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An Environmental Scan of Bioburden Reduction and Control Practices in Tissue Banking

A background document prepared in support of the Bioburden Reduction and Control Leading Practice Development initiative 2014-2015

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Contents

Purpose

The purpose of this environmental scan is to identify current practices relating to bioburden reduction and control in bone, connective tissue, cardiovascular and skin allograft tissue banks in Canada, the United States (U.S.) and Europe.

The goal of Canadian Blood Services' *Bioburden Reduction and Control Leading Practices* initiative is to create national agreement on recommendations for bioburden reduction and control practices in tissue banking in Canada. Key representatives from the Canadian bone, connective tissue, cardiovascular and skin banking community as well as recognized experts outside of the tissue community will be convened to a consensus forum to review and evaluate evidence and current practices in bioburden reduction and control, and to identify and recommend leading practices to improve the safety of tissue allografts produced in Canada.

Introduction and Background

While Canadian regulations and standards provide general requirements for reducing contamination risks they do not detail specific processes. Similarly, although the American Association of Tissue Banks' (AATB) Standards for Tissue Banking and the Food and Drug Administration's Code of Federal Regulations (FDA 21 CFR) Part 1270 have specific requirements to reduce contamination risks, these requirements allow for variability in the area of bioburden control practices employed by individual tissue banks.

On February 8-9, 2012, representatives from the Canadian eye and tissue banking community joined Canadian Blood Services and international colleagues for a workshop focused on the development of leading practices in four areas: data collection and sharing, donor identification and referral, donor tissue specifications, and bioburden reduction during tissue recovery. In preparation for this event a survey of Canadian tissue banks was undertaken. The results confirm variability in bioburden reduction and control practices in Canadian tissue banking operations.[1] Recommendations proposed by the participants applicable to bioburden discussions included:

- Form an expert advisory committee of clinicians and scientists to assess the available evidence and establish standardized national guidelines/criteria based on evidence.
- Initiate a comprehensive, evidence informed conversation with additional stakeholders such as microbiologists which is necessary to define and standardize best practice.
- Work with all provinces and stakeholders to make standardization a practice norm and share nationally standardized practices.
- Clarify what terms like validation and yield mean in the context of significant or non-significant pathogens.

Based on the feedback from the tissue community, Canadian Blood Services prioritized the development of leading practices in bioburden reduction and control. This environmental scan is

[1] Eye and Tissue Banking in Canada: A Leading Practices Workshop February 8 and 9, 2012 - Canadian Survey Responses

an important tool to identify current practices within Canada, and between Canadian banks and those in the U.S. and Europe.

Scope

This report will discuss tissue recovery, transport, storage, processing, disinfection, bacterial log reduction, sterilization methods, sterility assurance levels (SALs), environmental monitoring, staff attire, microbial sampling The focus of this environmental scan report is limited to analysis of current Canadian, U.S. and European bioburden reduction practices beginning with tissue donor preparations prior to tissue recovery, and the subsequent steps in processing through to the release of allografts prior to distribution. Only those practices specific to bone, connective, skin and cardiovascular tissue have been assessed as part of this report.

This report does not address any practices related to ocular tissues, amniotic membrane, decellularization of heart valves, donor cause of death, medical and social history screening, blood testing for transmissible infections, laboratory testing methods, the donor physical exam, autopsies, surgical bone banking or tissue allograft biovigilance programs.

Methods

Tissue banks in Canada, the United States and Europe were surveyed to determine bioburden reduction and control practices they employ. Electronic surveys were administered, completed and returned by email using SurveyMonkey software (SurveyMonkey Inc., Palo Alto, California, USA. www.surveymonkey.com). Copies of surveys were sent with the initial request so they could see the types of questions and the magnitude of the work required and select the appropriate staff person to complete the surveys.

Five surveys were developed:

- Environmental Monitoring, Clean Rooms, & Sterilizers survey (37 questions)
- Tissue Recovery survey (39 questions)
- Bone Processing and Validation survey (48 questions)
- Skin Processing and Validation survey (79 questions)
- Cardiovascular Tissue Processing and Validation survey (33 questions)

All questions were asked in a multiple choice format. The opportunity to provide comments or add further details was possible in most questions. Participant feedback was varied. Some participants completed a survey in less than 15 minutes while others took 1 to 1.5 hours to complete the same survey. Some rated the survey “frustrating” and difficult” when others rated the same as “fairly easy” or “rather enjoyable and interesting”.

The survey questions are presented in Appendices 1-5.

Tissue banks were contacted prior to survey distribution to determine their willingness and ability to participate. Canadian participation was very high, with 12 of 12 banks responding, however a number of U.S. banks declined to participate or provided only limited information. Some U.S. tissue banks declined to complete the survey because they considered part or much

of the information sought by the survey to be proprietary, or for other reasons. European participation in the survey varied; eight tissue banks provide full or partial responses. One Australian tissue bank responded; their response to the survey questions was varied and for the most part was not included in the analysis. Some tissue banks were unable to participate electronically but provided answers that were entered manually. From some tissue banks, key information was obtained by personal communication with tissue bank management and medical directors and from other public materials (e.g., marketing materials, publications, lectures, meeting presentations etc.). Information obtained in this manner was verified and entered manually into the survey. Individual survey results and the identity of the tissue banks were kept confidential by a third party; results were unlinked from the surveyed tissue bank and prepared for analysis by Canadian Blood Services.

Note: *Survey responses are analyzed by the number of banks selecting that specific answer out of the total number of banks answering that question. The denominator is the number of the banks answering the question, as opposed to the number of banks surveyed.*

Tissue Recovery Survey

This survey was sent to all 12 of the Canadian tissue banks and recovery agencies. 100% (n=12) were completed or partially completed. Questions were completed by eleven Canadian tissue banks and partially completed by a heart valve processing center that reported it does not perform recoveries.

Thirty U.S. tissue banks and recovery agencies were invited to participate in the Tissue Recovery survey; thirteen agreed and were sent surveys; eleven were completed.

This survey was sent to six European tissue banks and was completed by five.

The survey was completed by one Australian tissue bank.

Environmental Monitoring & Clean Room Survey

This survey was sent to 11 Canadian tissue banks that process tissue. 100% were partially or fully completed. Nine of the banks process bone, one processes skin only, and one processes heart valves only.

Twenty-one U.S. tissue banks were invited to participate in the Environmental Monitoring & Clean Room survey; six agreed to participate (four bone processors, one skin only, one heart valves only) but only five completed or partially completed the questions (four bone and one skin only processor). Two additional U.S. bone banks provided answers to several questions but did not complete the entire surveys. Their answers were entered manually.

This survey was partially completed by eight European tissue banks: five process bone, five process cardiovascular tissue and three process skin.

Cardiovascular Processing and Validation Survey

This survey was sent to four Canadian tissue banks that process and distribute heart valve allografts; 100% of the banks either completed or partially completed the survey.

This survey was not completed by the two U.S. heart valve processing tissue banks. Select data was collected from each tissue bank's staff through personal communication and entered into the survey. Information was also extracted from company brochures, package inserts and confirmed in a recent published survey.

Only four European heart valve tissue banks completed the survey, however, heart valve decontamination data from 17 recently surveyed European tissue banks was confirmed by contacting them and entering answers into the survey.

Bone Processing and Validation Survey
This survey was sent to the nine Canadian tissue banks that process bone. 89% (n=8) either partially or fully completed the survey. Of the eight participating in the survey, six also process connective tissues (tendon, ligament), and completed connective tissue processing and validation questions.

This survey was also sent to eight U.S. bone banks that that agreed to complete survey; six of eight fully or partially completed the survey, four banks completed questions about connective tissue processing, and two of eight banks did not complete the bone processing survey but provided answers to many of the key questions. Their answers were entered into the survey based on personal communication and recent documents provided by these two bone banks (package inserts, pamphlets describing processing, scientific publications).

A Bone Processing and Validation survey was sent to seven European bone banks; six completed the survey.

Skin Processing and Validation Survey
This survey was sent to the five Canadian tissue banks that recover, process and provide skin allografts. All five banks either partially or fully completed the survey. All five process cryopreserved skin and one also processes (stores) fresh refrigerated skin.

This survey was also sent to nine U.S. skin banks that agreed to participate; eight either partially or fully completed the survey. Of these, three process only dermis allografts, five process split-thickness cryopreserved skin, and two process fresh refrigerated skin.

This survey was also sent to four European skin processing tissue banks that agreed to participate, however, only two banks completed parts of the survey because this survey addressed skin processing by cryopreservation and by refrigerated storage in antibiotic solutions. European skin banks do not process and store skin in the manner addressed by the survey questions but instead mainly use high concentrations, i.e. 50 to 85%, of glycerol during long term refrigerated storage of nonviable skin.

Executive Summary

Viral, bacterial, and fungal infections have been transmitted via transplantation of organs, tissue allografts such as bone, skin, corneas, and heart valves, and cells such as islets, hematopoietic

stem cells, and semen.[2] For more than 20 years, no infectious disease transmission has been reported from processed, freeze dried allografts (except dura) using a validated process that ensures microbial and viral safety. Disease transmission has been associated with fresh, frozen or cryopreserved allografts.[3] Tissue banking activity in Canada is primarily focused on processing fresh, frozen and cryopreserved allografts.

The importance of bioburden reduction processes in tissue recovery and bone, cardiac and skin processing with respect to reducing the quantitative load of bacteria or fungus in allografts is fundamental to patient safety and is well documented in literature.[4]

There are many layers of bioburden reduction and control strategies and processes. These include minimizing contamination risks during tissue recovery and processing, controlled recovery and processing environments, reducing or eliminating bioburden through validated disinfection and sterilization procedures, microbial sampling and testing and environmental monitoring.

Tissue Recovery

Prior to the recovery of tissue from any donor, selection and qualification of a tissue recovery site contributes to minimizing contamination during donor tissue recoveries by preparing and continuously monitoring recovery sites for facility cleanliness and air quality. Prior to each recovery, sites are inspected to ensure that pre-established acceptance criteria are met.

Canadian tissue banks almost exclusively use hospital operating rooms (ORs) for tissue recoveries, sites known to control for environmental contamination. While all responding U.S. banks recover tissues at hospital ORs, an alternative practice using dedicated recovery facilities at tissue banks and medical examiner facilities accounts for the majority of tissue recoveries. This practice is not prevalent in Canada with only one tissue bank recovering in a dedicated recovery site. In the U.S. approximately half of those surveyed also report use of sites where control of environmental contamination is more challenging e.g. hospital morgues and funeral homes

As part of selecting a recovery environment, good practice involves the implementation of an environmental monitoring program. An environmental monitoring program identifies and monitors viable microbes and non-viable particulates from the recovery site surfaces, from recovery staff surfaces and in the circulating air so that preventative and corrective actions can be implemented to control the environment should contaminants exceed established limits. The survey identified that the frequency of environmental monitoring of recovery sites is greater among U.S. banks than Canadian banks with a higher focus on monthly monitoring and use of more numerous monitoring methods, such as settling plates, touch plates etc.

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[2] Eastlund, Ted (1995). Infectious disease transmission through cell, tissue and organ transplantation: Reducing the risk through donor selection. Cell Transplantation Volume 4 Issue 5 September – October 1995 Pages 455-477

[3] Eastlund T. et al (2011). Working Group Other Tissues (non ocular). Project NOTIFY Exploring Vigilance Notification for Organs, Tissues and Cells, A global consultation February 7-9,2011

[4] Ty Endean, DO, Allograft Tissue Transplantation and Sterilization Techniques. Managing Infection Control, August 2006

A contributing factor to contamination during recovery and processing is bioburden introduced by staff. Common practices were reported by all Canadian, U.S. and European recovery services and tissue banks. Almost all require their recovery and processing staff to wear the same protective and barrier attire worn by most hospital operating room staff and also require double gloving.

In addition to staff attire, non-single use equipment, supplies and instrumentation utilized during recovery and processing of tissue can introduce contaminants into the sterile field. Steam sterilization is the most common method of sterilization used by tissue banks for equipment and instrumentation in Canada, the U.S. and Europe. As with recovery and processing environments, sterilizers must be monitored for effectiveness at various intervals. Industry standards call for monitoring each equipment and supply load with chemical indicators and biologic indicators at least weekly[5].

Canadian and U.S. tissue banks report similar types of skin disinfectants applied prior to tissue recovery. Chlorhexadine is the most commonly used skin disinfectant for skin recoveries by Canadian, European, and U.S. recovery programs; however Canadian and European programs use it less than U.S. programs for bone, connective tissue and cardiovascular recoveries.

The means for obtaining pre-processing or recovery samples for culturing are similar for U.S., European and Canadian programs; swabbing is the most common method of sampling recovered donor bone and connective tissue for microbial testing in Canada, Europe, and in the U.S.

The use and value of postmortem blood cultures as a surrogate means to determine donor infection has been debated within the tissue banking community. Bacterial sepsis occurring near the time of death is a contraindication to tissue donation, however, postmortem; tissues may become contaminated from endogenous microbes and from exogenous contamination during the surgical recovery of donated tissues. Blood cultures become positive after death as part of bacterial translocation and bodily decomposition therefore a positive postmortem blood culture loses its predictive value for documenting pre-mortem donor sepsis. Although suitable deceased tissue donors can have no evidence of being clinically infected at the time of death, postmortem blood cultures at the time of tissue recovery are frequently positive, with rates commonly as high as 23% to 39%[3-5]. There is a sharp difference between the practices among Canadian and European tissue banks and U.S. tissue banks regarding obtaining postmortem blood cultures from tissue donors. 50% (n=6) of Canadian tissue banks and 20% (n=2) of European tissue banks obtain postmortem blood cultures from tissue donors as compared to 0% of U.S. tissue banks surveyed.

Controlled Environments

Once tissues have been recovered from a qualified recovery site, they must be processed in an appropriate processing environment. Although there is no current U.S. FDA guidance or an

[5] Association for the Advancement of Medical Instrumentation, American National Standards Institute. Comprehensive guide to steam sterilization and sterility assurance in health care facilities. ANSI/AAMI ST79-2006 and ANSI/AAMI/A1:2008. Arlington, VA: Association for the Advancement of Medical Instrumentation, 2008

AATB standard for cleaning and disinfecting clean rooms used specifically for donor tissue allograft processing, results of the surveys show that all except three of the 26 Canadian, U.S. and European tissue banks that were surveyed report having procedures that require disinfection of the processing area between each donor. Most tissue banks report having established cleaning procedures for their clean rooms; however, there is discrepancy in the number of banks that reported validating the effectiveness of those products and procedures. Just over half of Canadian tissue banks and half of the reporting European tissue banks reported that they have conducted validation studies of their cleaning and disinfection agents and procedures as compared to most of the reporting U.S. tissue banks.

The use of a clean room is necessary for processing tissue. The processing clean room (or laminar flow hood/biological safety cabinet) operates in a manner which minimizes introduction, generation and retention of airborne particles and microbes and is monitored to control the concentration of airborne particles. All reporting U.S. tissue banks that process bone and connective tissue do so within a class 100 environment as compared to approximately two thirds of reporting Canadian banks and less than half of reporting European tissue banks.

Monitoring of surfaces, air, equipment, supplies and staff are required. Tracking and trending of data and alert and action levels are used to signal the need for intervention. Survey answers demonstrate a wide and variable application and scheduling of environmental monitoring techniques. For instance, approximately one quarter of Canadian tissue banks do not perform microbial or non-viable particulate monitoring of the environment within which tissue is processed; whereas, all reporting U.S. and European tissue banks perform this monitoring of the areas within which tissue is processed. The methods used to perform environmental monitoring are considerably variable with tissue banks using a wide variety of techniques from passive air sampling in the form of settling plates to active air sampling. Some banks use touch plates to sample table surfaces and staff, whereas other banks use swabs for sample collection.

Tissue Processing

No Canadian or U.S. tissue banks reported pooling of tissue during tissue processing, however, one European bank reported pooling bone.

Bone: Bone processing in North America is most commonly a multi-step bioburden reduction process involving mechanical cleaning of tissue following by a decontamination step(s). There is great variation between Canadian, U.S and European practices in tissue decontamination. The majority of U.S. banks use antibiotics, peroxide, alcohol and detergents in cleaning, while there is less use of these processes in Canadian banks. All reporting European (except one) and all reporting U.S. tissue banks indicated using hydrogen peroxide as compared to only about a third of Canadian tissue banks. Although U.S banks all reported use of alcohol during tissue decontamination, only about a third of Canadian banks reported using alcohol and half of the European banks reported its use. The use of advanced and patented proprietary processes is also prevalent in U.S. banks and one European bank whereas no Canadian banks used patented or proprietary processes.

The same variation in practice exists for decontamination of tendon and ligament allografts (connective tissue). Two European banks reported using peroxide in their decontamination process for connective tissue whereas none of the Canadian or U.S. banks reported its use. Similar variation was reported for the use of detergents and alcohol.

Cardiac: As with bone and connective tissue processing and decontamination, there is great variation in microbial decontamination of heart valves. All tissue banks use an antibiotic "cocktail" consisting of multiple different antibiotics, however, those cocktails varies greatly in types of antibiotics used as well as the number of different antibiotics used. On average, the "antibiotic cocktail" used by Canadian tissue banks for heart valve processing combines fewer antibiotics than those used by U.S. and European tissue banks. In addition, the use of anti-fungal compounds is more prevalent in Europe as opposed to North American banks. Similarly, the duration and incubation temperature reported by surveyed tissue banks varies. Over half (61%) of tissue banks reported incubating heart valves at 2-8°C for approximately 24 hours during processing. However, all reporting U.S. banks incubate at 37°C, representing a significant portion of the world production of cardiac valves.

Skin: The choice of antibiotics used for decontamination of split thickness skin allografts also varies; however, gentamicin was identified as most commonly used by reporting banks.

Cleaning, decellularizing and disinfecting acellular dermis allografts uses different processing methods involving removing cells by hypotonic cell lysis, detergents, and endonucleases (removes DNA, RNA). U.S. skin processors decontaminate and sterilize acellular dermis allografts by antibiotic exposure and terminal radiation or by treatment with ethanol and peracetic acid. Some U.S. banks reported combining decontamination steps with terminal sterilization by applying low dose radiation.

Limited European data was obtained given that European banks process and store skin allografts in high concentrations of glycerol until transplantation; therefore the survey questions were not applicable to their practices.

Microbial Sampling and Testing

Bone: Survey questions did not address sampling methods or general microbial testing for bone and connective tissue during processing as samples are not often taken at this time.

Cardiac: Sampling for microbial testing occurs at critical points during heart valve processing and is performed by Canadian, U.S., and European heart valve processing tissue banks; but sampling sites and methods vary. Canadian and European tissue banks sample the transport fluid prior to processing whereas U.S. banks filter the transport fluid and sample the filter. Another key difference identified is that Canadian and European tissue banks sample the excised heart valves; however, U.S. banks sample the rinsate solution.

Skin: A variety of sampling techniques for microbial testing (transport fluid sample, swabbing skin, and immersion of pieces of skin) are used to sample recovered skin prior to antibiotic exposure by each of the Canadian and U.S. skin banks. The type of sampling of recovered skin for microbial testing is variable for both U.S. and Canadian banks but all perform testing prior to processing and antibiotic exposure. The survey did identify one particular difference; Canadian banks do not perform quantitative microbial bioburden studies of unprocessed split thickness skin, however two thirds of U.S. banks do. However, only one third of those banks have established upper bioburden limits for tissue acceptable for processing. Both the U.S. and Canada have identified unacceptable pathogens that would be cause for discard.

Mycobacteria: All reporting Canadian and U.S. tissue banks test finished skin allografts for bacteria and fungi, however, not all tissue banks reported testing for *Mycobacterium tuberculosis* (mycobacteria). In contrast, all U.S. banks reported testing cardiovascular allografts for mycobacteria as compared to none of Canadian tissue banks, however, one quarter of Canadian tissue banks reported testing bone allografts for mycobacteria as opposed to none of the U.S tissue banks. Overall, testing for mycobacteria is not common practice with only seven out of 39 (19%) reporting tissue banks indicating that they test. Notably, the use of the anti-tuberculosis antibiotic, streptomycin, is not common with only five (10%) of tissue banks using this particular antibiotic.

Bacteriostasis and fungistasis: Bacteriostasis and fungistasis are potential problems for bone, tendon, articular cartilage, heart valve, cartilage, and skin allografts that are exposed to antibiotics and disinfectants during processing. Allografts can contain residues of decontaminants used during processing such as antibiotics or peroxide. These residues can interfere with post-processing microbial testing, leading to a falsely-negative final sterility test. All U.S tissue banks reported performing bacteriostasis and fungistasis testing on bone and connective tissue allografts, however only half of Canadian and European banks reported performing this test. Of the four Canadian bone banks that do not perform bacteriostasis and fungistasis testing, two use antibiotics and disinfectants that could leave residues that could interfere with final microbial testing, reduce sensitivity and be at increased risk of having falsely negative final test results and, therefore, are at risk of releasing a contaminated allograft.

All respondents of the surveyed tissue banks that process cardiovascular tissue reported performing bacteriostasis and fungistasis testing during their validation studies.

Despite using antibiotics during processing of cryopreserved and fresh refrigerated skin allografts, bacteriostasis and fungistasis testing is much less prevalent in Canadian tissue banks than in U.S. tissue banks.

Sterilization

Terminal sterilization of a donor bone allograft after it is sealed in its final package can eliminate allograft-based microbes that survive bone disinfection and can eliminate environmentally-based microbes that may have contaminated the allograft during processing. All of the U.S. tissue banks responding apply terminal radiation to donor bone and connective tissue allografts

as compared to about one third of Canadian tissue banks and two thirds of European tissue banks. Of those tissue banks performing terminal sterilization with radiation on bone, radiation doses for donor bone allografts are reportedly lower in the U.S. than used in Canada or Europe, however, dosages used for tendons across U.S., Canadian and European banks are variable.

Final Sterility Testing

Following tissue processing by a tissue bank, a final sterility test is performed and there must be no growth for the release of the allograft into inventory for potential patient use. The final sterility test method of five U.S. and five European tissue banks is uniform. All of the reporting U.S. and European tissue banks employ sensitive standard sterility test methods used specifically for sterility testing. Canadian practice varies between programs and from that of the U.S. and Europe. Only a third of reporting Canadian tissue banks reported that their final sterility testing employed sensitive standard sterility test methods used specifically for sterility testing. Half of Canadian tissue banks reported using hospital based microbiology labs that use less sensitive rapid clinical methods designed for non-cadaveric patient samples.

Validation studies

Validation studies are performed to demonstrate that a process will consistently achieve the desired results intended. Questions were asked of tissue banks not to evaluate how thorough their validation studies were but merely to detect evidence to support that their overall decontamination process was validated. These questions included such topics as: inoculation of unprocessed tissue, bacteria inoculum representing the various types of bacteria (aerobic, spore forming etc.), quantitation of bioburden before and after processing, log kill during several time points (individual processing steps), the use of worst case settings and sterility assurance level (SAL) selected and achieved.

All U.S. and 80% of European reporting tissue banks have validated their overall bone and connective tissue decontamination process. Although many Canadian banks reported having performed validation studies of their bone and connective tissue bioburden reduction process, very few of the banks answered questions pertaining to the details of their studies. There are notable differences that were reported by Canadian tissue banks as compared to U.S. and European tissue banks: all reporting U.S. and European tissue banks performed quantitative microbial testing of final bone allografts as compared to only half of reporting Canadian tissue banks. All reporting U.S. and European tissue banks calculated a SAL compared to about one third of reporting Canadian tissue banks. No reporting Canadian tissue banks have assessed viral load as compared to all reporting European tissue banks and about two thirds of reporting U.S. tissue banks.

None of the reporting Canadian or U.S. tissue banks that process skin allografts reported validating their *fresh refrigerated* skin decontamination process; however most reporting U.S tissue banks have validated their *cryopreserved* skin decontamination process whereas most reporting Canadian tissue banks have not.

Similar discrepancies in validation study performance was reported of the overall heart valve allograft decontamination process with all responding U.S. tissue banks reporting that they have

validated their process as compared to a quarter of reporting Canadian tissue banks. Of the Canadian tissue banks providing details of their validations studies only a third reported calculating a SAL, a third reported calculating a log reduction and two thirds reported not using worst case settings in their protocols. The number of European tissue banks reporting and the number of questions answered was so small the results are not informative or representative.

In general, this environmental scan validated that there is variation in all areas of bioburden reduction and control processes among Canadian, U.S. and European tissue banks across each tissue group.

Survey Results of Individual Topics

Selecting and Qualifying Tissue Recovery Sites

Tissue Type:	Bone, connective, cardiovascular and skin
Process:	Tissue recovery
Sub Process:	Selecting and qualifying recovery sites
Data Source:	Tissue Recovery survey questions numbered 5-9

Scope

This report of survey results pertains to minimizing contamination during donor tissue recoveries, one of the bioburden reduction and control practices of tissue donations in Canada, US and Europe. Recovery site practices are designed to minimize contamination during donor tissue recoveries and by selecting, preparing and monitoring recovery sites for improved facility cleanliness and air quality.

Introduction and Overview

In the immediate postmortem interval some tissues become contaminated from exogenous microbes and from external contamination during the surgical removal of donated cadaver tissues. This has been a source of contamination responsible for infections in recipients of the tissue allografts. Tissue bank practices have been implemented to reduce this risk.

A recovery site can contribute to contamination. Some recovery sites have controlled environments and reduced risk of airborne contamination depending on the purpose of the location. Other recovery sites, such as funeral homes and hospital morgues, were not built to control microbial contamination.

Recoveries also take place in hospital operating rooms (ORs) and dedicated specially constructed tissue recovery rooms at tissue banks or forensic (medical examiner) facilities. These are designed to control the risk of external contamination. Recovery sites, regardless of the location, are cleaned and prepared prior to each use.

Tissue banks evaluate, select and prepare recovery sites based on predetermined bioburden risk criteria, prior to first use and each subsequent use. Some tissue banks test a recovery site for air and surface contamination as part of qualifying the recovery site prior to first use and conduct environmental monitoring at intervals thereafter.

Results

A *Tissue Recovery* survey was sent to eleven Canadian tissue banks and the single Canadian recovery (only) service; all either fully or partially completed the survey.

Thirty U.S. tissue banks and recovery agencies were invited to participate in the survey. Twelve agreed to participate and a *Tissue Recovery* survey was sent to seven tissue banks and five recovery (only) agencies; 11 of the 12 either fully or partially completed the survey.

A *Tissue Recovery* survey was sent to seven European tissue recovery services and tissue banks; four completed parts of the survey.

The *Tissue Recovery* survey was completed by one Australian tissue bank.

Table 1: *Qualifying a recovery site to prevent contamination*

Question	Canada	U.S.	Europe	Australia
Do you have written qualifying requirements for a tissue recovery site?				
Yes	10 of 12	11 of 11	3 of 4	0 of 1
No	2 of 12	0 of 11	1 of 4	1 of 1
Are your recovery site requirements the same as those published by AATB?				
Yes	10 of 10	11 of 11	1 of 4	NA
No	0 of 10	0 of 11	3 of 4	NA
If not the same as AATB which of the following are required by your procedures regarding qualifying a new tissue recovery site prior to using it for the first time?				
Adequate floor and tabletop space to allow aseptic recovery procedures	NA	NA	3 of 3	NA
Adequate lighting for physical assessment and tissue recovery	NA	NA	3 of 3	NA
Access to a suitably located hand-washing area for hand/forearm surgical scrub or wash	NA	NA	3 of 3	NA
A controlled air-flow system in the recovery area with no direct access to the outside of the building from the recovery room at any time during, before or after tissue recovery	NA	NA	1 of 3	NA
Wall, floor and work surfaces that are easily cleaned and in good state of repair	NA	NA	2 of 3	NA
No viable signs of insects, rodents or other pests	NA	NA	2 of 3	NA
Absence of standing fluids or contaminated water in the room or can be rectified prior to recovery	NA	NA	2 of 3	NA
Working surfaces that are capable of being cleaned and disinfected prior to recovery of tissue	NA	NA	3 of 3	NA

Question	Canada	U.S.	Europe	Australia
Prior to each recovery, is the recovery room inspected for meeting qualification requirement and the results documented?				
Yes	10 of 10	11 of 11	2 of 4	0 of 1
No	0 of 10	0 of 11	2 of 4	1 of 1

Each entry represents the number of tissue banks selecting the specific question out of the total number of banks answering the specific questions. Not all questions were answered by tissue banks.
NA = Not Answered
NC = No "comment" or "other" entered

Table 2: *Tissue recovery sites*

Question	Canada	U.S.	Europe	Australia
At which sites are tissues recovered?				
Dedicated recovery room at the tissue bank facility	1 of 12	9 of 11	3 of 5	1 of 1
Dedicated recovery room at a medical examiner's facility	0 of 12	8 of 11	0 of 5	0 of 1
Funeral homes, mortuary	0 of 12	5 of 11	3 of 5	0 of 1
Hospital morgue	1 of 12	6 of 11	5 of 5	0 of 1
Hospital operating room	12 of 12	11 of 11	3 of 5	1 of 1
Other: only skin and corneas can be recovered in a morgue	NC	NC	1 of 5	0 of 1
Other: only hospital operating room during organ procurement	NC	NC	NC	1 of 1

Each entry represents the number of tissue banks selecting the specific question out of the total number of banks answering the specific questions. Not all questions were answered by tissue banks.
NC = No "comment" or "other" entered

Analysis

Qualification of tissue recovery sites:

83% (n=10) of Canadian tissue banks report having written qualifying criteria for approving a new recovery site and that the criteria are the same as set by AATB (adequate space, lighting, surgical scrub area, controlled air, controllable access, cleanable surfaces, and cleanliness), with 17% (n=2) of Canadian tissue banks having no written qualification criteria. 83% (n=10) of Canadian banks also report checking a recovery site against required criteria prior to each use and recording results as compared to 17% (n=2) who do not.

100% (n=11) of U.S. tissue banks surveyed report being AATB accredited and report following the recovery site qualification expectations and monitoring.

75% (n=3) of European tissue banks report having written qualifying criteria for approving a new recovery site and only one bank uses criteria as set by the AATB (100% adequate space, 100% lighting, 100% surgical scrub area, 33% controlled air and controllable access, 66% cleanable surfaces and cleanliness). 25% (n=1) of European tissue banks reported having no written qualification criteria. 50% (n=2) of European banks also report checking a recovery site against required criteria prior to each use and recording results.

The one Australian tissue bank that responded does not have written qualifying requirements for a tissue recovery site nor did they report checking a recovery site against required criteria prior to each use and recording results.

Tissue recovery sites:
100% (n=12) of Canadian tissue banks and recovery agencies reported using hospital operating rooms for tissue recoveries, a site known to control for environmental contamination. 8% (n=1) also use a site dedicated to recoveries located at the tissue bank, 8% (n=1) use a hospital morgue, 0% use a funeral home and 0% use dedicated sites at medical examiner facilities.

100% (n=11) of U.S. tissue banks and recovery agencies surveyed use hospital operating rooms. 82% (n=9) also use sites built and dedicated for recoveries located at the tissue bank, 55% (n=6) use a hospital morgue, 45% (n=5) use a funeral home and 73% (n=8) use dedicated sites at medical examiner facilities.

60% (n=3) of European tissue banks reported using hospital operating rooms for tissue recoveries, a site known to control for environmental contamination. 60% (n=3) also use a site dedicated to recoveries located at the tissue bank, 100% (n=5) use a hospital morgue, 60% (n=3) use a funeral home and 0% use dedicated sites at medical examiner facilities. 20% (n=1) use a morgue for skin and cornea only recovery.

The Australian tissue bank reported using hospital operating rooms and a site dedicated to recoveries located at the tissue bank for tissue recoveries. They also reported using the hospital operating room during organ procurement, a site known to control for environmental contamination.

The survey sought identification of what type of recovery site is used but did not seek how often each recovery site is used.

Conclusions and Key Learning Points

1. Tissue banks in the U.S., Canada, and Europe have practices to reduce the risk of external contamination during tissue recoveries. 83% (n=10) of tissue banks in Canada, 100% of the surveyed U.S. tissue banks, and 75% (n=3) of European tissue banks have written qualifying criteria for approving a new recovery site. All of the banks in Canada and the U.S. reported that the criteria are the same as set by the AATB (adequate space, lighting, surgical scrub area, controlled air, controllable access, cleanable surfaces, and cleanliness). Prior to each recovery, the sites are inspected to assure that criteria are met. Qualification of the recovery site varies in European tissue banks.

2. In the U.S. approximately half of those surveyed report use of sites where control of environmental contamination is more challenging (hospital morgues and funeral homes), while only one program in Canada recovered from a morgue.

3. Canadian tissue banks almost exclusively use hospital ORs for tissue recoveries, sites known to control for environmental contamination. In the U.S. all 11 surveyed banks recover tissues at hospital ORs.

4. 10% of Canadian tissue banks (n=2) do not have written criteria for qualifying a recovery facility.

5. The practice of recovery in purpose-built and dedicated sites is highly prevalent in the U.S. with 82% (n=9) recovering in dedicated recovery sites at tissue banks and 73% (n=8) using dedicated sites at medical examiner facilities.

6. The practice of recovery in purpose-built and dedicated sites, hospital operating rooms, and funeral homes are the same in Europe (60% (n=9). Recovering in a hospital morgue was the most prevalent (100%).

7. The practice of recovery in purpose-built and dedicated sites is not prevalent in Canada with only 8% (n=1) recovering in a dedicated recovery site at a tissue bank.

Note: All statements about practices are based on the number of survey respondents, not the absolute number of banks.

Attire Worn by Tissue Recovery and Bone Processing Staff

Tissue Type:	Bone, connective, cardiovascular and skin
Process:	Tissue recovery and processing (bone and connective)
Sub Process:	Bioburden control by use of tissue recovery and bone processing staff attire
Data Source:	Environmental Monitoring and Clean Room survey question numbered 20 and 21 Tissue Recovery Survey question number 31

Scope

This is a report pertaining to attire worn by tissue recovery and bone processing staff. Tissue recovery and processing staff attire reduces the risk of donated tissues from becoming contaminated by tissue bank staff.

Introduction and Overview

The human body is a source of microbial contamination. The outermost layer of skin is made up of flattened dead squamous epithelial cells that are shed and replaced every day[1]. Approximately 10 million of these skin particulates are disseminated into the air daily and 10% contain viable bacteria[2]. Microbes shed from skin, hair[3] and mucous membranes of operating room staff and surgeons can cause wound infections in surgical patients. Similarly, this mechanism represents a risk for contaminating donated tissue by tissue recovery and processing staff. Surgical attire helps contain bacterial shedding and is important in controlling environmental contamination of donated tissues.

The attire worn by tissue recovery and bone processing staff provides the same contamination control as the attire required by hospital operating room staff[4]. Tissue recovery and bone processing staff surgical attire is personal protective equipment that not only provides them a barrier from being exposed to potentially infectious donor blood and tissue but also provides a barrier reducing the risk of staff contaminating the surgically recovered tissue**.** Face masks, eye shields, head covers, gloves and gowns worn tight at the wrists protect from splashes of blood and bodily fluids and reduce exposing skin and shedding of bacteria into the operative environment. Double gloving reduces the risk of self-puncture and laceration by 70% to 87% in comparison to wearing a single-glove[5-8]. Double gloving during surgery is a recommended practice of the Association of Peri-Operative Registered Nurses[4] and other U.S. hospital and medical professional organizations.

Results

A *Tissue Recovery* survey was sent to 11 Canadian tissue banks and one recovery agency; eleven completed the survey.

A *Tissue Recovery* survey was sent to 12 U.S. tissue banks and recovery agencies that agreed to provide information; eleven completed the survey.

A *Tissue Recovery* survey was sent to five European tissue banks; three completed the survey (a skin bank, a heart valve bank and a tissue recovery service).

An *Environmental Monitoring and Cleanroom* survey that asked about bone processing staff attire was sent to nine Canadian tissue banks; all nine completed the survey.

An *Environmental Monitoring and Cleanroom* survey was sent to six U.S. tissue banks with processing facilities and answers to questions 20 and 21 were obtained.

An *Environmental Monitoring and Cleanroom* survey was sent to five European tissue banks; five completed the survey.

The *Environmental Monitoring and Cleanroom* survey was completed by one Australian tissue bank.

Table 1: *Staff attire during tissue recoveries and during bone processing*

Question	Canada	U.S.	Europe	Australia
What attire does your recovery staff wear?				
Sterile gown over street clothes	1 of 12	0 of 11	0 of 5*	0 of 1
Sterile gown over surgical attire	11 of 12	11 of 11	5 of 5*	1 of 1
Sterile gloves, one pair	1 of 12	0 of 11	2 of 5*	0 of 1
Sterile gloves, two pair (double gloving)	11 of 12	11 of 11	3 of 5*	1 of 1
Disposable shoe covering (“booties”)	11 of 12	11 of 11	3 of 5*	0 of 1
Hair covering	12 of 12	11 of 11	5 of 5*	1 of 1
Face mask	12 of 12	11 of 11	4 of 5*	1 of 1
Eye protective glasses/shield	11 of 12	11 of 11	3 of 5*	1 of 1
Other: Cut resistant/Kevlar gloves	NA	4 of 11	NA	NA
Other: T5 helmet and shield during bone recovery	1 of 12	NA	NA	NA
Comment: plastic booties used (protective boots)	NA	NA	2 of 5	NA

Each entry represents the number of tissue banks selecting the specific question out of the total number of banks answering the specific questions.

**Answered by one recovery service, one skin bank, one heart valve bank*

NA = No Answer

Question	Canada	U.S.	Europe	Australia
What attire does your staff wear during *bone* processing?				
Sterile gown over street clothes	0 of 9	0 of 6	0 of 5	0 of 1
Sterile gown over surgical attire	9 of 9	6 of 6	4 of 5	1 of 1

Sterile gloves, one pair	1 of 9	0 of 6	0 of 5	0 of 1
Sterile gloves, two pair (double gloving)	8 of 9	6 of 6	4 of 5	1 of 1
Disposable shoe covering (“booties”)	8 of 9	6 of 6	4 of 5	0 of 1
Hair covering	8 of 9	6 of 6	5 of 5	1 of 1
Face mask	8 of 9	6 of 6	5 of 5	1 of 1
Eye protective glasses/shield	7 of 9	6 of 6	4 of 5	1 of 1
Other: clean gown, apron, double glove	0 of 9	0 of 6	1 of 5	NA
Other: process HV only	1 of 9	NA	NA	NA
Others: Process in OR, femoral head)	1 of 9	NA	NA	NA
Other: Do no bone processing	1 of 9	NA	NA	NA
Other: T4 hood	1 of 9	NA	NA	NA
Other: For retrieval, no processing	1 of 9	NA	NA	NA
Other: cut gloves	NA	NA	1 of 5	NA
Is double gloving required during processing?				
Yes	7 of 11	6 of 7	4 of 7**	1 of 1
No	1 of 11	1 of 7	3 of 7**	0 of 1
Other: OR policy	1 of 11	NA	NA	NA
Other: During bone recovery only	1 of 11	NA	NA	NA
Other: not applicable, collect femoral heads from live donors, no bone processing	1 of 11	NA	NA	NA

***Two of four process bone and double glove, one skin bank and one heart valve processor reported no double gloving*
NA = No Answer

Analysis

Eleven of 12 Canadian, 12 of 12 U.S., five of five European, and the Australian tissue banks and recovery services reported that their recovery staff wears the full array of surgical attire that is standard for hospital surgical staff.

One Canadian tissue bank reported that their recovery staff wear a sterile gown over street clothes and that they do not require recovery or bone processing staff to double glove.

Of the four European tissue banks reporting, two reported that they require double gloving of bone processing staff, whereas, the skin and heart valve processing facilities did not. Eight of nine Canadian tissue banks, six of six U.S. tissue banks with processing facilities, three of five European tissue banks, and the Australian require double gloving by processing/recovery staff.

Conclusions and Key Learning Points

1. Except for a few outliers, almost all of the surveyed Canadian, U.S., European, and Australian recovery services and tissue banks require their recovery and bone processing staff to wear the same protective and barrier attire worn by most hospital operating room staff and require double gloving.

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Autoclave Sterilizer for Sterile Equipment and Supplies

Tissue Type:	Bone, connective, cardiovascular and skin
Process:	Tissue processing
Sub Process:	Use of autoclave steam sterilization and monitoring its effectiveness
Data Source:	Environmental Monitoring survey questions numbered 31-37

Scope

This is a report of survey results pertaining to the control of bioburden and prevention of cross-contamination by sterilizing equipment and supplies that might come in contact with donor tissue during processing. This report addresses the monitoring of in-house steam sterilizers and autoclaves.

Introduction and Overview

The processing of donor tissue in clean rooms requires sterile equipment and supplies that come in contact with the tissue allografts being processed. Tissue banks often have in-house autoclaves to ensure sterility of equipment and supplies. Industry standards call for monitoring each equipment and supply load with chemical indicators and biologic indicators at least weekly[1].

Biological indicators are the best monitors of the sterilization process because they measure the lethality of the sterilizer directly by using the most resistant microorganisms (i.e. Bacillus spores), and not by merely testing the physical (measuring temperature, pressure) and chemical (color change chemical indicators) conditions necessary for sterilization. The U.S. Public Health Service's Centers for Disease Control and Prevention calls for lesser use of biological indicator monitoring if used for non-implantable supplies and equipment than for implantable objects: "If a sterilizer is used frequently (e.g., several loads per day), daily use of biological indicators allows earlier discovery of equipment malfunctions or procedural errors and thus minimizes the extent of patient surveillance and allograft recall needed in the event of a positive biological indicator. Each load should be monitored if it contains implantable objects"[2]**.**

Results

An *Environmental Monitoring* survey was sent to 11 Canadian tissue banks that process tissue; 11 surveys were returned either complete or partially complete. Of the 11 respondents nine process bone only or bone in addition to skin or heart valves, one processes skin only, and one processes heart valves only.

An *Environmental Monitoring* survey was sent to 21 U.S. tissue banks, however, only five either completed or partially completed the survey (four bone and one skin only processor). Two

additional U.S. tissue banks responded that they use sterilizers but did not answer further related questions and did not complete the surveys. Only four banks answered all of the sterilizer related questions.

An *Environmental Monitoring* survey was sent to eight European tissue banks. Eight responded with completed or partially completed surveys.

The *Environmental Monitoring* survey was completed by one Australian tissue bank.

Table 1: *In-house sterilizers and quality monitoring*

Question	Canada	U.S.	Europe	Australia
Do you have an in-house sterilizer(s)?				
Yes	8 of 11	6 of 7	5 of 8	1 of 1
No	3 of 11	1 of 7	3 of 8	0 of 1
Which of the following types of sterilizer(s) are used?				
Steam sterilizer	7 of 7	4 of 4	5 of 5	1 of 1
Ethylene oxide sterilizer	2 of 7	0 of 4	1 of 5	0 of 1
NovaSterilis (Supercritical CO_2)	0 of 7	0 of 4	1 of 5	0 of 1
Comment: Hydrogen peroxide vapor	1 of 7	NC	NC	0 of 1
Comment: “I don’t know – OR policy”	1 of 7	NC	NC	0 of 1
Which effectiveness tests are included with every batch?				
Biologic indicators	5 of 7	2 of 4	2 of 6	1 of 1
Bowie-Dick test	4 of 7	1 of 4	3 of 6	0 of 1
Other chemical indicator	5 of 7	3 of 4	2 of 6	1 of 1
Other: Bowie-Dick test only required once per day	NA	NA	NA	1 of 1
None of the above	0 of 7	1 of 4	1 of 6	0 of 1
Comment: Class 5 chemical integrator (chemical indicator)	NC	1 of 4	NC	0 of 1
Comment: “I don’t know”	1 of 7	NC	NC	0 of 1
Do your procedures require that each day the in-house sterilizer is used, that you run a biologic indicator as a positive control?				
Yes	6 of 7	3 of 4	2 of 6	1 of 1
No	1 of 7	1 of 4	4 of 6	0 of 1
Do you use a moist heat/steam sterilizer (autoclave)?				
Yes	7 of 7	4 of 4	6 of 6	1 of 1
No	0 of 7	0 of 4	0 of 6	0 of 1
Question	**Canada**	**U.S.**	**Europe**	**Australia**
Do you employ a Bowie-Dick test with steam sterilizers?				
Yes	6 of 6	3 of 4	4 of 5	1 of 1
No	0 of 6	1 of 4	1 of 5	0 of 1
How often are sterilizers monitored with biologic indicators?				
Each batch	3 of 5	0 of 3	1 of 2	1 of 1

Daily	2 of 5	3 of 3	1 of 2	0 of 1
Comment: Bowie-dick test every day of use	1 of 5	NC	NC	0 of 1

Each entry represents the number of banks selecting that specific answer out of the total number of banks answering that question.
NC = No Comment entered

Analysis

Of all the tissue banks surveyed, 74% (n=20) of tissue banks reported using in-house sterilizers with 62% (n=17) using autoclaves (steam sterilizers).

Of the 11 Canadian tissue banks, 73% (n=8) reported use of sterilizers. Of seven tissue banks answering the remainder of the questions, 71% (n=5) use biologic indicators with every batch as compared to 50% (n=2) of U.S. tissue banks and 33% (n=2) of European tissue banks. 71 % (n=5) Canadian tissue banks uses a chemical indicator with each batch and one did not know what their practice is.

Of seven surveyed U.S. tissue banks, 86% (n=6) report use of in-house sterilizers; of these, 67% (n=4) report use of autoclaves. Two of four use biologic indicators with each batch, one uses a biologic indicator daily and one uses it every three months along with a chemical indicator with each batch.

Of eight surveyed European tissue banks, 86% (n=5) report use of in-house sterilizers; of these, all report use of autoclaves. Two of six use biologic indicators with each batch, 33% (n=2) use a biologic indicator daily as a control along with the Australian tissue bank.

The Bowie-Dick test was used in autoclaves by 100% (n=6) of reporting Canadian tissue banks, 75% (n=3) of U.S. tissue banks, and 80% (n=4) of European tissue banks. A biologic indicator was reported to be used in each batch by 60% (n=3) of Canadian, none of U. S., 50% (n=1) of European tissue banks and the Australian tissue bank.

Conclusions and Key Learning Points

1. Monitoring processes and frequency of monitoring of sterilizers for effectiveness varies among tissue banks.

2. 87% (n=14) of tissue banks reported they use a Bowie Dick test for steam sterilization.

3. 71% (n=5) of Canadian tissue banks use biologic indicators with every batch as compared to 50% (n=2) of U.S. and 33% (n=2) of European tissue banks.

4. 29% (n=2) of Canadian tissue banks do not use biologic indicators or do not know what their practice is.

5. The Australian tissue bank uses an in-house autoclave with a biologic indicator with every batch and a positive control each day.

Note: All statements about practices are based on the number of survey respondents, not the absolute number of banks.

Reference

1. Association for the Advancement of Medical Instrumentation, American National Standards Institute. Comprehensive guide to steam sterilization and sterility assurance in health care facilities. ANSI/AAMI ST79-2006 and ANSI/AAMI/A1:2008. Arlington, VA: Association for the Advancement of Medical Instrumentation, 2008.

2. Rutala WA, Weber DJ, and the Healthcare Infection Control Practices Advisory Committee. Guideline for Disinfection and Sterilization in Healthcare Facilities, 2008. U. S. Public Health Service. Centers for Disease Control and Prevention.

Skin Preparation Prior to Recovery

Tissue Type:	Bone, connective, cardiovascular and skin
Process:	Tissue recovery
Sub Process:	Skin disinfection prior to tissue recovery
Data Source:	Tissue Recovery survey questions numbered 11, 18, and 24 (skin preparation).

Scope

This report of tissue bank survey results pertains to minimizing contamination during donor tissue recovery by means of donor skin disinfection steps prior to tissue recovery. This environmental scan asked specific questions about the different types of preparation and disinfection of the donor's skin prior to removal of tissues.

Introduction and Overview

Microbial contamination of donated tissue can arise from the recovery site environment, from recovery procedures, from the donor's skin and from the recovery staff during tissue recovery. Recovery staff utilizes aseptic surgical technique, sterile barrier attire, sterile instrumentation and sterile supplies. Prior to incision, the donor's skin at surgical sites and surrounding areas is cleaned and prepared with disinfectants. By sterile draping of the body into separate zones, then first recovering tissues from zones least likely to be contaminated and then sequentially recovering from zones more likely to be contaminated, the risk of cross-contamination is lessened. By recovering individual tissues in a standard numbered sequence, the tissues recovered subsequent to one that is later determined to be contaminated can be identified as being at higher risk of contamination from potentially contaminated instruments and gloves.

67% (n=8) of the 12 Canadian tissue banks and recovery agencies surveyed are AATB accredited. 100% of the 11 U.S. tissue banks and recovery agencies surveyed are AATB accredited. The accredited tissue banks follow AATB requirements for isolation draping, zoned recoveries and sequencing. The *Tissue Recovery* survey did not ask questions about draping, zoned recoveries and sequencing and therefore we cannot infer the practice of the non-AATB accredited programs.

Results

A *Tissue Recovery* survey was sent to 12 Canadian tissue banks and recovery agencies. The survey questions were completed by 11 Canadian tissue banks and partially completed by a heart valve processing center that reported it does not perform recoveries.

Thirty U.S. tissue banks and recovery agencies were invited to participate in the *Tissue Recovery* survey; 13 agreed and were sent surveys. Of the 13 surveys sent, 85% (n=11) were completed.

Five European tissue banks and recovery services agreed to complete the *Tissue Recovery* survey; four partially completed the surveys (one bank recovering bone and skin, one bank recovering skin only and one bank recovering hearts for valves only).

The *Tissue Recovery* survey was completed by one Australian tissue bank.

Table 1: *Disinfecting the Skin Prior to Surgical Removal of Tissue Allografts*

Question	Canada	U.S.	Europe	Australia
What preparation of the skin donor site is applied prior to skin removal?				
Soap	2 of 7	3 of 11	3 of 4	1 of 1
Chlorhexadine	6 of 7	11 of 11	3 of 4	1 of 1
Iodophor, e.g. povidone-iodine, betadine	3 of 7	2 of 11	2 of 4	0 of 1
Alcohol	5 of 7	10 of 11	1 of 4	0 of 1
Mineral Oil	0 of 7	1 of 11	0 of 4	0 of 1
Other: Javex (non-chlorine bleach)	NC	1 of 11	NC	0 of 1
Prior to bone recovery?				
Soap	3 of 10	2 of 11	3 of 3	1 of 1
Chlorhexadine	5 of 10	9 of 11	2 of 3	1 of 1
Iodophor, e.g. povidone-iodine, betadine	4 of 10	5 of 11	1 of 3	0 of 1
Alcohol	5 of 10	9 of 11	1 of 3	0 of 1
Prior to heart recovery?				
Soap	2 of 8	1 of 10	2 of 3	1 of 1
Chlorhexadine	4 of 8	9 of 10	3 of 3	1 of 1
Iodophor, e.g. povidone-iodine, betadine	3 of 8	5 of 10	1 of 3	0 of 1
Alcohol	6 of 8	7 of 10	2 of 3	0 of 1
Other: Ioban	NC	1 of 10	NC	NC

Each entry represents the number of tissue banks selecting the specific question out of the total number of banks answering the specific questions.
NC = No "other" Comment
NA = Not Answered

Analysis

Surveys were completed by 12 Canadian tissue banks, 11 U.S. tissue banks and recovery agencies, four European tissue banks and the results therefore describe common practices in these countries. Participation in the *Tissue Recovery* survey by only one Australian bank concludes that the results are insufficient to infer customary practice patterns in Australia.

Chlorhexadine is used for skin disinfection prior to removing skin allografts in 86% (n=6) of surveyed tissue banks and recovery agencies in Canada, 100% (n=11) in the U.S., and 75% (n=3) in Europe, and in the one Australian tissue bank. Chlorhexadine is less commonly used as a skin disinfectant prior to removing bone allografts in Canada (50%, n=5) than in the U.S. (82%, n=9) and Europe (66%, n=3). Similarly, chlorhexadine is less commonly used prior to whole heart recoveries in Canada (50%, n=4) than in the U.S. (90%, n=9) and Europe (100%, n=3).

43% (n=3) of Canadian tissue banks reported the use of iodophors for skin disinfection prior to skin recoveries, 40% (n=4) prior to bone recoveries and 38% (n=3) prior to whole heart recoveries. The use of iodophors is of similar frequency to the U.S. and European respondents.

Conclusions and Key Learning Points

1. Canadian, U.S., and European tissue banks report similar types of skin disinfectants applied prior to tissue recovery.

2. Chlorhexadine is the predominant reagent used as a skin disinfectant for skin recoveries by Canadian, U.S., and European recovery programs, followed closely by alcohol.

3. Canadian programs use chlorhexadine as a skin disinfectant in bone recoveries less frequently than U.S. and European programs; 50% versus 82% and 66% respectively.

4. Canadian programs use chlorhexadine as a skin disinfectant in cardiac recoveries less frequently than U.S. and European programs; 50% versus 90% and 100% respectively.

5. Chlorhexadine is used by the Australian program for skin preparation for skin, bone and heart recovery.

6. Iodophors are used in recoveries at similar frequencies by Canadian, U.S., and European programs.

Note: All statements about practices are based on the number of survey respondents, not the absolute number of banks.

Pre-Processing Microbial Testing of Bone and Connective Tissue

Tissue Type:	Bone and connective
Process:	Tissue recovery
Sub Process:	Pre-processing microbial testing of recovered bone and connective tissue (prior to exposure to antibiotics)
Data Source:	Tissue Recovery survey questions numbered 17, 19, 20 and 21

Scope

This is a report of survey results pertaining to microbial testing of recovered tissue prior to processing and exposure to antibiotics, disinfectants or sterilants.

Introduction and Overview

Donor tissues can become contaminated from the environment or staff during the recovery process. Tissues can also become contaminated prior to recovery during the natural decomposition of the body beginning at the time when blood circulation ceases; this process is called “bacterial translocation”. Bacterial translocation begins with bacterial overgrowth in the intestine followed by the spread of intestinal microbes from intra-luminal to extra-luminal sites into lymphatic and blood vessels, mesenteric lymph nodes and other tissues and organs.

Pre-processing microbial testing of recovered donor tissue is required by CSA[1], U.S. FDA[2] and AATB[3]. Swabbing each recovered tissue provides qualitative results (identification of genus and species) and is the most common method of sampling and culturing of recovered bone and connective tissue. Recently some tissue banks have switched to a quantitative estimate of each donor’s incoming bioburden load by sampling and culturing recovered bone and connective tissue in a growth medium, extracting microbes and quantifying microbial growth as colony forming units (CFU).

Results

A *Tissue Recovery* survey was sent to 11 Canadian tissue banks and one Canadian tissue recovery agency; eleven respondents completed questions 17 and 19 while ten completed questions 20 and 21. Respondents included four multi-tissue banks that process bone and connective tissue and five tissue banks that process bone only.

A *Tissue Recovery* survey was sent to 12 U.S. tissue banks and recovery agencies that agreed to complete the survey; eleven completed questions 17 and 19. Five U.S. tissue banks and recovery agencies completed questions 20 and 21 (six banks did not respond to these questions).

A *Tissue Recovery* survey was completed by two European tissue banks and an Australian tissue bank.

Table 1**:** *Microbial testing of recovered bone and connective tissues prior to exposure to antibiotics*

Question	Canada	U.S.	Europe	Australia
Which of the following types of skeletal tissue does your facility recover?				
Bone	11 of 11	11 of 11	2 of 2	1 of 1
Osteochondral, cartilage for **"**fresh" refrigerated cartilage allografts	3 of 11	11 of 11	0 of 2	0 of 1
Other cartilage	2 of 11	6 of 11	2 of 2	1 of 1
Ligaments	4 of 11	10 of 11	1 of 2	0 of 1
Tendons	9 of 11	11 of 11	2 of 2	1 of 1
Meniscus	2 of 11	9 of 11	2 of 2	1 of 1
Other: Fascia	1 of 11	NA	1 of 2	0 of 1
Are sampling and testing of recovered bone tissue performed to detect microbial growth prior to exposure to antibiotics?				
Yes, by recovery staff	11 of 11	8 of 11	1 of 2	1 of 1
Yes, but by processing lab staff not recovery staff	*1 of 11	6 of 11	0 of 2	0 of 1
No	0 of 11	0 of 11	0 of 2	0 of 1
Other: En-bloc knee recoveries cultured by recovery staff	NA	1 of 11	NA	NA
Other: Fresh articular grafts are cultured by processor	NA	1 of 11	NA	NA
Other: not all bone is treated with antibiotics. Testing is performed at the point of processing	NA	NA	1 of 2	NA
What recovered bone tissues are sampled for microbial growth?				
Each individual tissue is sampled	10 of 10	4 of 5	1 of 2	1 of 1
Representative tissues are sampled	0 of 10	1 of 5	1 of 2	0 of 1
What method is used for sampling of recovered bone tissues for microbial growth before being exposed to disinfectants and antibiotics?				
Swabbing each individual recovered tissue	6 of 10	4 of 5	2 of 2	1 of 1
Immersion of groups of recovered tissues in growth medium, filtering the extraction fluid, incubating the filter	2 of 10	1 of 5	0 of 2	0 of 1
Other**:** Bone Chip	1 of 10	NA	NA	NA
Other: Two chips or portions from each bone graft	1 of 10	NA	NA	NA

Each entry represents the number of tissue banks selecting the specific question out of the total number of banks answering the specific questions.

NA = Not Answered

** One bank checked yes to both answer options*

Analysis

Sampling of recovered bone for microbial testing is performed by recovery staff of all 11 reporting Canadian tissue banks but only eight of 11 U.S. and one of two European reporting tissue banks and recovery agencies.

Sampling of recovered bone is reportedly performed by processing staff at more tissue banks in the U.S (six of 11) than in Canada (one of 11).

When asked whether individual or representative samplings are taken, individual bone tissue is reported to be sampled by all 11 Canadian tissue banks, four of five U.S. tissue banks, one of two European tissue banks, and the Australian tissue bank.

Sampling is performed by bone swabbing for most tissue banks (six of ten Canadian, four of five U.S., two of two European banks, and the Australian bank).

Alternative sampling is performed by two of 11 Canadian tissue banks and one of five U.S. tissue banks. The alternative sampling process reported in the U.S. includes the immersion of groups of recovered bones in a growth medium, filtering the extraction fluid and incubating the filter. Two of 11 Canadian tissue banks sample by using bone chips.

Conclusions and Learning Points

1. Swabbing is the most common method of sampling recovered donor bone and connective tissue for microbial testing in Canada, U.S., Europe and Australia.

2. 20% of Canadian (n=2) tissue banks obtain and test bone chips from recovered donor bone and connective tissue. One of five U.S. tissue banks sample recovered donor bone by immersing large groups of bone in growth medium and obtaining both quantitative and qualitative incoming bioburden results.

Note: All statements about practices are based on the number of survey respondents, not the absolute number of banks.

References

1. Health Products and Food Branch Guidance Document. Guidance Document for Cell, Tissue and Organ Establishments - Safety of Human Cells, Tissues and Organs for Transplantation. Published by authority of the Canadian Minister of Health. Date adopted 06/18/2013 with minor revisions 08/26/2013. (Bacteriological testing – tissues. 27. An establishment that retrieves tissue, except ocular tissue, must perform bacteriological testing in accordance with section 14.3 of the tissue standard, except for section 14.3.2.8. Samples may either be collected from each individual tissue or may be obtained using a sampling strategy that represents all the tissues received from a particular donor. Indirect sampling can be accomplished by either a swabbing method or a fluid extraction method. Direct sampling is usually accomplished by placing samples of tissue directly into growth media.).

2. U.S. FDA 21 CFR 1271.265(a)] (a) Receipt. You must evaluate each incoming HCT/P for the presence and significance of microorganisms and inspect for damage and contamination. You must determine whether to accept, reject, or place in quarantine each incoming HCT/P, based upon pre-established criteria designed to prevent communicable disease transmission.

3. Standard K2.210. Standards for Tissue Banking. American Association of Tissue Banks. McLean, Virginia, USA. (Requires that appropriate pre-sterilization/pre-disinfection cultures from each donor shall be obtained and evaluated).

Postmortem Donor Blood Cultures and Timing of Tissue Recovery

Tissue Type:	Bone, connective, cardiovascular and skin
Process:	Tissue recovery
Sub Process	Postmortem donor blood cultures and timing of recovery
Data Source:	Tissue Recovery survey questions numbered 2-4

Scope

This is a report of survey results pertaining to minimizing microbial contamination of recovered tissues by performing postmortem donor blood cultures and by recovering tissues as soon as possible after death.

Introduction and Overview

Bacteremia, as documented by a positive blood culture, is the diagnostic test for bacterial sepsis in hospitalized patients. Bacterial sepsis occurring near the time of death is a contraindication to tissue donation. Postmortem blood cultures have been performed as a surrogate to assess the occurrence of sepsis at the time of death.

Postmortem, tissues may become contaminated from endogenous microbes and from exogenous contamination during the surgical recovery of donated tissues.

An important contributor to microbial contamination of recovered tissue is the expected postmortem bacterial overgrowth in the intestine and the spread of intestinal microbes from the intestine into lymphatic and blood vessels, mesenteric lymph nodes and other tissues and organs as part of the normal postmortem decomposition of the body[1]. This is called "translocation": the movement of viable bacteria from the intestine to other bodily sites. The intestinal epithelial barrier is very fragile and the translocation process begins immediately after cessation of circulation[2]. In an effort to mitigate translocation, tissue recoveries should take place as soon as possible after death and the body should be cooled as soon as possible to slow down microbial spread and proliferation. AATB standards require that tissue recoveries start within 24 hours of death if the body is stored refrigerated.

Blood cultures become positive after death as part of bacterial translocation and bodily decomposition therefore a positive postmortem blood culture loses its predictive value for documenting pre-mortem donor sepsis. Although suitable deceased tissue donors can have no evidence of being clinically infected at the time of death, postmortem blood cultures at the time of tissue recovery are frequently positive, with rates commonly as high as 23% to 39%[3-5].

Positive blood culture rates in tissue donors increase as the time interval between when the time the cultures were taken and the time of death becomes longer, especially when this interval is greater than 24 hours[6.7]. Wilson, et.al found that 54% of non-donor deaths had positive postmortem blood cultures even when the cause of death was not related to an infection[8].

Consequently, postmortem blood cultures are not required or recommended by AATB standards or U.S. governmental regulations and the practice has declined.

Results

A *Tissue Recovery* survey was sent to 12 Canadian tissue banks and recovery agencies; 11 banks completed the survey. A heart valve processing center that does not perform recoveries also answered one recovery question.

A *Tissue Recovery* survey was sent to 13 U.S. tissue banks; 11 banks completed the survey.

A *Tissue Recovery* survey was sent to seven European tissue banks; five banks completed the survey.

The *Tissue Recovery* survey was completed by one Australian tissue bank.

Tables 1 and 2 give raw survey data/responses pertaining to the interval between donor death and tissue recovery and the practice of collecting a post-mortem blood culture.

Table 1: *Interval between death and tissue recovery*

Question	Canada	U.S.	Europe	Australia
How long after asystole can non-ocular tissue be recovered if the body was cooled within 12 hrs and refrigerated?				
Within 12 hours	0 of 12	0 of 11	1 of 5*	0 of 1
Within 15 to 24 hours	0 of 12	1 of 11	0 of 5	0 of 1
Within 24 hours[+]	12 of 12	10 of 11	3 of 5	1 of 1
Within 48 hours	0 of 12	0 of 11	1 of 5	0 of 1
How long after asystole can non-ocular tissue be recovered if the body is stored at room temperature?				
Within 6-11 hours	0 of 11	0 of 11	1 of 5*	0 of 1
Within 12 hours	2 of 11	0 of 11	4 of 5	1 of 1
Within 15 hours	9 of 11	11 of 11	0 of 5	0 of 1

Each entry represents the number of tissue banks selecting the specific question out of the total number of banks answering the specific questions.

** Answered by a heart valve bank*

Table 2: *Post-mortem blood cultures in tissue donors*

Question	Canada	U.S.	Europe	Australia
Do you require postmortem blood cultures of the donor?				
Yes	6 of 12	0 of 11	2 of 5*	0 of 1
No	6 of 12	11 of 11	3 of 5	1 of 1

The entry represents the number of banks selecting the specific answer out of the total number of banks answering the question

** Answered by a tissue recovery service*

Analysis

Survey results show that 96% (n=28) of tissue banks recover tissue within the postmortem time limits set by AATB standards, except one European tissue bank. All Canadian and U.S. tissue banks, and the Australian tissue bank recover tissue (or start recoveries) within 24 hours of death when the donor body is stored under refrigerated conditions and within 15 hours of death if the donor body is stored at room temperature.

Some tissue banks selecting the answer "recover tissue within 24 hours" follow their standard practice requiring that tissue recovery *begin within 24 hours*. Others selecting this answer may have a practice requiring *recovery* of all tissue within 24 hours. The survey question did not specify the *start of recovery* within 24 hours or the *completion of recovery* within 24 hours therefore there is room for interpretation and the banks answered the question based on the closest fitting answer to their process.

50% (n=6) of Canadian tissue banks obtain postmortem blood cultures from deceased tissue donors. In comparison, 0% of U.S. tissue banks surveyed and 20% (n=2) of surveyed European tissue banks obtain postmortem blood cultures.

Conclusions and Key Learning Points

1. All Canadian, U.S. and European tissue banks and the Australian tissue bank who were surveyed, report performing tissue recoveries within the time limits set by AATB.

2. There is a sharp difference between the practices among Canadian tissue banks and the other banks surveyed (U.S., European and Australian tissue banks) regarding obtaining postmortem blood cultures from tissue donors. 50% of Canadian tissue banks (n=6) obtain postmortem blood cultures from tissue donors as compared to 0% of U.S. and Australian, and 20% of European tissue banks surveyed.

Note: All statements about practices are based on the number of survey respondents, not the absolute number of banks.

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Recovery Site Environmental Monitoring

Tissue Type:	Bone, connective, cardiovascular and skin
Process:	Tissue recovery
Sub Process:	Recovery site environmental monitoring
Data Source:	Tissue Recovery survey questions numbered 32-39

Scope

This report of survey results pertains to minimizing contamination during tissue recovery in relation to environmental monitoring of recovery sites, one of the bioburden control practices of tissue donation in Canada, the U.S. and Europe.

Introduction and Overview

In the immediate post-mortem interval some tissues become contaminated from endogenous microbes and others from external contamination during the surgical removal of donated tissues. Because enteric organisms make up a part of the contamination found on recovered tissues, a portion of microbial contamination is presumed of endogenous origin, related to postmortem bacterial translocation. It is unknown whether the origin of contamination is predominantly endogenous or is due to external contamination from the recovery site. For contamination originating from the recovery site environment, benefits will result from implementing a recovery site air quality program which includes good environmental monitoring practices. An environmental monitoring program identifies and monitors viable microbes and non-viable particulates from the recovery site and recovery staff surfaces and in the circulating air so that preventative and corrective actions can be implemented to control the environment should contaminants exceed established limits.

Environmental monitoring practices include surface sampling and monitoring as well as passive and active air sampling and monitoring. *Passive* air sampling can be performed using media filled petri dishes exposed to the environment for a specific duration of time. The limitation of passive air sampling is that it does not quantify the particles per volume of air. *Active* air sampling is performed by drawing in predetermined volumes of air so that particles can be counted per cubic foot or litre. *Surface* sampling can be performed using contact plates. One method involves using petri plates which are pressed to a surface and can reveal the number of organisms per surface area sampled. Surface sampling can also be performed using swabs which are rubbed over a surface. This method can determine the type of microorganism present by sub-culturing it to media. The limitation for swabbing is the swab picks up only a portion of the bacteria present and is qualitative, not quantitative.

Historical data of surface, air and staff contamination types and quantities enable recognition of upward trends. Established alert levels and action levels signal when preventative and corrective actions need to be implemented.

Results

A *Tissue Recovery* survey was sent to each of Canada's eleven tissue banks and its single recovery agency; 11 completed the survey. A heart valve processing center that does not perform recoveries, answered one recovery question, resulting in 12 facilities participating in that question.

Thirty U.S. tissue banks and recovery agencies were invited to participate in the *Tissue Recovery* survey; 13 agreed and were sent surveys, 10 of which were completed.

A *Tissue Recovery* survey was sent to seven European banks; five banks completed the survey.

Participation in the *Tissue Recovery* survey by only one Australian bank concludes that the results are insufficient to infer customary practice patterns in Australia.

Table 1: *Survey results pertaining to environmental monitoring of recovery sites*

Question	Canada	U.S.	Europe	Australia
Did your tissue bank perform microbial or particulate environmental monitoring as part of the evaluation and qualification of the tissue recovery site prior to first use?				
Yes	3 of 12	6 of 10	3 of 5	1 of 1
No	9 of 12	4 of 10	2 of 5	0 of 1
At initial evaluation of the recovery site, what type of environmental monitoring was performed?				
Touch plates of surfaces	1 of 3	4 of 6	3 of 3	1 of 1
Touch plates of employees	2 of 3	1 of 6	0 of 3	1 of 1
Swabs of surfaces	1 of 3	1 of 6	1 of 3	0 of 1
Swabs of employees	0 of 3	2 of 6	0 of 3	0 of 1
Passive air monitoring (settling plates)	1 of 3	3 of 6	3 of 3	1 of 1
Active air sampling, particulate counts	2 of 3	1 of 6	1 of 3	1 of 1
Active air sampling, viable particulates (microbial growth)	2 of 3	0 of 6	2 of 3	1 of 1
Other: Determined by hospital operating room policy	1 of 3	NC	NC	NC
Other: Environmental monitoring only at in-house recovery site	NC	1 of 6	NC	NC
Other: "Routine validation of environmental controls"	NC	1 of 6	NC	NC
Is environmental monitoring of a recovery site performed periodically?				
Yes	3 of 12	5 of 10	3 of 5	1 of 1
No	9 of 12	5 of 10	2 of 5	0 of 1

Question	Canada	U.S.	Europe	Australia
When performed periodically at a recovery site, what type of environmental monitoring is performed?				
Touch plates of surfaces	1 of 3	4 of 5	3 of 3	1 of 1
Touch plates of employees	2 of 3	0 of 5	1 of 3	1 of 1
Swabs of surfaces	1 of 3	2 of 5	1 of 3	0 of 1
Swabs of employees	0 of 3	0 of 5	0 of 3	0 of 1
Passive air monitoring (settling plates)	1 of 3	3 of 5	3 of 3	1 of 1
Active air sampling, particulate counts	2 of 3	1 of 5	1 of 3	1 of 1
Active air sampling, viable particulates (microbial growth)	2 of 3	0 of 5	2 of 3	1 of 1
Other: Determined by hospital operating room policy	1 of 3	NC	NC	NC
How often do you perform microbial environmental monitoring?				
Every donor	0 of 3	0 of 5	0 of 3	1 of 1
Once a month	0 of 3	4 of 5	0 of 3	0 of 1
Once a year	0 of 3	0 of 5	1 of 3	0 of 1
Other: Once every three months	2 of 3	NC	2 of 3	0 of 1
Other: Every six months	NC	1 of 5	NC	0 of 1
Other: As determined by OR policy	1 of 3	NC	NC	0 of 1
Other: Pre or post recovery only	NC	1 of 5	NC	0 of 1
Other: Not during the recovery procedure	NC	1 of 5	NC	0 of 1
Other: Settle plates only, other samples periodically	NC	NC	NC	1 of 1
Do you track and trend environmental monitoring data obtained at the recovery sites?				
Yes	2 of 3	5 of 5	3 of 3	1 of 1
No	1 of 3	0 of 5	0 of 3	0 of 1
Do you have environmental monitoring alert or action levels established for your tissue recovery data?				
Yes, alert levels	0 of 3	0 of 5	1 of 3	0 of 1
Yes, action levels	1 of 3	1 of 5	1 of 3	0 of 1
Yes, both	2 of 3	3 of 5	0 of 3	1 of 1
No	0 of 3	1 of 5	1 of 3	0 of 1
How did you establish those levels?				
Based on evaluation of historical data	1 of 3	0 of 5	NC	0 of 1
Based on industry-accepted values	2 of 3	3 of 5	1 of 2	0 of 1
Based on knowledge from previous company employment	0 of 3	1 of 5	NC	0 of 1
Other: Determined by hospital operating room policy	1 of 3	NC	NC	0 of 1
Other: Established by microbiologist	NC	NC	NC	0 of 1
Other: Based on air-quality GMP specifications	NC	NC	1of 2	0 of 1
Other: Based on EU classifications	NC	NC	NC	1 of 1

Each entry represents the number of tissue banks selecting that specific answer out of the total number of banks that answered the question. Several tissue banks completed surveys but skipped questions.
NC = No "other" or "comment" entered

Analysis

A larger portion of U.S. and European tissue banks than Canadian banks perform some degree of microbial or particulate environmental monitoring as part of the evaluation and qualification of their tissue recovery sites prior to first use.

25% (n=3) of Canadian, 60% (n=6) of U.S. and 60% (n=32) of European banks and the Australian tissue bank perform some degree of environmental monitoring of their recovery sites prior to first use. In addition, similar percentages of banks report that they perform environmental monitoring periodically thereafter.

The types and frequency of environmental monitoring of recovery sites are quite variable depending on the tissue bank.

Of those performing environmental monitoring, 80% (n=4) of U.S. banks perform recovery site environmental monitoring monthly as compared to 0% of Canadian and 0% of European banks. 66% (n=2) of Canadian and European banks report performing environmental monitoring quarterly and one Canadian bank performs environmental monitoring as determined by hospital operating room policy. One European bank performs environmental monitoring annually. The Australian tissue bank performs environmental monitoring on every donor recovery.

100% (n=4) of U.S. banks and 100% (n=3) of European banks performing periodic recovery site environmental monitoring use surface contact (touch) plates as compared to 33% (n=1) of Canadian banks.

The majority of U.S. banks 75% (n=3) and 100% (n=3) of European banks use passive air monitoring (settling plates) as compared to 33% (n=1) of Canadian banks.

Conclusions and Key Learning Points

1. 25% (n=3) of Canadian banks perform some level of recovery site environmental monitoring as compared to 60% (n=6) of U.S. banks and 60% (n=3) of European banks, both for initial evaluation of a prospective recovery site and for periodic monitoring thereafter.

2. Frequency of environmental monitoring is greater among U.S. banks and the Australian bank than Canadian and European banks with a higher focus on monthly monitoring and use of more numerous monitoring methods, such as settling plates, touch plates etc.

3. The majority of Canadian, U.S. and European banks report adoption of tracking and trending data with alert and action levels in place based on industry-accepted values. The Australian tissue bank report adoption of tracking and trending data with alert and action levels in place based on EU classifications.

4. Although contamination of recovered tissues is common, it is unknown whether contamination is endogenous and related to postmortem translocation or external contamination from the environment. A critical review of this topic and related scientific

studies can provide evidence for developing a recommended best practice for environmental monitoring of recovery sites.

Note: All statements about practices are based on the number of survey respondents, not the absolute number of banks.

Transportation of the Recovered Heart

Tissue Type:	Cardiovascular
Process:	Tissue recovery
Sub Process:	Conditions during transport of recovered heart from recovery site to processing facility
Data Source:	Tissue Recovery survey questions numbered 25-27

Scope

This report pertains to placing the recovered heart into a cold isotonic solution and maintaining low temperature conditions during shipping from the recovery site to the processing facility where heart valves will be excised, decontaminated and prepared for clinical use. This report does not address qualifying the shipping container, validating the transport process, or the conditions in which the heart, blood vessels or pericardium are temporarily stored and transported.

Introduction and Overview

Currently, heart valves are recovered by aseptic removal of the whole heart, placing it into a sterile isotonic transport solution and transporting it to the processing facility at a low temperature to suppress microbial proliferation and preserve cellular viability. Transportation can be of short or long duration. Distance is short when recovered in the region of the processing facility. In the U.S. cardiac processing has been centralized in a small number of processing facilities and therefore it is common in the U.S. to ship recovered whole hearts from distant sites across many states over long distances, requiring overnight air courier services.

This report addresses the conditions of transport used during transport of the recovered heart to the processing facility

Results

A *Tissue Recovery* survey was sent to 11 Canadian tissue banks and a single recovery (only) service; 11 either partially or fully completed the survey. One tissue bank that processes heart valves does not recover hearts but answered question 27. Seven of the 11 facilities reported that they recover hearts and completed the pertinent survey questions.

A *Tissue Recovery* survey was sent to 12 U.S. tissue banks and recovery (only) agencies; 11 completed the surveys but only 10 recover hearts and completed questions 25 through 27 regarding recovered heart temporary storage and transportation.

A *Tissue Recovery* survey was sent to seven European tissue recovery services; five partially completed the survey.

The *Tissue Recovery* survey was completed by one Australian tissue bank.

Table 1: *Transport conditions during shipment of recovered hearts to the processing facility*

Question	Canada	U.S.	Europe	Australia
Which of the following types of tissue does your facility recover?				
Heart	7 of 11	10 of 11	3 of 5	1 of 1
Blood vessels	0 of 11	9 of 11	3 of 5	0 of 1
Other: Pericardium	1 of 11	1 of 11	NA	NA
Other: Descending thoracic aorta	NA	1 of 11	NA	NA
Other: We do not recover, we process recovered hearts for heart valves	1 of 11	NA	NA	NA
Other: Not specified	NA	NA	2 of 5	NA
Into what fluid is the recovered whole heart placed for temporary storage and transport to the processing lab?				
Antibiotics	0 of 7	0 of 10	0 of 3	0 of 1
RPMI	0 of 7	1 of 10	0 of 3	0 of 1
Saline	1 of 7*	9 of 10	1 of 3	0 of 1
Other: Medium 199	0 of 7	0 of 10	0 of 3	1 of 1
Other: Not specified	NA	NA	1 of 3	NA
Comment: Lactated Ringers solution	6 of 7*	NA	NA	NA
Comment: Hanks solution	1 of 7	NA	NA	NA
What temperature is the transport fluid in which the recovered heart is placed?				
Room temperature (ambient)	0 of 7	1 of 10	1 of 3	0 of 1
Chilled, refrigerated, or wet ice	7 of 7	9 of 10	2 of 3	1 of 1
At which temperature condition is the recovered heart temporarily stored and transported to the processing facility?				
Wet ice	8 of 8**	10 of 10	2 of 3	1 of 1
Gel cold/freezer packs	0 of 8**	0 of 10	1 of 3	0 of 1
Dry ice	0 of 8**	0 of 10	0 of 3	0 of 1
Insulated ambient, room temperature	0 of 8**	0 of 10	0 of 3	0 of 1

Each entry represents the number of tissue banks selecting the specific question out of the total number of banks answering the specific questions.

**One recovery service uses both saline and Lactated Ringers (likely depending upon to which valve processor the heart is sent)*

***One heart valve processor does not recover hearts but answered the question, resulting in eight answers entered instead of seven.*

NA = No Answer

Analysis

100% (n=10) of the reporting U.S. tissue banks and 100% (n=8) of the reporting Canadian tissue banks and 66% (n=2) of the reporting European tissue banks, ship whole hearts to the processing facility on wet ice. One European bank completing these questions transports the heart on gel cold/freezer packs. Nine of eleven U.S. banks and three of five European tissue banks recover blood vessels. No reporting Canadian banks recover blood vessels.

Six of seven reporting Canadian tissue banks and recovery services place the whole heart in Lactated Ringers solution as their transport fluid whereas nine of ten U.S. tissue recovery services and one of three use chilled saline. None of the reporting tissue banks in Canada, the U.S. or the Europe add antibiotics to the heart transport fluid. The Australian tissue bank reported using medium 199 for temporary transport fluid.

Conclusions and Key learning Points

1. Tissue banks and tissue recovery services in Canada, the U.S. and Europe ship the recovered whole heart to the heart valve processor on wet ice to keep the heart cold.

2. There is a difference in the choice of a heart transport fluid with 86% (n=6) of Canadian heart valve recovery programs using Lactated Ringers and 90% (n=9) of U.S. recovery programs using isotonic saline and the Australian tissue bank reported using medium 199.

Note: All statements about practices are based on the number of survey respondents, not the absolute number of banks.

Cleaning and Disinfecting the Tissue Processing Clean Room

Tissue Type:	Bone, connective, cardiovascular and skin
Process:	Tissue processing
Sub Process:	Cleaning and disinfecting the clean room
Data Source:	Environmental Monitoring and Clean Room survey questions numbered 23-30

Scope

This is a report of survey results pertaining to current practices regarding the cleaning and disinfecting of clean rooms used by tissue allograft processing facilities in Canada, the United States and Europe. This environmental scan addresses the practices taken by tissue banks to clean and disinfect tissue processing clean rooms after each use to prevent microbial cross contamination from one donor processing lot to another and to prevent contamination from the processing environment to the tissue allograft being produced.

Introduction and Overview

Industry standards and the U.S. FDA provide guidance for clean rooms in which sterile products, such as injectable and intravenous pharmaceuticals and sterile medical devices, are produced. Guidance includes the following recommendations: that cleaning procedures are described in the procedure manual, that effectiveness of disinfectants and cleaning procedures is measured, that disinfectants should be effective against the normal microbial flora recovered from the facility, that a sporicidal disinfectant is used and that validation studies of disinfectants show adequate removal of potential contaminants from surfaces. There is no current U.S. FDA guidance or an AATB standard for cleaning and disinfecting clean rooms used specifically for donor tissue allograft processing.

Survey questions were presented, not to evaluate how thorough their clean room cleaning and disinfection procedures are, but to sample some of the elements of their practices.

Results

An *Environmental Monitoring and Clean Room* survey was sent to 11 Canadian tissue banks; all 11 either fully or partially completed the survey. Of the 11 respondents four Canadian multi-tissue banks process bone, five banks process bone only, one processes heart valves only and one processes skin only.

An *Environmental Monitoring and Clean Room* survey was sent to four U.S. multi-tissue banks that process bone; all four either fully or partially completed the survey. Two additional U.S. tissue banks provided answers to clean room questions but did not complete the survey.

Answers from these two additional tissue banks were entered into the survey based on personal communication. Surveys were also sent to one U.S. skin (only) bank and one heart valve (only) processing facility. The survey was completed by the skin bank but not by the heart valve processor.

An *Environmental Monitoring and Clean Room* survey was sent to eight European tissue banks who agreed to complete the survey; all either fully or partially completed the survey.

The *Environmental Monitoring and Clean Room* survey was completed by one Australian tissue bank.

Table 1: *Survey data pertaining to cleaning the tissue processing clean room*

Question	Canada	U.S.	Europe	Australia
Do your procedures require cleaning (using a soap or detergent) of the processing area between each donor?				
Yes	9 of 11	7 of 7	7 of 8	0 of 1
No	2 of 11	0 of 7	1 of 8	1 of 1
Comment: As required by hospital OR	1 of 11	NC	NC	NC
Comment: Terminal cleaning done every night in hospital OR	1 of 11	NC	NC	NC
Comment: We use a hospital OR and orthopedic surgeons	1 of 11	NC	NC	NC
Do your procedures require disinfection (using a disinfectant or sporicide) of the processing area between each donor?				
Yes	11 of 11	7 of 7	8 of 8	1 of 1
No	0 of 11	0 of 7	0 of 8	0 of 1
Do your procedures include cleaning and disinfection of the processing area after use at end of each work day?				
Yes	9 of 11	6 of 7	5 of 5	1 of 1
No	2 of 11	1 of 7	0 of 5	0 of 1
When cleaning the processing area at the end of the day, what staff are employed?				
In house employees who clean fulltime	8 of 9	2 of 5	3 of 8	1 of 1
In house employees who are also tissue processing staff	4 of 9	3 of 5	3 of 8	1 of 1
Employees of an outside company	0 of 9	1 of 5	3 of 8	0 of 1
Comment: Hospital housekeeping staff	1 of 9	NC	NC	NC

Question	Canada	U.S.	Europe	Australia
When processing areas are cleaned, does your procedure include cleaning and disinfection of floors and all horizontal surfaces?				
Yes	10 of 11	7 of 7	8 of 8	1 of 1
No	1 of 11	0 of 7	0 of 8	0 of 1
Comment: Floors cleaned according to hospital schedule, horizontal surfaces cleaned each processing.	1 of 11	NC	NC	NC
Comment: Cleaning done as per hospital policies	1 of 11	NC	NC	NC
Do procedures require sequence cleaning from clean areas to dirty areas?				
Yes	8 of 9	6 of 7	8 of 8	1 of 1
No	1 of 9	1 of 7	0 of 8	0 of 1
Do procedures require a two-step cleaning process that begins with cleaning (using a detergent) and is followed by a microbicidal process for disinfection?				
Yes	5 of 9	6 of 7	6 of 8	0 of 1
No	4 of 9	1 of 7	2 of 8	1 of 1
Was an internal validation/qualification performed for use of your cleaning and disinfecting agents?				
Yes	5 of 9	6 of 7	4 of 8	0 of 1
No	4 of 9	1 of 7	4 of 8	1 of 1

Each entry represents the number of banks selecting the specific answer out of the total number of banks answering the specific question. Many questions were skipped by several banks
NC = No "comment" or "other" remarks entered

Analysis

82% (n=9) of Canadian tissue banks have procedures that require cleaning (using soap or detergent) of the processing area between each donor as compared to 100% of U.S. tissue banks (n=7) and 88% (n=7) of European tissue banks and the Australian tissue bank.

27% (n=3) of Canadian tissue banks report processing donor tissue in a hospital operating room, leaving the cleaning and disinfection of the facility to the hospital staff. In response to another question, each of the 26 Canadian, U.S., and European tissue banks and the Australian tissue bank reported that were surveyed reported having procedures that require disinfection (using a disinfectant or sporicide) of the processing area between each donor.

56% (n=5) of Canadian tissue banks have written procedures that require cleaning of their clean rooms using a detergent, followed by a microbicidal process for disinfection as compared to 71% (n=5) of U.S. tissue banks and 75% (n=6) of European tissue banks

Almost all tissue banks reported having procedures that require cleaning and disinfection of clean room floors and all horizontal surfaces i.e. 91% (n=10) of Canadian, 100% (n=7) of U.S. and 100% (n=8) of European tissue banks and the Australian tissue bank, however, 18% (n=2) of Canadian tissue banks reported that cleaning is according to hospital schedule and policy.

Most commonly, tissue banks report that clean room cleaning is accomplished by in-house staff that clean full time or are tissue bank processing staff. One of five U.S. and three of eight European tissue banks report using employees from an outside company. One Canadian tissue bank reported that clean room cleaning is done by hospital housekeeping staff.

56% (n=5) of Canadian tissue banks reported that they have performed an internal validation or qualification for use of cleaning and disinfection agents as compared to 86% (n=6) of U.S. banks and 50% (n=4) of European banks.

Conclusions and Key Learning Points

1. All except four of the 27 Canadian, U.S. and European tissue banks and the Australian tissue bank that were surveyed report having procedures that require disinfection of the processing area between each donor.

2. 27% (n=3) of Canadian tissue banks report processing donor tissue allografts in a hospital operating room and leaving the cleaning and disinfection of the tissue processing area to the hospital staff and their policies.

3. 44% (n=4) of Canadian tissue banks reported that they have not conducted validation studies of their cleaning and disinfection agents as compared to 14% of U.S. tissue banks and 50% of European tissue banks. 86% of U.S. tissue banks have validated their cleaning and disinfection agents. The Australian tissue bank reported that they have not conducted validation studies of their cleaning and disinfection agents.

Note: All statements about practices are based on the number of survey respondents, not the absolute number of banks.

Controlling and Monitoring Air Quality during Tissue Processing

Tissue Type:	Bone, connective, cardiovascular and skin
Process:	Tissue processing and validation
Sub Process:	Air quality during tissue processing in clean rooms and laminar flow hoods
Data Source:	Environmental Monitoring and Clean room survey questions numbered 2 -19

Scope

This is a report of survey results pertaining to the air quality of the environment in which tissues are directly exposed during processing.

Introduction and Overview

A clean room is an environment with a low level of environmental pollutants such as dust, airborne microbes, aerosol particles, and chemical vapors. More accurately, a clean room has a controlled level of contamination that is specified by the number of particles per cubic meter at a specified particle size. For example, the ambient air outside in a typical urban environment contains 35,000,000 particles per cubic meter in the size range 0.5 µm and larger in diameter. This corresponds to an ISO 9 clean room, while an ISO 1 clean room allows no particles in that size range and only 12 particles per cubic meter of 0.3 µm and smaller.

Industry standards and the FDA provide guidance for clean rooms in which sterile products are produced. The processing clean room (or laminar flow hood/biological safety cabinet) operates in a manner which minimizes introduction, generation and retention of airborne particles and microbes and is monitored to control the concentration of airborne particles. Monitoring of surfaces, air, equipment, supplies and staff are required. Tracking and trending of data and alert and action levels are used to signal the need for intervention.

During processing of donor tissue allografts, the air in which the tissue is in immediate contact should be controlled and maintained. The surrounding support or secondary area in which the clean room (or laminar flow hood/sanitary cabinet) is entered or housed should also have a controlled environment. It can have a lower air quality.

The clean room should be provided with clean filtered air with positive pressure so it flows out rather than into the critical processing area. The air should be vented to the outside and not recycled.

Results

An *Environmental Monitoring Clean Room* survey was sent to 11 Canadian tissue banks that process tissue and was either fully or partially completed by all 11 tissue banks. Nine tissue banks process bone or bone plus additional tissues such as cardiac or skin, one tissue bank processes skin only and one tissue bank processes heart valves only.

Twenty one U.S. tissue banks were invited to participate in the *Environmental Monitoring Clean* valves only) and each were sent an *Environmental Monitoring Clean Room* survey; five completed some or all of the questions (four bone and one skin only processor). Two additional U.S. tissue banks provided answers to several questions but did not complete the surveys. Their answers were entered manually.

An *Environmental Monitoring Clean Room* survey was sent to eight European tissue banks; all eight were partially completed.

The *Environmental Monitoring and Clean Room* survey was completed by one Australian tissue bank.

Table 1: *Survey results pertaining to tissue processing and clean room air quality and environmental monitoring*

Question	Canada	U.S.	Europe	Australia
Do you process cardiovascular, skin, bone or connective tissue/soft tissue in a cleanroom?				
Yes	6 of 11	6 of 7	8 of 8	1 of 1
No	5 of 11	1 of 7	0 of 8	0 of 1
What cleanroom air quality is maintained?				
ISO Class 4 (~US Class 10)	1 of 6	0 of 5	0 of 7	0 of 1
ISO Class 5 (~US Class 100)	3 of 6	5 of 5	3 of 7	0 of 1
ISO Class 6 (~US Class 1000)	1 of 6	0 of 5	1 of 7	0 of 1
ISO Class 7 (~US Class 10,000)	1 of 6	0 of 5	4 of 7	1 of 1
ISO Class 8 (~US Class 100,000)	0 of 6	0 of 5	1 of 7	0 of 1
Does your cleanroom have positive pressure with air flowing from the processing cleanroom into adjacent areas?				
Yes	5 of 5	5 of 6	8 of 8	1 of 1
No	0 of 5	1 of 6	0 of 8	0 of 1
Is the cleanroom air exhausted to the outside via a non-recirculating system?				
Yes	4 of 4	3 of 6	6 of 8	0 of 1
No	0 of 4	3 of 6	2 of 8	1 of 1

Question	Canada	U.S.	Europe	Australia
Clean room filtered air exchanges take place at the following rates:				
1-5 air exchanges per hour	0 of 2	0 of 5	1 of 8	0 of 1
6-9 air exchanges per hour	0 of 2	0 of 5	1 of 8	0 of 1
10 air exchanges per hour	0 of 2	0 of 5	2 of 8	0 of 1
11-20 air exchange per hour	0 of 2	1 of 5	0 of 8	0 of 1
More than 20 air exchanges per hour	2 of 2	4 of 5	4 of 8	1 of 1
Do you use any laminar flow hoods (biological safety cabinets) for processing?				
Yes	7 of 11	5 of 5	6 of 8	1 of 1
No	4 of 11	0 of 5	2 of 8	0 of 1
What is the air quality maintained within the laminar air flow hood/biological safety cabinet?				
ISO Class 4 (~US Class 10)	0 of 5	0 of 4	2 of 6	0 of 1
ISO Class 5 (~US Class 100)	5 of 5	4 of 4	4 of 6	0 of 1
Other: EU Grade A	0 of 5	0 of 4	0 of 6	1 of 1
What air quality is maintained in the room which contains the laminar air flow hoods/biologic safety cabinets used for tissue processing?				
Air is not filtered and is uncontrolled for particulates	2 of 5	0 of 4	0 of 6	0 of 1
ISO Class 5 (~US Class 100)	1 of 5	2 of 4	2 of 6	0 of 1
ISO Class 7 (~US Class 10,000)	2 of 5	1 of 4	3 of 6	0 of 1
ISO Class 8 (~US Class 100,000)	1 of 5	0 of 4	1 of 6	0 of 1
Unknown	1 of 7	1 of 4	0 of 6	0 of 1
Other: EU Grade B	0 of 7	0 of 4	0 of 6	1 of 1
Does your tissue bank perform microbial or non-viable particulate monitoring of the environment within which tissue is processed?				
No	3 of 11	0 of 7	0 of 8	0 of 1
Yes, at initial evaluation and qualification of a new tissue clean room	0 of 11	3 of 7	2 of 8	0 of 1
Yes, periodically	8 of 11	7 of 7	7 of 8	0 of 1
Yes, at each recovery operation	0 of 11	0 of 7	0 of 8	1 of 1
Other: continuous	NA	NA	1 of 8	0 of 1

Question	Canada	U.S.	Europe	Australia
What type of environmental monitoring is performed?				
Touch plates of surfaces	5 of 8	3 of 5	6 of 8	1 of 1
Touch plates of employees	4 of 8	3 of 5	2 of 8	1 of 1
Swabs of surfaces	4 of 8	3 of 5	2 of 8	0 of 1
Swabs of employees	0 of 8	1 of 5	0 of 8	0 of 1
Passive air monitoring (settling plates)	4 of 8	4 of 5	6 of 8	1 of 1
Active air sampling, particulate counts A	5 of 8	4 of 5	3 of 8	1 of 1
Active air sampling viable particulates (microbial growth)	5 of 8	4 of 5	5 of 8	1 of 1
Comment: Two hour blood agar settling plates	NA	NA	1 of 8	0 of 1
Comment: Permanent counting of particles at work area	NA	NA	1 of 8	0 of 1
How often do you perform microbial or particulate environmental monitoring during processing?				
Each donor	0 of 8	1 of 5	3 of 7	1 of 1
Once a day	0 of 8	0 of 5	0 of 7	0 of 1
Once a week	1 of 8	1 of 5	0 of 7	0 of 1
Once a month	3 of 8	2 of 5	0 of 7	0 of 1
Once every three months	3 of 8	1 of 5	3 of 7	0 of 1
Once a year	1 of 8	0 of 5	0 of 7	0 of 1
Other: Hospital monitors air quality in OR	1 of 8	NA	NA	NA
Other: At rest and during processing	1 of 8	NA	NA	NA
Other: Every batch	NA	NA	1 of 7	NA
Other: once every three weeks	NA	NA	1 of 7	NA
Note: settle plates per donor; touch and active fortnightly; non-viable 6 monthly; employees annually	NA	NA	NA	1 of 1
Do you track and trend clean room environmental monitoring data?				
Yes	7 of 7	6 of 6	8 of 8	1 of 1
No	0 of 7	0 of 6	0 of 8	0 of 1
Do you have environmental monitoring alert or action levels?				
Yes, alert levels	1 of 7	3 of 6	2 of 8	0 of 1
Yes, action levels	2 of 7	3 of 6	3 of 8	0 of 1
Yes, both	6 of 7	6 of 6	5 of 8	1 of 1
No	0 of 7	0 of 6	0 of 8	0 of 1
If alert or actions levels are specified, how did you establish those levels?				
Based on evaluation of historical data	2 of 7	6 of 6	3 of 8	0 of 1

Question	Canada	U.S.	Europe	Australia
Based on industry-accepted values	4 of 7	1 of 6	4 of 8	0 of 1
Based on knowledge from previous company employment	2 of 7	0 of 6	0 of 8	0 of 1
Other: based on GMP specs	NA	NA	1 of 8	0 of 1
Other: based on EU classification levels	NA	NA	NA	1 of 1
Are any environmental testing, sampling locations described in written procedures?				
Yes	8 of 8	7 of 7	8 of 8	1 of 1
No	0 of 8	0 of 7	0 of 8	0 of 1
Are settling plates used as part of environmental monitoring?				
Yes	5 of 8	6 of 7	7 of 8	1 of 1
No	3 of 8	1 of 7	1 of 8	0 of 1
How often are settling plates used in the critical environment where tissue is processed?				
Every donor	0 of 3	2 of 4	3 of 6	1 of 1
Once a day	0 of 3	0 of 4	0 of 6	0 of 1
Every 2 to 7 days	0 of 3	0 of 4	1 of 6	0 of 1
Every 15-31 days	2 of 3	2 of 4	2 of 6	0 of 1
Used as needed, but without a schedule	1 of 3	0 of 4	0 of 6	0 of 1
Comments: two times per year, based on previous results	0 of 3	0 of 4	1 of 6	NA
During what time are settling plates used in the processing area?				
When no operations are taking place	3 of 4	2 of 3	2 of 7	0 of 1
At beginning of operations	1 of 4	0 of 3	1 of 7	0 of 1
During operations	2 of 4	1 of 3	6 of 7	1 of 1
At the end of operations	0 of 3	0 of 3	1 of 7	0 of 1

Each entry represents the number of tissue banks selecting the specific question out of the total number of banks answering the specific questions.
NA = No Answer

Table 2: *Canadian bone bank processing air quality (from individual survey responses)*

Bone Bank	Processing Air Quality	Use Laminar Flow hood?	Adjacent, Surrounding Room Air Quality
A	Class 2 (<class 100)	Yes	Not filtered, not controlled
B	Class 10	No	NA
C	Class 100	Yes	Class 100
D	Class 100	Yes	Class 10,000
F	Class 100	Yes	Class 10,000
G	Class 100	Yes	Class 10,000

H	Class 10,000	Yes	NA
K	Unknown. Bone is processed and packaged in the hospital operating room	No	NA
I	Unknown. No processing. Bone recovered in hospital OR by orthopedic surgeons, fashioned cultured and packaged in the OR.	No	NA
Heart valves only	Class 100	Yes	Not filtered, not controlled
Skin only	Class 100	Yes	Class 100

NA = No Answer

Table 3: *European tissue banks' air quality (from individual survey responses)*

Tissue Bank	Clean Room	Laminar Flow Hood	Area containing laminar flow hood or clean room
Bone bank A*	Class 10,000^{+}	Not used	Class 10,000
Skin bank	Class 100,000	Class 100^{+}	Class 100,000
Bone bank B*	Class 10,000	Class 10^{+}	Class 10,000
Heart valve bank A	Class 100,000	Class 100^{+}	Class 100,000
Heart valve bank B	Class 100^{+}	Not used	Class 10,000
Bone, Skin, Heart bank A	Class 100	Class 100	Class 100
Bone and Heart bank A	Class 10,000	Class 100	Class 10,000
Bone, Skin, Heart bank B	US Class 100+	Class 10	Class 100

**Terminally sterilize bone allografts*

$^{+}$ *Site of tissue processing*

Table 4: *Australian tissue bank air quality*

Tissue Bank	Clean Room	Laminar Flow Hood	Area containing laminar flow hood or clean room
Bone, skin, heart valve bank	Class 10,000^{+}	EU Grade A	EU Grade B

Analysis

67% (n=6) of Canadian bone banks process bone within a class 100 environment or better. One processes in a class 10,000 environment. Two Canadian bone banks reported fashioning and packaging bone in a hospital operating room. Hospital operating room air quality and environmental monitoring was determined and provided by the hospital at the hospital's schedule, with specifications unknown by the tissue bank. In contrast, 100% (n=4) of reporting U.S. bone banks process tissue in a class 100 environment.

In Europe, banks processes in a class 100 to class 10,000 environment clean rooms and class 10 to class 100 environment laminar flow hood/biological safety cabinet. The Australian tissue bank reported processes in a class 10,000 cleanroom.

73% (n=8) of Canadian bone, skin and cardiac processing tissue banks reported maintaining air quality in critical processing areas (clean rooms or laminar flow hoods) at class 100 or better as compared to 100% (n=5) of U.S. banks and 100% (n=8) of European banks.

100% (n=5) of responding Canadian tissue banks and the Australian tissue bank reported positive pressure in clean rooms with air flowing from the processing area into adjacent areas as compared to 83% (n=5) of U.S. tissue banks and 100% (n=8) of European tissue banks. Air is exhausted to the outside and not recycled by 100% (n=4) of responding Canadian tissue banks and the Australian tissue bank, as compared to 50% (n=3) of the U.S. tissue banks and 75% (n=6) of the European tissue banks. Clean room filtered air exchange rates are >20 per hour at 100% (n=2) of reporting Canadian tissue banks and the Australian tissue bank, 80% (n=4) of U.S. tissue banks and 50% (n=4) of European tissue banks.

64% (n=7) Canadian tissue banks, 100% (n=5) of reporting U.S. tissue banks and 75% of reporting European tissue banks use laminar flow hoods (biologic safety cabinets) for some or all tissue processing. Of those using laminar flow hoods, air quality was maintained at class 100 or better at all five reporting Canadian tissue banks, each of the four reporting U.S. tissue banks and all of the reporting European tissue banks.

73% (n=8) of Canadian tissue banks and the Australian tissue bank monitor particulate or microbial counts in the environment within which tissue is processed, compared to 100% (n=7) of U.S. tissue banks and100% (n=8) of European tissue banks. Each of the seven Canadian, six U.S. and eight European tissue banks and the Australian tissue bank that reported monitoring the processing environment also tracked and trended the data with alert and action levels.

Conclusions and Key Learning Points

1. 100% of the four reporting U.S. tissue banks that process bone do so within a class 100 environment. In comparison, 67% (n=6) of nine reporting Canadian tissue banks that process bone do so in an environment of class 100 or better.

2. 27% (n=3) of Canadian tissue banks that process bone do so within an environment above class 100 (less clean). Two of the three process in a hospital operating room. One Canadian and the Australian tissue bank operates within a class 10,000 environment.

3. 27% (n=3) of the 11 Canadian tissue banks do not perform microbial or non-viable particulate monitoring of the environment within which tissue is processed; whereas, 100% (n=7) of U.S. and 100% (n=8) of European tissue banks and the Australian tissue bank perform this monitoring of the areas within which tissue is processed.

4. Survey answers demonstrate a wide and variable application and scheduling of environmental monitoring techniques.

Note: All statements about practices are based on the number of survey respondents, not the absolute number of banks.

Cleaning and Decontaminating Bone and Connective Tissue Allografts

Tissue Type:	Bone and connective
Process:	Tissue processing
Sub Process:	Cleaning and decontaminating bone and connective tissue allografts
Data Source:	Bone Processing and Validation survey questions numbered 3-7

Scope

This is a report of survey results pertaining to bone cleaning and disinfection steps taken by tissue banks.

Introduction and Overview

Large-scale modern bone processing within the United States utilizes environmentally controlled facilities, cleanroom technology and advanced technologies in the manufacture of allografts. Processes are designed to meet quality requirements of the U.S. FDA's good manufacturing practices, regulatory requirements, accreditation requirements and customer/product specifications.

Bone processing steps are designed to cut, sculpt, mill and fashion allografts into the shapes and sizes desired by surgeons, to eliminate contamination of infectious organisms and to promote incorporation and engraftment by removing extraneous connective tissues, fat, and histo-incompatible marrow, blood and other cells that would otherwise need to be catabolized and removed by the recipient.

All of the large FDA-registered, AATB-accredited, U.S. tissue banks process bone and connective tissue with fully validated processes in clean room environments. They have many bone and connective tissue processing and bioburden reduction features in common: aseptic processing, mechanical and chemical removal of marrow, fat, blood cells as well as exposure to detergents, alcohol, hydrogen peroxide and antibiotics. Some U.S. tissue banks vary by using their own patented devices, such as a patented rotating sonication device containing allografts subjected to sequential detergent, peroxide, disinfectants, alcohol and/or antibiotics[1.] Another large U.S. tissue bank processes bone within a device that uses cycles of high pressure and vacuum with sequential addition and removal of solvent and chemical disinfectants[2-4].

Results

Eight of nine Canadian tissue banks completed questions 3-7 of the *Bone Processing and Validation* survey.

Bone Processing and Validation surveys were sent to six U.S. tissue banks that agreed to and did complete the surveys, including questions 3-6. Two additional U.S. tissue banks provided

answers to questions 3-6 but did not complete the *Bone Processing and Validation* survey. Answers were entered into the survey based on personal communication and recent documents provided by these two tissue banks (package inserts, pamphlets describing processing, scientific publications [5-8]). Only two of six U.S. tissue banks answered question 7.

Surveys were sent to six European tissue banks, six completed questions 3-6 of the survey but did not answer question 7.

The *Bone Processing and Validation* survey was completed by one Australian tissue bank.

Table 1: *Bone Cleaning and Decontamination: A Comparison of Canada, U.S. and Europe*

Question	Canada	U.S.	Europe	Australia
What type of bone and connective tissue does your facility process?				
Bone, deceased donor	8 of 8	6 of 6	6 of 6	1 of 1
Bone, living donor	2 of 8	0 of 6	6 of 6	1 of 1
Demineralized bone products	0 of 8	0 of 6	2 of 6	1 of 1
Tendon	7 of 8	6 of 6	6 of 6	1 of 1
Ligament	3 of 8	6 of 6	4 of 6	0 of 1
Fascia	4 of 8	6 of 6	3 of 6	0 of 1
Cryopreserved osteochondral allograft	0 of 8	0 of 6	0 of 6	1 of 1
“Fresh” refrigerated osteochondral	3 of 8	3 of 6	1 of 6	0 of 1
“Fresh” refrigerated osteoarticular	1 of 8	2 of 6	0 of 6	0 of 1
Other cartilage	0 of 8	0 of 6	2 of 6	0 of 1
Meniscus	2 of 8	1 of 6	4 of 6	1 of 1
Amnion	1 of 8	NA	5 of 6	0 of 1
During processing of traditional bone allografts (excluding demineralized allograft) which of the following steps and treatments are used to reduce bioburden?				
Mechanical or chemical processes to remove marrow, cells, fat	7 of 8	6 of 6	4 of 6	0 of 1
Alcohol	3 of 8	6 of 6	3 of 6	0 of 1
Hydrogen peroxide	3 of 8	6 of 6	5 of 6	1 of 1
Detergents	1 of 8	5 of 6	1 of 6	0 of 1
Antibiotics	4 of 8	6 of 6	3 of 6	1 of 1
Iodophor, e.g. povidone-iodine, betadine	1 of 8	0 of 6	0 of 6	0 of 1
Polyoxyethylene (PEG)	0 of 8	1 of 6	1 of 6	0 of 1
Proprietary methods	0 of 8	5 of 6	0 of 6	0 of 1
Other: Supercritical CO_2	0 of 8	0 of 6	1 of 6	0 of 1
Note: varies on tissue from nothing to antibiotics to hydrogen peroxide	NA	NA	NA	1 of 1

Question	Canada	U.S.	Europe	Australia
What proprietary bone processing methods are used at your facility?				
No, none of the following	8 of 8	1 of 6	5 of 6	1 of 1
Allowash®	0 of 8	3 of 6	0 of 6	0 of 1
Allowash XG®	0 of 8	1 of 6	0 of 6	0 of 1
Advanced Tissue Processing (ATP)	0 of 8	1 of 6	0 of 6	0 of 1
BioCleanse®	0 of 8	0 of 6	0 of 6	0 of 1
Tutoplast	0 of 8	0 of 6	0 of 6	0 of 1
Other: AlloTrue™	0 of 8	1 of 6	0 of 6	NA
Other: NovaSterilis – Supercritical CO_2 by a UDHE machine	0 of 8	0 of 6	1 of 6	NA
Other: Clearant Process®	0 of 8	0 of 6	1 of 6	NA
What type of alcohol is used during bone processing?				
None	5 of 8	0 of 6	3 of 6	1 of 1
Isopropyl alcohol/isopropanol	3 of 8	5 of 6	0 of 6	0 of 1
Denatured ethanol	0 of 8	1 of 6	1 of 6	0 of 1
Ethanol	0 of 8	0 of 6	2 of 6	0 of 1
Methanol	0 of 8	0 of 6	0 of 6	0 of 1
Which antibiotics are used for bone processing?				
None, no antibiotics are used	4 of 8	0 of 6	3 of 6	0 of 1
Gentamicin	4 of 8	2 of 6	2 of 6	1 of 1
Bacitracin	4 of 8	4 of 6	0 of 6	0 of 1
Polymyxin B	0 of 8	5 of 6	1 of 6	0 of 1
Amphotericin B	0 of 8	1 of 6	1 of 6	0 of 1
Penicillin	0 of 8	0 of 6	0 of 6	0 of 1
Primaxin	0 of 8	0 of 6	1 of 6	0 of 1
Proprietary cocktail	0 of 8	0 of 6	0 of 6	0 of 1
Other: Vancomicine plus Tobramicine plus Cotrimoxazole	0 of 8	0 of 6	1 of 6	NA
During cleaning and rinsing of tissue allografts, what type of water is used?				
Water prepared on site	1 of 5*	0 of 2**	0 of 4	0 of 1
Water purchased commercially	3 of 5*	2 of 2**	1 of 4	1 of 1
USP water for injection	1 of 5*	1 of 2**	3 of 4	0 of 1
USP purified water	0 of 5*	0 of 2**	0 of 4	0 of 1
Deionized	0 of 5*	0 of 2**	0 of 4	0 of 1
Distilled	0 of 5*	0 of 2**	0 of 4	0 of 1
Reverse osmosis (RO) water	0 of 5*	0 of 2**	0 of 4	0 of 1
Other: not applicable	NA	NA	1 of 4	NA

Each entry represents the number of tissue banks selecting the specific question out of the total number of banks answering the specific questions.

***** *Only 5 of the 8 Canadian bone banks answered this question*

** *Only 2 of the 6 U.S. bone banks answered the question*

NA = Not Answered

Table 2: *Bone cleaning, decontamination steps: Canadian bone banks*

Bone Bank	Debride, de-fat & de-cell	Detergent	H_2O_2	Alcohol	Antibiotic
A	Yes	No	No	No	No
B	Yes	No	No	No	No
C	Yes	No	No	No	Bacitracin Gentamicin Cefazolin
D	Yes	No	Yes	Yes	Bacitracin Gentamicin Cefazolin
E	No	No	No	No	Bacitracin Gentamicin Cefazolin
F	Yes	No	Yes	Yes	Bacitracin Gentamicin Cefazolin
G	Yes	No	No	No	No
H	Yes	Yes	Yes	Yes	No

Table 3: *Bone cleaning, decontamination steps: U.S. bone banks*

Bone Bank	Debride, de-fat & de-cell	Detergent	H_2O_2	Alcohol	Antibiotic
A	Yes	Yes	Yes	Yes	Polymyxin Bacitracin
B	Yes	Yes	Yes	Yes	Gentamicin Imipenem Amphotericin
C	Yes	Yes	Yes	Yes	Polymyxin Bacitracin
D	Yes	Yes	Yes	Yes	Polymyxin Bacitracin
E	Yes	No	Yes	Yes	Polymyxin Gentamicin
F	Yes	Yes	Yes	Yes	Polymyxin Bacitracin

Table 4: *Bone cleaning, decontamination steps: European bone banks*

Bone Bank	Debride, de-fat & de-cell	Detergent	H_2O_2	Alcohol	Antibiotic
A	Yes	Yes	Yes	Yes	Gentamicin
B	Yes	No	Yes	Yes	No
C	No	No	No	No	Vancomicine Tobramicine Cotrimoxazole
D	Yes	No	Yes	Yes	Polymyxin Gentamicin Primaxin Amphotericin

Table 5: *Bone cleaning, decontamination steps: Australia bone bank*

Bone Bank	Debride, de-fat & de-cell	Detergent	H_2O_2	Alcohol	Antibiotic
A	Yes	No	Yes	No	Gentamicin

Table 1 depicts the bone decontamination and decellularizing chemicals and steps used at eight Canadian tissue banks, six of the large U.S. tissue banks, six European tissue banks and one Australian tissue bank.

Survey completion by eight of nine Canadian tissue banks, the six large U.S. tissue banks, and six European tissue bank would thereby represent the most common practices in those countries; but, the survey participation of only one Australian bank is insufficient to infer customary practice patterns in Australia.

Analysis

88% (n=7) of reporting Canadian tissue banks reported at least minimal bone processing (Tables 1 and 2). Canadian banks' practices varied in their use of antibiotics, peroxide, alcohol and detergents. One Canadian bank uses an iodophor (contains iodine) to aid decontamination.

Of the eight reporting Canadian tissue banks that completed the *Bone Processing and Validation* survey, one has indicated that it does not apply a cleaning step that would include the removal of marrow, cells, or fat from bone. 63% (n=5) indicated they use no alcohol or peroxide, while 88% (n=7) use no detergent. 50% (n=4) of Canadian tissue banks who completed the survey use no antibiotics while the other half use bacitracin and gentamicin soaks.

Each of the six reporting U.S. banks use extensive mechanical and chemical processes in cleaning and removing cells, marrow and fat from bone with most using antibiotics, peroxide, alcohol and detergents (Tables 1 and 3).

All six reporting U.S. banks clean bone with processes including water jets, mechanical agitation, centrifugation, sonication with detergents and peroxide, 100% (n=6) use peroxide, 100% (n=6) use antibiotics, 83% (n=5) use alcohol (four use isopropanol and one uses a specially denatured ethanol) and 83% (n=5) use detergents. 83% (n=5) of U.S. banks use Polymyxin B; 66% (n=4) combine bacitracin with Polymyxin and 33% (n=2) combine bacitracin with gentamicin.

66% (n=4) of reporting U.S. banks use a proprietary technology called AlloWash®; a patented prescribed sequence and concentrations of detergent, peroxide, alcohol and antibiotics. One bank calls its similar proprietary technology Advanced Tissue Processing while another uses similar steps called AlloTrue™, but within a rotating sonication device[1]. Yet another bank uses another proprietary technology called BioCleanse® which includes a device with oscillating pressure and vacuum to enhance penetration of similar cleaning and disinfecting agents. This bank did not participate in the survey[2-4].

Five of the six reporting European banks, 83% (n=5) use extensive mechanical and chemical processes in cleaning and removing cells, marrow and fat from bone. Three of the reporting European banks use antibiotics in processing. 83% (n=5) of reporting European banks use hydrogen peroxide, 16% (n=1) use supercritical CO_2, 16% (n=1) use Polyoxyethylene (PEG) and 16% (n=1) of reporting European banks use proprietary technology (Clearant Process®) (Tables 1).

The Australian bank use hydrogen peroxide and antibiotics in cleaning and removing cells, marrow and fat from bone (Table 1).

Conclusions and Key Learning Points

1. Bone processing in North America is most commonly a multi-step bioburden reduction process.

2. The six U.S. tissue banks surveyed and all except one of the eight Canadian tissue banks surveyed clean bone by mechanical removal of marrow, cells or fat and exposing it to various combinations of detergents, oxidants, solvents, antibiotics or disinfectants.

3. The majority of reporting U.S. banks use antibiotics, peroxide, alcohol and detergents in cleaning, while there is less use of these processes in Canadian banks.

4. The Australian bank use antibiotics and peroxide in cleaning.

5. 100% (n=6) of reporting U.S. banks utilize antibiotics as compared to 50% (n=4) of reporting Canadian banks and 50% (n=3) of reporting European banks.

6. 83% (n=5) of U.S. banks use Polymyxin B as compared to 0% of the Canadian banks.

7. 100% (n=6) of U.S. and European banks (n=2) utilize hydrogen peroxide as compared to 38% (n=3) of Canadian banks.

8. 100% (n=6) of U.S. banks utilize alcohol as compared to 38% (n=3) of Canadian banks. 50% of the European banks surveyed use alcohol.

9. 83% (n=5) of U.S. banks utilize detergents as compared to 13% (n=1) of Canadian banks and 16% (n=1) of European banks.

10. The use of advanced and patented proprietary processes is prevalent in U.S. banks. No Canadian banks used patented or proprietary processes.

Note: All statements about practices are based on the number of survey respondents, not the absolute number of banks.

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Decontaminating Connective Tissue (Tendon, Ligament) Allografts

Tissue Type:	Bone and connective
Process:	Tissue processing
Sub Process:	Cleaning and decontaminating connective tissue (tendon, ligament) allografts
Data Source:	Bone Processing and Validation survey questions numbered 42 and 43

Scope

This is a report of survey results pertaining to the cleaning and decontamination of tendons and ligaments recovered from a donor's leg. Allografts include the patellar tendon (actually a ligament that connects the patella with the tibia with bone blocks at each end containing lipid, blood and cells), the Achilles tendon (connects the calcaneus bone with the gastrocnemius muscle with a bone block at one end) and other tendons such as the semi-tendinosis, gracilis, tibialis, and peroneus longus. These allografts are commonly used to replace torn or ruptured anterior and posterior cruciate ligaments at the knee and other areas.

Introduction and Overview

Although deceased tissue donors have been screened and may have no evidence of being clinically infected at the time of death, testing of recovered connective tissue frequently reveals bacterial and fungal contamination.

Recovered tendons can become contaminated from the recovery site environment, but an important contributor to microbial contamination of recovered tendons is the expected postmortem spread of intestinal microbes to extra-luminal sites such as lymphatic and blood vessels, mesenteric lymph nodes, and to tissues and organs as part of the normal postmortem decomposition of the body.

Tendons are decontaminated by a process that is similar to that used to decontaminate bones, but often is less vigorous. Some evidence suggests an adverse impact on the tendon from processing chemicals such as peroxide, depending on the concentration and length of exposure. Brief exposure to peroxide has no effect[1] but prolonged exposure can reduce tensile strength by up to 15%[2].The extent of tendon processing to reduce bioburden depends on the tissue bank and varies. Like bone, tendons may be exposed to several sequential steps of detergents, alcohol, antibiotics, etc., but with a reduced concentration or duration of exposure or eliminating a step such as peroxide altogether.

Results

Eight of the nine Canadian bone banks who received Bone Processing and Validation surveys, either fully or partially completed the surveys. Of the eight completing the survey, six processed connective tissues (tendon, ligament), and completed questions numbered 42 and 43.

Surveys were sent to four U.S. bone banks that agreed to complete them. All four banks fully or partially completed the surveys, including questions numbered 42 and 43. Two additional large U.S. bone banks that did not complete Bone Processing and Validation surveys provided answers to many of the questions, but not the questions numbered 42 and 43.

Surveys were sent to eleven European bone banks (tendon processing and sterilization questions included); six completed the survey including questions numbered 42 and 43.

The survey was completed by one Australian tissue bank.

Table 1: *Tendon and ligament processing*

Question	Canada	U.S.	Europe	Australia
Does your facility process connective tissue (tendon, ligament)?				
Yes	6 of 8	4 of 4	6 of 6	1 of 1
No	2 of 8	0 of 4	0 of 6	0 of 1
Which of the following types of bioburden reduction processing steps are used for soft/connective tissue?				
Antibiotics or chemicals	3 of 6	2 of 4	4 of 6	1 of 1
Antibiotics or chemical and ionizing radiation	0 of 6	3 of 4	1 of 6	1 of 1
Alcohol	2 of 6	3 of 4	3 of 6	0 of 1
Peroxide	0 of 6	0 of 4	2 of 6	0 of 1
Detergents	1 of 6	2 of 4	2 of 6	0 of 1
Proprietary method only	0 of 6	0 of 4	0 of 6	0 of 1
Proprietary method (Supercritical CO_2)	0 of 6	0 of 4	1 of 6	0 of 1
None of the above	*1 of 6	1 of 4	0 of 6	0 of 1

Each entry represents the number of tissue banks selecting the specific question out of the total number of banks answering the specific questions.

utilizes ionizing radiation without antibiotics or chemicals

Analysis

Five of six Canadian and three of four U.S. tissue banks that were surveyed reported processing tendons using one or more of the following: alcohol, antibiotics, and detergents. None of the Canadian or U.S. bone banks reported using peroxide for tendon processing. The six European tissue banks use antibiotics, alcohol, peroxide, or supercritical CO^2 for tendon processing. 75% (n=3) of the U.S. tissue banks use ionizing radiation in tendon processing as compared to 17% (n=1) of Canadian banks and 17% (n=1) of European banks. The Australian bank reported using antibiotics and ionizing radiation in tendon processing.

Conclusions and Key Learning Points

1. There is considerable variation in the use of chemicals and antibiotics by tissue banks to decontaminate tendons.

2. 100% (n=2) of European bone banks surveyed use peroxide for tendon processing compared to 0% of the Canadian and 33% of the U.S. banks.

3. 75% (n=3) of U.S. banks and the Australian bank use ionizing radiation in tendon processing compared to 17% (n=1) of Canadian banks and 17% (n=1) of the European banks.

4. 100% (n=4) of U.S. banks use detergents in tendon processing compared to 17% (n=1) of Canadian banks and 33% (n=2) of European banks.

5. 75% (n=3) of U.S. banks use alcohol in tendon processing compared to 33% (n=2) of Canadian banks and 50% (n=3) of European banks.

Note: All statements about practices are based on the number of survey respondents, not the absolute number of banks.

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Heart Valve Decontamination

Tissue Type:	Cardiovascular
Process:	Tissue processing
Sub Process:	Heart valve antibiotic decontamination
Data Source:	Cardiovascular Processing and Validation survey questions numbered 4, 6, and 7

Scope

This report of survey results pertains to current donor heart valve microbial decontamination steps as one of the bioburden reduction and control practices of tissue banks in Canada, the United States and Europe. This scan pertains to antibiotics, anti-mycotics and combinations used for microbial decontamination of heart valves as well as the temperature and duration of their incubation.

Introduction and Overview

Current bioburden reduction practices during donor heart valve processing are designed to: identify and eliminate contaminating microbes, monitor bioburden reduction by microbial testing at critical points, prevent further contamination and produce a finished heart valve allograft free from infectious organisms. One important processing step is the decontamination of excised heart valves by their immersion in solutions of multiple antibiotics.

Donor heart valve processing steps vary among tissue banks but they utilize most of the following processes and steps. The aseptically recovered and iced heart is examined and dissected in a clean room or laminar flow hood environment with air quality and surfaces monitored and controlled for non-viable particulates and viable microbes. Processing is performed in either a class 100 (Grade A, ISO 5) clean room or a class 100 biological safety cabinet (laminar flow hood) that is located within a room of lesser, but controlled, air quality.

The cold heart undergoes macroscopic evaluation and dissection to excise the heart valves and their outflow conduits. Excised valves are sized and examined for meeting anatomic, mechanical and functional specifications. Because approximately 10% to 27%[1,2,4,5] of recovered heart valves show some growth of bacteria, and 1% to 3%[1,3] show growth of fungi, an antibiotic decontamination (or disinfection) step follows dissection which is then followed by rinsing, final sterility testing and cryopreservation.

Antibiotic decontamination involves exposure to multiple antibiotics at a specific temperature for a specific duration. Variables contributing to antibiotic effectiveness include the types and

concentrations of antibiotics and the duration and temperature of the incubation period at which the heart valve is exposed to the antibiotics.

Anti-fungal compounds are less commonly used, partly due to their harmful effect on cellular viability but also due to their ability to cause antibiotic resistance by permitting growth of resistant fungal clones.

Results

A *Cardiovascular Processing and Validation* survey was sent to four Canadian tissue banks that recover, process and distribute donor heart valve allografts; all four completed or partially completed the survey.

Cardiovascular Processing and Validation surveys were not directly completed by two U.S. tissue banks but select data was collected from each tissue bank's staff through personal communication and entered into the survey. Information was also provided by company brochures, package inserts[6-9] and confirmed in a recent published survey[10].

Decontamination data from 17 recently surveyed European tissue banks was confirmed by contacting them and entering answers into the survey[11].

The *Cardiovascular Processing and Validation* survey was completed by one Australian tissue bank.

Tables 1 through 4 describe survey results pertaining to heart valve decontamination by four Canadian, two U.S., 17 European, and one Australian tissue banks.

Table 1: *Survey results for heart valve decontamination in Canada, United States and Europe*

Question	Response	Canada	U.S.	Europe	Australia	Total
For heart valve disinfection, which antibiotics are used?	Vancomycin	3 of 4	2 of 2	15 of 17	0 of 1	22 of 24
	Gentamicin	2 of 4	1 of 2	7 of 17	0 of 1	11 of 24
	Cephalosporin: cefazolin, cefoxitin (Mefoxitin), cefoperazone (Cefobid, Cefazone), cefotaxime, cefuroxime, ceftazidime	4 of 4	1 of 2	6 of 17	0 of 1	11 of 24
	Amphotericin B	0 of 4	1 of 2	7 of 17	0 of 1	9 of 24
	Polymyxin B	0 of 4	1 of 2	5 of 17	0 of 1	7 of 24
	Ciprofloxacin	0 of 4	1 of 2	5 of 17	0 of 1	6 of 24
	Colistin (Polymyxin E),Colistimethate (colimycin, ColyMycin M)	2 of 4	0 of 2	4 of 17	0 of 1	6 of 24
	Lincomycin (Lincocin)	0 of 4	1 of 2	4 of 17	0 of 1	5 of 24
	Amikacin	0 of 4	0 of 2	4 of 17	0 of 1	4 of 24
	Imipenum	0 of 4	0 of 2	0 of 17	0 of 1	1 of 24
	Meropenum	0 of 4	1 of 2	0 of 17	0 of 1	1 of 24

Question	Response	Canada	U.S.	Europe	Australia	Total
For heart valve disinfection, which antibiotics are used? (continued from above)	Fluconazole	0 of 4	0 of 2	1 of 17	0 of 1	1 of 24
	Ampicillin & sulbactam (Unisyn)	0 of 4	0 of 2	2 of 17	0 of 1	2 of 24
	Metronidazole (Flagyl)	0 of 4	0 of 2	3 of 17	0 of 1	3 of 24
	Nystatin (antifungal)	0 of 4	0 of 2	2 of 17	0 of 1	3 of 24
	Clindamycin	0 of 4	0 of 2	1 of 17	0 of 1	1 of 24
	Penicillin	0 of 4	0 of 2	1 of 17	1 of 1	1 of 24
	Timentin (ticarcillin & clavulanate)	0 of 4	0 of 2	1 of 17	0 of 1	1 of 24
	Piperacillin/tazobactam (Tazocin, Zosyn)	0 of 4	0 of 2	1 of 17	0 of 1	1 of 24
	Piperacillin	0 of 4	0 of 2	1 of 17	0 of 1	1 of 24
	Streptomycin	0 of 4	0 of 2	1 of 17	1 of 1	2 of 24
	Nystatin	0 of 4	0 of 2	2 of 17	0 of 1	2 of 24
	Ketoconazole	0 of 4	0 of 2	1 of 17	0 of 1	1 of 24
What is the temperature during disinfection of heart valves?	2-8°C	3 of 4	0 of 2	11 of 17	0 of 1	16 of 24
	22-26°C	0 of 4	0 of 2	2 of 17	0 of 1	2 of 24
	20-30°C	0 of 4	0 of 2	2 of 17	0 of 1	2 of 24
	33–38°C	1 of 4	2 of 2	2 of 17	1 of 1	5 of 24
What is the length of incubation during heart valve disinfection?	5-6 hrs	0 of 4	0 of 2	1 of 17	0 of 1	1 of 24
	6-8 hrs	0 of 4	0 of 2	0 of 17	1 of 1	1 of 24
	18-24 hrs	1 of 4	0 of 2	4 of 17	0 of 1	7 of 24
	24±2 hrs	3 of 4	1 of 2	10 of 17	0 of 1	14 of 24
	30-38 hrs	0 of 4	1 of 2	0 of 17	0 of 1	1 of 24
	48 hrs	0 of 4	0 of 2	1 of 17	0 of 1	1 of 24
	72 hrs	0 of 4	0 of 2	1 of 17	0 of 1	1 of 24

Each entry represents the number of tissue banks selecting the specific question out of the total number of banks answering the specific questions.

***Table 2**: Antibiotics used in heart valve decontamination, United States, December 2013*

Tissue Bank	Antibiotic	Medium	Duration	Temperature
A	Vancomycin Gentamicin Polymyxin B Cefoxitin (Mefoxitin) Lincomycin Meropenem Ciprofloxacin	NA	24±2 hrs	37±2°C
B	Vancomycin Amikacin Imipenem Amphotericin B Fluconazole	NA	30-38 hrs	~37°C

NA = Not asked of U.S banks

***Table 3**: Canadian heart valve decontamination, 2013*

Tissue Bank	Antibiotic	Medium	Duration	Temperature
A	Vancomycin Cefoxitin Lincomycin Colimycin M	RPMI	24±2 hrs	4°C
B	Vancomycin Cefoxitin Gentamicin	DMEM	18-26 hrs	33-38°C
C	Gentamicin Cephazolin	HBSS	24±2 hrs	4°C
D	Vancomycin Cefoxitin Lincomycin Colimycin M	RPMI	24±2 hrs	4°C

***Table 4:** European heart valve decontamination, 2012-2013 by bank[11]*

Tissue Bank	Antibiotics	Medium	Duration	Temperature
A	Cefoxitin Vancomycin Polymyxin Clindamycin Amphotericin B	NA	24 hrs	5°C (2-8°C)
B	Penicillin Vancomycin Streptomycin Amphotericin B	RPMI	24 hrs	5°C (2-8°C)
C	Mefoxitin Lincocin Colistin Vancomycin	NA	18-24 hrs	6°C
D	Amikacin Metrodinazol Flu cytosine Vancomycin Ciprofloxacin	NA	18-24 hrs	5°C (2-8°C)
E	Amphotericin Ciprofloxacin Vancomycin Gentamicin	HBSS	21-24 hrs	22°C
F	Lincomycin Vancomycin Polymyxin B	M199	48 hrs	4°C

Tissue Bank	Antibiotics	Medium	Duration	Temperature
G	Gentamicin Vancomycin Clindamycin Colistin Ampicilin & Sulbactam	RPMI	24 hrs	4°C
H	Cefuroxime Gentamicin Amphotericin B Ciprofloxacin Vancomycin Colistin	NA	24 hrs	37°C
I	Amphotericin B Gentamicin Metronidazol Ciprofloxacin Vancomycin	RPMI	24 ±2 hrs	4°C
J	Amphotericin B Ketoconazol Colistin Vancomycin Gentamicin	NA	24 hrs	5°C (2-8°C)
K	Polymyxin B Vancomycin Cefoxitin or Cefotaxime Lincomycin	RPMI-1640	24 hrs	4°C
L	Amikacin Cefuroxime Timentin Vancomycin Polymyxin B Nystatin	M199	18-24 hrs	20-30°C
M	Vancomycin Gentamycin Clindamycin	RPMI	18-24 hrs	4°C
N	Amikacin Ampicilin & Sulbactam Cefperazon Fluconazol Amphotericin B	M199	24 hrs	20-30°C

Tissue Bank	Antibiotics	Medium	Duration	Temperature
O	Amikacin Vancomycin Ciprofloxacin Metronidazole Flu cytosine	NA	5-6 hrs	37°C
P	Vancomycin Polymyxin Ceftazidime Lincomycin	RPMI	72 hrs	4°C
Q	Tazocin (Piperacillin + Tazobactam) Vancomycin Nystatin Vancomycin	NA	24 hrs	20°C (18-22°C)

NA = Not Answered

***Table 5**: Antibiotics used in heart valve decontamination, Australia, 2013*

Tissue Bank	Antibiotic	Medium	Duration	Temperature
A	Penicillin Streptomycin	Medium 199	6-8 hrs	37±2°C

Analysis

Antibacterial agents

The most commonly used antibiotic for donor heart valve decontamination, always in combination with other antibiotics, is vancomycin. Vancomycin is used in 84% (n=22) of surveyed tissue banks including 75% (n=3) of Canadian tissue banks, 100% (n=2) of U.S. tissue banks, and 89% (n=17) of European tissue banks (Table 1).

A cephalosporin is used in 42% (n=11) of surveyed tissue banks including 100% (n=4) of Canadian tissue banks, 50% (n=1) of U.S. tissue banks and 31% (n=6) of European tissue banks (Table 1).

Gentamicin is used in 42% (n=11) of surveyed tissue banks including 50% (n=2) of Canadian tissue banks, 50% (n=1) of U.S. tissue banks and 42% (n=8) of European tissue banks (Table 5).

Antifungal agents

Of 19 European tissue banks surveyed in 2011 by de By et al and partially resurveyed and confirmed in 2013 (Table 4), 63% (n=12) use antifungal antibiotics in their decontamination step (amphotericin by eight banks, nystatin by four banks, fluconazole by one bank, ketoconazole by one bank, and flu cytosine by one bank).

Two European tissue banks (Table 4) and one U.S. tissue bank (Table 2) combine two antifungal compounds in their “antibiotic cocktails”. In contrast, one of the two U.S. tissue banks and all four Canadian tissue banks do not use antifungals (Tables 2, 3).

Anti-mycobacterial agents

Of 19 European tissue banks, only two uses streptomycin in its decontamination process (Table 4). None of the two U.S. tissue banks or the four Canadian tissue banks use streptomycin or any other anti-mycobacterial antibiotics (Tables 2, 3).

Antibiotic combinations

Table 4 shows that in Europe a mean of 4.6 antibiotics (range of 3-6) are combined for use in the decontamination step. Of the two surveyed U.S. tissue banks, one combines five antibiotics and the other combines seven antibiotics (Table 2). The four Canadian tissue banks surveyed (Table 3) use a mean of 3.2 antibiotics (range 2-4). Two Canadian tissue banks use the same four antibiotics, incubation temperatures and duration. On average, Canadian tissue banks use fewer antibiotics in their “antibiotic cocktail” than tissue banks in the U.S. and Europe. A combination of only two antibiotics is used at one of four Canadian tissue banks (Table 3).

Incubation temperature

61% (n=16) of tissue banks carry out heart valve incubation in antibiotics at 2-8°C, 15% (n=4) at 22-30°C (room temperature) and 22% (n=5) at 33-38°C (body temperature). 75% (n=3) of Canadian tissue banks and 68% (n=13) of European tissue banks use 4-5°C (range 2-8°C) as the incubation temperature (Table 3, 4). Neither of the two U.S. banks incubates heart valves at refrigerated temperatures (Table 1, 2).

The following tissue banks disinfect valves at “physiologic” body temperatures i.e. 37°C, 33-38°C: 25% (n=1) of Canadian tissue banks, 100% (n=2) of U.S. tissue banks and 10% (n=2) European tissue banks.

Duration of antibiotic exposure

Table 1 summarizes that 83% (n=19) of tissue banks listed in Tables 2-4 incubate heart valves for 18 to 26 hours, including 100% (n=4) of Canadian tissue banks, 50% (n=1) of U.S. tissue banks and 80% (n=17) of European tissue banks. 53% (n=14) of the 26 tissue banks incubate heart valves for 24 hours (Table 1). The Australian bank incubate heart valves for 6-8 hours at 33-38°C. Two of 19 European tissue banks decontaminate for 48-72 hours and one U.S. tissue bank incubates heart valves for 30-38 hours.

Duration of antibiotic incubation is 6-8 hours at one of 19 European tissue banks. This shorter duration is associated with incubation at bodily temperatures (~33-38°C) rather than cold temperatures (4-6°C).

Conclusions and Key Learning Points

1. There is a very wide variety of antibiotics in use for donor heart valve processing in North America and Europe. The types of antibiotics, incubation times and temperatures chosen by Canadian tissue banks mimic the diversity seen worldwide.

2. Vancomycin is used in 84% (n=22) of the surveyed tissue banks that process heart valves. One Canadian tissue bank does not use vancomycin.

3. On average, the “antibiotic cocktail” used by Canadian tissue banks for heart valve processing combines fewer antibiotics than those used by U.S. and European tissue banks. Canadian tissue banks use an average of 3.2 different antibiotics as compared to the European tissue banks using an average of 4.6 varieties and the U.S. banks using an average of 6.0 varieties.

4. 61% (n=16) of all tissue banks incubate heart valves in antibiotics at 2-8°C for approximately 24 hours during processing, 15% (n=4) incubate at room temperature 20-30°C and 19% (n=5) incubate at 37°C.

5. 100% of the U.S. tissue banks surveyed (n=2) incubate heart valves in antibiotics at 37°C.

6. Most tissue banks in Europe, but not in North America, use an antifungal compound in their antibiotic cocktail. The use of an anti-mycobacterium antibiotic during heart valve disinfection (such as streptomycin used for tuberculosis) is rare.

Note: All statements about practices are based on the number of survey respondents, not the absolute number of banks.

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Microbial Decontamination of Skin Allografts

Tissue Type:	Skin
Process:	Tissue processing
Sub Process:	Split thickness skin allograft microbial decontamination
Data Source:	Skin Processing and Validation survey questions numbered 4, 29 and 30

Scope

This is a report of survey results pertaining to current donor skin allograft microbial decontamination steps as a bioburden reduction and control practice of skin processing facilities in Canada, the United States and Europe. This report addresses decontamination of both cryopreserved and fresh refrigerated donor skin as processed in North America and excludes donor skin stored at refrigerated temperatures in 85% glycerol, as processed in Europe.

Introduction and Overview

Current bioburden reduction practices during donor skin allograft processing are designed to eliminate and prevent further contamination, and produce a finished allograft as free as possible from infectious organisms. After recovery, skin is stored in an antibiotic solution for a variable period of time. The use of antibiotics in decontaminating recovered skin is the subject of this environmental scan.

Cryopreserved donor skin allografts with bacterial contamination have been implicated in causing high fevers and Pseudomonas sepsis[1]. No cases have been published since this paper was published despite wide use of skin allografts in patients with severe burns and burn-related immune suppression.

Two types of donor split thickness skin allografts, cryopreserved and fresh refrigerated skin, are provided to hospitals by tissue banks in North America. After skin is recovered from the donor it is then placed in an antibiotic solution for storage until it is cryopreserved and stored frozen or it is stored in the refrigerator and released to a hospital for a burn patient, usually within 14 days of storage. Cryopreservation is the most common method of skin preservation and storage in North America.

Antibiotic decontamination takes place while donor skin is being stored at refrigerated conditions for a variable period of time. Hypothermic conditions are chosen for immediate short-term storage to preserve skin cell viability and discourage microbial proliferation. Paradoxically, many antibiotics are not effective at these temperatures. When provided as refrigerated skin, the allograft might be used within a few days if clinical need is great or be immersed in antibiotics for up to a week or two if not needed by patients. To maintain skin viability during refrigerated storage the skin medium is exchanged with fresh medium at regular intervals.

When provided as cryopreserved skin, the exposure to antibiotics is shorter since viability declines in storage. To maximize viability, cryopreservation takes place while the skin is fresh.

Results

A *Skin Processing and Validation* survey was sent to five Canadian tissue banks that recover, process and distribute donor skin allografts; all five surveys were returned either complete or partially complete. All five responding tissue banks process cryopreserved skin and one also processes (stores) fresh refrigerated skin.

A *Skin Processing and Validation* survey was sent to nine U.S. tissue banks that recover, process and distribute donor skin allografts. Eight of nine surveys were returned either complete or partially complete; three respondents process only dermis allografts, five process split-thickness cryopreserved skin, and two process fresh refrigerated skin.

A *Skin Processing and Validation* survey was sent to four European tissue banks; only one survey was returned but almost all questions were unanswered because the survey addressed skin processing by cryopreservation and by refrigerated storage in antibiotic solutions. European tissue banks do not process and store skin in the manner addressed by the survey. European tissue banks mainly use high concentrations, 50 to 85%, of glycerol for disinfection and preservation during long term refrigerated storage of nonviable skin. The other three European tissue banks did not complete the survey for the same reason.

The single European tissue bank completing the survey uses 85% glycerol during refrigerated storage. High concentrations of glycerol have antimicrobial activity and serve as a decontamination step for European tissue banks.

The *Skin Processing and Validation* survey was completed by one Australian tissue bank.

Table 1: *Antibiotics used during temporary refrigerated storage prior to cryopreservation*

Question	Canada	U.S.	Europe	Australia
Which antibiotics are used during cryopreserved skin processing?				
Gentamicin	4 of 5	4 of 5	1 of 1	0 of 1
Cephazolin	2 of 5	1 of 5	0 of 1	0 of 1
Vancomycin	2 of 5	1 of 5	1 of 1	0 of 1
Bacitracin	1 of 5	0 of 5	0 of 1	0 of 1
Ciefoxitin/Mefoxitin	1 of 5	0 of 5	0 of 1	0 of 1
Streptomycin	1 of 5	0 of 5	0 of 1	1 of 1
Kanamycin	0 of 5	1 of 5	0 of 1	0 of 1
Ciprofloxacin	0 of 5	1 of 5	0 of 1	0 of 1
Oxacillin	0 of 5	1 of 5	0 of 1	0 of 1
Other: Amphotericin B	NA	1 of 5	0 of 1	0 of 1
Other: Bactrim	NA	1 of 5	0 of 1	0 of 1
Polymyxin B	0 of 5	0 of 5	1 of 1	0 of 1

Lincomycin	0 of 5	0 of 5	0 of 1	0 of 1
Nystatin (antifungal)	0 of 5	0 of 5	1 of 1	0 of 1
Meropenem	0 of 5	0 of 5	0 of 1	0 of 1
Timentin	0 of 5	0 of 5	0 of 1	0 of 1
Clindamycin	0 of 5	0 of 5	0 of 1	0 of 1
Cefoperazone	0 of 5	0 of 5	0 of 1	0 of 1
Cefataxime	0 of 5	0 of 5	0 of 1	0 of 1
Cefuroxime	0 of 5	0 of 5	0 of 1	0 of 1
Piperacillin	0 of 5	0 of 5	0 of 1	0 of 1
Fluconazole (antifungal)	0 of 5	0 of 5	0 of 1	0 of 1
Ketoconazole (antifungal)	0 of 5	0 of 5	0 of 1	0 of 1
Proprietary antibiotic cocktail	0 of 5	0 of 5	0 of 1	0 of 1
Other: Penicillin	0 of 5	0 of 5	0 of 1	1 of 1

Each entry represents the number of tissue banks selecting the specific question out of the total number of banks answering the specific questions.

Table 2: *Antibiotics used during fresh refrigerated split thickness skin allograft storage*

Question	Canada	U.S.	Australia
Which antibiotics are used during fresh refrigerated skin processing?			
Streptomycin	1 of 1	0 of 2	0 of 1
Gentamicin	0 of 1	2 of 2	0 of 1
Vancomycin	0 of 1	1 of 2	0 of 1
Nystatin (antifungal)	0 of 1	1 of 2	0 of 1
Polymyxin B	0 of 1	0 of 2	0 of 1
Lincomycin	0 of 1	0 of 2	0 of 1
Cephazolin	0 of 1	0 of 2	0 of 1
Bacitracin	0 of 1	0 of 2	0 of 1
Cefoxitin/Mefoxitin	0 of 1	0 of 2	0 of 1
Kanamycin	0 of 1	0 of 2	0 of 1
Ciprofloxacin	0 of 1	0 of 2	0 of 1
Oxacillin	0 of 1	0 of 2	0 of 1
Meropenem	0 of 1	0 of 2	0 of 1
Timentin	0 of 1	0 of 2	0 of 1
Clindamycin	0 of 1	0 of 2	0 of 1
Cefoperazone	0 of 1	0 of 2	0 of 1
Cefataxime	0 of 1	0 of 2	0 of 1
Cefuroxime	0 of 1	0 of 2	0 of 1
Piperacillin	0 of 1	0 of 2	0 of 1
Fluconazole (antifungal)	0 of 1	0 of 2	0 of 1
Ketoconazole (antifungal)	0 of 1	0 of 2	0 of 1
Proprietary antibiotic cocktail	0 of 1	0 of 2	0 of 1

What is the maximum storage period for fresh refrigerated skin?			
7 days or less	0 of 1	0 of 2	0 of 1
8-13 days	0 of 1	1 of 2	0 of 1
14 days	1 of 1	1 of 2	0 of 1

Each entry represents the number of tissue banks selecting the specific question out of the total number of banks answering the specific questions.

Analysis

The tables depict survey data pertaining to the types of antibiotics in which donor skin is temporarily stored.

Gentamicin is the most common antibiotic chosen for use in cryopreserved donor skin; sometimes used alone or in combination with others. Gentamicin is used by 80% (n=4) of Canadian tissue banks and 100% (n=4) of U.S. tissue banks who responded to the survey. One respondent indicated adding an additional antibiotic to gentamicin while another indicated adding three other antibiotics.

100% of the U.S. tissue banks (n=2) reported using Gentamicin for fresh donor skin. Only one Canadian tissue bank provides fresh split thickness donor skin allografts; the same tissue bank also provides cryopreserved skin. This tissue bank uses only one antibiotic, streptomycin, in its cryopreserved and fresh refrigerated skin decontamination and temporary storage process.

European tissue banks use 85% glycerol and refrigerated storage. High concentration glycerol has anti-microbial activity and serves as a decontamination process.

Conclusions and Key Learning Points

1. Gentamicin, an antibiotic used clinically chiefly for infections due to Gram negative bacteria, is used by most tissue banks that recover, process and distribute donor skin allografts in North America.

2. Other antibiotics were added by some tissue banks but the choice and type was variable.

Note: All statements about practices are based on the number of survey respondents, not the absolute number of banks.

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Cleaning, Decellularizing and Disinfecting Dermis Allografts

Tissue Type:	Skin
Process:	Tissue Processing
Sub Process:	Decellularizing and decontaminating dermis allograft
Data Source:	Skin Processing and Validation survey questions numbered 50-60

Scope

This environmental scan reports survey results of tissue banks in Canada, the U.S. and Europe pertaining to the processes used to decellularize, decontaminate, sterilize and store human acellular dermis allografts.

Introduction and Overview

Acellular dermis is a versatile allograft used for patients with severe burns, abdominal wall, breast, and pelvic reconstructions and for treatment of non-healing wounds and skin ulcers. Acellular dermis is derived from full thickness skin allograft donations by removing the epidermis and all the cells from the underlying dermis. The remaining extracellular dermal matrix proteins provide a structurally-sound connective tissue sheet which serves as a scaffold for the patient's own cells to repopulate, incorporate, and re-vascularize. As an acellular tissue, the absence of donor cells lessens the chance of the patient developing an inflammatory or an immune response to the graft.

Results

Skin Processing and Validation surveys were sent to five Canadian skin banks and each of them completed all or part of the survey. None of the five reporting Canadian skin banks process or provide human dermis allografts or provided answers to questions numbered 50 through 60 pertaining to dermis allograft processing.

Of seven U.S. skin processors providing data for the *Skin Processing and Validation* survey, six answered question number 50 about processing dermis allografts. Three reported that they processed dermis and their answers to questions 50 through 60 make up the data for this report.

Surveys were sent to six European skin processing facilities who agreed to participate. Only two surveys were returned; the skin banks processes split-thickness skin and dermis allografts.

The survey was completed by one Australian tissue bank.

Table 1**:** *Survey results pertaining to acellular dermis allograft processing (Australia does not process acellular, decellularized dermis allograft)*

Question	Canada	U.S.	Europe
Does your facility process acellular, decellularized dermis allograft?			
Yes	0 of 5	3 of 6	2 of 2
No	5 of 5	3 of 6	0 of 2
During dermis processing which of the following treatments are applied?			
Soaks in hypertonic fluid	NA	2 of 3	1 of 2
Hypotonic lysis	NA	1 of 3	1 of 2
Antibiotics	NA	2 of 3	2 of 2
Enzymes to remove cells such as Trypsin	NA	0 to 3	1 of 2
Nucleases, endonucleases to degrade DNA/RNA such as recombinant endonuclease, Benzonase®, Pulmozyme® or others	NA	1 of 3	1 of 2
Detergents such as polysorbate-20, Triton X 100, Tween 80 or others	NA	0 of 3	1 of 2
Anionic detergents such as N-lauryl sarcosinate or sodium dodecyl sulfate (SDS)	NA	2 of 3	1 of 2
Alcohol	NA	1 of 3	1 of 2
Hydrogen peroxide	NA	1 of 3	0 of 2
Radiation	NA	2 of 3	0 of 2
Proprietary steps	NA	1 of 3	0 of 2
Other: Low concentration sodium hydroxide, 50% and 85% glycerol	NA	0 of 3	1 of 2
Other: Peracetic acid	NA	1 of 3	0 of 2
During processing of acellular dermis allografts, which of the follow antibiotics are used?			
No antibiotics are used	NA	1 of 3	0 of 2
Vancomycin	NA	2 of 3	1 of 2
Lincomycin/Linocin	NA	1 of 3	0 of 2
Gentamicin	NA	1 of 3	1 of 2
Polymyxin B	NA	1 of 3	1 of 2
Streptomycin	NA	1 of 3	1 of 2
Amphotericin B	NA	1 of 3	0 of 2
Nystatin (antifungal)	NA	0 of 3	1 of 2
Other: Penicillin	NA	0 of 3	1 of 2

Question	Canada	U.S.	Europe
Under which condition is dermis stored?			
Terminal radiation and ambient temperature/room temperature storage	NA	1 of 3	1 of 2
In alcohol stored at ambient temperature/room temperature storage	NA	1 of 3	0 of 2
Freeze-dried and stored in ambient temperature storage	NA	1 of 3	0 of 2
In glycerol and stored at refrigerated temperatures	NA	0 of 3	1 of 2
Other: glycerol, stored at ambient temperature	NA	1 of 3	0 of 2
Which of the following sterilization methods are applied as part of dermis processing?			
None of the following	NA	2 of 3	1 of 2
Ethylene oxide gas	NA	0 of 3	0 of 2
NovaSterilis (Supercritical CO_2)	NA	0 of 3	0 of 2
Other: Peracetic Acid	NA	1 of 3	0 of 2
Other: Gamma irridiation	NA	0 of 3	1 of 2
Do you apply radiation to dermal allografts?			
Yes	NA	2 of 3	1 of 2
No	NA	1 of 3	1 of 2
What type of radiation?			
Gamma radiation	NA	1 of 2	1 of 2
Electron beam radiation	NA	1 of 2	0 of 2
Does your processing include radiation to some or all incoming dermis prior to processing?			
Yes, applied to all	NA	0 of 3	0 of 2
No	NA	3 of 3	2 of 2
Is radiation applied as a final step, an end point of dermis processing in its final package (terminal sterilization)?			
Yes, applied to all	NA	2 of 3	1 of 2
Yes, depending on the results of pre-processing or in-processing microbial test results or other indications	NA	0 of 3	0 of 2
No	NA	1 of 3	1 of 2
What is the minimum dose of radiation that is used as a final dermis treatment (terminal sterilization)?			
Between 1.0 and 1.5 MRad (10 and 15 kGy)	NA	0 of 3	NA
1.5 MRad (15 kGy)	NA	1 of 3	NA
2.0 MRad (20 kGy)	NA	1 of 3	NA
2.5 MRad (25 kGy)	NA	NA	1 of 1

Each entry represents the number of tissue banks selecting the specific question out of the total number of banks answering the specific questions.

NA = Not Answered

Table 2**:** *Variations of decellularizing and decontamination steps during dermis processing by four skin processing facilities*

	Tissue Bank					
	U.S.-1	**U.S.-2***	**U.S.-2***	**U.S.-3**	**EU-1**	**EU-2**
Hypertonic Fluid Soaks	No	Yes	Yes	Yes	No	Yes
Hypotonic Lysis of Cells	No	Yes	Yes	Yes	No	Yes
Nuclease	Yes (Benzonase)	No	No	No	No	Yes
Detergents	Yes (N-lauryl sarcosinate)	Yes	Yes	No	No	Yes
	Tissue Bank					
	U.S.-1	**U.S.-2***	**U.S.-2***	**U.S.-3**	**EU-1**	**EU-2**
Antibiotics	Vancomycin Lincomycin Gentamicin Polymyxin B	None	None	Vancomycin Streptomycin Amphotericin	Streptomycin Penicillin	Yes
Alcohol	No	Yes	Yes	No	No	Yes
Sodium Hydroxide	No	No	No	No	Yes	No
Sterilization Step	Low Dose Gamma Radiation	Peracetic Acid	Peracetic Acid	E-Beam Radiation	No	Gamma irradiation
Storage	Glycerol, Room Temp	Ethanol Dip*, Room Temp	Freeze-dried* Room Temp	Room Temp	Glycerol (50%, 85%) Refrigerator	Terminal radiation and room temp

**Two acellular dermis allografts produced by same tissue bank, one is freeze-dried*

Analysis

Removal of cells from dermis is accomplished by hypertonic/hypotonic cell lysis by two of three U.S. tissue banks, with detergents by two U.S. banks and endonuclease enzymes by one. Two of three U.S. banks decontaminate the dermis with antibiotics and one of three with ethanol. Two of three U.S. banks sterilize the dermis by radiation (one with gamma, one with electron beam radiation) and one of three by chemical sterilization using peracetic acid.

All three U.S. banks store dermis at room temperature; one uses lyophilization, one dips the dermis in ethanol and one stores dermis in glycerol.

One of the two European bank removals cells from dermis by hypertonic/hypotonic cell lysis, with detergents and endonuclease enzymes. The bank decontaminate the dermis with antibiotics and ethanol. The other European bank did not provide processing details but stores the acellular dermis similar to their split-thickness skin allografts. Their processing includes antibiotic exposure and refrigerated storage in 85% glycerol.

Conclusions and Key Learning Points

1. Acellular dermis is processed using a wide variety of methods such as removing cells by hypotonic cell lysis, detergents, and endonucleases (removes DNA, RNA).

2. U.S. skin processors decontaminate and sterilize dermis by antibiotic exposure and terminal radiation (two banks: one using electron beam, one using gamma radiation) or by ethanol and peracetic acid (one bank).

3. Two of three U. S. banks sterilize dermis combining decontamination steps with terminal sterilization by applying low dose radiation: 1.5 MRad (15 kGy) and 2.0 MRad (20 kGy).

4. European skin processor decontaminate and sterilize dermis by antibiotic exposure and terminal radiation, applying a 2.5 MRad (25 kGy) dose

Note: All statements about practices are based on the number of survey respondents, not the absolute number of banks.

Pooling of Tissue during Processing

Tissue Type:	Bone, connective, cardiovascular and skin
Process:	Tissue processing
Sub Process:	Tissue pooling
Data Source:	Bone Processing and Validation survey question number 2, Skin Processing and Validation survey question number 2, Cardiovascular Processing and Validation survey question number 2

Scope

This report addresses pooling of tissue from two or more donors during processing of bone, connective tissue, skin and heart valves.

Introduction and Overview

Pooling of recovered tissue from more than one donor during the cleaning and disinfection steps of processing can reduce expenses. It can also lead to the contamination of the entire pool when tissue from one donor contains an infectious organism not present in the other donors. Avoiding tissue pooling from more than one donor is a very important bioburden reduction and control step.

Over 60 years ago, studies of transfusion recipients during WWII showed that 0.8% of recipients of blood from a single donor developed jaundice (hepatitis B); whereas, jaundice developed in 5% of those receiving plasma made by pooling donations from many donors[1]. In the early 1980's, HIV and HCV developed in 80 to 90% of severe hemophilia A patients receiving freeze-dried, purified Factor VIII coagulant concentrates derived from large pools of plasma from thousands of paid U.S. plasma donors. Approximately half of all persons with hemophilia contracted HIV during the 1980's [2-4].

In the field of tissue transplantation, pooling of dura allografts during processing was associated with nearly 200 fatal cases of dura-related Creutzfeldt-Jakob Disease (CJD) transmissions, mostly in Japan[5,6]. More recently two additional cases of CJD, the latest taking place in Canada, were reported following use of a different brand of dura allograft made by a different processor[7-9].

One U.S. tissue bank patented a tissue pooling procedure and has pooled bone allografts during processing[10,11]. During July 25 – 28, 2000, the U.S. FDA inspected this bank and documented that they were pooling bones from more than one donor during processing[12]. In January, 2001, the U.S. FDA published proposed Good Tissue Practices which prohibited pooling of tissues during processing[13]. AATB standards also prohibit pooling of tissues[14]. Canadian standards[15] and the U.S. FDA's Final Rule, Good Tissue Practices, effective in 2005[16], also prohibit pooling of tissue from donors during processing.

Pooling of tissue remains an issue today. Survey results show that one tissue bank that processes bone in Europe reported pooling of tissue from more than one donor during bone processing in 2013.

Results

A *Bone Processing and Validation* survey was sent to nine Canadian tissue banks that process bone and connective tissue; eight of nine banks completed question number two of the survey.

A *Bone Processing and Validation* survey was sent to four U.S tissue banks that process bone and connective tissue; all four completed question number two of the survey. Two additional U.S. tissue banks provided answers to question number two but did not complete the survey.

A *Bone Processing and Validation* survey was sent to six European tissue banks that process bone and connective tissue; six of the six banks completed question number two of the survey.

A *Cardiovascular Processing and Validation* survey was sent to four Canadian tissue banks that recover, process and distribute heart valve allografts; all four completed question number two of the survey.

A *Cardiovascular Processing and Validation* survey was not directly completed by two U.S. tissue banks but the answer to question number two was collected from each tissue bank's staff through personal communication.

A *Cardiovascular Processing and Validation* survey was sent to six European tissue banks that process heart valves; four returned the completed survey and answered question number two. The answer to question number two from 15 other recently surveyed European tissue banks was obtained by contacting them and entering their verbal answers into the survey[17].

A *Skin Processing and Validation* survey was sent to five Canadian tissue banks that recover, process and distribute skin allografts; all five banks completed question number two of the survey.

A *Skin Processing and Validation* survey was sent to seven U.S. tissue banks; five banks that process split-thickness skin and two banks that process dermis. The five banks producing split-thickness grafts completed question number two. The two banks that process dermis did not complete the survey but were willing to answer some of the survey questions. Their verbal answers were entered into the survey results, including their answers to question number two.

A *Skin Processing and Validation* survey was sent to six European tissue banks that process skin; two banks returned the survey and completed question number two.

The surveys were completed by one Australian tissue bank.

Table 1: *Survey data from question number two about pooling tissue during processing*

Question	Canada	U.S.	Europe	Australia
Does your facility pool/co-mingle bone from two or more donors?				
Yes	0 of 8	0 of 6	1 of 6	0 of 1
No	8 of 8	6 of 6	5 of 6	1 of 1
Does your facility pool/co-mingle cardiovascular tissue from two or more donors?				
Yes	0 of 4	0 of 2	0 of 19	0 of 1
No	4 of 4	2 of 2	19 of 19	1 of 1
Does your facility pool/co-mingle skin from two or more donors?				
Yes	0 of 5	0 of 7	0 of 2	0 of 1
No	5 of 5	7 of 7	2 of 2	1 of 1

Each entry represents the number of tissue banks selecting the specific question out of the total number of banks answering the specific questions.

Analysis

The results show that bone, skin and heart valve tissue processors in the U.S., Canada and the one bank from Australia do not pool tissue from two or more donors. The same was true for European skin and cardiovascular processors that were surveyed; however, one of six European tissue banks surveyed reported pooling of bone from two or more donors during processing and sterilization.

Conclusions and Key Learning Points

1. No Canadian (n=8) or U.S. (n=6) or Australian (n=1) bone banks reported pooling tissue during processing.

2. One of six European bone banks reported pooling tissue during processing.

3. No Canadian (n=4), U.S. (n=2) or European (n=19) or Australian (n=1) cardiac banks reported pooling tissue during processing.

4. No Canadian (n=5), U.S. (n=7) or European (n=2) or Australian (n=1) skin banks reported pooling tissue during processing.

Note: All statements about practices are based on the number of survey respondents, not the absolute number of banks.

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Bacteriostasis and Fungistasis Testing: Bone and Connective Tissue Allografts

Tissue Type:	Bone and connective
Process:	Tissue processing
Sub process:	Bacteriostasis and fungistasis testing
Data Source:	Bone Processing and Validation survey questions numbered 1, 3, 5, 6 and 19

Scope

This is a report pertaining to bacteriostasis and fungistasis testing of finished bone, osteochondral, articular cartilage and tendon allografts.

Introduction and Overview

Bacteriostasis and fungistasis are potential problems for bone, tendon, articular cartilage, heart valve, cartilage, and skin allografts that are exposed to antibiotics and disinfectants during processing. Allografts can contain residues of decontaminants used during processing such as antibiotics or peroxide. These residues can interfere with post-processing microbial testing, leading to a falsely-negative final sterility test. In this case, there exists the potential to distribute a contaminated allograft and, when transplanted, a recipient could develop a serious infection. Tissue banks can test for this interference (bacteriostasis and fungistasis testing) and, if present, implement preventive steps.

In bacteriostasis and fungistasis testing the product in question is inoculated with known numbers of a bacteria culture and a fungi culture. Any product that shows no growth or slowed growth is considered bacteriostatic or fungistatic.

Although deceased tissue donors have been screened and may have no evidence of being clinically infected at the time of death, the tissue donated after death often acquires postmortem contamination by bacteria and fungi. Tissue banks report that four to 53% of donated bone, cartilage and tendon tissues recovered from deceased tissue donors are contaminated by bacteria or fungi[1-5].

Bone and connective tissue allografts commonly undergo a variety of bioburden reduction processing steps depending on the individual tissue bank. Many tissue banks process bone extensively; cleaning and decontaminating with detergents, peroxide, alcohol and antibiotics with or without a terminal sterilizing treatment. Other allografts, such as fresh viable articular cartilage and osteochondral allografts, are not extensively disinfected but are temporarily stored in a refrigerated antibiotic culture medium. Because cellular viability is thought to be important, they do not undergo a terminal sterilization process (e.g. radiation). Despite final rinsing,

antibiotic residues can remain in bone, tendon and cartilage allografts and can interfere with final microbial testing. This mechanism has been responsible for fatal and non-fatal cases of bacterial infections transmitted through osteochondral, cartilage and tendon allografts.

By detecting interfering substances through bacteriostasis and fungistasis testing, simple and practical strategies for their removal prior to final sterility testing can be incorporated into procedures.

Results

Bone Processing and Validation surveys were sent to nine Canadian tissue banks; eight of the nine banks returned surveys and completed part or all of the questions.

Bone Processing and Validation surveys were sent to four U.S. tissue banks that agreed to complete surveys; all four completed most or all questions in the survey.

Two additional large U.S. tissue banks that did not complete the entire survey did provided answers to several questions through direct communication. A number of questions were also completed by extracting information from package inserts, pamphlets describing processing, and scientific publications[6-10] from these two banks.

Surveys were sent to eleven European tissue banks; six completed part or all of the questions.

The *Bone Processing and Validation* survey was completed by one Australian tissue bank.

Table 1: *Bone processing and bacteriostasis & fungistasis testing*

Question	Canada	U.S.	Europe	Australia
Which of the following bone and connective tissues does your facility process?				
Bone, deceased donor	8 of 8	6 of 6	6 of 6	1 of 1
Bone, live donor	NA	NA	6 of 6	1 of 1
Demineralized bone	0 of 8	6 of 6	4 of 6	1 of 1
Tendon	7 of 8	6 of 6	6 of 6	1 of 1
Ligament	3 of 8	6 of 6	4 of 6	0 of 1
Fascia	4 of 8	4 of 6	3 of 6	0 of 1
“Fresh” refrigerated osteochondral allograft	3 of 8	3 of 6	1 of 6	0 of 1
“Fresh” refrigerated articular cartilage	1 of 8	2 of 6	0 of 6	0 of 1
Cryopreserved osteochondral allograft	0 of 8	2 of 6	0 of 6	1 of 1
Other cartilage	0 of 8	2 of 6	2 of 6	0 of 1
Meniscus	2 of 8	1 of 6	4 of 6	1 of 1
Amnion	1 of 8	0 of 6	5 of 6	0 of 1
Mesenchymal stem cells	0 of 8	2 of 6	0 of 6	0 of 1

Question	Canada	U.S.	Europe	Australia
During processing of traditional bone allografts which of the following are used to reduce bioburden?				
Mechanical or chemical processes to remove marrow, cells, fat	7 of 8	6 of 6	4 of 6	0 of 1
Alcohol	3 of 8	6 of 6	3 of 6	0 of 1
Hydrogen Peroxide	3 of 8	6 of 6	5 of 6	1 of 1
Detergents	1 of 8	4 of 6	1 of 6	0 of 1
Antibiotics	4 of 8	6 of 6	3 of 6	1 of 1
Iodophor, e.g. povidone-iodine, betadine	1 of 8	0 of 6	0 of 6	0 of 1
Polyoxyethylene	0 of 8	1 of 6	1 of 6	0 of 1
Other: Supercritical CO_2	NA	NA	1 of 6	0 of 1
What proprietary bone processing methods are used at your facility?				
No	8 of 8	1 of 6	5 of 6	1 of 1
Allowash®	0 of 8	3 of 6	0 of 6	0 of 1
Allowash XG®	0 of 8	1 of 6	0 of 6	0 of 1
Advanced Tissue Processing	0 of 8	1 of 6	0 of 6	0 of 1
Other: AlloTrue™	0 of 8	1 of 6	0 of 6	0 of 1
BioCleanse®	0 of 8	0 of 6	0 of 6	0 of 1
Tutoplast®	0 of 8	0 of 6	0 of 6	0 of 1
Other: NovaSterilis – Supercritical CO_2 by a UDHE machine	NA	0 of 6	1 of 6	NA
Other: Clearant®	NA	NA	1 of 6	NA
What type of alcohol is used during bone processing?				
None	5 of 8	0 of 6	3 of 6	1 of 1
Isopropyl alcohol/isopropanol	3 of 8	5 of 6	0 of 6	0 of 1
Denatured ethanol	0 of 8	1 of 6	1 of 6	0 of 1
Ethanol	0 of 8	0 of 6	2 of 6	0 of 1
Which antibiotics are used for bone processing?				
None, no antibiotics are used	4 of 8	0 of 6	3 of 6	0 of 1
Gentamicin	4 of 8	2 of 6	2 of 6	1 of 1
Bacitracin	4 of 8	4 of 6	0 of 6	0 of 1
Polymyxin B	0 of 8	5 of 6	1 of 6	0 of 1
Amphotericin	0 of 8	1 of 6	1 of 6	0 of 1
Primaxin	0 of 8	1 of 6	1 of 6	0 of 1
Other: Vancomicine plu Tobramicine plus Cotrimoxazole	0 of 8	0 of 6	1 of 6	0 of 1
Has your facility performed bacteriostasis or fungistasis testing of bone allografts?				
Yes, during validation studies	4 of 8	6 of 6	3 of 6	NA
No	4 of 8	0 of 6	3 of 6	NA

Each entry represents the number of tissue banks selecting the specific question out of the total number of banks answering the specific questions.

NA = Not Asked

Bacteriostasis and fungistasis testing is performed by each of the six U.S. tissue banks surveyed and each of the two European tissue banks returning surveys. In Canada, four of the eight tissue banks perform bacteriostasis and fungistasis testing (Table 1).

Because some Canadian tissue banks do not perform bacteriostasis and fungistasis testing, Table 2 was constructed to show whether the tissue banks that use disinfectants and antibiotics during processing of traditional bone allografts also test for bacteriostasis and fungistasis.

Because each of the U.S. and European banks that were surveyed use disinfectants or antibiotics or both and perform bacteriostasis and fungistasis testing, data about their processing was not presented in tabular form.

Table 2: *Use of disinfectants, antibiotics, and bacteriostasis and fungistasis testing: Canadian tissue banks*

Bank	Detergent	H_2O_2	Alcohol	Antibiotic	Terminal Radiation	Perform final sterility test?	Bacteriostasis and Fungistasis tested?	Provide fresh osteoarticular, osteochondral?
A	No	No	No	No	Yes	Yes	No	No
B	No	No	No	Yes*	Yes	Yes	No	Yes
C	No	No	No	Bacitracin Gentamicin Cefazolin	No	Yes	Yes	Yes
D	No	Yes	Yes	Bacitracin Gentamicin Cefazolin	No	Yes	No	Yes
E	No	No	No	Bacitracin Gentamicin Cefazolin	No	Yes	Yes	No
F	No	Yes	Yes	Bacitracin Gentamicin Cefazolin	No	Yes	Yes	No
G	No	No	No	No	No	Yes	No	No
H	Yes	Yes	Yes	No	Yes	No	Yes	No

**Provides fresh osteochondral grafts that are temporarily stored in a growth medium containing antibiotics and which are not irradiated. This tissue bank does not employ antibiotic soaks in other processing as these grafts are irradiated.*

Table 2 depicts survey data pertaining to testing for bacteriostasis and fungistasis by each of the Canadian tissue banks, along with whether they use disinfectants or antibiotics that could carryover and affect sterility testing. Of the four Canadian tissue banks not performing bacteriostasis and fungistasis testing, one tissue bank uses antibiotics or disinfectants during traditional bone processing and one tissue bank uses antibiotics in the processing of “fresh” refrigerated osteochondral allografts.

Analysis

100% of reporting U.S tissue banks (n=6) perform bacteriostasis and fungistasis testing as compared to 50% (n=4) of the reporting Canadian tissue banks and 50% (n=3) of the reporting European banks (Table 1).

Survey results show that 50% (n=4) of the eight surveyed Canadian tissue banks use antibiotic soaks with an additional tissue bank (n=1) using antibiotics in the storage medium for fresh osteochondral grafts.

38% (n=3) of the Canadian tissue banks surveyed apply terminal radiation. These three tissue banks do not employ antibiotic soaks.

Of the five banks using antibiotic soaks two do not perform bacteriostasis and fungistasis testing; Banks B and D (highlighted in Table 2).

Bank B:

- does not apply terminal radiation to fresh osteochondral grafts
- uses antibiotics in the storage medium for fresh osteochondral grafts
- does not perform bacteriostasis or fungistasis testing to determine whether residual antibiotics cause a falsely negative final testing

Bank D:

- does not apply terminal radiation
- uses disinfectants and antibiotics during standard bone processing
- does not perform bacteriostasis or fungistasis testing to determine whether residual antibiotics cause a falsely negative final testing

Conclusions and Key Learning Points

1. Of the four Canadian bone banks that do not perform bacteriostasis and fungistasis testing, two use antibiotics and disinfectants that could leave residues that could interfere with final microbial testing and reduce sensitivity.

2. Bacteriostasis and fungistasis testing are important when processing tissues with antibiotics and disinfectants. Guidance for tissue banks should be considered.

3. Bank B and Bank D maybe at increased risk of having falsely negative final test results and, therefore, are at risk of releasing a contaminated allograft.

Note: All statements about practices are based on the number of survey respondents, not the absolute number of banks.

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Bacteriostasis and Fungistasis Testing: Heart Valves

Tissue Type:	Cardiovascular
Process:	Tissue processing
Sub Process:	Bacteriostasis and fungistasis testing
Data Source:	Cardiovascular Processing and Validation survey question number 17

.

Scope

This report pertains to bacteriostasis and fungistasis testing of finished (final packaging) heart valve allografts.

Introduction and Overview

Bacteriostasis and fungistasis are potential problems for bone, tendons, articular cartilage, heart valves, cartilage, and skin allografts that are exposed to antibiotics and disinfectants during processing. Heart valve allografts can contain residues of antibiotics used for decontamination during processing. These antibiotic residues can interfere with post-processing microbial testing, leading to a falsely-negative final sterility test of a heart valve. The potentially contaminated heart valve could then be released and distributed which may then lead to a serious infection in the recipient. Tissue banks can test for this interference through bacteriostasis and fungistasis testing and, if present, implement preventive steps.

In bacteriostasis and fungistasis testing the product in question is inoculated with known numbers of a bacteria culture and a fungi culture. Any product that shows no growth or slowed growth is considered bacteriostatic or fungistatic.

Although deceased tissue donors have been screened and may have no evidence of being clinically infected at the time of death, the tissue donated after death often acquires postmortem contamination by bacteria and fungi. Tissue banks report that 10 to 27% of donated hearts and heart valves taken from deceased tissue donors are contaminated by bacteria[1, 2] and, 1-3%, by fungi[1, 3].

Decontamination of heart valves involves incubation in antibiotics for a predetermined amount of time, usually about 24 hours. Despite thorough washing and rinsing, residues of antibiotics and other substances can remain on the allograft. These substances can suppress growth without killing bacteria and fungi that are present. These interfering substances are considered bacteriostatic and fungistatic. As a consequence the final sterility test result can be falsely-negative and the allograft may be released for use in patients. After implantation, the interfering substances dissipate, microbes can proliferate and an infection can develop. This mechanism has been reported to be responsible for at least one fatal bacterial infection, a life-threatening

fungal infection and significant morbidity due to allograft-related infections in many other recipients.

By detecting interfering substances through bacteriostasis and fungistasis testing, practical strategies to effectively dilute, neutralize or remove these bacteriostatic and fungistatic residues can be incorporated into procedures.

Results

A *Cardiovascular Processing and Validation* survey was sent to four Canadian cardiovascular tissue processing facilities that recover, process and distribute heart valve allografts. All four completed a portion of or all of the survey.

Two U.S. cardiovascular tissue processing facilities did not agree to complete the survey, however, through personal communication they responded to survey question number 17.[4,5]

A *Cardiovascular Processing and Validation* survey was sent to six European cardiovascular tissue processing facilities, four of which completed them.

The *Cardiovascular Processing and Validation* survey was completed by one Australian tissue bank.

Table 1: *Bacteriostasis and Fungistasis Testing Performed by* Cardiovascular Tissue *Processing Facilities in Canada, the U.S. and Europe*

Question	Canada	U.S.	Europe	Australia
Has your facility performed bacteriostasis and fungistasis testing of tissue allografts?				
Yes, during validation studies	4 of 4	2 of 2	2 of 4	1 of 1
No	0 of 4	0 of 2	2 of 4	0 of 1

Each entry represents the number of tissue banks selecting the specific question out of the total number of banks answering the specific questions.

The table shows that all North American cardiovascular tissue processors and two of the four surveyed European and the Australian cardiovascular tissue processors used bacteriostasis and fungistasis testing when validating processing procedures.

Analysis

N/A

Conclusions and Key Learning Points

1. All respondents of the surveyed cardiovascular tissue processing facilities performed bacteriostasis and fungistasis testing during their validation studies, a key step in ensuring the safety of heart valve allografts.

Note: All statements about practices are based on the number of survey respondents, not the absolute number of banks.

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5. Personal communication with Greg Ray, MD, CryoLife Medical Director, Dec 18, 2013,

Bacteriostasis and Fungistasis Testing: Skin Allografts

Tissue Type:	Skin
Process:	Tissue processing
Sub Process:	Bacteriostasis and fungistasis testing
Data Source:	Skin Processing and Validation survey questions numbered 1, 3, 4, 14, 15, 28, 29, 32, and 34.

Scope

This report addresses bacteriostasis and fungistasis testing for substances that can interfere with final microbial testing of split-thickness skin allografts.

Introduction and Overview

The surface of skin donated after death is not sterile and can contain bacteria and fungi despite cleansing and preparing with disinfectants such as chlorhexadine. In the U.S. and Canada, recovered donor skin is temporarily stored in antibiotic solutions at refrigerated temperatures until it is cryopreserved or is distributed as "fresh" refrigerated skin allografts. This antibiotic exposure reduces the skin's microbial load. These processing methods are also designed to maintain skin cell viability. Viable skin is preferred by burn surgeons in the U.S. and Canada.

In Europe, skin is routinely stored at refrigerated temperatures in high concentrations (e.g. 85%) of glycerol for comparatively long periods. This exposure to high concentrations of glycerol reduces the skin's microbial load but cellular viability is not maintained.

Despite rinsing, residues of antibiotics and disinfectants can remain in the allograft and suppress growth of bacteria and fungi, if present, without eliminating them. These interfering substances are considered bacteriostatic and fungistatic. As a consequence the final sterility test result can be falsely-negative and the allograft may be released for use in patients. After implantation, the interfering substances dissipate, microbes can proliferate and an infection can develop.

Skin allografts are commonly used as a temporary skin substitute in patients with extensive severe burns. The allografts are applied to non-sterile body surfaces. Final microbial testing of skin allografts, prior to final packaging, should be as sensitive as possible so highly virulent, pathogenic microbes can be reliably identified and, if present, the allograft discarded.

By detecting interfering substances through bacteriostasis and fungistasis testing it is possible to implement procedures using substances and devices that effectively dilute, neutralize or remove bacteriostatic and fungistatic residues, resulting in a more sensitive final microbial test.

Results

A *Skin Processing and Validation* survey was sent to five Canadian tissue banks that recover, process and distribute skin allografts. All five tissue banks completed part or all of the survey.

A *Skin Processing and Validation* survey was sent to seven U.S. tissue banks that process split-thickness skin. Five agreed to participate and completed some of the survey questions. These tissue banks in Canada and the U.S. process cryopreserved skin and "fresh" refrigerated skin.

A *Skin Processing and Validation* survey was sent to five European tissue banks that process skin. Five surveys were returned but responses to almost all questions were not provided because the survey addressed skin processing by cryopreservation and by refrigerated storage in antibiotic solutions. European tissue banks do not process and store skin in the manner addressed by the survey. European tissue banks mainly use high concentrations, 50-85%, of glycerol for disinfection and preservation during long term refrigerated storage of nonviable skin.

The *Skin Processing and Validation* survey was completed by one Australian tissue bank.

Table 1: *Survey questions about bacteriostasis and fungistasis testing of cryopreserved and fresh skin allografts*

Question	Canada	U.S.	Europe	Australia
Do you process cryopreserve split thickness skin at your facility?				
Yes	5 of 5	5 of 5	1 of 5	1 of 1
No	0 of 5	0 of 5	4 of 5	0 of 1
What microbial tests are performed following cryopreserved skin processing at the time of final packaging?				
Bacteria	5 of 5	5 of 5	1 of 5	1 of 1
Fungi	5 of 5	5 of 5	1 of 5	1 of 1
Mycobacterium	1 of 5	2 of 5	0 of 5	0 of 1
Has your facility ever performed bacteriostasis of fungistasis studies of cryopreserved skin?				
Yes	0 of 5	2 of 5	0 of 5	0 of 1
Yes, during validation studies	1 of 5	2 of 5	0 of 5	1 of 1
No	4 of 5	1 of 5	1 of 5	0 of 1

Question	Canada	U.S.	Europe	Australia
Do you process and provide "fresh" refrigerated split thickness skin?				
Yes	1 of 5	2 of 5	0 of 5	0 of 1
No	4 of 5	3 of 5	5 of 5	1 of 1
What microbial tests are performed on "fresh" refrigerated skin allograft?				
Bacteria	1 of 1*	2 of 2*	NA	0 of 1
Fungi	1 of 1	2 of 2	NA	0 of 1
Mycobacteria	0 of 1	0 of 2	NA	0 of 1
n/a	0 of 1	0 of 2	NA	1 of 1
Has your facility performed bacteriostasis or fungistasis testing of fresh refrigerated skin?				
Yes	0 of 1*	2 of 2*	NA	1 of 1
No	1 of 1	0 of 2	NA	0 of 1

Each entry represents the number of tissue banks selecting the specific question out of the total number of banks answering the specific questions.
**Only one Canadian and two U.S. skin banks surveyed provide "fresh", refrigerated skin allografts.*
NA = Not Answered

Table 2: *Skin bacteriostasis and fungistasis testing and antibiotics used*

Skin Bank	Antibiotics	Bacteriostasis/ Fungistasis Tested?
Canadian Banks - Cryopreserved Skin		
A	Vancomycin Gentamicin Cephalosporin	No
B	Gentamicin Cephalosporin	Yes
C	Vancomycin Gentamicin	No
D	Vancomycin Gentamicin Cephalosporin	No
E	Streptomycin	No

Skin Bank	Antibiotics	Bacteriostasis/ Fungistasis Tested?
U.S. Banks - Cryopreserved Skin		
A	Gentamicin	Yes
B	Gentamicin Kanamycin Cephazolin	Yes
C	Gentamicin Oxacillin	Yes
D	Gentamicin	Yes
E	Vancomycin Ciprofloxacin Bactrim Amphotericin	No
European Banks - Cryopreserved Skin		
A	Vanocomycin Polymyxin B Gentamicin Nystatin	Yes
Australian Bank – Cryopreserved Skin		
A	Penicillin Streptomycin	Yes
Canadian Banks - Fresh, Refrigerated Skin		
A	NA	NA
B	NA	NA
C	NA	NA
D	NA	NA
E	Streptomycin	No
U.S. Banks - Fresh, Refrigerated Skin		
A	Gentamicin	Yes
B	NA	NA
C	NA	NA
D	Gentamicin Nystatin	Yes
E	NA	NA

NA = Not Answered

Analysis

80% (n=4) of responding U.S. tissue banks and the Australian tissue bank perform bacteriostasis and fungistasis testing on cryopreserved skin as compared to 20% (n=1) of Canadian tissue banks.

The types of antibiotics used vary between banks.

100% (n=2) of responding U.S. tissue banks perform bacteriostasis and fungistasis testing on “fresh” refrigerated skin as compared to 0% of responding Canadian tissue banks or the Australian tissue bank.

Conclusions and Key Learning Points

1. Despite using antibiotics during processing of cryopreserved and fresh refrigerated skin allografts, bacteriostasis and fungistasis testing is much less prevalent in Canadian tissue banks and European tissue banks than in U.S. tissue banks or the one Australian tissue bank.

Note: All statements about practices are based on the number of survey respondents, not the absolute number of banks

Condition of Transport of Heart Valve Allograft from Tissue Bank to Hospital

Tissue Type:	Cardiovascular
Process:	Tissue processing
Sub Process:	Transport of donor heart valve allograft from tissue bank to hospital
Data Source:	Cardiovascular Processing and Validation survey question number 19

Scope

This report pertains to maintaining low temperature storage for donor heart valves during shipping from the processing facility to the hospital for transplantation. This survey question addresses how storage temperatures are maintained during shipment.

Introduction and overview

Storage of cryopreserved donor heart valve allografts aims at maintaining cellular viability. This can be assured when the temperature is colder than -130°C where all metabolic activity ceases.

Immersion in liquid nitrogen provides a temperature of -196°C. AATB standards require that donor heart valve storage temperatures do not get warmer than -100°C. The temperature of the vapor above liquid nitrogen is -150°C to -196°C near the liquid surface but rises to -95°C or warmer at the top[1]. Some mechanical, electrically-powered freezers can provide a temperature of -140°C more evenly throughout the device.

Liquid nitrogen can become contaminated with bacteria and viruses[2-4]. After reports that hepatitis B virus-contaminated liquid nitrogen storage caused transmission of infection to six recipients of frozen bone marrow[3,4], the vapor phase of liquid nitrogen became a common choice for storage of donor heart valves allografts.

When donor heart valve allografts are shipped to a hospital, a safe temperature must be maintained. To maintain storage temperatures, transporting heart valves is commonly performed in the vapor phase of liquid nitrogen in “dry shippers” but in Europe, dry ice is also used[5-7]. The temperature of dry ice is -78°C.

Results

A *Cardiovascular Processing and Validation* survey was sent to the four Canadian tissue banks; all four completed or partially completed the survey, including question #9.

A *Cardiovascular Processing and Validation* survey was sent to the two U.S. heart valve processing tissue banks but they declined to directly complete the survey. Selected data, including answers to question #9, were collected from each tissue bank's staff through personal communication and entered into the survey[8, 9].

A *Cardiovascular Processing and Validation survey* was sent to several European tissue banks; three were returned partially complete.

The *Cardiovascular Processing and Validation* survey was completed by one Australian tissue bank.

Table 1: *Maintaining safe temperatures during shipment of donor heart valve allografts*

Question	Canada	U.S.	Europe	Australia
Under which conditions are heart valves shipped/transported to the hospital?				
Dry Shipper (Vapor phase of liquid nitrogen)	4 of 4	2 of 2	1 of 3	1 of 1
Submerged in liquid nitrogen in a liquid nitrogen container	0 of 4	0 of 2	0 of 3	0 of 1
Dry ice (solid CO_2)	0 of 4	0 of 2	3 of 3	0 of 1

Each entry represents the number of tissue banks selecting the specific question out of the total number of banks answering the specific questions.

Analysis

100% of the Canadian tissue banks (n=4), 100% of the U.S. (n=2) tissue banks, and 33% (n=1) of the European tissue banks and the Australian tissue bank that responded to the survey reported shipping donor heart valves in the vapor phase of liquid nitrogen. The responding European tissue banks reported shipping donor heart valves in dry ice.

Conclusions and Key learning Points

1. Tissue banks in North America and the one Australian tissue bank ship heart valves in a "dry shipper" maintaining vapor phase liquid nitrogen temperatures.

2. Although only three European tissue banks returned the survey, its method of shipment on dry ice is not uncommon when compared with other European tissue banks reporting their practices in the medical literature[5-7].

Note: All statements about practices are based on the number of survey respondents, not the absolute number of banks.

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8. Personal communication with Perry Lang, Vice President, LifeNet Health, November 15, 2013.

9. Personal communication with Greg Ray, MD, CryoLife medical director, Dec 18, 2013.

Split Thickness Skin Allograft Cryopreservation

Tissue Type:	Skin
Process:	Tissue processing
Sub Process:	Cryopreserved split thickness skin allograft freezing, storage and shipment
Data Source:	Skin Processing and Validation survey questions numbered 6-12, 28 and 79

Scope

This report addresses the types of freezing methods used and the conditions under which frozen donor skin is stored and transported.

Introduction and Overview

Storing skin at ultra-cold temperatures with cryoprotectant additives can maintain skin viability during storage as well as help prevent microbial growth. Donor skin allografts are an essential therapy for patients experiencing severe burns over large portions of their body surface**.** Viable donor skin allografts are perishable during refrigerated storage with gradual loss of viability within weeks. Growth of any contaminating bacteria and fungi is slowed but not fully prevented. If stored frozen at ultra-cold temperatures skin cellular metabolism and microbial growth stops. The use of cryoprotectants such as glycerol or dimethylsulphoxide (DMSO) prevents cellular damage during the freezing process and maintains post-thaw viability of skin cells (and bacteria).

Results

A *Skin Processing and Validation* survey was sent to five Canadian tissue banks; each completed all or part of the survey. All five respondents indicated that they process, store and distribute cryopreserved donor skin.

A *Skin Processing and Validation* survey was sent to eight U.S. tissue banks; seven completed part or all of the survey questions. Of the seven respondents, two indicated they process only dermis allografts and the remaining five respondents indicated they process split-thickness cryopreserved allografts. Each of the five banks producing split-thickness allografts completed all or part of survey questions numbered 6-12 (see Table 1); of those five, two also process fresh refrigerated skin (question 28, not in Table 1).

A *Skin Processing and Validation* survey was sent to two European tissue banks; they completed the survey but indicated they process and store skin at refrigerated temperatures in 50-85% concentrations of glycerol (question 79), as do most European tissue banks. None of the Canadian or U.S. tissue banks process or store skin in high concentrations of glycerol at refrigerated temperatures (question 79).

The survey was completed by one Australian tissue bank.

Table 1: *Freezing, storage and shipping cryopreserved skin allografts*

Question	Canada	U.S.	Europe	Australia
Does your facility use glycerol as a cryopreservative during skin cryopreservation?				
Yes	2 of 5	5 of 5	0 of 2	0 of 1
No	3 of 5	0 of 5	2 of 2	1 of 1
What concentration of glycerol is used in cryopreservation of split thickness skin?				
Between 1 and 10%	1 of 2	1 of 4*	NA	NA
10%	1 of 2	2 of 4*	NA	NA
Between 10-15%	0 of 2	1 of 4*	NA	NA
15%	0 of 2	0 of 4*	NA	NA
n/a	0 of 2	0 of 4	NA	NA
Does your facility use DMSO as a cryopreservative?				
Yes	3 of 5	0 of 5	1 of 2	1 of 1
No	2 of 5	5 of 5	1 of 2	0 of 1
What concentration of DMSO is used?				
7.5%	1 of 3	NA	0 of 1	0 of 1
10%	2 of 3	NA	0 of 1	1 of 1
Greater than 20%	0 of 3	NA	1 of 1	0 of 1
What is your freezing method for cryopreserved split thickness skin?				
Controlled-rate, electronically programmed freezing	3 of 5	4 of 5	1 of 1	1 of 1
Controlled-rate, insulated heat-sink box method in mechanical freezer	2 of 5	0 of 5	0 of 1	0 of 1
Controlled-rate, insulated heat-sink box method in dry ice (solid CO_2)	0 of 5	1 of 5	0 of 1	0 of 1
Dry ice (solid CO_2)	0 of 5	0 of 5	0 of 1	0 of 1
Under which conditions does your facility store cryopreserved split thickness skin?				
Vapor phase of liquid nitrogen	2 of 5**	1 of 5	1 of 1	1 of 1
Submerged in liquid nitrogen	0 of 5	0 of 5	0 of 1	0 of 1
Dry Ice (solid CO_2)	0 of 5	0 of 5	0 of 1	0 of 1
Mechanical freezer colder than -140°C	0 of 5	1 of 5	0 of 1	0 of 1
Mechanical freezer colder than - 100°C	0 of 5	0 of 5	0 of 1	0 of 1
Mechanical freezer at temperature colder than -40°C	4 of 5**	3 of 5	0 of 1	0 of 1

**One of the five U.S. skin banks using glycerol did not answer this question*.
***One skin bank stores some skin at -40°C and other skin in vapor phase liquid nitrogen (-196°C).*
NA = Not Answered

Question	Canada	U.S.	Europe	Australia
Under what condition is cryopreserved split thickness skin shipped/transported?				
Dry Shipper (vapor phase of liquid nitrogen)	0 of 5	0 of 5	0 of 1	1 of 1
Dry Ice (Solid $C0_2$)	5 of 5	5 of 5	1 of 1	0 of 1
Wet Ice	0 of 5	0 of 5	0 of 1	0 of 1

Each entry represents the number of tissue banks selecting the specific question out of the total number of banks answering the specific questions.
NA = Not Answered

Analysis

At 60% (n=3) of reporting Canadian tissue banks, 80% (n=5) of reporting U.S. tissue banks and one of the reporting European tissue bank and the Australian tissue bank, the freezing rate of skin allografts is electronically-controlled with gradual addition of liquid nitrogen. Two of five reporting Canadian tissue banks control the freezing rate by initially freezing skin allografts in an insulated heat-sink box in a mechanical freezer. One of five reporting U.S. tissue banks controls the freezing rate by using an insulated heat-sink box in dry ice.

100% (n=5) of reporting U.S. tissue banks use glycerol as a cryopreservative at concentrations between 1-10% (n=1), 10% (n=2) and between 10-15% (n=1). One U.S. bank did not answer the question.

In Canada 40% (n=2) of reporting tissue banks use glycerol at concentrations between 1-10% (n=1) and 10% (n=1). 60% (n=3) of reporting Canadian tissue banks use DMSO as a cryopreservative as compared to 0% of reporting U.S. tissue banks.

In Europe and Australia the reporting tissue banks use DMSO as a cryopreservative at a concentration of greater than 20% and 10%, respectively.

80% (n=4) of reporting Canadian tissue banks and 60% (n=3) of reporting U.S. tissue banks store cryopreserved donor skin in a mechanical freezer at -40°C or colder. Two of five reporting Canadian tissue banks, one of five reporting U.S. tissue banks, one of the reporting European tissue banks, and the Australian tissue bank, store skin in the vapor phase of liquid nitrogen. One reporting U.S. tissue bank stores skin in a mechanical freezer at -140°C or colder.

Each of the reporting Canadian, U.S., and European tissue banks uses dry ice (solid CO_2) to ship cryopreserved skin to hospitals. Australian tissue bank uses a dry shipper (vapor phase of liquid nitrogen).

Conclusions and Key Learning Points

1. The most common type of freezing of split-thickness donor skin allografts in North America is by cryopreservation using electronically controlled-rate freezing. When used with a cryoprotectant this maintains cellular viability.

2. 100% (n=5) of U.S. tissue banks use glycerol as a cryopreservative as compared to 40% (n=2) of Canadian tissue banks.

3. The European, Australian, and 60% of Canadian tissue banks use DMSO as a cryopreservative as compared to 0% of U.S. tissue banks.

4. The most common donor skin storage condition used by North American tissue banks is in a mechanical freezer at -40°C or colder. Vapor phase liquid nitrogen storage is used by at least one bank in Canada, US, Europe, and Australia.

5. Each of the Canadian, U.S., and European tissue banks ship cryopreserved skin using dry ice. The Australian tissue bank uses a dry shipper (vapor phase liquid nitrogen).

Note: All statements about practices are based on the number of survey respondents, not the absolute number of banks.

Bone Allograft Terminal Sterilization

Tissue Type:	Bone and connective
Process:	Tissue processing
Sub Process:	Terminal sterilization of bone allografts
Data Source:	Bone Processing and Validation survey questions numbered 4, 8, 9, and 12-14

Scope

This is a report of survey results pertaining to the processing of traditional bone allografts (for example: large structural bone grafts, struts, wedges and cancellous) by the use of terminal sterilization. This report does not pertain to tendons, ligaments or demineralized bone. It addresses the type of sterilization method but not the validation method, dose determination, containers to maintain sterility or labeling.

Introduction and Overview

Although suitable deceased tissue donors have no evidence of being clinically infected at the time of death, testing of recovered donor bone frequently reveals bacterial and fungal contamination.

Microbial testing of recovered donor bone *before processing* is important because it permits monitoring of incoming bioburden so the capacity of the validated bioburden reduction and sterilization process is not exceeded. It also permits identifying virulent pathogens on recovered donor bone, permitting its discard or special processing or sterilization steps to eliminate the microbe(s).

According to the U.S. FDA's Current Good Tissue Practices [21 CFR 1271.220(c)], terminal sterilization should achieve a sterility assurance level (SAL) of 10^{-6} if used with tissues having pre-processing cultures that are either: positive with enteric or pathogenic microorganisms, positive for *Clostridium* of *Streptococcus pyogenes* (group A strep) or positive for "other microorganisms difficult to eliminate".

Terminal sterilization of a donor bone allograft, after it is sealed in its final package, can eliminate allograft-based microbes that survive bone disinfection and can eliminate environmentally-based microbes that may have contaminated the allograft during processing. There are several methods of terminal sterilization of donor bone attaining a SAL of 10^{-6} that are currently used in the U.S. and in Europe. These methods include: mechanical and chemical cleaning and disinfection followed by low dose gamma radiation i.e. ≤ 2 MRad/≤ 20 kGy (examples of these proprietary methods are AlloWash XG®, AlloTrue™, Advanced Tissue Processing, GraftShield™ and BioCleanse®), high dose radiation, i.e. ≥ 2.5 MRad/ ≥ 25.0 kGy,

with radioprotectants (this proprietary method is known as the Clearant Process®), supercritical CO_2, high dose radiation, i.e. ≥ 2.5 MRad/ ≥ 25.0 kGy, without radioprotectants, low dose heat (known as the Marburg Lobator system) and chemical sterilization (using peracetic acid).

Results

A *Bone Processing and Validation* survey was sent to nine Canadian tissue banks; eight of the nine Canadian tissue banks fully or partially completed the survey.

A *Bone Processing and Validation* survey was sent to six U.S. tissue banks; four of six fully or partially completed the survey. The remaining two of six tissue banks did not complete the survey but provided answers to many of the key questions. Several answers were entered into the survey based on personal communication and recent documents provided by the two banks such as package inserts, pamphlets describing processing, scientific publications[1-7].

A *Bone Processing and Validation* survey was sent to eleven European tissue banks; six completed the survey.

The *Bone Processing and Validation* survey was completed by one Australian tissue bank.

The survey results of tissue banks in Canada and in the U.S. represent current practices in these countries; whereas, due to small numbers participating, the European tissue bank results are not necessarily representative of current European practices. One major tissue bank in the U.S. did not provide bone processing information for the survey, but its proprietary bone cleaning process, like the other major U.S. tissue banks participating in the survey, has a multistep bone cleaning and disinfecting process followed by terminal radiation in final packaging with a resultant SAL of 10^{-6}.

Table 1: *Terminal sterilization by bone banks in Canada, U.S. and Europe*

Question	Canada	U.S.	Europe	Australia
Does your facility use any of the following proprietary bone processing methods?				
No	8 of 8	1 of 6	1 of 6	1 of 1
AlloWash®	0 of 8	3 of 6	0 of 6	0 of 1
AlloWash XG®	0 of 8	1 of 6	0 of 6	0 of 1
Advanced Tissue Processing	0 of 8	1 of 6	0 of 6	0 of 1
BioCleanse®	0 of 8	0 of 6	0 of 6	0 of 1
Clearant Process®	0 of 8	0 of 6	1 of 6	0 of 1
Tutoplast®	0 of 8	0 of 6	0 of 6	0 of 1
AlloTrue™	0 of 8	1 of 6	0 of 6	0 of 1
NovaSterilis (supercritical CO_2 by a high pressure extractor machine)	0 of 8	0 of 6	1 of 6	0 of 1

Each entry represents the number of tissue banks selecting the specific question out of the total number of banks answering the specific questions.

Question	Canada	U.S.	Europe	Australia
Which sterilization methods are applied to your bone processing?				
None	5 of 8	0 of 6	2 of 6	0 of 1
Gamma radiation	3 of 8	6 of 6	4 of 6	1 of 1
Electron beam radiation	0 of 8	*1 of 6	0 of 6	0 of 1
Dry heat	0 of 8	0 of 6	0 of 6	0 of 1
Ethylene oxide gas	0 of 8	0 of 6	0 of 6	0 of 1
NovaSterilis (supercritical CO_2)	0 of 8	0 of 6	1 of 6	0 of 1
Moist heat (steam)	0 of 8	0 of 6	0 of 6	0 of 1
Clearant Process®	0 of 8	0 of 6	1 of 6	0 of 1
**One bank uses both gamma and electron beam radiation, with electron being applied to non-traditional bone allografts,*				
Does your facility apply radiation to bone prior to processing?				
Yes	0 of 8	0 of 6	0 of 6	0 of 1
No	8 of 8	6 of 6	5 of 6	1 of 1
Is radiation applied to bone at your facility as a final step in its final package?				
Yes, applied to all	3 of 8	4 of 6	2 of 6	0 of 1
Yes, depending on the results of pre-processing microbial tests or other indications	0 of 8	2 of 6	1 of 6	1 of 1
No	5 of 8	0 of 6	0 of 6	0 of 1
What type of radiation is used for traditional bone allografts?				
Gamma	3 of 3	6 of 6	4 of 4	1 of 1
Electron beam	0 of 3	0 of 6	0 of 4	0 of 1
What is minimum dose of radiation used as terminal sterilization of bone?				
< 1.0 MRad (< 10.0 kGy)	0 of 3	0 of 6	0 of 4	0 of 1
1.0 MRad (< 10.0 kGy)	0 of 3	1 of 6	0 of 4	0 of 1
1.0 to 1.5 MRad (< 10.0-15.0 kGy)	0 of 3	1 of 6	0 of 4	0 of 1
1.5 MRad (< 15.0 kGy)	0 of 3	2 of 6	0 of 4	0 of 1
1.5 to 1.75 MRad (< 12.0-20.0 kGy)	0 of 3	0 of 6	0 of 4	0 of 1
1.75 MRad (< 17.5 kGy)	0 of 3	0 of 6	0 of 4	0 of 1
1.75 to 2.0 MRad (< 17.5-20.0 kGy)	0 of 3	0 of 6	0 of 4	0 of 1
2.0 MRad (< 20.0 kGy)	1 of 3	1 of 6	0 of 4	0 of 1
2.0 to 2.5 MRad (< 20.0-25.0 kGy)	0 of 3	1 of 6	0 of 4	0 of 1
2.5 MRad (< 25.0 kGy)	0 of 3	0 of 6	2 of 4	1 of 1
> 2.5 MRad (> 25.0 kGy)	2 of 3	0 of 6	2 of 4	0 of 1

Each entry represents the number of tissue banks selecting the specific question out of the total number of banks answering the specific questions.

Analysis

100% (n=6) of reporting U.S. tissue banks and 66% (n=4) of reporting European tissue banks apply terminal radiation to donor bone allografts as compared to 38% (n=3) of reporting Canadian tissue banks.

67% (n=2) of reporting Canadian tissue banks and 100% (n=4) of reporting European tissue banks applying terminal radiation use “high dose” gamma radiation (> 2.5 MRad) as compared to 0% of reporting U.S. tissue banks applying terminal radiation who use “lower dose” gamma radiation.

Reporting U.S. tissue banks applying radiation use lower doses than reporting Canadian and European tissue banks: four of six U.S. tissue banks using a dose of 1.5 MRad (15.0 kGy) or lower. Of the six reporting U.S. tissue banks using relatively low dose radiation (<2.5 MRad), the doses used were quite variable with only two reportedly using the same radiation dose.

83% (n=5) of reporting U.S. tissue banks and 33% (n=2) of reporting European tissue banks apply proprietary processes, all in combination with radiation, while 0% of reporting Canadian tissue banks use any proprietary processes.

The reporting Australian tissue bank apply a radiation dose lower than reporting Canadian and half of the European tissue banks: using a dose of 2.5 MRad (<25 kGy).

Conclusions and Key Learning Points

1. 100% of U.S. and 66% European tissue banks responding (n=10) apply terminal radiation to donor bone allografts as compared to 38% (n=3) of Canadian tissue banks.

2. Terminal radiation doses for donor bone allografts are higher in Canadian, European and Australian tissue banks than those used in the U.S.

3. The use of proprietary processes are absent in Canadian tissue banks while 88% (n=7) of U.S. and European tissue banks apply proprietary processes.

Note: All statements about practices are based on the number of survey respondents, not the absolute number of banks.

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Connective Tissue (Tendon and Ligament) Sterilization by Gamma Radiation

Tissue Group:	Bone and connective
Process:	Tissue processing
Sub process:	Sterilizing connective tissue (tendon and ligament) allografts
Data Source:	Bone Processing and Validation survey questions numbered 42, 44, 46, and 47

Scope

This is a report of survey results pertaining to the sterilization of tendons and ligaments during processing. Allografts include the patellar tendon (a ligament that connects the patella with the tibia) with attached bone blocks at each end containing lipid, blood and cells), the Achilles tendon (connects the calcaneus bone with the gastrocnemius muscle) with a bone block attached at one end and other tendons such as the semi-tendinosis, gracilis, tibialis, and peroneus longus. These allografts are usually used to repair torn or ruptured anterior and posterior cruciate ligament reconstructions at the knee and other areas. Questions related to the possible impact on the quality or efficacy of allografts treated with radiation were not included in this survey.

Introduction and Overview

Although deceased tissue donors may have no evidence of being clinically infected at the time of death, testing of recovered connective tissue frequently reveals bacterial and fungal contamination.

Microbial testing of recovered connective tissue before processing is important. The same microbes causing infections in recipients of tendon allografts have been identified in samples obtained immediately after recovering the tissue from the donor. In addition, by identifying virulent pathogens on recovered connective tissue, it can be discarded or sterilization steps can be applied to eliminate the microbe.

Although recovered connective tissue can become contaminated from the incision or the recovery environment, an important contributor to microbial contamination of recovered connective tissue is the expected post-mortem spread of intestinal microbes to extra-luminal sites, lymphatic and blood vessels, mesenteric lymph nodes and other tissues and organs as part of the normal post-mortem decomposition process in the body.

Gamma radiation of the tendon and ligament allografts after they are sealed in the final packaging (terminal sterilization) can eliminate microbes that survive disinfection and environmental microbes that may have contaminated the allografts during processing.

Results

A *Bone Processing and Validation* survey (includes connective tissue questions) was sent to nine Canadian tissue banks; eight banks fully or partially completed the survey.

A *Bone Processing and Validation* survey was sent to eight U.S. tissue banks; six tissue banks either fully or partially completed the survey. The remaining two U.S. tissue banks did not complete the survey but provided answers to several of the key questions. Some answers were entered into the survey based on personal communication and recent documents provided by these two tissue banks (package inserts, pamphlets describing processing, scientific publications) [1-4].

A *Bone Processing and Validation* survey *was* sent to eleven European tissue banks; six completed the survey.

The *Bone Processing and Validation* survey was completed by one Australian tissue bank.

Table 1: *Tendon Sterilization by Gamma Radiation*

Question	Canada*	U.S.*	Europe*	Australia
Does your facility process tendons, ligaments?				
Yes	6 of 8	6 of 6	4 of 6	1 of 1
No	2 of 8	0 of 6	0 of 6	0 of 1
Is radiation applied to tendons/ligaments prior to processing?				
Yes	0 of 6	0 of 6	0 of 6	NA
No	6 of 6	6 of 6	6 of 6	NA
Is radiation applied as a final step of ligament/tendon processing in its final package (terminal sterilization)?				
Yes, applied to all	1 of 6	3 of 4	2 of 6	0 of 1
Yes, some, not all	1 of 6	1 of 4	1 of 6	0 of 1
No	0 of 6	0 of 6	3 of 6	1 of 1
What is the minimum dose of radiation that is used as a final treatment of soft tissue/connective tissue?				
Less than 1.0 MRad (10 kGy)	0 of 2*	0 of 4	1 of 3	0 of 1
1.0 MRad (10 kGy)	0 of 2	1 of 4	0 of 3	0 of 1
Between 1.0 and 1.5 MRad (10 – 15 kGy)	1 of 2	1 of 4	0 of 3	0 of 1
1.5 MRad (15 kGy)	0 of 2	1 of 4	0 of 3	0 of 1
2.0 MRad (20 kGy)	1 of 2	1 of 4	0 of 3	0 of 1
2.5 MRad (25kGy)	0 of 2	0 of 4	1 of 3	0 of 1
Greater than 2.5 MRad (25 kGy)	0 of 2	0 of 4	1 of 3	0 of 1
n/a	0 of 2	0 of 4	0 of 3	1 of 1

Each entry represents the number of tissue banks selecting the specific question out of the total number of banks answering the specific questions.

Analysis

No tissue banks surveyed apply irradiation prior to processing.

Two of six reporting Canadian tissue banks apply radiation to tendons and ligaments, while all four responding U.S. tissue banks and half of the surveyed European tissue banks apply radiation to tendons and ligaments. The two reporting Canadian tissue banks use doses of 1.0-1.5 MRads and 2.0 MRads (10.0-20.0 kGy). None of the four U.S. tissue banks use the same dose but all use similarly low doses: 1.0, 1.0-1.5, 1.5 and 2.0 MRads (10.0-20.0 kGy). The reporting European tissue banks apply either a low dose (less than 1.0 MRad or a higher dose 2.5 MRad or greater. The Australian tissue bank does not irradiate tendons or ligaments.

Conclusions and Key Learning Points

1. 100% of responding U.S. tissue banks (n=4) and 50% of responding European tissue banks (n=3) apply terminal sterilization (radiation) to tendons and ligaments as compared to 33% (n=2) of Canadian tissue banks.

2. Radiation doses chosen for terminal sterilization of tendons and ligaments were highly variable, but all doses used by Canadian and U.S. tissue banks for tendon and ligament allografts are considered “low dose” radiation, between 1.0-2.0 MRads (10.0-20.0 kGy).

Note: All statements about practices are based on the number of survey respondents, not the absolute number of banks.

References

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Microbial Testing:
Split Thickness Skin Allografts

Tissue Type:	Skin
Process:	Tissue recovery
Sub Process:	Microbial testing of split thickness skin allografts
Data Source:	Skin Processing and Validation survey questions numbered 3, 13-18, 28, 31-34 and 79

Scope

This is a report of survey results pertaining to microbial testing of split-thickness skin allografts prior to, during and following processing steps at facilities in the United States and Canada.

Introduction and Overview

Split-thickness skin allografts are critically important in the management of large severe burn injuries. In North America, recovered split-thickness skin allografts are placed in an antibiotic solution for storage until they are either cryopreserved for long-term frozen storage or are stored in a refrigerator until released to a hospital for a burn patient, usually within 14 days of storage. To maintain skin viability during refrigerated storage the storage medium is exchanged with fresh medium at regular intervals.

In 1976, bacterially-contaminated, cryopreserved human skin allografts were reported to have caused high fevers and Pseudomonas sepsis in a severely burned child. No cases have been reported since despite wide use of skin allografts in patients with severe burns and burn-related immune suppression. There have been no reports of skin allografts transmitting fungal or mycobacterial infections.

Results

A *Skin Processing and Validation* survey was sent to five skin banks in Canada; surveys were partially completed by all five, however, responses were not provided to several questions.

A *Skin Processing and Validation* survey was sent to nine skin banks in the U.S.; fully or partially completed surveys were received from seven banks (five split-thickness skin banks, two dermis processors), however, responses were not provided to several questions.

A *Skin Processing and Validation* survey was sent to three skin banks in Europe; a completed survey was returned by two.

A *Skin Processing and Validation* survey was sent and returned to a skin bank in Australia.

Table 1: *Microbial Testing of Cryopreserved and "Fresh" Refrigerated Skin Allograft*

Question	Canada	U.S.	Europe	Australia
Do you process cryopreserved split thickness skin at your facility?				
Yes	5 of 5	5 of 5	1 of 2	1 of 1
No	0 of 5	0 of 5	1 of 2	0 of 1
What is routinely sampled for microbial testing before or during processing and immediately prior to cryopreserving split thickness skin?				
The transport fluid bathing the recovered unprocessed skin during temporary storage and transportation to the facility.	1 of 5	1 of 5	0 of 1	1 of 1
Swabbing of each zone of recovered skin.	1 of 5	0 of 5	0 of 1	1 of 1
A small piece of each sheet of recovered unprocessed skin.	1 of 5	2 of 5	0 of 1	0 of 1
A small piece of recovered unprocessed skin from each anatomical site.	1 of 5	3 of 5	0 of 1	0 of 1
For cryopreserved skin, a sample of the cryopreservation fluid after exposure to skin while in the final package,	1 of 5	2 of 5	0 of 1	1 of 1
Other: Representative sample piece the first, last and every fifth skin graft produced.	2 of 5	0 of 5	0 of 1	0 of 1
Other: Each unit of skin is swabbed after processing.	1 of 5	0 of 5	0 of 1	0 of 1
Other: Sample of co-processed antibiotic soaked skin that was trimmed during processing	0 of 5	1 of 5	0 of 1	0 of 1
Other: Not specified	0 of 5	0 of 5	1 of 1	0 of 1
What microbial tests are performed following cryopreserved skin processing at the time of final packaging?				
Bacteria	5 of 5	5 of 5	1 of 1	1 of 1
Fungi	5 of 5	5 of 5	1 of 1	1 of 1
Mycobacterium	1 of 5	2 of 5	0 of 1	0 of 1
Has your facility ever performed bacteriostasis or fungistasis studies of cryopreserved skin?				
Yes	0 of 5	2 of 5	0 of 1	0 of 1
Yes, during validation studies	1 of 5	2 of 5	0 of 1	1 of 1
No	4 of 5	1 of 5	1 of 1	0 of 1

Each entry represents the number of tissue banks selecting the specific question out of the total number of banks answering the specific questions.

Question	Canada	U.S.	Europe	Australia
Does your facility have a list of virulent bacteria or fungi which, if found on incoming unprocessed skin, is cause for discard or other use instead of processing for transplantation?				
Yes	4 of 4	5 of 5	1 of 1	1 of 1
No	0 of 4	0 of 5	0 of 1	0 of 1
Have you performed quantitative microbial bioburden studies of incoming, unprocessed, spit thickness skin which will be cryopreserved?				
Yes	0 of 4	2 of 3	0 of 1	0 of 1
No	4 of 4	1 of 3	1 of 1	1 of 1
Did you establish upper limits of bacterial bioburden levels which are acceptable for processing?				
Yes	NA	1 of 3	0 of 1	0 of 1
No	NA	2 of 3	1 of 1	1 of 1
Do you process and provide "fresh" refrigerated split thickness skin?				
Yes	1 of 5	2 of 7	0 of 2	0 of 1
No	4 of 5	5 of 7	2 of 2	1 of 1
What is your maximum storage period for "fresh" refrigerated skin?				
8-13 days	0 of 1	1 of 2	NA	0 of 1
14 days	1 of 1	1 of 2	NA	0 of 1
Greater than 15 days	0 of 1	0 of 2	NA	0 of 1
n/a	0 of 1	0 of 2	NS	1 of 1
What is routinely sampled for microbial testing during "fresh" refrigerated split thickness skin processing or storage?				
The transport fluid bathing the recovered unprocessed skin during temporary storage and transportation to the processing facility.	1 of 1	1 of 2	NA	0 of 1
Swabbing of each zone of recovered skin.	0 of 1	0 of 2	NA	0 of 1
A small piece of each sheet of recovered unprocessed skin.	1 of 1	0 of 2	NA	0 of 1
A small piece of recovered unprocessed skin from each anatomical site.	1 of 1	2 of 2	NA	0 of 1
A sample of the storage fluid immediately prior to shipment to a patient in a hospital.	0 of 1	0 of 2	NA	0 of 1
Other: Piece of recovered skin each time medium is changed	1 of 1	0 of 2	NA	0 of 1
n/a	0 of 1	0 of 2	NA	1 of 1

Question	Canada	U.S.	Europe	Australia
What microbial tests are performed with samples identified above?				
Bacteria	1 of 1	2 of 2	NA	0 of 1
Fungi	1 of 1	2 of 2	NA	0 of 1
Mycobacteria	0 of 1	0 of 2	NA	0 of 1
n/a	0 of 1	0 of 2	NA	1 of 1
Has your facility performed bacteriostasis or fungistasis testing of fresh refrigerated skin?				
Yes	0 of 1	2 of 2	NA	0 of 1
Yes, during validation studies	0 of 1	0 of 2	NA	1 of 1
No	1 of 1	0 of 2	NA	0 of 1
Do you have a list of virulent bacteria or fungi which, if found on recovered unprocessed skin is cause for discard?				
Yes	1 of 1	2 of 2	1 of 1	1 of 1
No	0 of 1	0 of 2	0 of 1	0 of 1
Does your facility provide skin stored in high concentrations of glycerol, e.g., 50-85%?				
Yes	0 of 4	0 of 6	1 of 2	0 of 1
No	4 of 4	6 of 6	1 of 2	1 of 1

NA = Not Answered

Analysis

All five reporting Canadian skin banks, five of seven surveyed U.S. skin banks, one of the European skin banks, and the Australian skin bank process cryopreserved skin allografts. Only one of five reporting Canadian banks and two of seven reporting U.S. banks provide "fresh" refrigerated skin allografts. One of the European skin banks completing the survey produces glycerol-preserved refrigerated skin allografts (typically used in Europe) but not cryopreserved or fresh refrigerated skin allografts (as is typically used in North America).

100% of responding Canadian banks (n=5), U.S. banks (n=5), European (n=1) and Australian (n=1) reported testing finished skin allografts for bacteria and fungi. 20% (n=1) of reporting Canadian banks and 40% (n=2) of reporting U.S. banks also test for *Mycobacterium tuberculosis*.

A variety of sampling techniques for microbial testing (transport fluid sample, swabbing skin, and immersion of pieces of skin) are used to sample recovered skin prior to antibiotic exposure by each of the reporting Canadian and U.S. skin banks. The type of sampling of skin allografts in final packaging prior to release was not reported by all skin banks.

Only three of the ten reporting Canadian and U.S skin banks that cryopreserve skin answered the question: "*Do you have a list of virulent bacteria or fungi which, if found on recovered unprocessed skin is cause for discard?".* All three have such a list.

Testing of cryopreserved skin for bacteriostasis and fungistasis is performed by 20% (n=1) of reporting Canadian banks compared to 80% (n=4) of reporting U.S. banks.
Fresh refrigerated skin is processed and provided by one of five reporting Canadian banks and two of seven reporting U.S. skin banks; each of these three banks has a maximum storage period of 14 days or less and each bank tests skin prior to processing with antibiotics. It is unclear whether they sample and test the skin immediately prior to release. Samples are tested for bacteria and fungi.

Testing of fresh refrigerated skin for bacteriostasis and fungistasis is performed by 0% of reporting Canadian banks and 100% (n=2) of reporting U.S.

Conclusions and Key Learning Points

1. The type of sampling of recovered skin for microbial testing is variable for U.S., European and Canadian skin banks but all perform testing prior to processing and antibiotic exposure.

2. All banks sample for bacteria and fungi testing but a few also test for Mycobacterium tuberculosis.

Note: All statements about practices are based on the number of survey respondents, not the absolute number of banks.

Microbial Testing:
Heart Valve Allograft Processing

Tissue Type:	Cardiovascular
Process:	Tissue processing
Sub Process:	Sampling for microbial testing during processing of heart valve allografts
Data Source:	Tissue Recovery survey question number 27 and Cardiovascular Processing and Validation survey question number 13

Scope

This is a report pertaining to the sampling of the donor heart valve tissues for microbial growth performed during processing.

Introduction and Overview

Tissue banks report 10 to 27% of donated hearts and heart valves recovered from deceased tissue donors are contaminated by bacteria[1, 2] and 1 to 3% by fungi[1, 3].

To maximize safety for recipients, heart valve testing and processing steps are designed to: identify and eliminate contaminating microbes, monitor bioburden reduction by microbial testing at critical points, prevent further contamination and produce a finished allograft free from infectious organisms.

Donor heart valve processing steps vary among tissue banks but they utilize most of the following processes and steps. The aseptically recovered and iced heart is examined and dissected in a clean room or laminar flow hood environment with air quality and surfaces monitored and controlled for non-viable particulates and viable microbes.

The cold heart undergoes macroscopic evaluation and dissection to excise the heart valves and their outflow conduits. Excised valves are examined for meeting anatomic and functional specifications and those not meeting established acceptance criteria are discarded. Because recovered heart valves are expected to harbour bacteria, an antibiotic decontamination (disinfection) step follows dissection which is then followed by rinsing, final sterility testing and cryopreservation. The disinfection step is important because heart valves cannot be terminally sterilized without rendering the tissue clinically unsuitable. Many tissue banks perform microbial testing at critical points of the bioburden reduction process to add confidence that their process is reliable and effective. Testing is typically performed from samples taken before and after the antibiotic disinfection step but source of tissue and type of sampling varies among the banks. This report identifies variation in the types of sampling and testing, for example, some test tissue biopsy samples or fluid samples directly; others filter the fluid and culture the filter.

Results

The *Cardiovascular Processing and Validation* survey was sent to four Canadian tissue banks that recover, process and provide donor heart valve allografts. All four completed or partially completed the portion of the survey pertaining to microbial sampling during processing (question 13).

The *Cardiovascular Processing and Validation* survey was not directly completed by the two U.S. heart valve tissue banks but survey question number 17 was asked from each tissue bank's staff through personal communication and entered into the survey[4, 5].

The *Cardiovascular Processing and Validation* survey was sent to four European heart valve tissue banks; four returned the completed surveys by email and the data was entered manually into the Survey Monkey database.

The *Tissue Recovery* survey was sent to each of Canada's 11 tissue banks and one recovery agency; 11 completed the survey and nine answered question 27.

Thirty U.S. tissue banks and recovery agencies were invited to participate in the *Tissue Recovery* survey; 13 agreed and were sent surveys, ten of which were completed, including question 27.

The *Tissue Recovery* survey was sent to six European tissue banks; six banks completed the survey, but only two tissue bank recovering hearts complete question 27.

The survey was completed by one Australian tissue bank.

Table 1: *Heart valve recovery and processing*

Question	Canada*	U.S.*	Europe*	Australia
Is sampling of the recovered heart performed by recovery staff to detect microbial growth and prior to exposure to antibiotics and processing?				
Yes, by recovery staff	1 of 9	0 of 10	0 of 2	0 of 1
No, not by recovery staff	7 of 9	10 of 10	2 of 2	1 of 1
Unknown	0 of 9	0 of 10	0 of 2	0 of 1
Other: Processing staff	3 of 9	2 of 10	0 of 2	0 of 1

**Each entry represents the number of tissue banks selecting the specific question out of the total number of banks answering the specific questions.*

Question	Canada*	U.S.*	Europe*	Australia
What is routinely sampled for microbial testing during heart valve processing?				
Direct culturing of the transport fluid in which the heart was received by the processing lab	4 of 4	0 of 2	5 of 6	0 of 1
Filtering the transport fluid and culturing the filter	0 of 4	2 of 2	0 of 6	1 of 1
Swabbing of the whole heart prior to exposure to antibiotics	0 of 4	0 of 2	0 of 6	0 of 1
The excised valve prior to exposure to antibiotics	1 of 4	0 of 2	3 of 6	0 of 1
Prior to exposure to antibiotics but after the heart valves have been dissected and rinsed, the rinsate is cultured	0 of 4	1 of 2	0 of 6	1 of 1
Co-processed cardiac tissues (e.g., conduit, myocardium) prior to exposure to antibiotics	2 of 4	1 of 2	2 of 6	0 of 1
Co-processed cardiac tissue (e.g., conduit, myocardium) after exposure antibiotics, rinsing and immersion in the cryopreservation fluid	3 of 4	0 of 2	4 of 6	1 of 1
The cryopreservation fluid containing the heart valve immediately prior to sealing the final package before the freezing process	2 of 4	1 of 2	4 of 6	1 of 1
Other: Prior to antibiotic disinfection but after companion tissue has been dissected and rinsed, the rinsate is cultured (as noted above)	NA	1 of 2	NA	0 of 1
Other: After antibiotic disinfection, heart valve rinsed, immersed and fluid filtered and filter cultured	1 of 4	2 of 2	1 of 6	0 of 1
Other: After antibiotic disinfection step, co-processed, companion tissue is ground in a stomacher and the fluid is cultured in BacTAlert system (as noted above)	NA	1 of 2	NA	0 of 1
Other: After antibiotic disinfection and rinsing, a heart valve tissue biopsy sample is cultured	1 of 4	0 of 2	1 of 6	0 of 1

**Each entry represents the number of tissue banks selecting the specific question out of the total number of banks answering the specific questions.*
NA = Not Answered or Not Asked

Analysis

Sampling of the donor heart at recovery is not commonly performed by reporting Canadian, U.S., European tissue banks or the Australian bank.

100% of reporting Canadian (n=4) and 83% (n=5) of reporting European tissue banks directly culture the transportation fluid as compared to 0% of reporting U.S. banks. Responding U.S. banks and Australian bank filter the transportation fluid and culture the filter; providing for a more sensitive culturing methodology.

Conclusions and Key Learning Points

1. Sampling for microbial testing at critical points during donor heart valve processing is commonly performed by Canadian, U.S., Australian and European tissue banks; but sampling sites and methods vary.

2. Each of the surveyed Canadian, U.S., Australian and European tissue banks perform microbial testing of the whole heart transport fluid prior to exposure to antibiotics.

3. 100% (n=2) of U.S. tissue banks and the Australian tissue bank filter the transport fluid and culture the filter as compared to 0% of Canadian and European tissue banks. Canadian and European tissue banks culture the fluid directly.

4. Canadian tissue banks perform almost as many microbial tests per valve processed (3.5) as do U.S. (4.0) and European tissue banks (3.75). *(Total number of pre and post disinfection tests performed divided by number of reporting banks)*

Note: All statements about practices are based on the number of survey respondents, not the absolute number of banks.

References

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3. Gall K, Smith S, Willmette C, Wong M, O'Brien M. Allograft heart valve sterilization: a six-year in-depth analysis of a twenty-five-year experience with low-dose antibiotics. J Thoracic Cardiovasc Surg, 1995;110:680–687.

4. Personal communication with Michael Bergevin, M.D., LifeNet Health medical director June 5, 2014.

5. Personal communication with Greg Ray, M.D, CryoLife medical director, Dec 18, 2013, June 5, 2014

Microbial Testing:
Testing Allografts for Mycobacterium tuberculosis

Tissue Type:	Bone, connective, skin and cardiovascular
Process:	Processing and validation
Sub Process:	Testing tissues for *Mycobacterium tuberculosis*
Data Source:	Cardiovascular Processing and Validation survey questions numbered 4 and 14 Skin Processing and Validation survey questions numbered 4, 14, 24, 29, 32 and 52 Bone Processing and Validation survey questions numbered 6 and 17

Scope

This is a report of survey results pertaining to final sterility testing of processed donor bone, connective tissue, cardiovascular and skin allografts. Testing is designed to identify growth of bacteria and fungi on all allografts tested prior to release into inventory and distribution to hospitals for clinical use. Testing may also specifically include identification for *Mycobacterium tuberculosis:* a pathogenic bacterial species responsible for most cases of tuberculosis, which in turn is a contraindication for tissue donation.

Introduction and Overview

In the 1950s, tuberculosis was transmitted to several patients through transplantation of frozen donor rib allografts recovered from donors with active tuberculosis. Several cases of tuberculosis, reported over 30 years ago, developed after a donor heart valve allograft was transplanted and transmission from the valve was suspected. One donor died with pneumonia and other donors had histories of treated tuberculosis infection. One case of Mycobacterium hominis transmission via an arterial allograft has been documented.

No cases of tuberculosis transmission through tissue transplantation have been reported recently, however, there was one unpublished case of a tissue donor who was not suspected of having tuberculosis but the results of an autopsy showed an active tuberculosis infection[1.] The donated tissues were discarded after the report of the autopsy was available.

Final sterility testing of finished donor bone, connective tissue, cardiovascular and skin allografts is designed to identify growth of bacteria and fungi prior to release into inventory and distribution to hospitals for clinical use. A few tissue banks also test for *Mycobacterium tuberculosis* which is the subject of this environmental scan report.

Results

A *Skin Processing and Validation* survey was sent to five Canadian, seven U.S. and four European tissue banks that recover, process and distribute donor skin allografts. All five Canadian tissue banks completed or partially completed the survey. Five of the seven U.S. tissue banks agreed to participate and submitted partially completed surveys. Only one European survey was returned but the question about mycobacteria testing was not answered.

A *Bone Processing and Validation* survey was sent to nine Canadian, eight U.S and seven European tissue banks that recover, process and distribute donor bone allografts. Eight of the nine Canadian banks returned completed or partially completed surveys. Six of the eight U.S. tissue banks completed or partially completed the survey and six of the seven European tissue banks returned completed or partially completed surveys.

A *Cardiovascular Processing and Validation* survey was sent to four Canadian tissue banks as well as six European tissue banks. All four Canadian and four European tissue banks completed or partially completed the surveys. Surveys were not directly completed by the two participating U.S. tissue banks but information about antibiotic use and microbial testing was collected from each tissue bank's staff through personal communication and entered into the survey.

The surveys were completed by one Australian tissue bank.

Table 1: *Final microbial testing of finished allografts: types of microorganisms sought*

Question	Canada	U.S.	Europe	Australia
Heart Valves				
What microbial tests are performed at or near final packaging of heart valves as end product testing?				
Bacterial culturing	4 of 4	2 of 2	4 of 4	1 of 1
Fungal/yeast culturing	4 of 4	2 of 2	19 of 19	1 of 1
Mycobacterium culturing	0 of 4	2 of 2	1 of 4	0 of 1
Cryopreserved Skin				
What microbial tests are preformed following cryopreserved skin processing at the time of final packaging?				
Bacterial culturing	5 of 5	5 of 5	1 of 1	1 of 1
Fungal culturing	5 of 5	5 of 5	1 of 1	1 of 1
Mycobacterium culturing	1 of 5	5 of 5	0 of 1	0 of 1

Each entry represents the number of tissue banks selecting the specific question out of the total number of banks answering the specific questions.

Question	Canada	U.S.	Europe	Australia
Fresh Refrigerated Skin				
What microbial tests are performed with samples from "fresh" refrigerated skin?				
Bacterial culturing	1 of 1	2 of 2	NA	0 of 1
Fungal culturing	1 of 1	2 of 2	NA	0 of 1
Mycobacterium culturing	0 of 1	0 of 2	NA	0 of 1
n/a	0 of 1	0 of 2	NA	1 of 1
Bone and Tendon Allografts				
What type of microbiologic testing of bone, tendon or ligament allografts is performed at or near final packaging?				
Bacterial culturing	7 of 8	5* of 6	5 of 6	1 of 1
Fungal/yeast culturing	5 of 8	5 of 6	4 of 6	1 of 1
Mycobacterium culturing	2 of 8	0 of 6	1 of 6	0 of 1
None, we use a validated radiation process and dosimetric release	1 of 8	2 of 6	1 of 6	0 of 1
None, we use a validated sterilization (NovaSterilis) and parametric release	0 of 8	0 of 6	1 of 6	0 of 1

NA = Not Applicable.

One bone bank performs final microbial tests on tendons but not bone allografts.

Table 2: *Testing for mycobacteria and use of streptomycin during processing of skin*

Skin Bank	Antibiotics	Final Test for Mycobacteria?
Cryopreserved Skin		
CN-A	Vancomycin Gentamicin Cephalosporin	No
CN-B	Gentamicin Cephalosporin	No
CN-C	Vancomycin Gentamicin	No
CN-D	Vancomycin Gentamicin Cephalosporin	Yes
CN-E	Streptomycin	No
US-A	Gentamicin	No
US-B	Gentamicin Kanamycin Cephazolin	Yes
US-C	Gentamicin Oxacillin	Yes
US-D	Gentamicin	No
US-E	Vancomycin Ciprofloxacin Bactrim Amphotericin	No
EU-A	Vanomycin Polymyxin B Gentamicin Nystatin	No
AU-A	Penicillin Streptomycin	No

Skin Bank	Antibiotics	Final Test for Mycobacteria?
Fresh, Refrigerated Skin		
CN-E	Streptomycin	No
US-A	Gentamicin	No
US-D	Gentamicin Nystatin	No

NA = Not Answered

Table 3: *Antibiotics used in decontamination of tissues at 54 facilities in Canada, the U.S., Europe, and Australia 2013*

Antibiotic	Heart Valve Banks (26 total)	Bone Banks (16 total, 13 Using Antibiotics)	Split-thickness Skin, Cryopreserved Banks (12 total)	Split-thickness Skin, Fresh Banks (3 total)	Dermis Banks (4 total, 4 used antibiotics)	Skin, Bone and Heart Valve Banks using Antibiotics* (49 of 59 total)
No antibiotic	0 of 26	7 of 21	0 of 12	0 of 3	1 of 5	8 of 64
Vancomycin	23 of 26*	0 of 15	4 of 12*	0 of 3*	3 of 4*	30 of 57*
Gentamicin	11 of 26	9 of 15	9 of 12	2 of 3	2 of 4	33 of 57
Polymyxin B	7 of 26	6 of 15	1 of 12	0 of 3	2 of 4	16 of 57
Amphotericin B (antifungal)	9 of 26	2 of 15	1 of 12	0 of 3	1 of 4	13 of 57
Bacitracin	0 of 26	8 of 15	1 of 12	0 of 3	0 of 4	9 of 57
Cefoxitin/Mefoxitin	7 of 26	0 of 15	1 of 12	0 of 3	0 of 4	8 of 57
Lincomycin/Lincocin	7 of 26	0 of 15	0 of 12	0 of 3	1 of 4	8 of 57
Cephazolin (Ancef)	1 of 26	4 of 15	3 of 12	0 of 3	0 of 4	8 of 57
Ciprofloxacin	6 of 26	0 of 15	1 of 12	0 of 3	0 of 4	7 of 57
Amakacin	5 of 26	0 of 15	0 of 12	0 of 3	0 of 4	5 of 57
Streptomycin	3 of 26	0 of 15	2 of 12	1 of 3	2 of 4	8 of 57
Colistin (Polymyxin E)	4 of 26	0 of 15	0 of 12	0 of 3	0 of 4	4 of 57
Clindamycin	3 of 26	0 of 15	0 of 12	0 of 3	0 of 4	3 of 57
Nystatin (antifungal)	3 of 26	0 of 15	1 of 12	1 of 3	0 of 4	5 of 57
Metronidazole (Flagyl)	3 of 26	0 of 15	0 of 12	0 of 3	0 of 4	3 of 57
Ampicillin, Sulbactam	2 of 26	0 of 15	0 of 12	0 of 3	0 of 4	2 of 57
Flu cytosine/ 5-fluorocytosine, (antifungal)	2 of 26	0 of 15	0 of 12	0 of 3	0 of 4	2 of 57
Fluconazole (antifungal)	2 of 26	0 of 15	0 of 12	0 of 3	0 of 4	2 of 57
Cefuroxime	2 of 26	0 of 15	0 of 12	0 of 3	0 of 4	2 of 57
Penicillin	2 of 26	0 of 15	1 of 12	0 of 3	1 of 4	4 of 57
Colimycin M	2 of 26	0 of 15	0 of 12	0 of 3	0 of 4	2 of 57
Piperacillin/tazobactam (Tazocin, Zosyn)	1 of 26	0 of 15	0 of 12	0 of 3	0 of 4	1 of 57
Amoxicillin	1 of 26	0 of 15	0 of 12	0 of 3	0 of 4	1 of 57
Timentin (Ticarcillin and Clavulanate),	1 of 26	0 of 15	0 of 12	0 of 3	0 of 4	1 of 57

Antibiotic	Heart Valve Banks (26 total)	Bone Banks (16 total, 13 Using Antibiotics)	Split-thickness Skin, Cryopreserved Banks (12 total)	Split-thickness Skin, Fresh Banks (3 total)	Dermis Banks (4 total, 4 used antibiotics)	Skin, Bone and Heart Valve Banks using Antibiotics* (49 of 59 total)
Oxacillin	0 of 26	0 of 15	1 of 12	0 of 3	0 of 4	1 of 57
Kanamycin	0 of 26	0 of 15	1 of 12	0 of 3	0 of 4	1 of 57
Cefoperazone/Cefibid/cefazone	1 of 26	0 of 15	0 of 12	0 of 3	0 of 4	1 of 57
Cefataxime	1 of 26	0 of 15	0 of 12	0 of 3	0 of 4	1 of 57
Primaxin (imipenem, cilastatin)	0 of 26	2 of 15	0 of 12	0 of 3	0 of 4	2 of 57
Ketoconazole (antifungal)	1 of 26	0 of 15	0 of 12	0 of 3	0 of 4	1 of 57
Meropenem	1 of 26	0 of 15	0 of 12	0 of 3	0 of 4	1 of 57
Ipipenem	2 of 26	0 of 15	0 of 12	0 of 3	0 of 4	2 of 57
Bactrim (Trimethoprim and Sulfamethoxazole)	0 of 26	0 of 15	1 of 12	0 of 3	0 of 4	1 of 57
Vancomicine	0 of 26	1 of 15	0 of 12	0 of 3	0 of 4	1 of 57
Tobramycine	1 of 26	1 of 15	0 of 12	0 of 3	0 of 4	2 of 57
Contrimoxazole	1 of 26	1 of 15	0 of 12	0 of 3	0 of 4	2 of 57
Total types of antibiotics used	30	10	14	3	8	43

**Data entered as the number of banks using that specific antibiotic out of the total number of banks using antibiotics.*

Analysis

When performing microbial testing of processed donor allografts, each of the surveyed tissue banks: skin (n=13), cardiovascular (n=26) and bone (n=16) in Canada, the U.S., Australia and Europe test for bacteria and fungi. Seven reporting tissue banks test specifically for mycobacteria, including *M. tuberculosis*.

Of the 47 tissue banks who completed the question about mycobacteria testing, 25% (n=12) reported performing testing for mycobacteria: 36% (n=3) of tissue banks providing cardiovascular allografts, 54% (n=6) of tissue banks providing cryopreserved skin, and 15% (n=3) of tissue banks providing bone allografts. None of the four tissue banks providing fresh refrigerated skin test for mycobacteria.

The survey demonstrated that an anti-tuberculosis antibiotic, streptomycin, is used in only 10% (n=8) of 57 tissue banks that use antibiotics during processing. Table 3 lists the antibiotics used by 57 tissue banks that were surveyed: 26 tissue banks providing cardiovascular tissue, 13 providing bone, 12 providing cryopreserved skin, three providing fresh refrigerated skin and four providing acellular dermis.

Tables 2 and 3 show that two of twelve (10%) tissue banks that provide cryopreserved donor skin, one of three (33%) providing fresh refrigerated skin, two of three (66%) providing dermis, and three of 26 (11%) providing cardiovascular tissue, use the anti-tuberculosis antibiotic, streptomycin, during processing.

Conclusions and Key Learning Points

1. 100% (n=2) of U.S. tissue banks providing donor cardiovascular allografts test for mycobacteria as compared to 0% of Canadian tissue banks.

2. 25% (n=2) of Canadian tissue banks providing donor bone allografts test for mycobacteria as compared to 0% of U.S. tissue banks.

3. Testing for mycobacteria is not common practice with only 25% (n=12) of 47 reporting tissue banks indicating that they test.

4. The use of the anti-tuberculosis antibiotic, streptomycin, is not common with 11% (n=11) of tissue banks using this particular antibiotic.

5. A critical analysis of tuberculosis and the risk management involved in producing and providing donor tissue allografts could give an evidence base for developing guidance to tissue banks about final allograft testing.

Note: All statements about practices are based on the number of survey respondents, not the absolute number of banks.

Reference

1. Eastlund T. PowerPoint Lecture: Sentinel Events in Tissue Banking & Tissue Transplantation: Why determining donor suitability is so important. AATB Tissue Donor Safety Workshop. McLean, Virginia, US. Jan 16, 2010

Sterility Test Method

Tissue Type:	Bone and connective
Process:	Processing and validation
Sub Process:	Final sterility testing method
Data Source:	Bone Processing and Validation question 18

Scope

This report of tissue bank survey results pertains to testing bioburden elimination following processing of donor bone and tendon allografts. For those tissue banks performing a final sterility test of processed tissues, a negative test is required prior to release for clinical use.

Introduction and Overview

Following tissue processing by a tissue bank, a final sterility test is performed and there must be no growth for the release of the allograft into inventory for potential patient use. In the U.S. the standard test method specifically for sterility testing of drugs, medical devices and tissue allografts is set in the U.S. (USP 71) and European Pharmacopeia and U.S. federal regulations (CFR 610.12). The method includes a 14 day incubation in two different growth promoting media and incubation at two temperatures.

Any growth in the final sterility test, even from a low virulence non-pathogen, is cause to discard the allograft as non-sterile (unless a lab error or a faulty material is proven). The method reliably identifies aerobic and anaerobic bacteria, molds and fungi, and slow growing bacteria.

In contrast to sterility testing, clinical testing at hospitals is less sensitive, often with samples incubated for only a few days and often using automated, non-culture based methods. Clinical hospital based microbial testing is designed to rapidly identify clinically significant microbial pathogens to aid in diagnosing infections in patients.

Results

A *Bone Processing and Validation* survey was sent to nine Canadian tissue banks; eight of the nine tissue banks either fully or partially completed the survey. Six Canadian tissue banks completed question 18.

A *Bone Processing and Validation* survey was sent to eight U.S. tissue banks; five of the eight either fully or partially completed the survey.

A *Bone Processing and Validation* survey was sent to eleven European tissue banks; five of the seven completed the survey.

The *Bone Processing and Validation* survey was completed by one Australian tissue bank.

The table below gives raw survey data/responses pertaining to methods used for microbial sterility testing of bone allografts.

Table 1: *Methods used for final sterility testing of finished bone allografts*

Question	Canada*	U.S.*	Europe*	Australia
For microbial "sterility" testing of final finished bone allografts which type of testing method is being performed?				
Method as described in EP, USP, US CFR with a 14 day incubation involving two growth media and two temperatures	1 of 6	5 of 5	3 of 5	0 of 1
Rapid automated non culture based microbial testing i.e. Bactec	0 of 6	0 of 5	1 of 5	0 of 1
Other validated rapid, non-culture based microbial tests	0 of 6	0 of 5	0 of 5	0 of 1
Performed by a hospital in their clinical microbiology lab by their standard clinical methods	3 of 6	0 of 5	0 of 5	0 of 1
Performed by a hospital but by sterility test method provided by the tissue bank	1 of 6	0 of 5	1 of 5	0 of 1
Performed by independent microbiology lab	1 of 6	0 of 5	0 of 5	0 of 1
Performed by the tissue bank	0 of 6	1 of 5	0 of 5	1 of 1
Other: method as per British Pharmacopoeia	NC	NC	NC	1 of 1
Comment: USP sterility test method used for quarterly bioburden and dose audits	NC	1 of 5	NC	NC
Comment: USP/CFR sterility testing performed on 10% of each batch	NC	1 of 5	NC	NC

**Each entry represents the number of tissue banks selecting the specific question out of the total number of banks answering the specific questions.NC = No "comment" or "other" entered.*

Analysis

100% (n=5) of responding U.S. tissue banks and 60% (n=3) of responding European tissue banks report using the standard sterility test that involves a 14 day incubation, two media types and two different incubation temperatures as compared to 16% (n=1) of the reporting Canadian tissue banks. 50% (n=3) of the responding Canadian tissue banks reported they send samples from processed bone for microbial testing at a hospital microbiology lab using their clinical methods, the same methods used for non-cadaveric patient samples, as compared to 0% of U.S. and 10% of European banks reporting. One Canadian tissue bank sends samples to an independent microbiology lab; however, type of sterility test method is not specified. The Australian tissue bank performs the microbial testing themselves using the British Pharmacopoeia method.

Conclusions and Key Learning Points

1. The final sterility test method of five U.S. and three European tissue banks is uniform. 100% of the reporting U.S. and 60% of reporting European tissue banks employ sensitive standard sterility test methods used specifically for sterility testing.

2. Canadian practice varies between programs and from that of the U.S. and Europe. Only 33% (n=2) of reporting Canadian tissue banks reported that their final sterility testing employed sensitive standard sterility test methods used specifically for sterility testing.

3. 50% (n=3) of Canadian tissue banks reported sending samples from finished bone allografts to hospital microbiology labs using less sensitive rapid clinical methods designed for non-cadaveric patient samples and identifying clinically significant organisms.

Note: All statements about practices are based on the number of survey respondents, not the absolute number of banks.

Validation of the Overall Bone and Connective Tissue Bioburden Elimination Process

Tissue Type:	Bone and connective
Process:	Bone processing and validation
Sub Process:	Validation of bone and connective tissue bioburden reduction processes
Data Source:	Bone Processing and Validation survey questions numbered 22-38

Scope

This is a report pertaining to validation of the overall cleaning, disinfecting and sterilization of donor bone allografts which is designed to eliminate viable microbes. It includes survey responses of tissue banks with extensive bone cleaning, disinfection and sterilization steps compared to those with less extensive processes.

Introduction and Overview

Although deceased tissue donors have been screened and may have no evidence of being clinically infected at the time of death, the tissue donated after death often acquires postmortem contamination by bacteria and fungi. Tissue banks report that four to 53% of donated bone, cartilage and tendon tissues recovered from deceased tissue donors are contaminated by bacteria or fungi [1-5].

Donor bone and connective tissue allografts commonly undergo a variety of bioburden reduction processing steps depending on the individual tissue bank. Processes range between some tissue banks that perform minimal processing with or without a terminal sterilization step and others that employ extensive cleaning and disinfection steps but do not apply a sterilization step. Lastly, other tissue banks process bone extensively; cleaning and decontaminating with detergents, peroxide, alcohol and antibiotics followed by a terminal sterilizing treatment of the allograft resulting in a sterility assurance level (SAL) of 10^{-6}.

This environmental scan addresses whether tissue banks have performed validation studies to demonstrate that their bioburden reduction process achieves expected results. Questions were asked of tissue banks not to evaluate how thorough their validation study was but merely to detect evidence to support that their overall bioburden reduction process was validated. These questions included such topics as: inoculation of unprocessed tissue, bacteria inoculum representing the various types of bacteria (aerobic, spore forming etc.), quantitation of bioburden before and after processing, log kill during several time points (individual processing steps), the use of worst case settings and SAL selected and achieved.

Results

A *Bone Processing and Validation* survey was sent to nine Canadian tissue banks; eight of the nine returned the survey either complete or partially complete.

A *Bone Processing and Validation* survey was sent to four U.S. tissue banks; all four returned the survey either complete or partially complete. Two additional U.S. tissue banks provided answers to several questions, including some questions pertaining to their validation of bone processing; however, they did not complete the entire survey. Answers were entered into the survey based on personal communication and recent documents provided by these two tissue banks including package inserts, pamphlets describing processing, bioburden reduction capacity, log kill, SALs and scientific publications [6-10].

A *Bone Processing and Validation* survey was sent to eleven European tissue banks; six returned the survey either complete or partially complete.

The *Bone Processing and Validation* survey was completed by one Australian tissue bank.

Table 1: *Validation of overall bone bioburden reduction and elimination process*

Question	Canada*	U.S.*	Europe*	Australia
Have you performed validation studies of your overall bioburden reduction process?				
Yes, for traditional bone allograft processing	5 of 8	5 of 6	3 of 6	0 of 1
Yes, for some but not all bone	0 of 8	1 of 6	2 of 6	0 of 1
Yes, for tendon and ligament processing	3 of 8	2 of 6	1 of 6	0 of 1
Yes, for demineralized bone products	0 of 8	0 of 6	1 of 6	0 of 1
No	2 of 8	0 of 6	1 of 6	1 of 1
Other: Bioburden tested before each batch sterilized	0 of 8	0 of 6	1 of 6	0 of 1
During your validation studies, did you inoculate incoming unprocessed bone with bacteria?				
Yes	2 of 3	2 of 4	2 of 5	1 of 1
No	1 of 3	2 of 4	3 of 5	0 of 1

**Each entry represents the number of tissue banks selecting the specific question out of the total number of banks answering the specific questions.*

Question	Canada*	U.S.*	Europe*	Australia
Please indicate the microbes used for inoculation?				
Staph epidermidis	0 of 2	0 of 2	2 of 3	0 of 1
Staph aureus	1 of 2	2 of 2	1 of 3	1 of 1
Clostridium sporogenes	1 of 2	0 of 2	0 of 3	1 of 1
Candida albicans	1 of 2	0 of 2	0 of 3	1 of 1
P. acnes	0 of 2	1 of 2	2 of 3	0 of 1
E. faecalis	0 of 2	2 of 2	1 of 3	0 of 1
C. sordellii	0 of 2	1 of 2	1 of 3	0 of 1
C. sporogenes	0 of 2	0 of 2	2 of 2	0 of 1
B. subtilis	0 of 2	2 of 2	0 of 3	1 of 1
Aspergillis niger	0 of 2	1 of 2	0 of 3	1 of 1
Other: E. coli	0 of 2	1 of 2	0 of 3	0 of 1
Other: P. aeruginosa	0 of 2	1 of 2	0 of 3	0 of 1
Was your bone processing validation study performed using bacteria recovered from your facility?				
Yes	0 of 3	2 of 4	2 of 5	0 of 1
No	3 of 3	2 of 4	3 of 5	1 of 1
As part of your bone processing validation studies, did you perform microbial/sterility testing of the final bone allograft?				
Yes, qualitative results; genus etc.	1 of 2	2 of 3	1 of 5	1 of 1
Yes, quantitative results	0 of 2	3 of 3	0 of 5	0 of 1
Both qualitative and quantitative	1 of 2	1 of 3	4 of 5	0 of 1
No	0 of 2	0 of 3	0 of 5	0 of 1
During your bacterial bioburden reduction validation studies, which of the following bone processing settings did you use?				
Typical full scale settings	1 of 2	1 of 3	4 of 5	1 of 1
Half scale settings	0 of 2	0 of 3	0 of 5	0 of 1
Worst case scenario	1 of 2	3 of 3**	2 of 5	0 of 1
Did you perform bacterial log reduction studies of individual bone processing steps?				
Yes	1 of 3	2 of 3	4 of 5	0 of 1
No	2 of 3	1 of 3	1 of 5	1 of 1
Upon completion of the bone processing validation did you calculate the log reduction capability?				
Yes	0 of 1	1 of 1	4 of 5	0 of 1
No	1 of 1	0 of 1	1 of 5	1 of 1

Question	Canada*	U.S.*	Europe*	Australia
What is the overall bacterial log reduction capacity of your validated bone cleaning decontamination process?				
0 to 3 log reduction	NA	0 of 2	0 of 4	0 of 1
4-6 log reduction	NA	1 of 2	1 of 4	0 of 1
7-10 log reduction	NA	0 of 2	2 of 4	0 of 1
11-19 log reduction	NA	0 of 2	0 of 4	0 of 1
>19 log reduction	NA	1 of 2	1 of 4	0 of 1
Other: 9.5 to 19.3 log reduction	NA	1 of 2	0 of 4	0 of 1
n/a	NA	0 of 2	0 of 4	1 of 1
What best describes your overall bacterial sterility assurance level achieved by your bone processing?				
We did not calculate a sterility assurance level	2 of 3	0 of 3	1 of 5	0 of 1
10^{-6} or better	0 of 3	2 of 3	1 of 5	0 of 1
Between 10^{-6} and 10^{-10}	1 of 3	0 of 3	1 of 5	0 of 1
10^{-10} or better	0 of 3	1 of 3	0 of 5	0 of 1
Unknown	NA	NA	0 of 5	NA
Other: 10^{-6}	NA	NA	1 of 5	NA
Other: don't claim sterility	NA	NA	NA	1 of 1
Have you established a periodic re-validation for your bone processing?				
Yes	1 of 3	1 of 1	3 of 5	0 of 1
No	2 of 3	0 of 1	2 of 5	1 of 1
Other: When radiation rods are replaced	1 of 3	0 of 1	0 of 5	NA
How often does this occur?				
Annually	NA	NA	1 of 2	NA
Every two years	NA	NA	1 of 2	NA
Did you inoculate incoming unprocessed bone with a virus(es)?				
Yes	0 of 3	2 of 3	2 of 6	0 of 1
No	3 of 3	1 of 3	4 of 6	1 of 1
Please indicate the viruses used?				
HIV	NA	2 of 2	1 of 1	NA
HAV	NA	2 of 2	1 of 1	NA
Bovine Diarrhea Virus (HCV substitute)	NA	2 of 2	1 of 1	NA
Porcine Parvovirus	NA	2 of 2	0 of 1	NA
PrV (HHV substitute)	NA	2 of 2	0 of 1	NA
PV-1	NA	1 of 2	0 of 1	NA
During your viral validation studies, what type of settings did you use?				
Typical full scale	NA	0 of 2	1 of 2	NA
Half scale	NA	0 of 2	0 of 2	NA
Worst case scenario (shortest time, low concentration)	NA	2 of 2	1 of 2	NA

Question	Canada*	U.S.*	Europe*	Australia
Did you perform viral log reduction studies of individual bone processing steps?				
Yes	NA	2 of 2	2 of 4	0 of 1
No	NA	0 of 2	2 of 4	1 of 1
What is your overall viral log reduction achieved?				
<3 log reduction	NA	0 of 2	0 of 2	NA
3	NA	0 of 2	0 of 2	NA
4-5 log reduction	NA	1 of 2	0 of 2	NA
6	NA	0 of 2	0 of 2	NA
7-9 log reduction	NA	0 of 2	1 of 2	NA
10-13 log reduction	NA	1 of 2	0 of 2	NA
≥14 log reduction	NA	0 of 2	1 of 2	NA
Total log reduction not calculated	NA	0 of 2	0 of 2	NA
What best describes your overall *viral* sterility assurance level achieved?				
We did not calculate a viral SAL	NA	0 of 2	0 of 3	NA
10^{-3} log reduction	NA	0 of 2	0 of 3	NA
10^{-3} to 10^{-6}	NA	0 of 2	0 of 3	NA
10^{-6}	NA	2 of 2	0 of 3	NA
10^{-6} to 10^{-10}	NA	0 of 2	1 of 3	NA
10^{-10}	NA	0 of 2	0 of 3	NA
10^{-10} to 10^{-14}	NA	0 of 2	0 of 3	NA
10^{-14} or better	NA	0 of 2	1 of 3	NA
Unknown	NA	0 of 2	1 of 3	NA

**Data entries are the number of tissue banks selecting that specific question out of the number of banks answering the question.*

***One tissue bank reported validation studies at both full scale settings and at worst case scenario settings*

NA = No Answer

Analysis

63% (n=5) of Canadian tissue banks surveyed reported performing studies to validate their overall donor bone bioburden reduction and elimination process as compared to 83% (n=5) of U.S. tissue banks and 83% (n=5) European tissue banks.

At least one of the U.S. tissue banks explained that their validation study was performed, not by themselves, but by the tissue bank from whom they obtained a licensed, validated processing technology.

As a whole, fewer Canadian, U.S., and European tissue banks reported validating their overall tendon bioburden reduction processing i.e. 30% (6 of 20) than their bone processing i.e. 65% (13 of 20).

Despite reporting to have performed validation studies, very few of the tissue banks answered the subsequent questions pertaining to the details of their validation studies. Several U.S. tissue banks use a proprietary, licensed, fully validated technology from another tissue bank and may not have the detailed validation information available. 67% (n=4) of U.S. reporting tissue banks use the proprietary AlloWash® processing technology. Canadian tissue banks do not use a licensed proprietary bone processing technology validated by an outside provider.

Bacterial Bioburden Reduction Validation

67% (n=2) of responding Canadian tissue banks and 50% (n=2) of responding U.S. tissue banks reported inoculating unprocessed bone with microbes as part of their validation study. Other than the insufficient inclusion of fungi, the selections of bacteria for their inoculum represent the various types of bacteria (aerobic, anaerobic, spore formers, gram positive and negative). Alternatively, 33% (n=1) of Canadian tissue banks, 50% (n=2) of U.S. tissue banks and 30% (n=3) of the European tissue banks who answered the question reported that they did not inoculate unprocessed donor bone with bacteria in their validation studies.

Of eleven Canadian, U.S., Australian and European tissue banks reporting their processing settings that were used during bacterial validation studies, 54% (n=6) reported using worst case scenario settings, which is appropriate for a proper validation study.

50% (n=1) of reporting Canadian tissue banks, 100% (n=3) of reporting U.S tissue banks and 80% (n=4) of reporting European tissue banks used post-processing quantitative microbial testing of the final donor bone allograft as part of their bacterial bioburden reduction process validation studies. The Australian reporting tissue bank uses qualitative microbial testing of the final donor bone allograft as part of their bacterial bioburden reduction process validation studies.

33% (n=1) of reporting Canadian tissue banks, 67% (n=2) of reporting U.S. tissue banks and 80% (n=4) of reporting European tissue banks documented log reductions of bacterial bioburden at each step of bone processing e.g. detergent, peroxide, antibiotic exposures.

33% (n=1) of reporting Canadian tissue banks, 100% (n=3) of reporting U.S. tissue banks and 40% (n=2) reporting European tissue banks reported attaining a bacterial SAL of 10^{-6} or better during their validation studies. 67% (n=2) of responding Canadian tissue banks reported that they did not calculate a SAL for processed bone allografts.

A periodic re-validation of bone bioburden reduction processing was established by 33% (n=1) of reporting Canadian tissue banks, 100% (n=1) of reporting U.S. tissue banks and 60% (n=3) of reporting European tissue banks.

Viral Bioburden Reduction Validation Studies

80% (n=4) of U.S. and European tissue banks answering question 34 reported inoculating bone with viruses as part of their bone processing validation studies. 0% of responding Canadian tissue banks reporting inoculated donor bone with viruses in their validation studies.

Of four tissue banks (two U.S. tissue banks and two European tissue banks) reporting the processing settings that were used during viral validation; 75% (n=3) reported using worst case scenario settings, which is appropriate for a proper validation study.

Of the four tissue banks reporting *viral* SALs (two U.S. tissue banks, and two European tissue banks) each reported attaining a *viral* SAL of 10^{-6} or better.

Conclusions and Key Learning Points

1. Many of the validation survey questions were not answered by Canadian tissue banks. Despite reporting having performed validation studies, very few of the reporting tissue banks answered the subsequent questions pertaining to the details of their validation studies.

2. 25% (n=2) of Canadian tissue banks did not validate their bioburden reduction processes as compared to 100% of U.S. tissue banks and 60% European tissue banks who all validated their processes.

3. 50% (n=1) of Canadian tissue banks reported using post-processing quantitative microbial testing of the final donor bone allograft as part of their bacterial bioburden reduction process validation studies as compared to 100% of U.S. (n=3) and 80% (n=4) of European tissue banks.

4. 66% (n=2) of Canadian tissue banks reported they did not calculate a sterility assurance level as compared to 33% of U.S. tissue banks and 10% of European tissue banks, all of which calculated sterility assurance levels.

5. 0% of Canadian tissue banks reported that they assessed viral bioburden reduction as part of their validation as compared to 66% of U.S. tissue banks (n=2) and 33% of European tissue banks (n=2) reporting.

6. Separate validation studies of the overall bioburden reduction process for tendon processing were reported by only 30% (6 of 20) of Canadian, U.S. and European tissue banks as compared to 65% (13 of 20) reporting validation of the overall bioburden reduction process for bone processing.

Note: All statements about practices are based on the number of survey respondents, not the absolute number of banks.

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Validation of Split Thickness Skin Allograft Microbial Decontamination Process

Tissue Type:	Skin
Process:	Skin processing and validation
Sub Process:	Validation of split thickness skin allograft microbial decontamination
Data Source:	Skin processing and validation survey questions numbered 17, 19-25 (cryopreserved) and 35-47 (refrigerated)

Scope

This report addresses whether skin banks in Canada and the U.S. have conducted validation studies of their decontamination and disinfection procedures designed to reduce and remove viable microbes from skin allografts during short term refrigerated storage. This report pertains to decontamination of both cryopreserved and fresh refrigerated skin as processed in Canada and the U.S. Skin stored at refrigerator temperatures in 85% glycerol, as practiced in Europe, is not in scope of this report.

Introduction and Overview

Although deceased tissue donors have been screened and may have no evidence of being clinically infected at the time of death, the tissue donated after death often acquires postmortem contamination by bacteria and fungi. Tissue banks report that 15.6% to 26.5% of donated skin recovered from deceased tissue donors is contaminated by bacteria[1-3] and 5.4% by fungi[1].

Because of this, tissue banks in Canada and the U.S. add antibiotics to the skin and storage solution for microbial growth suppression and decontamination during short term storage in refrigerated conditions. Although the antibiotic, gentamicin, is used by most Canadian and U.S. tissue banks, the combination of other antibiotics in an antibiotic mixture varies among tissue banks. Cryopreserved donor skin allografts with bacterial contamination have been implicated in causing high fevers and Pseudomonas sepsis[4].

This report addresses whether tissue banks have performed validation studies to demonstrate that their microbial growth suppression and decontamination process attains its expected purpose.

Survey questions were presented, not to evaluate how thorough their validation study was, but to sample some of the elements of their validation study, i.e., inoculation of bacteria onto unprocessed skin, whether the inoculum represents the spectrum of expected contaminants, bioburden quantification before and after processing, log kill during several time points during processing, the use of worst case processing settings and the sterility assurance level (SAL) selected and achieved.

Results

A *Skin Processing and Validation* survey was sent to five Canadian tissue banks that recover, process and distribute donor skin allografts; all five either partially or fully completed the survey. All five tissue banks process cryopreserved skin and one also processes (stores) fresh refrigerated skin. One tissue bank did not complete any questions pertaining to validation of skin bioburden reduction.

A *Skin Processing and Validation* survey was sent to nine U.S. skin banks; eight either partially or fully completed the survey. Of these eight tissue banks three process only dermis allografts, five process split-thickness cryopreserved skin, and two process fresh refrigerated skin. Many survey questions were left unanswered.

A *Skin Processing and Validation* survey was sent to four European skin processing tissue banks; one survey was returned but almost all questions were unanswered because the survey addressed skin processing by cryopreservation and by refrigerated storage in antibiotic solutions. Survey questions did not address the practices performed by European tissue banks. The other three European tissue banks did not complete the survey for the same reason.

European skin banks mainly use high concentrations of glycerol, ranging from 50-85%, for its preservative actions during long term refrigerated storage of nonviable skin. The recently reported antimicrobial and anti-viral effects of high concentrations of glycerol during skin storage[5-7] do not represent a decontamination process and have not required validation.

The *Skin Processing and Validation* survey was completed by one Australian tissue bank.

Table 1: *Survey data pertaining to validation studies of the process to decontaminate cryopreserved and "fresh" refrigerated skin at Canadian and U.S. skin banks*

Question	Canada	U.S.	Europe	Australia
Cryopreserved Skin				
Have you performed validation studies of your overall bioburden reduction process for cryopreserved skin?				
Yes	1 of 4	4 of 5	0 of 1	0 of 1
No	3 of 4	1 of 5	1 of 1	1 of 1
Have you performed quantitative microbial bioburden studies of incoming unprocessed split thickness skin which will be cryopreserved?				
Yes	0 of 4	3 of 4	0 of 1	0 of 1
No	4 of 4	1 of 4	1 of 1	1 of 1

Question	Canada	U.S.	Australia
During your validation studies of your overall bioburden reduction process cryopreserved skin, did you inoculate skin with bacteria?			
Yes	1 of 2	2 of 3	0 of 1
No	1 of 2	1 of 3	1 of 1
Please indicate the microbes used for inoculation?			
Streptococcus faecium	0 of 1	0 of 1	0 of 1
Proprionibacterium	0 of 1	0 of 1	0 of 1
Enterococcus faecalis	0 of 1	0 of 1	0 of 1
Clostridium sordellii	0 of 1	1* of 1	0 of 1
Staph epidermidis	1 of 1	0 of 1	0 of 1
Staph aureus	1 of 1	1* of 1	1 of 1
Bacillus subtills	0 of 1	0 of 1	1 of 1
Bacillus pumulus	0 of 1	0 of 1	0 of 1
Was your skin processing validation study performed using bacteria recovered from your facility?			
Yes	0 of 2	1 of 3	0 of 1
No	2 of 2	2 of 3	1 of 1
During your validation studies, which processing setting did you use?			
Typical full scale setting	0 of 1	1 of 1	1 of 1
Half cycle settings (e.g. Half of the normal contact times)	0 of 1	0 of 1	0 of 1
Worst case scenario (e.g. shorten times of exposure to antibiotics, lowest concentrations, etc.)	1 of 1	0 of 1	0 of 1
Did you perform bacterial log reduction studies?			
Yes	1 of 2	2 of 3	0 of 1
No	1 of 2	1 of 3	1 of 1
What is the overall bacterial log reduction capacity of your cryopreserved skin antibiotic decontamination process?			
0-3 log reduction	NA	1 of 1	NA
4-6 log reduction	NA	0 of 1	NA
“Fresh” Refrigerated Skin			
Have you performed validation studies of your overall bioburden reduction process for “fresh” refrigerated split thickness skin?			
Yes	0 of 1	0 of 1	0 of 1
No	1 of 1	1 of 1	1 of 1

Each entry represents the number of tissue banks selecting the specific question out of the total number of banks answering the specific questions.

**Both entries are from same bank*

No responses from Europe

Analysis

25% (n=1) of responding Canadian tissue banks reported performing studies to validate their overall skin decontamination process for cryopreserved skin. This tissue bank also reported

inoculating skin with two bacteria, conducting the validation study using worse case processing settings and calculating overall log reduction capacity. 75% (n=3) of responding Canadian skin banks reported they have not conducted a validation study of the overall decontaminating process.

In contrast to Canadian banks, 80% (n=4) of responding U.S. tissue banks reported conducting validation studies of their decontamination process of cryopreserved skin. However, the U.S banks, like the Canadian banks, reported little to none of the details of the validation studies. Two reported inoculating skin with bacteria and conducting log reduction studies (one attained a bacterial log reduction of between 0 and three logs). The single U.S. tissue bank answering the question reported “typical full scale settings” as its validation study processing settings (worst case settings are more rigorous).

The type and number of bacteria selected for the inoculum by the Canadian and the U.S. skin banks did not represent the spectrum used in a typical validation study.

Neither the Canadian or U.S. responding tissue banks that process fresh refrigerated skin have performed validation studies of their antibiotic decontamination process.

It should be noted that inconsistencies exist in some data as some tissue banks reported they did not perform validation studies but then proceeded to answer some specific validation related questions.

Conclusions and Key Learning Points

1. The validation of decontamination processes for cryopreserved skin occurs much more frequently in U.S. banks than in Canadian banks.

2. 75% (n=3) of the Canadian skin banks reported they did not validate their decontamination process for cryopreserved skin.

3. 80% (n=4) of the U.S. skin banks reported they did validate their decontamination process for cryopreserved skin.

4. None of the Canadian or U.S. skin banks reported validating their fresh refrigerated skin decontamination process.

Note: All statements about practices are based on the number of survey respondents, not the absolute number of banks.

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Validation of the Overall Heart Valve Bioburden Reduction Process

Tissue Type:	Cardiovascular
Process:	Tissue processing
Sub Process:	Validation of the overall heart valve bioburden reduction process
Data Source:	Cardiac Processing and Validation survey questions 14 and 18-30

Scope

This is a report pertaining to validation studies of decontamination and disinfection procedures designed to eliminate viable microbes from donor heart valve allografts conducted by tissue banks in Canada, the U.S. and Europe.

Introduction and Overview

Although deceased tissue donors have been screened and may have no evidence of being clinically infected at the time of death, the tissue donated after death often acquires postmortem microbial contamination. Tissue banks report that 10% to 27% of donated hearts and heart valves recovered from tissue donors are contaminated by bacteria[1,2] and 1% to 3% by fungi[1,3].

Due to this postmortem contamination, donor heart valve allografts undergo an antibiotic decontamination step. The composition and concentration of the antibiotic mixture and the temperature and duration of incubation varies among tissue banks. This environmental scan addresses whether banks have performed validation studies which demonstrate that their decontamination processes achieve expected results.

Survey questions were intended not to evaluate how thorough a validation study was but to sample some of the elements of the validation study; for example, inoculation of bacteria and viruses into unprocessed valves, whether the inoculum represents the spectrum of expected contaminants, bioburden quantification before and after processing, log kill during variable time points during processing, use of worst case settings, sterility assurance level (SAL) targeted and attained, etc.

Results

A *Cardiovascular Processing and Validation* survey was sent to four Canadian tissue banks that recover, process and distribute donor cardiovascular allografts. Each of the four banks returned partially completed surveys.

Two U.S. tissue banks conducted decontamination validation studies but provided little to no information about study details. Answers were entered into the survey based on personal communication.

Of the six European tissue banks sent surveys, three partially completed the survey were completed.

The *Cardiovascular Processing and Validation* survey was completed by one Australian tissue bank.

Table 1: *Validation of heart valve decontamination process*

Question	Canada*	U.S.*	Europe*	Australia
Have you performed validation studies of your overall heart valve bacterial bioburden reduction process?				
Yes	3 of 4	2 of 2	0 of 3	0 of 1
No	1 of 4	0 of 2	3** of 3	1 of 1
Have you performed quantitative bacterial bioburden studies of incoming unprocessed heart or heart valve tissues?				
Yes	1 of 4	NA	0 of 3	0 of 1
No	3 of 4	NA	3 of 3	1 of 1
During validation studies of your overall bioburden reduction process did you inoculate incoming unprocessed heart valve tissue with bacteria?				
Yes	2 of 3	1 of 1	1 of 1	0 of 1
No	1 of 3	0 of 1	0 of 1	0 of 1
Other: inoculate the filter for the transport solution (includes antibiotic)	NA	NA	NA	1 of 1
What microbes were used for inoculation?				
Proprionbacterium acnes	1 of 2	NA	1 of 1	0 of 1
Staph epidermidis	1 of 2	NA	NA	0 of 1
Streptococcus faecium	NA	NA	NA	0 of 1
Enterococcus faecalis	NA	NA	NA	0 of 1
Clostridium sordellii	NA	NA	1 of 1	0 of 1
Staph aureus	1 of 2	NA	1 of 1	1 of 1
Bacillus subtilis	1 of 2	NA	1 of 2	1 of 1
Other: C. albicans	1 of 2	NA	NA	0 of 1
Other: E. coli	1 of 2	NA	NA	0 of 1
Other: Bacillus cereus	1 of 2	NA	NA	0 of 1
Other: Penicillium	1 of 2	NA	NA	0 of 1
Other: Lacobacillus	1 of 2	NA	NA	0 of 1
Other: Streptococcus agalactiae	1 of 2	NA	NA	0 of 1
Was your validation study performed using bacteria recovered from your facility?				
Yes	1 of 2	NA	0 of 2	0 of 1
No	1 of 2	NA	2 of 2	0 of 1
Other: in house only Micrococcus and	NA	NA	NA	1 of 1

Diptheroids spp				
As part of processing validation studies, did you perform microbial/sterility testing of the finished heart valve allograft?				
Yes, qualitative results, genus, etc.	3 of 3	NA	1 of 2	0 of 1
Yes, quantitative bioburden testing results	1 of 3	NA	0 of 2	0 of 1
Yes, both of the above	1 of 3	NA	0 of 2	0 of 1
No	0 of 3	NA	1 of 2	1 to 1

Question	Canada*	U.S.*	Europe*	Australia
During your validation studies, which heart valve processing setting did you use?				
Typical full scale settings	2 of 3	NA	NA	0 of 1
Half cycle settings	0 of 3	NA	NA	0 of 1
Worst case scenario	1 of 3	NA	NA	0 of 1
Other: don't claim bioburden redulation	NA	NA	NA	1 of 1
Did you calculate the log reduction capability of the overall process?				
Yes	1 of 3	NA	0 of 2	0 of 1
No	2 of 3	NA	2 of 2	1 of 1
What is your overall bacterial log reduction capacity of your validated heart valve cleaning, decontamination process?				
We haven't calculated an overall log reduction	0 of 1	NA	NA	1 of 1
0 to 3 log reduction	1 of 1	NA	NA	0 of 1
4 to 6 log reduction	0 of 1	NA	NA	0 of 1
What is your overall bacterial sterility assurance level achieved by your heart valve processing?				
We have not calculated a sterility assurance level	2 of 3	NA	2 of 2	1 of 1
Between 10^{-2} and $10^{-2.9}$	0 of 3	NA	NA	0 of 1
Between 10^{-3} and 10^{-4}	1 of 3	NA	NA	0 of 1
Between 10^{-4} and 10^{-5}	0 of 3	NA	NA	0 of 1
10^{-6}	0 of 3	NA	NA	0 of 1
Have you established a periodic revalidation plan for heart valve processing?				
Yes	1 of 3	NA	1 of 2	0 of 1
No	2 of 3	NA	1 of 2	1 of 1
How often does this occur?				
Twice a year	NA	NA	0 of 1	NA
Annually	NA	NA	1 of 1	NA
Every two years	NA	NA	0 of 1	NA

**Each entry represents the number of tissue banks selecting the specific question out of the total number of banks answering the specific questions.*
*** Internal inconsistency: reported not to have performed validation study but subsequent answers report a validation step taken (inoculating bacteria)*
NA = No answer

Analysis

75% (n=3) of Canadian and 100% (n=2) of U.S. responding tissue banks reported performing studies to validate their overall donor heart valve decontamination process. Of the three Canadian tissue banks conducting validation studies: only one reported quantifying incoming bioburden, two of three reported inoculating unprocessed tissue with bacteria, one reported appropriate use of worst case scenario decontamination settings, one calculated bioburden log reduction and one calculated a SAL of 10^{-3}.

Despite five of six North American tissue banks reporting that they performed validation studies, very few of the tissue banks answered the subsequent questions numbered 23-28 pertaining to the details of their validation studies. Of the five, only four answered the question about inoculating bacteria into the tissue as part of the decontamination validation study (three of the four inoculated bacteria: two Canadian banks and one U.S. bank).

A periodic re-validation of their donor heart valve decontamination process was established by one of three responding Canadian tissue banks.

The responding European tissue banks reported they did not conduct a validation study but one reported inoculating tissue with bacteria (a common validation study component).

Conclusions and Key Learning Points

1. 75% (n=3) of responding Canadian tissue banks reported that their heart valve decontamination process was validated as compared to 100% (n=2) of responding U.S. tissue banks.

2. 66% (n=2) of Canadian tissue banks reported their corresponding validations did not report a SAL.

3. 66% (n=2) of Canadian tissue banks reported their corresponding validations did not use worst case settings.

4. Of the 3 Canadian tissue banks reporting validations, only one reported calculating a log reduction.

5. The number of European tissue banks reporting and the number of questions answered was so small the results are not informative or representative.

Note: All statements about practices are based on the number of survey respondents, not the absolute number of banks.

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Validation Studies for Tissue Bank Processes Other than the Overall Bioburden Reduction Process

Tissue Type:	Bone, connective, skin and cardiovascular
Process:	Tissue processing and validation
Sub Process:	Validation studies for processes other than the overall bioburden reduction process
Data Source:	Bone Processing and Validation survey questions 40-41, Cardiovascular Processing and Validation survey questions 31-32, Skin Processing and Validation survey questions 26-27, 48-49, and 77-78

Scope

This is a report of survey results pertaining to the validation studies performed by tissue banks that process donor bone, connective, skin and cardiovascular tissue in Canada, the U.S. and, to a limited degree, Europe. This report addresses validation studies performed in areas other than the overall donor tissue bioburden reduction process.

Introduction and Overview

A validation study demonstrates that a procedure reliably results in the desired outcome. Validation shows that the procedure or process is effective, i.e., that you have established by objective evidence that a process described in written standard operating procedures consistently produces a result or product meeting its predetermined specifications.

Tissue banks commonly perform validation studies for critical procedures, methods and processes during tissue recovery, processing, disinfecting and sterilizing, storage and transport. The U.S. Food and Drug Administration (FDA) recommend validating critical processes and have issued guidance. The American Association of Tissue Banks (AATB) is also currently in the process of developing a comprehensive validation guidance document.

This scan provides a listing of reported validation studies, not an analysis of their design, thoroughness or adequacy of the studies. The scan will show what different processes have been validated by responding tissue banks and gives an indication as to the level of control the tissue banks have over their processes.

Tables 1-5 depict survey data pertaining to validation studies reported by Canadian, U.S. and European tissue banks that process donor bone, connective, skin and cardiovascular tissue as well as Canadian and U.S. tissue banks who reported not performing any validation studies.

Results

Validation studies performed by tissue banks that process donor bone and connective tissue

A *Bone Processing and Validation* survey was sent to nine Canadian tissue banks; eight of the nine returned the survey either fully or partially completed.

A *Bone Processing and Validation* survey was sent to four U.S. tissue banks; all four either fully or partially completed the survey. Two additional large U.S. tissue banks provided answers to several questions, including questions about their validation of donor bone processing. They did not complete the *Bone Processing and Validation* survey however their results to specific questions were entered manually into the database.

A *Bone Processing and Validation* survey was sent to eleven European tissue banks; six returned the survey either fully or partially complete.

A *Bone Processing and Validation* survey was sent and returned to an Australian tissue bank, however, the results may not be representative of practices in the Australian tissue community.

Validation studies performed by tissue banks that process cardiovascular tissue

A *Cardiovascular Processing and Validation* survey was sent to four Canadian tissue banks that perform recovery, processing and distribution of donor cardiovascular tissue; all four either fully or partially completed the survey.

A *Cardiovascular Processing and Validation* survey was sent to two U.S. tissue banks that perform recovery, processing and distribution of donor cardiovascular tissue; neither bank completed the survey, however, many survey questions were answered by the tissue banks through personal communication and the answers were entered manually into the survey database[1,2]. Neither answered questions pertaining to the various types of validations performed.

A *Cardiovascular Processing and Validation* survey was sent to four European tissue banks that process donor cardiovascular tissue; two returned the completed surveys by email and the data was entered into the database. One of the two did not answer questions pertaining to “other” validation studies.

Validation studies performed by tissue banks that perform skin processing

A *Skin Processing and Validation* survey was sent to five Canadian tissue banks that perform recovery, processing and distribution of donor skin allografts; all five either fully or partially completed the survey. All five banks process cryopreserved donor skin and one also processes fresh refrigerated donor skin.

A *Skin Processing and Validation* survey was sent to nine U.S. tissue banks; eight either fully or partially completed the survey. Of these, four process donor dermis allografts, five process donor split-thickness cryopreserved skin and two process donor fresh refrigerated skin.

A *Skin Processing and Validation* survey was sent to five European tissue banks. One survey was returned but almost all questions were unanswered because the survey addressed donor skin processing by cryopreservation and by refrigerated storage in antibiotic solutions. European tissue banks do not process and store skin in the manner addressed by the survey. European tissue banks mainly use high concentrations (50% to 85%) of glycerol for its disinfectant and preservative actions during long term refrigerated storage of nonviable skin. The other three European tissue banks did not complete the survey for the same reason.

The surveys were completed by one Australian tissue bank.

Table 1: *Bone bank validation studies of processes other than their overall bioburden reduction process*

Question	Canada*	U.S.*	Europe*	Australia
Have you performed other validation studies of bone or connective tissue processing, storage or transport?				
Yes	5 of 8	6 of 6	5 of 6	1 of 1
No	3 of 8	0 of 6	1 of 6	0 of 1
What type(s) of bone tissue validation studies have been performed?				
Cleaning and re-sterilization of processing equipment	3 of 5	5 of 6	3 of 5	0 of 1
In-house sterilizers	3 of 5	5 of 6	2 of 5	1 of 1
Final package/container	4 of 5	6 of 6	5 of 5	1 of 1
Residual antibiotics	0 of 5	2 of 6	2 of 5	0 of 1
Residual lipids	1 of 5	2 of 6	3 of 5	0 of 1
Residual blood cells (hemoglobin)	0 of 5	2 of 6	2 of 5	0 of 1
Residual cells	0 of 5	2 of 6	3 of 5	0 of 1
Residual chemicals such as alcohol	0 of 5	2 of 6	2 of 5	0 of 1
Bacteriostasis or fungistatis studies	0 if 5	0 of 6	2 of 5	1 of 1
Allograft biocompatibility studies	0 of 5	3 of 6	2 of 5	0 of 1
Allograft pyrogen testing	0 of 5	1 of 6	0 of 5	0 of 1
Stability studies to determine storage period and expiration date	3 of 5	5 of 6	4 of 5	0 of 1
Osteoinduction activity of bone	0 of 5	4 of 6	3 of 5	1 of 1
Mechanical testing and physical testing of bone	0 of 5	4 of 6	3 of 5	0 of 1
Penetrance of alcohol, detergent, peroxide or other disinfection chemicals	0 of 5	2 of 6	1 of 5	0 of 1
Allograft shipping containers and transport process	4 of 5	6 of 6	4 of 5	1 of 1
Sterility testing of the final allograft	2 of 5	5 of 6	3 of 5	0 of 1
Dosimetric release after radiation without routine testing of each of the final allografts	1 of 5	5 of 6	1 of 5	1 of 1

Question	Canada*	U.S.*	Europe*	Australia
What type(s) of bone tissue validation studies have been performed?				
Parametric release of allografts after a multistep process not necessarily including radiation	0 of 5	0 of 6	0 of 5	0 of 1
Computer software	1 of 5	6 of 6	0 of 5	1 of 1

**Each entry represents the number of tissue banks selecting the specific question out of the total number of banks answering the specific questions.NA = No Answer*

Table 2: *Cardiovascular tissue bank validation studies of processes other than their overall donor heart valve bioburden reduction process*

Question	Canada*	U.S.*	Europe*	Australia
Have other validation studies been performed to show your processing and methods accomplish their intended purpose?				
Yes	3 of 4	2 of 2	1 of 2	1 of 1
No	1 of 4	0 of 2	1 of 2	0 of 1
What types(s) of validation studies have been performed?				
Validation of cleaning and re-sterilization of processing equipment	3 of 3	NA	0 of 1	1 of 1
In-house sterilizers used for critical equipment and supplies	3 of 3	NA	0 of 1	1 of 1
Final package/container validation	3 of 3	NA	1 of 1	1 of 1
Allograft studies of residual antibiotics	3 of 3	NA	0 of 1	0 of 1
Allograft studies of residual DMSO	3 of 3	NA	0 of 1	0 of 1
Allograft pyrogen testing	0 of 3	NA	0 of 1	0 of 1
Stability studies to determine storage period and expiration date	1 of 3	NA	0 of 1	0 of 1
Mechanical testing and physical testing of heart valves	0 of 3	NA	0 of 1	0 of 1
Allograft shipping containers and transport process	3 of 3	NA	0 of 1	1 of 1
Computer software	3 of 3	NA	0 of 1	1 of 1

**Each entry represents the number of tissue banks selecting the specific question out of the total number of banks answering the specific questions.NA = No Answer*

Table 3: *Tissue bank validation studies of cryopreserved donor skin processes other than their overall bioburden reduction process*

Question	Canada*	U.S.*	Europe*	Australia
Have you performed other validation studies of cryopreserved split thickness skin allografts?				
Yes	3 of 5	3 of 4	NA	1 of 1
No	2 of 5	1 of 4	NA	0 of 1
What other validation studies have been performed to show your cryopreserved skin processing and methods accomplish their intended purpose?				
Validation of cleaning and re-sterilization of processing equipment	1 of 3	0 of 2	NA	1 of 1
In-house sterilizers used for critical equipment and supplies	0 of 3	0 of 2	NA	0 of 1
Allograft studies for residual antibiotics	0 of 3	0 of 2	NA	0 of 1
Allograft residual cryoprotectants such as DMSO or glycerol	0 of 3	0 of 2	NA	0 of 1
Allograft studies of cell viability	0 of 3	1 of 2	NA	0 of 1
Microbiologic testing of the final allograft	1 of 3	1 of 2	NA	0 of 1
Final package/container validation	0 of 3	1 of 2	NA	0 of 1
Stability studies to determine storage period	0 of 3	0 of 2	NA	0 of 1
Skin shipping containers and transport process	0 of 3	1 of 2	NA	0 of 1
Computer software	1 of 3	0 of 2	NA	0 of 1

** *Each entry represents the number of tissue banks selecting the specific question out of the total number of banks answering the specific questions.NA = No Answer*

Table 4: *Tissue bank validation studies of fresh refrigerated skin processes other than their overall bioburden reduction process*

Question	Canada*	U.S.*	Europe*	Australia
Have you performed other validation studies of "fresh" refrigerated split thickness skin allografts?				
Yes	NA**	1 of 1^{+}	NA	1 of 1
No	NA	0 of 1^{+}	NA	0 of 1

Question	Canada*	U.S.*	Europe*	Australia
What other validation studies have been performed to show that "fresh" refrigerated split thickness skin processing accomplishes its intended purpose?				
Validation of cleaning and re-sterilization of processing equipment	0 of 1	2 of 2	NA	1 of 1
In-house sterilizers used for critical equipment and supplies	0 of 1	2 of 2	NA	1 of 1
Allograft studies of cell viability	0 of 1	1 of 2	NA	0 of 1
Microbiologic testing of the final allograft	1 of 1	2 of 2	NA	1 of 1
Final package/container validation	0 of 1	1 of 2	NA	1 of 1
Stability studies to determine storage period	0 of 1	0 of 2	NA	0 of 1
Skin shipping containers and transport process	0 of 1	2 of 2	NA	1 of 1
Computer software	0 of 1	1 of 2	NA	1 of 1

NA = No Answer

Each entry represents the number of tissue banks selecting the specific question out of the total number of banks answering the specific questions. The single Canadian bank processing fresh refrigerated skin skipped this question but in the following question listed its "other" validation study it completed.*

[+] Of the two surveyed U. S. skin banks that process fresh skin, one skipped this question but did report on its "other" validation studies in the next question.

Table 5: *Tissue bank validation studies of* dermis*; processes other than their overall bioburden reduction process*

Question	Canada*	U.S.*	Europe*	Australia
Have you performed other validation studies of your dermis processing or storage?				
Yes	NA	4 of 4	2 of 2	1 of 1
No	NA	0 of 4	0 of 2	0 of 1
What other validation studies have been performed to show your processing and methods accomplish their intended purpose?				
Validation of cleaning and sterilization of processing equipment validation.	NA	2 of 2[+]	1 of 2	1 of 1
In-house sterilizers	NA	0 of 2	0 of 2	1 o f1
Final package/container validation	1 of 2**	2 of 2	2 of 2	1 of 1
Allograft studies of residual antibiotics and chemicals	NA	1 of 2	2 of 2	0 of 1
Allograft studies of residual cells	NA	1 of 2	2 of 2	0 of 1
Allograft studies of residual chemicals such as alcohol, detergents, etc.	NA	1 of 2	2 of 2	0 of 1

Question	Canada*	U.S.*	Europe*	Australia
Allograft biocompatibility studies	NA	0 of 2	2 of 2	0 of 1
Allograft pyrogen testing	NA	0 of 2	1 of 2	0 of 1
Stability studies to determine storage period and expiration date	NA	1 of 2	2 of 2	0 of 1
Mechanical and physical testing	NA	1 of 2	2 of 2	0 of 1
Allograft shipping containers and transport process	2 of 2**	1 of 2	2 of 2	1 of 1
Sterility testing of the final allograft	1 of 2**	1 of 2	1 of 2	0 of 1
Dosimetric release after radiation without routine testing of each final allograft	NA	0 of 2	1 of 2	0 of 1
Parametric release of allografts after a multistep process not necessarily including radiation	NA	0 of 2	0 of 2	0 of 1
Computer software	2 of 2**	1 of 2	0 of 2	1 of 1

**Each entry represents the number of tissue banks selecting the specific question out of the total number of banks answering the specific questions.*

***erroneous entries: these two Canadian tissue banks reported no dermis processing but may have answered these questions because the question did not specify dermis even though it was in a group of 27 dermis processing questions.*

[+]of the four surveyed tissue banks that processed dermis, only two answered the question about the types of validation studies performed.

NA = No Answer

Analysis

Validation studies performed by tissue banks that process donor bone and connective tissue

63% (n=5) of responding Canadian tissue banks reported performing studies to validate various processes other than their overall bone bioburden reduction process as compared to 100% (n=6) of responding U.S. tissue banks, 83% (n=5) of responding European tissue banks, and the Australian tissue bank.

Reporting U.S. tissue banks and European tissue banks and the Australian tissue bank perform validation studies more frequently than reporting Canadian tissue banks in the following areas:

- post-processing bone osteoinduction activity with 67% (n=4) of reporting U.S. tissue banks and 60% (n=3) of European tissue banks and the Australian tissue bank performing studies compared to 0% of reporting Canadian tissue banks
- post-processing bone mechanical properties with 67% (n=4) of reporting U.S. tissue banks and 60% (n=3) of reporting European tissue banks performing compared to 0% of reporting Canadian tissue banks
- penetration of processing reagents such as alcohol, detergents, or peroxide with 67% (n=4) of reporting U.S. tissue banks and 10% (n=1) European tissue banks performing as compared to 0% of reporting Canadian tissue banks

- computer software with 100% (n=6) of reporting U.S. tissue banks performing as compared to 20% (n=1) of reporting Canadian tissue banks and 0% of reporting European tissue banks.

There were no specific tissue bank processes reported where Canadian tissue banks perform validation studies more than U.S. tissue banks.

Validation studies by tissue banks that process cardiovascular tissue

75% (n=3) of Canadian tissue bank respondents reported performing studies to validate various processes and methods other than their overall heart valve bioburden reduction process as compared to100% (n=2) of U.S. tissue bank respondents. The number of European tissue bank respondents was too small for meaningful comparison. The single Canadian tissue bank not conducting any "other" validation studies was also the only Canadian tissue bank that had not conducted a validation study of its overall bioburden reduction process.

The following processes were scientifically validated by all three of the responding Canadian tissue banks performing "other" validation studies:

- cleaning and sterilization of processing equipment
- performance of in-house sterilizers
- final packaging
- residual antibiotics
- residual DMSO
- allograft shipping containers and
- transport process
- computer software

The types of "other" validation studies performed by U.S. tissue banks were not reported.

Validation studies by tissue banks that perform skin processing

Although 60% of reporting Canadian (n=3), 75% of reporting U.S. (n=3) tissue banks and the Australian tissue bank that produce cryopreserved skin reported conducting validation studies of processes other than their overall bioburden reduction process, very few validation studies were reported. A total of three validation studies were reported by the three reporting Canadian tissue banks that perform validation studies. Of the two U.S. tissue bank respondents a total of four validation studies were indicated. Only one validation study was reported by the Australian tissue bank.

Fresh Refrigerated Skin

Only one validation study was reported by the single reporting Canadian skin bank that processes fresh refrigerated skin.

Two U.S. skin banks that were surveyed and produce fresh refrigerated skin reported conducting several validation studies on a range of processes. Both conducted validation studies on the following:

- cleaning and re-sterilizing processing equipment
- function of in-house sterilizers
- microbiologic testing of the final allograft
- shipping containers and transport

The Australian tissue bank that was surveyed and produce fresh refrigerated skin reported conducting several validation studies on a range of processes. They conducted validation studies on the following:

- cleaning and re-sterilizing processing equipment
- function of in-house sterilizers
- microbiologic testing of the final allograft
- shipping containers and transport
- computer software

Dermis

None of the five reporting Canadian tissue banks process human donor dermis allografts or provided answers to questions pertaining to dermis allograft validation studies.

Only two of the reporting U.S. tissue banks that process dermis answered the question about the various types of validation studies they performed. The two reporting U.S. and the one reporting European tissue banks that process dermis and that answered these questions reported validation studies on 12 different processes and methods. The Australian tissue bank performed five different validation studies for dermis processing and methods.

Conclusions and Key Learning Points

1. In tissue banks that process bone, connective tissue, cryopreserved skin and cardiovascular tissue, more Canadian than U.S. tissue banks reported that they did not perform validation studies in areas other than their overall bioburden reduction process.

    a. 37% (n=3) of Canadian tissue banks reported they did not conduct any “other” validation studies in the areas listed as compared to 0% of U.S and 17% (n=1) of European tissue banks who all conducted additional validations.

    b. 25% (n=1) of Canadian tissue banks that process cardiovascular tissue answered that they did not conduct any “other” validation studies in the areas listed as compared to 0% of U.S and 50% (n=1) of European tissue banks who all conduct additional validations.

    c. 20% (n=2) Canadian tissue banks that process skin answered that they have not conducted any “other” validation studies for cryopreserved skin as compared to 25% (n=1) of U.S tissue banks who reported they did not conduct any “other” validation studies.

2. Acellular dermis, a relatively new donor tissue allograft, has been the subject of a wide range of “other” validation studies by the U.S., European, and Australian tissue banks who produce them.

Note: All statements about practices are based on the number of survey respondents, not the absolute number of banks.

References

1. Personal communication with Perry Lang, Vice President, LifeNet Health, November 15, 2013.

2. Personal communication with Greg Ray, MD, CryoLife medical director, Dec 18, 2013.

Appendix A: Bone Processing and Validation Survey Questions

1. Which of the following bone and connective tissues does your facility process?

    - Bone, deceased donor
    - Bone, live donor
    - Demineralized bone products
    - Tendon
    - Ligament
    - Fascia
    - “Fresh” refrigerated osteochondral allograft
    - Cryopreserved osteochondral allograft
    - “Fresh” refrigerated articular cartilage
    - Other cartilage
    - Meniscus
    - Mesenchymal stem cells or other bone forming cells with or
    - without combining with bone or other carrier
    - Dura
    - Amnion
    - Other (please specify)

2. During bone processing, does your facility pool/comingle bone from two or more donors?

    - Yes
    - No

3. During processing of traditional bone allografts (excluding demineralized allograft) which of the following steps and treatments are used to reduce bioburden?

    - Mechanical or chemical processes to remove marrow, cells, fat
    - Alcohol
    - Hydrogen peroxide
    - Detergents such as TritonX,
    - tri(nbutyl)
    - phosphate (TNBP),
    - polyoxyethylensorbitan monooleate (Tween 80), polysorbate80,
    - polysorbate20,
    - Nlauroyl
    - sarconate, or other.
    - Polyoxyethylene/polyethylene glycol
    - Antibiotics
    - Iodophor, e.g., povidoneiodine,
    - betadine
    - Acetone
    - Ether
    - Sodium hydroxide
    - Vapor phase hydrogen peroxide
    - Proprietary methods
    - Other nonproprietary steps, please specify

4. Does your facility use any of the following proprietary bone processing methods?

    - No, none of the following
    - AlloWash
    - AlloWash XG
    - Advanced Tissue Processing (ATP)
    - BioCleanse
    - Clearant
    - Tutoplast
    - NovaSterilis (supercritical CO2)
    - Other (please specify)

5. Does your facility use any of the following types of alcohol during bone processing?

    - No, none of the following
    - Isopropyl alcohol/isopropanol
    - Ethanol
    - Denatured ethanol
    - Methanol
    - Other (please specify)

6. Which of the following type of antibiotics are used for bone processing?

    - None, no antibiotics are used
    - Polymyxin B
    - Gentamicin
    - Bacitracin
    - Penicillin
    - Primaxin (imipenem and cilastatin)
    - Amphotericin B
    - Proprietary antibiotic “cocktail”
    - Other (please specify)

7. During cleaning and rinsing of tissue allografts during processing, what type of water is used (check all that apply)?

    - Water prepared on site
    - Water purchased commercially
    - USP purified water
    - USP water for injection
    - Deionized
    - Distilled
    - Reverse osmosis (RO) water
    - Other (please specify)

8. Which of the following sterilization methods are applied before, during or after some or all of your bone allograft processing (check all that apply)?

    - None of the following
    - Gamma radiation
    - Electron beam radiation
    - Dry heat
    - Ethylene oxide gas
    - NovaSterilis (supercritical CO2)
    - Moist heat/steam (autoclave)
    - Other (please specify)

9. Does your facility apply radiation (gamma or electron beam) to some or all incoming musculoskeletal tissue (bone, cartilage) PRIOR TO PROCESSING?

    - Yes, applied to all
    - Yes, depending on the results of recovery or other preprocessing
    - microbial test results or other indications
    - No
    - Other (please specify)

10. Which of the following types of radiation is used?

    - Gamma radiation
    - Electron beam radiation
    - Other (please specify)

11. What best describes the minimum dose of radiation that is used PRIOR to bone processing?

    - Less than 1.0 MRad (10 kGy)
    - MRad (10 kGy)
    - Between 1.0 and 1.5 MRad (10 and 15 kGy)
    - 1.5 MRad (15 kGy)
    - Between 1.5 and 1.75 MRad (15 and 17.5 kGy)
    - 1.75 MRad (17.5 kGy)
    - Between 1.75 to 2.0 MRad (17.5 and 2.0 kGy)
    - MRad (20 kGY)
    - Between 2.0 and 2.5 MRad (20 and 25 kGy)
    - 2.5 MRad (25 kGy)
    - Greater than 2.5 MRad (25 kGy)

12. Is radiation applied at your facility as a final step, an end point of processing in its final package (terminal sterilization)?

    - Yes, applied to all
    - Yes, depending on the results of preprocessing or in-processing microbial test results or other indications
    - No
    - Other (please specify)

13. What type of radiation is used as a final allograft treatment/terminal sterilization for traditional bone allografts?

    - Gamma radiation
    - Electron beam radiation
    - Other (please specify)

14. What best describes your minimum dose of radiation that is used as a FINAL bone allograft treatment?

    - Less than 1.0 MRad (10 kGy)
    - MRad (10 kGy)
    - Between 1.0 and 1.5 MRad (10 and 15 kGy)
    - 1.5 MRad (15 kGy)
    - Between 1.5 and 1.75 MRad (15 and 17.5 kGy)
    - 1.75 MRad (17.5 kGy)
    - Between 1.75 to 2.0 MRad (17.5 and 2.0 kGy)
    - MRad (20 kGY)
    - Between 2.0 and 2.5 MRad (20 and 25 kGy)
    - 2.5 MRad (25 kGy)
    - Greater than 2.5 MRad (25 kGy)

15. Do you have a validated bone sterilization process that includes radiation and dosimetric release without the need for final sterility testing?

    - Yes
    - No
    - Comments?

16. Do you have a validated bone sterilization process, other than radiation, and parametric release or release with biologic indicators, without the need for final sterility testing?

    - Yes
    - No
    - Comment?

17. What type(s) of microbiologic testing of bone, tendon, or ligament allografts is performed at or near final packaging (end-product testing or final sterility testing) and must have acceptable results before allografts or batches are released for distribution(check all that apply)?

    - Bacterial culturing
    - Fungal/yeast culturing
    - Mycobacterium culturing
    - None, we use a validated radiation process and dosimetric release.
    - None, we use a validated sterilization process, other than radiation, and parametric release or release with biological indicators.
    - Other (please specify)

18. For microbial “sterility" testing of final finished bone allograft, which type of testing method is being performed?

    - Unknown method
    - Method as described in European Pharmacopoeia 2.6.1, US Pharmacopeia, chapter 71 or US Code of Federal Regulations 610.12 with a 14 day incubation involving two growth media and two temperatures.
    - Rapid automated non-culture based microbial testing involving colorimetric detection of CO2 release or ATP bioluminescence, e.g., BacTAlert or BACTEC systems.
    - Other validated rapid, non-culture based microbial tests
    - Performed by a hospital in their clinical microbiology lab by their standard clinical methods.
    - Performed by a hospital but by sterility test methods provided by the tissue bank
    - Performed by independent microbiology lab
    - Performed by tissue bank
    - Other (please specify)

19. Has your facility ever performed bacteriostasis or fungistasis testing of bone allografts after processing?

    - No
    - Yes, during validation studies
    - Yes, periodically
    - Yes, other times
    - Other (please specify)

20. Have you performed quantitative bacterial or fungal bioburden studies of incoming unprocessed bone tissues?

    - Yes
    - No
    - Other (please specify)

21. Has your facility established bacterial bioburden alert or action levels or bioburden limits for unprocessed, incoming bone tissue?

    - Yes
    - No

22. Have you performed validation studies of your overall bioburden reduction process (check all that apply)?

    - Yes, for traditional bone allograft processing
    - For some but not for all bone processing
    - Yes, for tendon and ligament processing
    - Yes, for demineralized bone products
    - Yes, for " fresh" refrigerated osteochondral and cartilage
    - No
    - Other (please specify)

23. During your validation studies of your overall bioburden reduction process, did you inoculate incoming unprocessed bone with bacteria?

    - Yes
    - No
    - Other (please specify)

24. Please indicate the microbes used for inoculation as part of your validation study (check all that apply).

    - Staph epidermitis
    - Staph aureus
    - Proprionibacterium acnes
    - Enterococcus faecalis
    - Clostridium sordellii
    - Clostridium sporogenes
    - Streptococcus faecium
    - Bacillus subtilis
    - Bacillus pumulus
    - Candida albicans
    - Aspergillus
    - Other (please specify)

25. Was your bone processing validation study performed using bacteria recovered from your facility?

    - No
    - Yes

26. As part of your overall bone processing validation studies, did you perform microbial/sterility testing of the finished bone allograft (check all that apply)?

    - Yes, qualitative results, genus etc
    - Yes, quantitative bioburden testing results
    - Both qualitative and quantitative
    - Other (please specify)

27. During your validation studies of your overall bacterial bioburden reduction process, which of the following bone processing settings did you use(check all that apply)?

    - Typical full scale settings
    - Half cycle settings (e.g., half of the normal contact time)
    - Worst case scenario (e.g., shortest times of alcohol, peroxide or antibiotic exposure, lowest concentrations etc)
    - Other (please specify)

28. Did you perform bacterial log reduction studies of individual bone processing steps, such as alcohol, detergents or peroxide?

    - Yes
    - No
    - Other (please specify)

29. Upon completion of the bone processing validation did you calculate the log reduction capability of the overall process?

    - Yes
    - No

30. What best approximation describes the overall bacterial log reduction capacity of your validated bone cleaning, decontamination process?

    - 0 to 3 log reduction
    - 4 to 6 log reduction
    - 7 to 10 log reduction
    - 11 to 19 log reduction
    - greater than 19 log reduction
    - Other (please specify)

31. What best describes your overall bacterial sterility assurance level achieved by your bone processing?

    - Between 10^2 and 10^3
    - 10^3
    - Between 10^3 and 10^6
    - 10^6
    - Between 10^6 and 10^{10}
    - 10^{10}
    - Between10^{10} and 10^{14}
    - 10^{14} or better
    - Unknown
    - Other (please specify)
    - We did not calculate a sterility assurance level

32. Have you established a periodic revalidation or requalification for your bone processing?

    - Yes
    - No

33. How often does this occur?

    - Twice a year
    - Annually
    - Every two years
    - Other (please specify)

34. Did you inoculate incoming unprocessed bone with virus and perform viral log reduction studies as part of validation studies of the entire process?

    - Yes
    - No

35. Please indicate the viruses used(check all that apply).

    - HTLV
    - HIV
    - Polio
    - Hepatitis A virus
    - Bovine diarrhea virus (HCV substitute)
    - Porcine parvovirus
    - PrV (HHV substitute)
    - WNV
    - Other (please specify)

36. During your validation studies of your overall viral bioburden reduction process, what type of bone processing settings did you use(check all that apply)?

    - Typical full scale settings
    - Half cycle settings (e.g., half of the normal contact time)
    - Worst case scenario (e.g., shortest times of alcohol, peroxide or antibiotic exposure, lowest concentrations etc)
    - Other (please specify)

37. Did you perform viral log reduction studies of individual bone processing steps, such as alcohol, detergents, or peroxide?

    - Yes
    - No

38. What approximation best describes your overall viral log reduction achieved by your bone processing?

    - <3 log reduction
    - 3 log reduction
    - 4-5 log reduction
    - 6 log reduction
    - 7-9 log reduction
    - 10-13 log reduction
    - 14 log reduction or better
    - Total log reduction was not calculated
    - Other (please specify)

39. What best describes your overall viral sterility assurance level achieved by your bone processing?

    - We did not calculate a sterility assurance level
    - Between 10[2] and 10[3]
    - 10[3] (10 to the 3 power)
    - Between 10[3] and 10[6]
    - 10[6] (10 to the 6 power)
    - Between 10[6] and 10[10]
    - 10[10] (10 to the minus 10 power)
    - Between10[10] and 10[14]
    - 10[14] or better
    - Unknown
    - Other (please specify)

40. Have you performed other validation studies of bone or connective tissue processing, storage or transport?

    - Yes
    - No

41. What type(s) of validation studies have been performed to show that your processing steps accomplish their intended purpose?

    - Validation of cleaning and re-sterilization of processing equipment validation (check all that apply).
    - In-house sterilizers used for critical equipment and supplies
    - Final package/container validation
    - Allograft studies of residual antibiotics
    - Allograft studies of residual lipid
    - Allograft studies of residual blood cells (Hemoglobin)
    - Allograft studies of residual cells
    - Allograft studies of residual chemicals such as alcohol etc
    - Bacteriostasis or fungistasis studies
    - Allograft biocompatibility studies
    - Allograft pyrogen testing
    - Stability studies to determine storage period and expiration date
    - Osteoinductivity of bone allograft
    - Mechanical testing and physical testing of bone
    - Penetrance of alcohol, detergent, peroxide or other disinfection chemicals
    - Allograft shipping containers and transport process
    - Sterility testing of the final allograft
    - Dosimetric release after radiation without routine testing of each final allografts
    - Parametric release of allografts after a multistep process not necessarily including radiation
    - Computer software
    - Other (please specify)

42. Does your facility process soft tissue/connective tissue (tendon, ligament, or fascia)?

    - Yes
    - No

43. What of the following types of bioburden reduction processing steps is used for soft tissue/connective tissue (tendon, ligament, fascia) processing (check all that apply)?

    - Antibiotics or chemicals
    - Antibiotic or chemicals and ionizing radiation (gamma or electron beam)
    - Alcohol
    - Peroxide
    - Detergents
    - Proprietary method only
    - Proprietary method and radiation (gamma or electron beam)
    - None of the above
    - Other (please specify)

44. Is radiation applied PRIOR TO PROCESSING of soft tissue/connective tissue (ligaments, tendons) to reduce bioburden?

    - Yes, applied to all
    - Yes, depending on the results of recovery or other preprocessing microbial test results or other indication
    - No
    - Other (please specify)

45. What best describes the minimum dose of radiation that is used PRIOR TO PROCESSING of soft tissue/connective tissue (ligaments, tendons)?

    - Less than 1.0 MRad (10 kGy)
    - MRad (10 kGy)
    - Between 1.0 and 1.5 MRad (10 and 15 kGy)
    - 1.5 MRad (15 kGy)
    - Between 1.5 and 1.75 MRad (15 and 17.5 kGy)
    - 1.75 MRad (17.5 kGy)
    - Between 1.75 to 2.0 MRad (17.5 and 2.0 kGy)
    - MRad (20 kGY)
    - Between 2.0 and 2.5 MRad (20 and 25 kGy)
    - 2.5 MRad (25 kGy)
    - Greater than 2.5 MRad (25 kGy)

46. Is radiation applied as a final step of soft tissue/connective tissue (ligament, tendon) processing in its final package (TERMINAL STERILIZATION)?

    - Yes, applied to all
    - Yes, but it depends on recovery or other preprocessing microbial test results or other indication
    - No
    - Other (please specify)

47. What best describes the minimum dose of radiation that is used as a FINAL treatment of soft tissue/connective tissue (ligament, tendon) allograft?

    - Less than 1.0 MRad (10 kGy)
    - MRad (10 kGy)
    - Between 1.0 and 2.5 MRad (10 and 15 kGy)
    - 1.5 MRad (15 kGy)
    - Between 1.5 and 1.75 MRad (15 and 17.5 kGy)
    - 1.75 MRad (17.5 kGy)
    - Between 1.75 to 2.0 MRad (17.5 and 2.0 kGy)
    - MRad (20 kGY)
    - Between 2.0 and 2.5 MRad (20 and 25 kGy)
    - 2.5 MRad (25 kGy)
    - Greater than 2.5 MRad (25 kGy)

48. Is your facility AATB accredited?

    - Yes
    - No

Appendix B: Cardiovascular Processing and Validation Survey Questions

1. Which of the following cardiovascular tissues does your facility process?

    - Heart valves
    - Cryopreserved aortic arch/thoracic aorta
    - Pericardium
    - Cryopreserved saphenous vein
    - Cryopreserved intra-abdominal arteries or veins
    - Refrigerated intra-abdominal arteries, e.g., iliac to aid organ transplants
    - Acellular/decellularized heart valves
    - Other (please specify)

2. During cardiovascular tissue processing, does your facility pool/comingle tissue from two or more donors?

    - Yes
    - No

3. At your facility what is the maximum permitted time between the donor time of death (asystole) and the time placed in the antibiotic disinfection solution (antibiotic cocktail)?

    - Less than 12 hours
    - Between 12 and 24 hours
    - 24 hours
    - Between 24 and 36 hours
    - 36 hours
    - Between 36 and 48 hours
    - 48 hours
    - Between 48 and 60 hours
    - 60 hours
    - Greater than 60 hours
    - Other (please specify)

4. During bioburden reduction processing of traditional cryopreserved heart valve allografts, which of the following antibiotics are used at your facility (check all that apply)?

    - Ciprofloxacin
    - Gentamicin
    - Lincomycin/Lincocin
    - Vancomycin
    - Polymyxin B
    - Penicillin
    - Cefoxitin/Mefoxitin
    - Meropenem
    - Imipenem

- Netilmicin
- Timentin (Ticarcillin and Clavulanate),
- Clindamycin
- Streptomycin
- Rifampin
- Amoxicillin
- Ampicillin/sulbactam (Unisyn)
- Metronidazole (Flagyl)
- Cefoperazone/Cefibid/cefazone
- Cefataxime
- Cefuroxime
- Colistin (polymyxin E)
- Amikacin
- Piperacillin/tazobactam (Tazocin, Zosyn)
- Amphotericin B (antifungal)
- Fluconazole (antifungal)
- Ketoconazole (antifungal)
- Nystatin (antifungal)
- Flucytosine/ 5fluorocytosine (antifungal)
- Proprietary antibiotic cocktail
- Other (please specify)

5. What are some of the ingredients of the antibiotic diluent used during heart valve decontamination and processing?

    - Medium 199
    - RPMI-1640
    - Hanks balanced salt solution
    - Dulbecco modified Eagle medium
    - Eagle's MEM
    - Saline
    - Human serum albumin
    - Fetal bovine (calf) serum
    - Other (please specify)

6. At which temperature is the antibiotic solution during disinfection of heart valves?

    - Refrigerated (approximately 4C, 0 to 4C [32F to 40F], 2C to 5C, 4 to 8C, or 0 to 10C [32F to 50F])
    - Room temperature, (approximately 18C to 25C [65F to 75F], approximately 21 to 25C)
    - Approximately 30C (Approximately 86F)
    - Approximately 37C, 35 to 39C, 33 to 38C (approximately 98.6F)
    - Other (please specify)

7. Which of the following describes your planned length of incubation during antibiotic disinfection of heart valves?

    - At least 6 hours
    - 6 hours
    - 6 to 8 hours
    - 6 to 12 hours
    - At least 12 hours
    - 12 hours
    - 18 to 24 hours
    - 24 hours
    - 24 plus or minus 2 hours
    - Between 24 to 36 hours
    - 36 hours
    - Between 36 and 48 hours
    - 48 hours
    - Greater than 48 hours
    - Other (please specify)

8. During cleaning and rinsing of heart valve allografts during processing, what type of fluid is used (check all that apply)?

    - Medium 199
    - RPMI
    - Saline
    - Balanced salt solution, Hanks or other
    - Dulbecco modified Eagle medium
    - Eagle MEM
    - USP water for injection
    - Other (please specify)

9. Have any cardiovascular tissues, rinsates, or final preservation fluid been tested for pyrogens?

    - Yes
    - Yes, during validation studies
    - Yes, periodically
    - No

10. What type of cryoprotectant/cryopreservative is used during heart valve processing?

    - Glycerol
    - DMSO
    - Other (please specify)

11. What is the concentration of DMSO is used?

    - Less than 7.5 %
    - 7.5%
    - Between 7.5 and 10%
    - 10%
    - Between 10 and 12.5%
    - 12.5%
    - Between 12.5 and 15%
    - 15%
    - Between 15 and 20%
    - 20%
    - Greater than 20%
    - Other (please specify)

12. Other than the cryopreservative, what is included in your cryopreservation fluid?
    - RPMI-1640
    - RPMI with glutamine
    - Dulbecco minimal essential medium
    - DMEM with HEPES buffer
    - Hanks balanced salt solution
    - HBSS with HEPES
    - TC199
    - Human albumin
    - Fetal bovine (calf) serum
    - Isotonic saline with Tris buffer
    - Antibiotic
    - Other (please specify

13. What is routinely sampled for microbial testing during heart valve processing (check all that apply)?

    - Direct culturing of the transport fluid in which the heart was received by the processing lab
    - Filtering the transport fluid and culturing the filter
    - Swabbing of the whole heart prior to exposure to antibiotics
    - The excised valve prior to exposure to antibiotics
    - Prior to exposure to antibiotics but after the heart valves have been dissected and rinsed, the rinsate is cultured
    - Co-processed cardiac tissues (e.g., conduit, myocardium) prior to exposure to antibiotics
    - Co-processed cardiac tissue (e.g., conduit, myocardium) after exposure antibiotics, rinsing and immersion in the cryopreservation fluid
    - The cryopreservation fluid containing the heart valve immediately prior to sealing the final package before the freezing process
    - Other (please specify)

14. What microbial tests are performed at or near final packaging of heart valves as end product testing (check all that apply)?

    - Bacterial culturing
    - Fungal/yeast culturing
    - Mycobacterium culturing
    - Other (please specify)

15. Have you performed quantitative bacterial or fungal bioburden studies of incoming unprocessed heart or heart valve tissues?

    - Yes
    - No
    - Other (please specify)

16. Did you establish upper limits of bacterial bioburden levels which are acceptable for processing?

    - Yes
    - No

17. Has your facility ever performed bacteriostasis or fungistasis testing of heart valve allografts after processing?

    - No
    - Yes, during validation studies
    - Yes, periodically
    - Yes, other times
    - Other (please specify)

18. Under which storage conditions are cryopreserved heart valves stored?

    - Vapor phase of liquid nitrogen
    - Submerged in liquid nitrogen
    - Dry ice (solid CO2)
    - Mechanical freezer at temperature colder than 100C
    - Mechanical freezer at temperature colder than 135C
    - Mechanical freezer at temperature colder than 140C
    - Other (please specify)

19. Under which conditions are heart valves shipped/transported to the hospital?

    - Dry shipper (vapor phase of liquid nitrogen)
    - Vapor phase of liquid nitrogen in a liquid nitrogen container
    - Submerged in liquid nitrogen in a liquid nitrogen container
    - Dry ice (solid CO2)
    - Other (please specify)

20. Have you performed validation studies of your overall heart valve bacterial bioburden reduction process?

    - Yes
    - No
    - Other (please specify)

21. During your validation studies of your overall bioburden reduction process, did you inoculate incoming unprocessed bone with bacteria?

    - Yes
    - No
    - Other (please specify)

22. Please indicate the microbes used for inoculation as part of your validation study (check all that apply).

    - Proprionibacterium acnes
    - Staph epidermidis
    - Streptococcus faecium
    - Enterococcus faecalis
    - Clostridium sordellii
    - Staph aureus
    - Bacillus subtilis
    - Bacillus pumulus
    - Other (please specify)

23. Was your heart valve processing validation study performed using bacteria recovered from your facility?

    - Yes
    - No
    - Other (please specify)

24. As part of your overall processing validation studies, did you perform microbial/sterility testing of the finished heart valve allograft (check all that apply)?

    - Yes, qualitative results, genus etc
    - Yes, quantitative bioburden testing results
    - Yes, both of the above
    - No
    - Other (please specify)

25. During your validation studies of your overall bacterial bioburden reduction process, which of the following heart valve processing settings did you use(check all that apply)?

    - Typical full scale settings
    - Half cycle settings (e.g., half of the normal contact time)
    - Worst case scenario (e.g., shortest times of alcohol, peroxide or antibiotic exposure, lowest concentrations etc)
    - Other (please specify)

26. Upon completion of the heart valve processing validation did you calculate the log reduction capability of the overall process?

    - Yes
    - No
    - Other (please specify)

27. What best describes the overall bacterial log reduction capacity of your validated heart valve cleaning, decontamination process?

    - We haven't calculated our overall log reduction
    - 0 to 3 log reduction
    - 4-6 log reduction
    - 7-10 log reduction
    - 11-19 log reduction
    - Greater than 19 log reduction
    - Other (please specify

28. What best describes your overall bacterial sterility assurance level achieved by your heart valve processing?

    - We have not calculated a sterility assurance level
    - Between 10^{-2} and $10^{-2.9}$
    - 10^{-3}
    - 10^{-4} to 10^{-5}
    - 10^{-6}
    - 10^{-7} to 10^{-9}
    - 10^{-10} to 10^{-13}
    - 10^{-14} or better
    - Other (please specify)

29. Have you established a periodic re-validation or re-qualification plan for your heart valve processing?

    - Yes
    - No

30. How often does this occur?

    - Twice a year
    - Annually
    - Every two years
    - Other (please specify)

31. Have you performed other validation studies of cardiovascular tissue processing, storage or transport?

    - Yes
    - No

32. What type(s) of validation studies have been performed to show that your processing steps accomplish their intended purpose?

    - Validation of cleaning and re-sterilization of processing equipment
    - In-house sterilizers used for critical equipment and supplies
    - Final package/container validation

- Allograft studies of residual antibiotics
- Allograft studies of residual cryopreservatives such as DMSO
- Allograft pyrogen testing
- Stability studies to determine storage period and expiration date
- Mechanical testing and physical testing of heart valves
- Allograft shipping containers and transport process
- Computer software
- Saphenous vein processing
- Intra-abdominal arteries or veins
- Acellular, decellularized heart valves
- Other (please specify)

33. Is your facility AATB accredited?

- Yes
- No

Appendix C: Skin Processing and Validation Survey Questions

1. What type of skin allograft is processed at your facility?

    - Split thickness skin
    - Dermis
    - Other (please specify)

2. During skin or dermis processing, does your facility pool/comingle tissue from two or more donors?

    - Yes
    - No
    - Other (please specify)

3. Do you process cryopreserved split thickness skin at your facility?

    - Yes
    - No
    - Other (please specify)

4. During processing of cryopreserved, split thickness skin allografts, which of the following antibiotics are used during skin processing or storage at your facility (check all that apply)?

    - Vancomycin
    - Polymyxin B
    - Lincomycin/Lincocin
    - Gentamcin
    - Nystatin (antifungal)
    - Bacitracin
    - Kanamycin
    - Cephazolin
    - Ciprofloxacin
    - Cefoxitin/Mefoxitin
    - Meropenem
    - Timentin (Ticarcillin and Clavulanate)
    - Clindamycin
    - Streptomycin
    - Cefoperazone/Cefibid/Cefazone
    - Cefataxime
    - Cefuroxime
    - Piperacillin/Tazobactam (Tazocin, Zosyn)
    - Fluconazole (antifungal)
    - Ketoconazole (antifungal)
    - Proprietary antibiotic cocktail
    - Other (please specify)

5. During cleaning and rinsing of skin allografts during processing, what type of fluid is used?

    - Saline
    - Hanks balanced salt solution
    - RPMI
    - Medium 199
    - Dulbecco modified Eagle medium
    - Eagle MEM
    - Other (please specify)

6. Does your facility use glycerol as a cryopreservative during split thickness skin cryopreservation?

    - Yes
    - No
    - Other (please specify)

7. What concentration of glycerol is used in cryopreservation of split thickness skin?

    - Between 1 and 10%
    - 10%
    - Between 10 and 15%
    - 15 %
    - Between 15 and 20%
    - 20%
    - Between 20 and 30%
    - 30%
    - Greater than 30%
    - Other (please specify)

8. Does your facility use DMSO as a cryopreservative during split thickness skin cryopreservation?

    - Yes
    - No

9. What concentration of DMSO is used in cryopreservation of split thickness skin?

    - Less than 7.5 %
    - 7.5%
    - Between 7.5 and 10%
    - 10%
    - Between 10 and 12.5%
    - 12.5%
    - Between 12 and 15%
    - 15%
    - Between 15 and 20%

- 20%
- Greater than 20%

10. What is your freezing method for cryopreserved split thickness skin?

    - Controlled rate, electronically programmed freezing
    - Controlled rate, insulated heat-sink box method in mechanical freezer
    - Controlled rate, insulated heat-sink method in dry ice (solid CO_2)
    - Dry ice (solid CO_2)
    - Other (please specify)

11. Under which conditions does your facility store cryopreserved split thickness skin?

    - Vapor phase of liquid nitrogen
    - Submerged in liquid nitrogen
    - Dry Ice (solid CO_2)
    - Mechanical freezer at temperature colder than 140 ^{o}C
    - Mechanical freezer at temperature colder than 100 ^{o}C
    - Mechanical freezer at temperature colder than 40 ^{o}C
    - Other (please specify)

12. Under what condition is cryopreserved split thickness skin shipped/transported?

    - Dry shipper (vapor phase of liquid nitrogen)
    - Vapor phase of liquid nitrogen in a liquid nitrogen container
    - Submerged in liquid nitrogen in a liquid nitrogen container
    - Dry ice (Solid CO_2)
    - Wet ice
    - Other (please specify)

13. What is routinely sampled for microbial testing before or during processing and immediately prior to cryopreserving split thickness skin (check all that apply)?

    - The transport fluid bathing the recovered unprocessed skin during temporary storage and transportation to the processing facility
    - Swabbing of each zone of recovered skin
    - A small piece of each sheet of recovered unprocessed skin
    - A small piece of recovered unprocessed skin from each anatomical site
    - For cryopreserved skin, a sample of the cryopreservation fluid after exposure to skin while in the final package
    - Other (please specify)

14. What microbial tests are performed following cryopreserved skin processing at the time of final packaging (check all that apply)?

    - Bacterial culturing
    - Fungal culturing
    - Mycobacterium culturing
    - Other (please specify)

15. Has your facility ever performed bacteriostasis of fungistasis studies of cryopreserved skin?

    - Yes
    - Yes, during validation studies
    - Yes, periodically
    - No

16. Does your facility have a list of virulent bacteria or fungi which if found on incoming unprocessed skin, is cause for discard or other use instead of processing for transplantation?

    - Yes
    - No

17. Have you performed quantitative microbial bioburden studies of incoming unprocessed spit thickness skin which will be cryopreserved?

    - Yes
    - No

18. Did you establish upper limits of bacterial bioburden levels which are acceptable for processing?

    - Yes
    - No

19. Have you performed validation studies of your overall bioburden reduction process for cryopreserved skin?

    - Yes
    - No

20. During your validation studies of your overall bioburden reduction process cryopreserved skin, did you inoculate incoming unprocessed skin with bacteria?

    - Yes
    - No

21. Please indicate the microbes used for inoculation as part of your validation study (Check all that apply)?
    - Streptococcus faecium
    - Proprionibacterium
    - Enterococcus faecalis
    - Clostridium sordellii
    - Staph epi
    - Staph aureus
    - Bacillus subtilis
    - Bacillus pumulus

22. Was your skin processing validation study performed using bacteria recovered from your facility?

    - Yes
    - No

23. During your validation studies of your overall bioburden reduction processing of cryopreserved skin, which of the following skin processing settings did you use (check all that apply)?

    - Typical full scale settings
    - Half cycle settings (e.g., half of the normal contact time)
    - Worst case scenario (e.g., shorten times of exposure to antibiotics, lowest concentrations, etc)?
    - Other (please specify)

24. Did you perform bacterial log reduction studies of the process?

    - Yes
    - No

25. What is the overall bacterial log reduction capacity of your cryopreserved skin antibiotic treatment decontamination process?

    - 0 to 3 log reduction
    - 4-6 log reduction
    - 7-10 log reduction
    - 11-19 log reduction
    - Greater than 19 log reduction

26. Have you performed other validation studies of cryopreserved split thickness skin allografts?

    - Yes
    - No

27. What other validation studies have been performed to show that your cryopreserved split thickness skin processing and methods accomplish their intended purpose (Check all that apply)?

    - Validation of cleaning and re-sterilization of processing equipment
    - In-house sterilizers used for critical equipment and supplies
    - Allograft studies of residual antibiotics
    - Allograft residual cryoprotectants such as DMS0 or glycerol
    - Allograft studies of cell viability
    - Microbiologic testing of the final allograft
    - Final package/container validation
    - Stability studies to determine storage period and expiration date

- Skin shipping containers and transport process
- Computer software
- Other (please specify)

28. Do you process and provide "fresh" refrigerated split thickness skin?

    - Yes
    - No

29. During processing of "fresh" split thickness skin allografts, which of the following antibiotics are used during skin processing or storage at your facility (check all that apply)?

    - Vancomycin
    - Polymyxin B
    - Lincomycin/Lincocin
    - Gentamicin
    - Nystatin (antifungal)
    - Bacitracin
    - Kanamycin
    - Cephazolin
    - Ciprofloxacin
    - Primaxin
    - Cefoxitin/Mefoxitin
    - Meropenem
    - Timentin (Ticarcillin and Clavulanate)
    - Clindamycin
    - Streptomycin
    - Cefoperazone/Cefibid/Cefazone
    - Cefatazime
    - Cefuroxime
    - Piperacillin/Tazobactam (Tazocin, Zosyn)
    - Fluconazole (antifungal)
    - Ketoconazole (antifungal)
    - Proprietary antibiotic cocktail
    - Other (please specify)

30. What is the maximum storage period for refrigerated "fresh" split thickness skin processed at your facility?

    - 7 days or less
    - 8 to 13 days
    - 14 days
    - 15 to 20 days
    - 21 days
    - Greater than 21 days

31. What is routinely sampled for microbial testing during "fresh" refrigerated split thickness skin processing or storage (Check all that apply)?

    - The transport fluid bathing the recovered unprocessed skin during temporary storage and transportation to the processing facility
    - Swabbing of each zone of recovered tissue prior to exposure to antibiotics
    - A small piece of each sheet of recovered unprocessed skin prior to exposure to antibiotics
    - A small piece of recovered unprocessed skin from each anatomical site prior to exposure to antibiotics
    - A sample of the storage fluid immediately prior to shipment to a patient in a hospital
    - Other (please specify)

32. What microbial tests are performed with samples identified in the previous question (Check all that apply)?

    - Bacterial culturing
    - Fungal culturing
    - Mycobacterium culturing
    - Other (please specify)

33. Does your facility have a list of specific bacteria or fungi which if found on incoming, unprocessed split thickness skin, would be cause for discard and not process for transplantation purposes?

    - Yes
    - No

34. Has your facility ever performed bacteriostasis of fungistasis studies of fresh refrigerated skin?

    - Yes
    - Yes, during validation studies
    - No
    - Other (please specify)

35. Have you performed validation studies of your overall bioburden reduction process for "fresh" refrigerated split thickness skin?

    - Yes
    - No

36. Did you perform quantitative bacterial or fungal bioburden studies of incoming unprocessed skin?

    - Yes
    - No

37. Did you establish upper limits of bacterial bioburden levels on unprocessed skin which are acceptable for processing?

    - Yes
    - No

38. During your validation studies of your bioburden reduction process, did you inoculate incoming, unprocessed, split thickness skin with bacteria?

    - Yes
    - No

39. Please indicate the microbes used for inoculation as part of your validation study (Check all that apply)?

    - Streptococcus faecium
    - Proprionibacterium
    - Enterococcus faecalis
    - Clostridium sordellii
    - Staph epi
    - Staph aureus
    - Bacillus subtilis
    - Bacillus pumulus
    - Other (please specify)

40. Was your skin processing validation study performed using bacteria recovered from your facility?

    - Yes
    - No
    - Other (please specify)

41. As part of your skin processing validation studies, did you perform microbial testing of the finished allograft?

    - Yes, qualitative results, genus etc.
    - Yes, quantitative bioburden testing results
    - No
    - Other (please specify)

42. During your validation studies of your bacterial bioburden reduction process, which of the following processing settings did you use (Check all that apply)?

    - Typical full scale settings
    - Half cycle settings (e.g., half of the normal contact time)
    - Worst case scenario (e.g., shortest times of antibiotic exposure, lowest concentrations,etc.)
    - Other (please specify)

43. Did you perform bacterial log reduction studies of "fresh" refrigerated split thickness skin processing?

    - Yes
    - No

44. What is the overall bacterial log reduction capacity of your refrigerated split thickness skin antibiotic treatment decontamination process?

    - 0 to 3 log reduction
    - 4-6 log reduction
    - 7-10 log reduction
    - 11-19 log reduction
    - Greater than 19 log reduction
    - Other (please specify)

45. Have you established a periodic revalidation or requalification for your tissue processing?

    - Yes
    - No

46. How often does this occur?

    - Twice a year
    - Annually
    - Every two years
    - Other (please specify)

47. Did you inoculate incoming unprocessed skin with virus and perform viral log reduction studies as part of validation studies of the entire process?

    - Yes
    - No

48. Have you performed other validation studies of "fresh" refrigerated split thickness skin allografts?

    - Yes
    - No

49. What other validation studies have been performed to show that your "fresh" refrigerated split thickness skin processing and methods accomplish their intended purpose (Check all that apply)?

    - Validation of cleaning and re-sterilization of processing equipment
    - In-house sterilizers used for critical equipment and supplies
    - Allograft studies of residual antibiotics
    - Allograft studies of cell viability
    - Microbiologic testing of the final allograft
    - Final package/container validation
    - Stability studies to determine storage period and expiration date
    - Skin shipping containers and transport process
    - Computer software
    - Other (please specify)

50. Does your facility process acellular, decellularized dermis allograft?

    - Yes
    - No

51. During dermis processing, which of the following treatments are applied (Check all that apply)?

    - Soaks in hypertonic fluid
    - Hypotonic lysis
    - Antibiotics
    - Enzymes to remove cells such as Trypsin
    - Nucleases, endonucleases to degrade DNA/RNA such as recombinant endonuclease, Benzonase, Pulmozyme or others
    - Detergents such as polysorbate20, Triton X 100, Tween 80 or others
    - Anionic detergents such as Nlauroyl sarconsinate (NLS) or sodium dodecyl sulfate (SDS)
    - Alcohol
    - Hydrogen peroxide
    - Sonication
    - Radiation
    - Proprietary steps
    - None of the above
    - Other (please specify)

52. During processing of acellular dermis allografts, which of the following antibiotics are used at your facility (Check all that apply)?

    - No antibiotics are used
    - Vancomycin
    - Lincomycin/Lincocin
    - Gentamicin
    - Ciprofloxacin
    - Polymyxin B
    - Cefoxtin/Mefoxitin
    - Meropenem
    - Timentin (Ticarcilln and Clavulanat)
    - Clindamycin
    - Streptomycin
    - Colitin (polymyxin E)
    - Amikacin
    - Amphotericin B (antifungal)
    - Fluconazole (antifungal)
    - Nystatin (antifungal)
    - Proprietary antibiotic cocktail
    - Other (please specify)

53. Under which conditions is dermis stored?

    - Terminal radiation and ambient temperature/room temperature storage
    - In alcohol stored at ambient temperature/room temperature storage
    - Freeze-dried and stored at ambient temperature storage
    - In glycerol and stored at refrigerated temperatures
    - Mechanical freezer, at 40C or colder
    - Mechanical freezer, at 100C or colder
    - Dry ice
    - Liquid nitrogen
    - Other (please specify)

54. Which of the following sterilization methods are applied as part of or after dermis processing (Check all that apply)?

    - None of the following
    - Ethylene oxide gas
    - NovaSterilis (Supercritical CO2)
    - Other (please specify)

55. Do you apply radiation to dermal allografts?

    - Yes
    - No

56. What type of radiation?

    - Gamma radiation
    - Electron Beam radiation

57. Does your processing include radiation to some or all incoming dermis prior to processing?

    - Yes, applied to all
    - Yes, depending on the results of recovery or other preprocessing microbial test results or other indications
    - No

58. What best describes then minimum dose of radiation that is used prior to processing dermis?

    - Less than 1.0 MRad (10 kGy)
    - MRad (10 kGy)
    - Between 1.0 and 1.5 MRad (10 and 15 kGy)
    - Between 1.5 and1.75 MRad (15 and 17.5 kGy)
    - 1.75 MRad (7.5 kGy)
    - Between 1.75 to 2.0 MRad (17.5 and 20 kGy)
    - MRad (20 kGy)
    - Between 2.0 and 2.5 MRad (20 and 25 kGy)
    - 2.5 MRad (25 kGy)
    - Greater than 2.5 MRad(25 kGy)

59. Is radiation applied as a final step, an end point of dermis processing in its final package (terminal sterilization)?

    - Yes, applied to all
    - Yes, depending on the results of preprocessing or in-processing microbial test results or other indications
    - No

60. What best describes the minimum dose of radiation that is used as a final dermis treatment (terminal sterilization)?

    - Less than 1.0 MRad (10 kGy)
    - MRad (10 kGy)
    - Between 1.0 and 1.5 MRad (10 and 15 kGy)
    - 1.5 MRad (15 kGy)
    - Between 1.5 and 1.75 MRad (15 and 17.5 kGy)
    - 1.75 MRad (17.5 kGy)
    - Between1.75 and 2.0 MRad (17.5 and 20 kGy)
    - MRad (20 kGy)
    - Between 2.0 to 2.5 MRad (20 to 25 kGy)
    - 2.5 MRad (25 kGy)
    - Greater than 2.5 MRad (kGy)

61. Have you performed bacterial or fungal bioburden studies of incoming unprocessed bone tissues?

    - Yes
    - No
    - Other (please specify)

62. Did you establish upper limits of bacterial bioburden levels of unprocessed thick skin which are acceptable for processing?

    - Yes
    - No

63. Do you perform some form of final sterility testing of the final finished dermis allograft?

    - Yes
    - No
    - No, we have a validated process with terminal radiation and dosimetric release that does not require final sterility testing
    - No, we have a validated process, that does not include terminal radiation, and does not require final sterility testing
    - Other (please specify)

64. Have you performed validation studies of your overall bioburden reduction process for dermis allograft?

    - Yes
    - No

65. During your validation studies of your overall bioburden reduction process, did you inoculate incoming unprocessed dermis with bacteria?

    - Yes
    - No
    - Other (please specify)

66. Please indicate the microbes used for inoculation as part of your validation study (check all that apply).

    - Streptococcus faecium
    - Proprionibacterium acnes
    - Enterococcus faecalis
    - Clostridium sordellii
    - Staph epi
    - Staph aureus
    - Bacillus subtilis
    - Bacillus pumulus
    - Other (please specify)

67. Was your dermis processing validation study performed using bacteria recovered from your facility?

    - Yes
    - No

68. As part of your overall dermis processing validation studies, did you perform microbial/sterility testing of the finished dermis allograft?

    - Yes, qualitative results, genus etc
    - Yes, quantitative bioburden testing results
    - Both of the above
    - No
    - Other (please specify)

69. During your validation studies of your overall bacterial bioburden reduction process, which of the following dermis processing settings did you use(check all that apply)?

    - Typical full scale settings
    - Half cycle settings (e.g., half of the normal contact time)
    - Worst case scenario (e.g., shortest times of alcohol, peroxide or antibiotic exposure, lowest concentrations etc)
    - Other (please specify)

70. Upon completion of the dermis processing validation did you calculate the bacterial log reduction capability of the overall process?

    - Yes
    - No

71. What best describes the overall bacterial log reduction capacity of your validated dermis cleaning, decontamination process?

    - 0 to 3 log reduction
    - 4-6 log reduction
    - 7-10 log reduction
    - 11-19 log reduction
    - Greater than 19 log reduction
    - Other (please specify)

72. What best describes your overall bacterial sterility assurance level achieved by your dermis processing?

    - We did not calculate a sterility assurance level
    - Between 10^{-2} and $10^{-2.9}$
    - 10^{-3}
    - 10^{-4} to 10^{-5}
    - 10^{-6}
    - 10^{-7} to 10^{-9}
    - 10^{-10} to 10^{-13}
    - 10^{-14} or better
    - Unknown
    - Other (please specify)

73. Did you inoculate incoming unprocessed dermis with virus and perform viral log reduction studies as part of validation studies of the entire process?

    - Yes
    - No

74. Please indicate the viruses used (check all that apply).

    - HTLV
    - HIV
    - Polio
    - Hepatitis A virus
    - Bovine diarrhea virus (HCV substitute)
    - Porcine parvovirus
    - PrV (HHV substitute)
    - WNV
    - Other (please specify)

75. What best describes your overall viral log reduction achieved by your dermis processing?

    - < 3 log reduction
    - 3 log reduction
    - 4-5 log reduction
    - 6 log reduction
    - 7-9 log reduction
    - 10-13 log reduction
    - 14 log reduction or better
    - Unknown
    - Other (please specify)

76. What best describes your overall viral sterility assurance level achieved by your dermis processing?

    - Between 10^{-2} and $10^{-2.9}$
    - 10^{-3}
    - 10^{-4} to 10^{-5}
    - 10^{-6}
    - 10^{-7} to 10^{-9}
    - 10^{-10} to 10^{-13}
    - 10^{-14} or better
    - We did not calculate a sterility assurance level
    - Other (please specify)

77. Have you performed other validation studies of your dermis processing or storage?

    - Yes
    - No

78. What other validation studies have been performed to show that your processing, storage or transportation procedures accomplish their intended purpose?

    - Validation of cleaning and re-sterilization of processing equipment validation
    - In-house sterilizers used for critical equipment and supplies
    - Final package/container validation
    - Allograft studies of residual antibiotics or other chemicals
    - Allograft studies of residual cells
    - Allograft studies of residual chemicals such as alcohol, detergents, etc
    - Allograft biocompatibility studies
    - Allograft pyrogen testing
    - Stability studies to determine storage period and expiration date
    - Mechanical testing and physical testing
    - Allograft shipping containers and transport process
    - Sterility testing of the final allograft
    - Dosimetric release after radiation without routine testing of each final allografts
    - Parametric release of allografts after a multistep process not necessarily including radiation

- Computer software
- Other (please specify)

79. Does your facility provide split thickness skin preserved in high concentrations of glycerol, e.g., approximately 50 to 85% glycerol?

    - Yes
    - No

80. Is your facility AATB accredited?

    - Yes
    - No

Appendix D: Tissue Recovery Survey Questions

1. Which of the following types of tissue does your facility recover (Check all that apply)?

   - Bone
   - Tendons/ligaments
   - Heart
   - Blood vessels
   - Skin
   - Eyes or cornea
   - Dura
   - Nerves
   - Other (please specify)

2. According to your written procedures, how long after death (asystole) can the donor body be stored before tissue must be recovered when the body has been refrigerated or cooled within 12 hours of asystole?

   - Within 12 hours of asystole
   - Within 13-14 hours
   - Within 15-24 hours
   - Within 16-23 hours
   - Within 24 hours
   - Within 25-35 hours
   - Within 36 hours
   - Within 37-47 hours
   - Within 48 hours
   - Greater than 48 hours
   - Comment?

3. According to your written procedures, how long after death (asystole) can the donor body be stored before tissue must be recovered when the body is not refrigerated but is stored at room or ambient temperature?

   - Within 6-11 hours of asystole
   - Within 12 hours
   - Within 13-14hours
   - Within 15 hours
   - Within 16-23 hours
   - Within 24 hours
   - Within 25-35 hours
   - Within 36 hours
   - Greater than 36 hours
   - Comment?

4. Do your procedures require postmortem blood cultures of the donor?
   - Yes
   - No

5. At which sites are tissue recoveries performed (Check all that apply)?

   - Dedicated recovery room at the tissue bank facility
   - Dedicated recovery room at a medical examiner's facility
   - Funeral homes, mortuary
   - Hospital morgue
   - Hospital operating room
   - Other (please specify)

6. Do you have written qualifying requirements for a tissue recovery site?

   - Yes
   - No

7. Are your recovery site requirements the same as those published by AATB in its guidance document (Prevention of Contamination and Cross-contamination at Recovery: Practices & Culture Results. No. 2, version 2, May 29, 2007)?

   - Yes
   - No

8. Which of the following are required by your procedures regarding qualifying a new tissue recovery site prior to using it for the first time (Check all that apply)?

   - Adequate floor and tabletop space to allow aseptic recovery procedures
   - Adequate lighting for physical assessment and tissue recovery
   - Access to a suitably located hand washing area for hand/forearm surgical scrub or wash
   - A controlled airflow system in the recovery area with no direct access to the outside of the building from the recovery room at any time during, before or after tissue recovery
   - Walls, floor and work surfaces that are easily cleaned and in a good state of repair
   - No visible signs of insects, rodents, or other pests
   - Absence of standing fluids or contaminated waste in the room or can be rectified prior to recovery
   - Working surfaces that are capable of being cleaned and disinfected prior to recovery of tissue
   - None of the above
   - Other (please specify)

9. Prior to each recovery, is the recovery room inspected for meeting qualification requirements and the results documented?

   - Yes
   - No

10. Does your tissue bank organization recover skin?

    - Yes
    - No

11. What preparation of the skin donor site is applied prior to skin removal (Check all that apply)?

    - Soap
    - Chlorhexadine
    - Iodophor, e.g. povidone iodine, betadine
    - Alcohol
    - Mineral Oil
    - Other (please specify)

12. What type of skin is recovered (Check all that apply)?

    - Split-thickness skin with a dermatome
    - Thick skin with a dermatome
    - Full thickness skin "free hand" with a scalpel
    - Other (please specify)

13. Are sampling and testing of recovered skin tissue performed to detect microbial growth prior to exposure to antibiotics and processing?

    - Yes, by recovery staff
    - Yes, but not by recovery staff
    - No
    - Unknown

14. What recovered skin tissues are sampled for microbial growth prior to exposure to antibiotics (Check all that apply)?

    - Each individual piece of skin
    - One piece of skin from each zone
    - Other representative tissues are sampled
    - Other (please specify)

15. Into what type of fluid is split thickness skin placed immediately after recovery?

    - Antibiotics
    - RPMI
    - Hanks balanced salt solution
    - Dulbecco minimum Eagle medium
    - Eagle MEM
    - Other (please specify)

16. Does your facility recover bone?

    - Yes
    - No

17. Which of the following types of skeletal tissue does your facility recover (Check all that apply)?

    - Bone
    - Osteochondral, cartilage for "fresh" refrigerated cartilage allografts
    - Other cartilage
    - Ligaments
    - Tendons
    - Meniscus
    - Other (please specify)

18. What preparation of the skin takes place prior to draping and recovery of bone?

    - Soap
    - Chlorhexadine
    - Iodophor, e.g., povidone iodine, betadine,
    - Alcohol

19. Are sampling and testing of recovered bone tissue performed to detect microbial growth prior to exposure to antibiotics and processing?

    - Yes, by recovery staff
    - Yes, but by processing lab staff not recovery staff
    - No
    - Other (please specify)

20. What recovered bone tissues are sampled for microbial growth?

    - Each individual tissue is sample
    - Representative tissues are sampled
    - Other (please specify)

21. What method is used for sampling of recovered bone tissues for microbial growth (bacterial, fungal) before being exposed to disinfectant, antibiotics, sterilants and processing?

    - Swabbing each individual recovered tissue
    - Immersion of groups of recovered tissues in growth medium, filtering the extraction fluid, incubating the filter
    - Other (please specify)

22. Does your facility recover heart or other cardiovascular tissue?

    - Yes
    - No
    - Other (please specify)

23. What type of cardiovascular tissue does your facility recover (Check all that apply)?

    - Whole heart
    - Aortic arch
    - Thoracic aorta
    - Intra-abdominal arteries or veins
    - Saphenous vein
    - Pericardium
    - Other (please specify)

24. What preparation of the skin is applied prior to draping and heart recovery (Check all that apply)?

    - Soap
    - Chlorhexadine
    - Iodophor, e.g. povidone iodine, betadine
    - Alcohol
    - Other (please specify)

25. Into what fluid is the recovered whole heart placed for temporary storage and transport to the processing lab?

    - Antibiotics
    - RPMI
    - Saline
    - Eagles MEM
    - Dulbecco modified Eagles medium
    - Other (please specify)

26. What temperature is the transport fluid in which the recovered heart is placed?

    - Room (ambient) temperature
    - Chilled, refrigerated or wet ice temperature
    - Other (please specify)

27. At which temperature condition is the recovered heart temporarily stored and transported to the processing facility?

    - Wet ice
    - Gel cold/freezer packs
    - Dry ice
    - Insulated ambient, room temperature
    - Other (please specify)

28. Is sampling of the recovered heart performed by recovery staff to detect microbial growth and prior to exposure to antibiotics and processing?

    - Yes, by recovery staff
    - No, not by recovery staff
    - Unknown
    - Other (please specify)

29. What type of sampling for microbial growth is performed by whole heart recovery staff?

    - Swabbing of the heart surface
    - Sampling of the transport fluid in which the heart has been placed
    - Other (please specify)

30. Have you validated your temporary storage and transportation method used to transport recovered tissue from the recovery site to the processing facility?

    - Yes
    - No
    - Comment?

31. During tissue recovery, recovery staff wear the following protective attire (Check all that apply):

    - Sterile gown over street clothes
    - Sterile gown over surgical attire
    - Sterile gloves using one pair
    - Sterile gloves using two pair (double gloving)
    - Disposable shoe coverings ("Booties")
    - Hair covering
    - Face masks
    - Eye protective glasses or eye shields
    - Other (please specify)

32. Did your tissue bank perform some degree of microbial or particulate environmental monitoring as part of the evaluation and qualification of the tissue recovery site prior to first use?

    - Yes
    - No

33. At initial evaluation of the recovery site, what type of environmental monitoring was performed (Check all that apply)?

    - Touch plates of surfaces
    - Touch plates of employees
    - Swabs of surfaces
    - Swabs of employees
    - Passive air monitoring (settling plates)
    - Active air sampling, particulate counts
    - Active air sampling, viable particulates (microbial growth)
    - Other (please specify)

34. Is environmental monitoring of a recovery site performed periodically?

    - Yes
    - No

35. When performed periodically at a recovery site, what type of environmental monitoring is performed (Check all that apply)?

    - Touch plates of surfaces
    - Touch plates of employees
    - Swabs of surfaces
    - Swabs of employees
    - Passive air monitoring (settling plates)
    - Active air sampling, particulate counts
    - Active air sampling, viable particulates (microbial growth)
    - Other (please specify)

36. How often do you perform microbial environmental monitoring during recovery?

    - Each donor
    - Once a day
    - Once a week
    - Once a month
    - Once every three months
    - Once a year
    - Other (please specify)

37. Do you track and trend environmental monitoring data obtained at the recovery sites?

    - Yes
    - No

38. Do you have environmental monitoring alert or action levels established for your tissue recovery data (Check all that apply)?

    - Yes, alert levels
    - Yes, action levels
    - Yes, both
    - No

39. How did you establish those levels (Check all that apply)?

    - Based on evaluation of historical data
    - Based on industry-accepted values
    - Based on knowledge from previous company employment
    - Other (please specify)

40. Is your facility AATB accredited?

    - Yes
    - No

Appendix E: Environmental Monitoring, Clean Rooms, Sterilizers Survey Questions

1. Which of the following types of tissue are processed at your facility?

    - Bone, DECEASED DONOR
    - Bone, LIVE DONOR, e.g., femoral head
    - Connective tissue/soft tissue, e.g., tendon, ligament
    - Heart valves or other cardiovascular tissue
    - Skin, including split thickness or dermis
    - Mesenchymal stem cells or other bone forming cells, with or without bone or other carrier
    - Dura
    - Amnion
    - Other (please specify)

2. Do you process cardiovascular, skin, bone or connective tissue/soft tissue in a cleanroom?

    - Yes
    - No

3. What cleanroom air quality is maintained?

    - ISO Class 4 (~US Class 10)
    - ISO Class 5 (~US Class 100)
    - ISO Class 6 (~US Class 1000)
    - ISO Class 7 (~US Class 10,000)
    - ISO Class 8 (~US Class 100,000)
    - Unknown
    - Comment?

4. Does your cleanroom have positive pressure with air flowing from the processing cleanroom into adjacent areas?

    - Yes
    - No
    - Unknown
    - Other (please specify)

5. Is the cleanroom air exhausted to the outside via a non-recirculating system?

    - Yes
    - No
    - Other (please specify)

6. Cleanroom filtered air exchanges take place at the following rates:

    - Less than one complete air exchange per hour
    - 1-5 air exchanges per hour
    - 6-9 air exchanges per hour
    - 10 air exchanges per hour
    - 11-20 air exchanges per hour
    - More than 20
    - Other (please specify)

7. Do you use any laminar flow hoods or biological safety cabinets for processing?

    - Yes
    - No

8. What is the air quality maintained within the laminar air flow hood/biologic safety cabinet?

    - ISO Class 4 (~US Class 10)
    - ISO Class 5 (~US Class 100)
    - ISO Class 6 (~US Class 1000)
    - ISO Class 7 (~US Class 10,000)
    - ISO Class 8 (~US Class 100,000)
    - Unknown
    - Other (please specify)

9. What air quality is maintained in the room which contains the laminar air flow hoods/biologic safety cabinets used for tissue processing?

    - Air is not filtered and is uncontrolled for particulates
    - ISO Class 4 (~US Class 10)
    - ISO Class 5 (~US Class 100)
    - ISO Class 6 (~US Class 1000)
    - ISO Class 7 (~US Class 10,000)
    - ISO Class 8 (~US Class 100,000)
    - Unknown
    - Other (please specify)

10. Does your tissue bank perform microbial (viable particulates) or nonviable particulate monitoring of the environment, e.g., air, staff, surfaces, within which tissue is processed?

    - No
    - Yes, at initial evaluation and qualification of a new tissue clean room
    - Yes, periodically
    - Yes, at each recovery operation
    - Other (please specify)

11. What type of environmental monitoring is performed (check all that apply)?

    - Touch plates of surfaces
    - Touch plates of employees
    - Swabs of surfaces
    - Swabs of employees
    - Passive air monitoring (settling plates)
    - Active air sampling, particulate counts
    - Active air sampling, viable particulates (microbial growth)
    - Other (please specify)

12. How often do you perform microbial or particulate environmental monitoring during processing?

    - Each donor
    - Once a day
    - Once a week
    - Once a month
    - Once every three months
    - Once a year
    - Other (please specify)

13. Do you track and trend environmental monitoring data obtained from clean room sites?

    - Yes
    - No

14. Do you have environmental monitoring alert or action levels established for your clean room data (check all that apply)?

    - Yes, alert levels
    - Yes, action levels
    - Yes, both
    - No

15. If alert or action levels are specified, how did you establish those levels?

    - Based on evaluation of historical data
    - Based on industry-accepted values
    - Based on knowledge from previous company employment
    - Other (please specify)

16. For any environmental testing, are sampling locations described in written procedures?

    - Yes
    - No

17. Are settling plates used as part of environmental monitoring?
    - Yes
    - No

18. How often are settling plates used in the critical environment where tissue is processed?

    - Every donor
    - Every shift
    - Once a day
    - Every 2-7 days
    - Every 8-14 days
    - Every 15-31 days
    - Used as needed but without a schedule
    - Other (please specify)

19. During what time of using the processing area are settling plates used (check all that apply)?

    - When no operations are taking place
    - At beginning of operations
    - During operations
    - At the end of operations
    - Other (please specify)

20. During bone processing, processing staff wear the following protective attire (check all that apply):

    - Sterile gown over street clothes
    - Sterile gown over surgical attire
    - Sterile gloves using one pair
    - Sterile gloves using two pair (double gloving)
    - Disposable shoe coverings (“Booties”)
    - Hair covering
    - Face masks
    - Eye protective glasses or eye shields
    - Other (please specify)

21. Is double gloving required?

    - Yes
    - No
    - Comment?

22. Which of the following is included at the hand washing sink near the processing room (check all that apply)?

    - Hands-free equipment
    - Sink
    - Towel
    - Soap dispensers
    - Alcohol waterless agents
    - Other (please specify)

23. Do your procedures require cleaning (using a soap or detergent) of the processing area between each donor?

    - Yes
    - No
    - Comment?

24. Do your procedures require disinfection (using a disinfectant or sporicide) of the processing area between each donor?

    - Yes
    - No
    - Comment?

25. Do your procedures include cleaning and disinfection of the processing area after use at end of each work day?

    - Yes
    - No

26. When cleaning the processing area at the end of the day, what staff are employed?

    - In house employees who clean fulltime
    - In house employees who are also tissue processing staff
    - Employees of an outside company
    - Other (please specify)

27. When processing areas are cleaned, does your procedure include cleaning and disinfection of floors and all horizontal surfaces?

    - Yes
    - No
    - Comment?

28. Do procedures require sequence cleaning from clean areas to dirty areas?

    - Yes
    - No

29. Do procedures require a two-step cleaning process that begins with cleaning (using a detergent) and is followed by a microbicidal process for disinfection?

    - Yes
    - No

30. Was an internal validation/qualification performed for use of your cleaning and disinfecting agents?

    - Yes
    - No
    - Comment?

31. Do you have an in-house sterilizer(s) for equipment or supplies that are for reuse in tissue processing clean rooms?

    - Yes
    - No

32. Which of the following types of equipment or supply sterilizer(s) are used at your facility (check all that apply)?

    - Steam sterilizer
    - Ethylene oxide sterilizer
    - NovaSterilis (Supercritical CO_2).
    - Other (please specify)

33. Which of the following effectiveness tests, if any, are included with each sterilizer run and every batch (check all that apply)?

    - Biologic indicators
    - Bowie-Dick test
    - Other chemical indicator
    - None of the above
    - Other (please specify)

34. Do your procedures require that each day the in-house sterilizer is used, that you run a biologic indicator as a positive control, i.e., not exposed to the sterilant but incubated with the others?

    - Yes
    - No

35. Do you use a moist heat/steam sterilizer (autoclave)?

    - Yes
    - No

36. Do you employ a Bowie-Dick test (color change if air is removed and replaced by steam) with steam sterilizers?

    - Yes
    - No

37. How often are sterilizers monitored with biologic indicators?

    - Each batch
    - Daily
    - Weekly
    - Other (please specify)

38. Is your facility AATB accredited?

    - Yes
    - No