cell therapy products, albeit of high complexity. As pioneers in gene therapy manufacturing since the 1990s [22], we introduced closed-system processing (from flask to bag-based cell culture) as a means to reduce product contamination [23]. These methods continue to be used and optimized for recent immunotherapies, including the manufacture of clinical grade Chimeric Antigen Receptor (CAR) T cells, TCR-transduced T cells, mesenchymal stromal cells, and multivirus-specific T cells [24-26].

Simultaneously, other safety aspects, including facility design, EM, and sterility testing methodologies, have evolved to accommodate these complex clinical manufacturing protocols. As part of a multipronged approach, we chose to study the utility of combining comprehensive sterility testing methods and EM data in a risk-based assessment protocol to ensure microbial safety for final product release.

Our prospective data demonstrate effective implementation of this protocol that has in turn streamlined data review and documentation for regulatory review. EM and facility data provided ancillary evidence for aseptic technique compliance during product manufacture. Although there is a lack of published data for using a risk-based strategy such as ours, another facility has reported best practices and approaches to mitigate infectious risks in cell therapy products [17]. Data from our routine facility EM are consistent with that reported previously [27]. Most organisms in our engineering-controlled facility appear to arise from personnel

Table 1
Prospective Annual Data on Positive Sterilities in the Cellular Therapy (CT) Laboratory by Systematic Investigation (October 2016 to September 2017)

Case No.	Human Cell Therapy Product	Bactec Bottles*	SDA†	Other	Additional Microbiology Data	Relevant Facility/ Equipment Deviations	Rationale for Infusion	True Positive?	Product Infused?	Associated Post Infusion Reaction /Infection?‡
1	Mononuclear cells		*Micrococcus luteus*		2 colonies, day 2 of 14 incubation	None identified	Possible laboratory contaminant	Unlikely	No (patient issues)	N/A
2	Mononuclear cells		*Fusarium* spp.		1 colony on edge of third quadrant, day 9 of 14 incubation	None identified	Probable laboratory contaminant	Unlikely	No (patient issues)	N/A
3	Lymphocyte	*Bacillus* spp.			Aerobic bottle only, day 1 of 14 incubation	None identified	Origin uncertain; additional sample unavailable to retest	Likely	No (no donor lymphocyte infusion stored, patient deceased)	N/A
4	Lymphocyte		*Moraxella* spp., *Dermacoccus* spp.		1 colony each, day 5 of 14 incubation	None identified	Probable laboratory contaminant	Unlikely	No (healthy donor feeder cells discarded)	N/A
5	Lymphocyte		*Ustilago* spp.		1 colony, edge of plate, day 9 of 14 incubation	None identified	Probable laboratory contaminant	Unlikely	Yes (before final results available)	None
6	Lymphocyte		*Botrytis* spp.		1 colony on edge of primary quadrant, day 11 of 14 incubation	None identified	Probable laboratory contaminant	Unlikely	No (see row below)	N/A
7	Lymphocyte			Rare gram-negative rods	Gram stain of final product	None identified	Two positive Gram stains Paired cultures were negative	Unlikely	No (equivocal Gram stain)	N/A
8	Lymphocyte		*Dermacoccus* spp.		1 colony, third quadrant, day 3 of 14 incubation	None identified	Probable laboratory contaminant	Unlikely	No (patient issues)	N/A
9	Lymphocyte		*Chaetomium* spp.		1 colony, second quadrant, day 1 of 14 incubation	None identified	Possible laboratory contaminant	Unlikely	Yes (before final result available)	None
10	Bone marrow		*Staphylococcus warneri*, *Rothia* spp.		Total of 3 colonies on quadrants 2-4, day 10 of 14 incubation	None identified	Probable laboratory contaminant	Unlikely	Yes (before final results available)	None
11	Bone marrow	*P. acnes*			Anaerobic bottle only, day 14 of 14 incubation	None identified	Skin contamination at the time of collection	Likely	Yes (before final results available)	None
12	Bone marrow	*Staphylcoccus caprae*			Anaerobic bottle only, day 1 of 14 incubation	None identified	Skin contamination at the time of collection	Likely	Yes (before final results available)	None
13	Bone marrow	*P. acnes*			Anaerobic bottle only, day 11 of 14 incubation	None identified	Skin contamination at the time of collection	Likely	Yes (with paired antibiotics)	None

* Bactec, aerobic and anaerobic blood culture bottles inoculated in the processing facility and submitted to the laboratory for incubation and continuous, automated monitoring on the Bactec FX instrument.
† SDA; fungal sterility test added in 2015 to improve detection of molds. Sample inoculated onto media in the laboratory.
‡ Data on pertinent immediate infusion reactions and clinical cultures collected on recipients up to 1 month after product infusion.

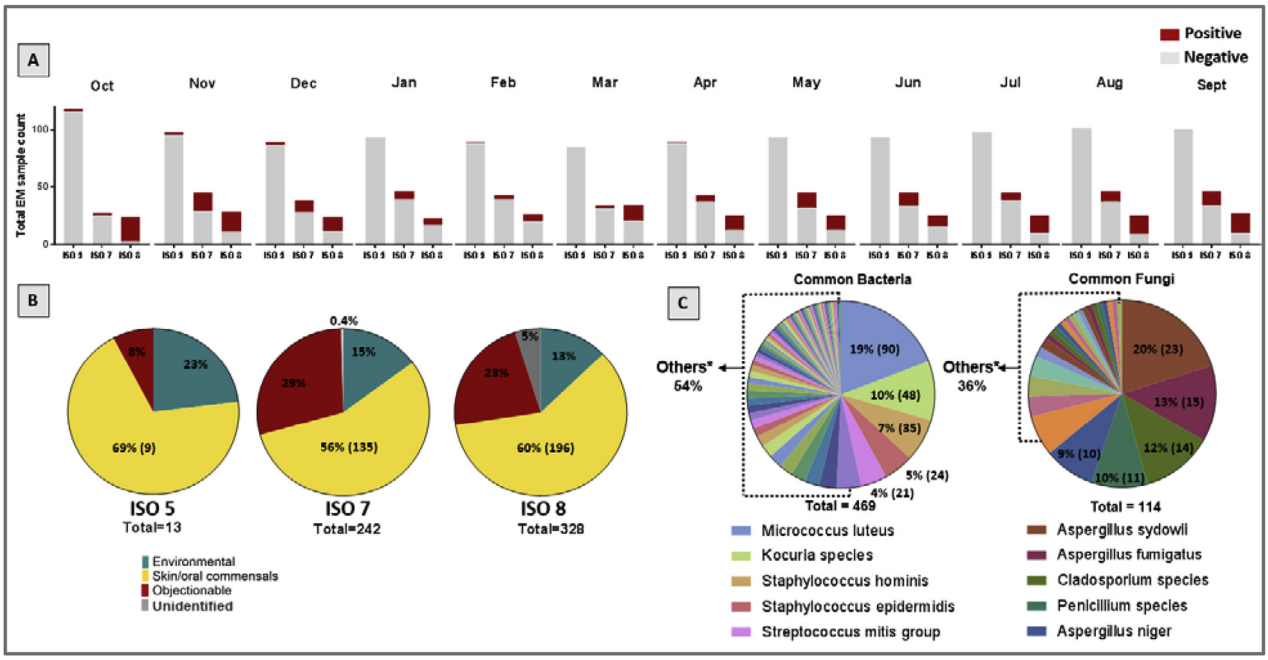

Figure 2. Prospective annual data on EM and microbial speciation in the Cellular Therapy (CT) laboratory (October 2016 to September 2017). (A) Total EM microbial counts, including positives (*red*) and negatives (*gray*) by ISO zones (5, 7, and 8) within the cell processing facility over the 1-year period. (B) Characteristics and distribution of the type of organisms identified in the 3 ISO zones (5, 7, and 8) during the 1-year period. (C) Common bacterial and fungal species identified in the facility across all ISO zones. *Species listed as "others" among bacteria and fungi are included in Supplementary Tables S4 and S5, respectively.

commensal flora that are generally considered nonpathogenic but can cause clinically significant infections in immunocompromised hosts. In our cohort no patients who received infusions with positive sterility products classified as false positive had transfusion-related infections. Ongoing data collected through the application of our assessment protocol will aid further in our risk management approach for product infusion status and the administering of antimicrobial prophylaxis, if needed, when a positive sterility test result is reported.

In conclusion, risk-based product sterility evaluation strategies performed prospectively are a streamlined approach for clinically sound decision-making before product infusion. In addition, they provide a comprehensive documentation framework for eventual safety assessments of these complex products by regulatory agencies. Our systematic and consistent microbial safety assessment may be useful for consideration in a recently proposed "minimum consensus package" for the standardized manufacture and testing of cell therapy products across institutions [28].

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SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at https://doi.org/10.1016/j.bbmt.2018.08.003.

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