Canadian Blood Services
Société canadienne du sang

Bioburden Reduction and Control In Tissue Banking

LEADING EVIDENCE BASED PRACTICE GUIDELINES FOR:

Tissue Recovery

Microbial Sampling

Processing of Musculoskeletal Tissue

Processing of Cardiac Tissue

Processing of Skin Tissue

FINAL REPORT
NOVEMBER 2016

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ACRONYMS AND ABBREVIATIONS

AAMI	Association for the Advancement of Medical Instrumentation
AATB	American Association of Tissue Banks
ANSI	American National Standards Institute
CBS	Canadian Blood Services
CFR	Code of Federal Regulations [USA]
CTO	Cells, Tissues, and Organs
CSA	Canadian Standards Association
DMSO	Dimethylsulphoxide
HCVA	Human cardiac valve allograft
OR	Operating room
PICO	Problem, Intervention, Comparison, Outcome
SAL	Sterility Assurance Level
WHO	World Health Organization

GLOSSARY

Decontamination: A process directed to inanimate objects such as equipment that reduces the number of viable cellular microorganisms but does not necessarily destroy all microbial forms such as spores and viruses.

Disinfection: A process directed to tissue that reduces the number of viable cellular microorganisms but does not necessarily destroy all microbial forms such as spores and viruses. Use of antibiotics, while not normally described as disinfection, is included.

Good Tissue Practice: Generated in this report when the scientific evidence, augmented by expert opinion, informed a practice where there was no reasonable comparative practice (versus a recommendation which recommends one practice over comparative practices).

Predictive Value: Positive and negative predictive values (PPV and NPV, respectively) describe the performance of a diagnostic or screening test and are the proportions of positive and negative results that are true positives and true negatives in a given population. Positive predictive value is the probability that donor with a positive screening test or a tissue with a positive microbial test, truly has the microbe being tested. Negative predictive value is the probability that donors with a negative screening test or tissues with a negative microbial detection test truly do not have the microbe.

Qualification: The process of establishing confidence that equipment, reagents, and ancillary systems are capable of consistently operating within established limits and tolerances. Process performance qualification is intended to establish confidence that the process is effective and reproducible.

Recommendation: A proposed best tissue bank practice generated in this report when the evidence supported a preferred practice over another.

Sensitivity: The sampling methods likelihood of detecting the presence of relevant microorganisms.

Specificity: The likelihood a testing methods result truly represents growth or non-growth of microorganisms and is not falsely positive or falsely negative.

Sterility Assurance Level (SAL): The probability of a single viable microorganism being detected on an allograft after sterilization (refer to AHSI/AAMI ST67:2003).

Sterilization: A validated process used to render tissue free from viable microorganisms; including spores (refer to ANSI/AAMI ST67.2003).

Terminal Sterilization: A validated process whereby finally-packaged tissue within its primary package is sterilized (refer to ANSI/AAMI ST67:2003).

Validation: The process of establishing documented evidence that provides a high degree of assurance that a specific process will consistently produce the predetermined outcome.

Verification: The confirmation by examination and provision of objective evidence that specific requirements have been fulfilled.

THEMES

Five working groups of experts were involved in developing evidence-based leading practice guidelines for Canadian tissue banks. Resources included: (a) systematic reviews of the published literature, (b) international environmental scan, a survey of tissue bank practices in North America, Europe and Australia, (c) analysis of regulatory documents, and (d) a review of documented disease transmissions. The experts developed recommendations, good tissue practices and research priorities in bioburden reduction and control in five areas: tissue recovery; microbial sampling; and musculoskeletal, cardiac and skin processing. The work was reviewed and adopted by an expert steering committee.

A number of themes evolved, including:

- *Inhibition:* Residual antibiotics and antifungals used during tissue processing or in incubation media can result in microbial inhibition and prevent detection of microorganisms that are present in or on tissue.

- *Sensitivity of sampling and specificity of culture method*: Sampling and culture techniques require validation of their sensitivity and specificity in detecting and identifying the range of microorganisms that could potentially contaminate tissue.

- *Validations*: Disinfection and sterilization procedures should be validated by quantification of bioburden reduction. Qualitative analysis is acceptable for process verification but should not be used as a surrogate for validation using quantitative log reduction.

- *Incubation*: Antibiotic disinfection of cardiovascular tissue should be conducted at a temperature of 37°C with a suitable broad spectrum antibiotic or mixture of antibiotics.

- *Sterilization methods*: Irradiation is the preferred methodology to sterilize nonviable musculoskeletal grafts. If irradiation methods are used, the recommended dose should be employed to preserve the structure and function of the tissue for its intended use. Irradiation is not recommended for viable refrigerated cartilage, split-thickness skin or cardiac grafts.

EXECUTIVE SUMMARY

The transplantation of a human tissue allograft introduces the risk of complications to the recipient including the fatal and nonfatal transmission of infectious organisms such as bacteria, fungi, viruses, parasites, and prions.

Canadian tissue banking originated in hospitals in the 1970s and '80s when surgical programs began to temporarily store autograft tissues and to look for sources of allograft tissues. Today, tissue banks are considered to be manufacturers of human biologics where donor tissue is processed and enhanced using good manufacturing practices and good tissue practices to optimize safety and clinical outcomes. As biological manufacturers of tissue allografts that present a risk of disease transmission, tissue bank practices that reduce and eliminate infectious organisms must be effective, evidence-based and validated.

In February 2012, the Canadian tissue community convened and identified the need for evidence-based leading practices to inform bioburden reduction and control (disinfection) processes.[1] In response, Canadian Blood Services facilitated the development of leading practice guidelines for bioburden reduction and control in five areas: tissue recovery; microbial sampling; and musculoskeletal, cardiac, and skin processing.

An expert steering group was convened. Significant resources were directed to the collection of evidence to inform leading practice discussions and recommendations, including (a) systematic reviews of the literature in each of the five topic areas, (b) an international environmental scan, a survey of tissue banks to determine the most commonly used current practices, (c) an analysis of regulations, and (d) an analysis of disease transmissions reported in tissue transplantation. Working groups made up of Canadian and U.S. experts in each of the five topic areas reviewed the scientific literature and developed and recommended evidence-based guidelines. These were presented to the Steering Committee for review, reflection, revision, and finally adoption as leading practice guidelines.

This report presents evidence-based bioburden reduction leading practice guidelines including 16 recommendations, 21 good tissue practices, and identification of 39 research priorities. In response to public and professional concern for preventing transmission of infectious disease by transplantation, these guidelines aim to support the provision of safe tissue for transplantation by standardizing practice using evidence-based scientific information.

A key finding in all areas of review was the lack of published scientific, clinical and tissue bank research to be used as evidence to inform specific tissue bank practices. In particular, it

[1] Canadian Blood Services (2012). http://www.organsandtissues.ca/s/english-expert/leading-practices-public-awareness-and-education-2

appears that a number of standards and practices now in place were developed using consensus opinion and anecdotal evidence instead of published scientific research. The lack of evidence may be in part due to the lack of research scientists and research funding within tissue banking. This lack of tissue bank-related scientific publications presents a challenge to the development of evidence-based leading practice and reduces the comprehensiveness, strength, thoroughness of our recommendations.

There are three significant outputs of the leading practice initiative:

1) Bioburden leading practice recommendations and good tissue practices to inform the Canadian tissue community and to advance standardized practice.
2) Recommendations to the Canadian Standards Association (CSA) for amendment of current standards, and therefore regulations, to align with the leading practice recommendations.
3) Research priorities to identify evidence gaps and provide researchers with insight into areas where evidence could inform and improve bioburden reduction practices.

The report recommends that tissue programs, standards organizations, regulators, researchers, and other stakeholders identify collaborative opportunities and potentially a consolidated approach to the implementation of these guidelines and to the generation of the additional evidence that may inform the standardization of practice to support the provision of safe tissue for transplantation.

RECOMMENDATIONS

A. Global

Recommendation 1: Disinfection procedures for musculoskeletal and cardiac tissue should be validated with quantification of log reduction, using challenge organisms. Qualitative analysis, such as calculation of discard and/or contamination rates, is acceptable for process verification but should not be used as a surrogate for the quantitative validation of log reduction.

Recommendation 2: For each tissue type, programs should consult microbiology experts and tissue bank experts, and current standard practices of other tissue banks to identify a comprehensive list of objectionable microbes that necessitate tissue discard when identified in the transport solution, recovery cultures or at any processing stage including final sterility testing. The list shall include, but be not limited to, *Clostridium* spp, *Streptococcus pyogenes*, *Staphylococcus aureus* and fungi. Pathogens that render tissue unacceptable for transplant should be documented in policies and procedures.

Recommendation 3: Programs considering the use of antifungals for tissue where cellular viability is required should carefully assess and consider the risks of their use. Many antifungals are cytotoxic and will reduce cellular viability.

Recommendation 4: Programs that process tissue with antibiotics should use broad spectrum antibiotics active against common contaminants and in a concentration and temperature effective to eliminate virulent or otherwise unacceptable microorganisms.

Recommendation 5: Programs that process tissue with antibiotics or antifungals or both should validate the rinsing method to be sure antimicrobial residues do not inhibit the detection of microorganisms.

B. Tissue Recovery

None

C. Microbial Sampling

Recommendation 6: Programs should validate the sensitivity of sampling methods used to obtain specimens for microbial culture to assess tissue bioburden.

Recommendation 7: Testing laboratories should determine the specificity of their final culture testing methods and quantify the negative predictive value, i.e., probability of a true negative culture result.

D. Processing of Musculoskeletal Tissue

Recommendation 8: Irradiation sterility testing should comply with the Radiation Sterilization Standards ANSI/AAMI/ISO 11137 and AAMI TIR 33 (soon to be ISO 13004). Irradiation is the preferred method for the terminal sterilization of nonviable musculoskeletal allografts.

Recommendation 9: The Sterility Assurance Level (SAL) that should be demonstrated following sterilization of musculoskeletal allografts is 10^{-6} SAL. Alternative SAL values can be considered for other tissue allografts based on evidence-based risk assessment.

Recommendation 10: Programs employing disinfection of musculoskeletal tissue by irradiation should consider the use of lower dosage (e.g., 12-17 kGy) irradiation and low temperature (dry ice conditions) in order to reduce potential negative biomechanical changes and clinical impact of terminal sterilization of musculoskeletal tissue by high dose irradiation (e.g. doses of > 20 kGy).

E. Processing of Cardiac Tissue

Recommendation 11: To reduce bioburden optimally, the temperature used during cardiac antimicrobial incubation should be 37°C. While antimicrobials may have some activity at lower temperatures, they are not as effective at lower temperatures and have a lower rate of microorganism kill.

F. Processing of Split Thickness Skin Tissue for Burn Treatment

Recommendation 12: Skin antibiotic disinfection processes should be validated. Quantitative validation of the bioburden log reduction using challenge organisms, which is accepted as an industry standard, is the preferred validation process.

Recommendation 13: To maintain cellular viability, terminal sterilization using processes such as irradiation or peracetic acid should not be employed on split thickness skin grafts used in burn treatment.

G. System

Recommendation 14: The focus of Canadian tissue banks is the provision of safe effective quality tissue allografts in adequate supplies; the provision of which requires research, publication and data sharing.

Recommendation 15: Surveillance programs such as the Cells Tissues and Organs Surveillance System (CTOSS) should provide, to Canadian programs, greater analysis and insight into their data to inform practice.

Recommendation 16: Canadian Blood Services and Héma-Québec, as established biologic manufacturers with infrastructure and core expertise supporting evidence-based scientific methods in the manufacture of biologics, should undertake and collaborate in an initiative to:

- Explore the development of a national tissue committee to support collaborations within the tissue community to maintain leading practices.
- Encourage the collection, analysis, and exchange of existing data on bioburden reduction and control.
- Develop analytics to inform quality improvement.
- Identify opportunities for collaborative and/or consolidated approaches to support the implementation of standardized leading practices.

- Advocate for research funding, including targeting the Canadian Institutes for Health Research (CIHR), to advance the scientific rigor of tissue manufacturing in Canada, linking to local vigilance and surveillance to advance evidence and improve practice.

GOOD TISSUE PRACTICES

A. Global

Good Tissue Practice 1: Tissue banks should track tissue recovery, in-processing tissue sampling, tissue processing environmental culture results, final sterility test results and contamination rates as well as the type of organisms identified, monitor trends, determine action levels and conduct root cause analysis to inform practice change, as required.

Good Tissue Practice 2: Programs should evaluate and monitor their bioburden reduction processes periodically and re-evaluate them after significant changes to practice.

B. Tissue Recovery

Good Tissue Practice 3: When determining the maximum tissue ischemic time from asystole to skin prep, programs should reference American Association of Tissue Banks (AATB) Guidance Document No. 7 "*Evaluation of Body Cooling*" and follow AATB Standard D5.400.

Good Tissue Practice 4: When developing standards of practice related to time requirements for body cooling, programs should reference AATB Guidance Document No. 7 "*Evaluation of Body Cooling"* and follow AATB Standard D5.400.

Good Tissue Practice 5: With respect to donor skin condition, programs should reference AATB Guidance Document No. 2 *"Prevention of Contamination and Cross-contamination at Recovery: Practices & Culture Results Requirement"* and follow AATB Standard D5.500 IV.

Good Tissue Practice 6: When developing protocols for skin preparation, programs should reference AATB Guidance Document No. 2 *"Prevention of Contamination and Cross-contamination at Recovery: Practices & Culture Results Requirement"* and follow AATB Standard D5.500.

Good Tissue Practice 7: When developing protocols for tissue recovery, programs should reference AATB Guidance Document No. 2 *"Prevention of Contamination and Cross-contamination at Recovery: Practices & Culture Results Requirement"* and follow AATB Standard D5.500.

Good Tissue Practice 8: With respect to tissue excision techniques to reduce contamination, programs should reference AATB Guidance Document No. 2 *"Prevention of Contamination and Cross-contamination at Recovery: Practices & Culture Results Requirement"* and follow AATB Standards D5.300/D5.310.

Good Tissue Practice 9: With respect to personnel that may have serious medical illnesses, programs should follow AATB standard J3.720.

Good Tissue Practice 10: With respect to personnel that have open skin lesions, programs should follow AATB standard J3.720.

C. Microbial Sampling

Good Practice Statement 11: Microbial testing by sampling and culturing of the tissue, pre- and post-processing, is the most direct measure of microbial contamination and should be performed. Evidence is lacking to support incremental tissue safety associated with the use of post-mortem

donor blood cultures as an additional screening test. Post-mortem donor blood cultures are not required or recommended.

Good Tissue Practice 12: The use of elution and swabbing methods to obtain samples for microbial testing avoids destructive testing and should be considered for most tissue applications.

Good Tissue Practice 13: Microbial identification testing should be performed on all positive cultures to determine the genus and species present during tissue recovery, processing, environmental monitoring and final sterility testing and as part of a program's quality management monitoring system.

Good Tissue Practice 14: Any pathogen found that cannot be eliminated during processing makes the tissue unacceptable for transplant and the pathogens should be documented in policies and procedures.

Good Tissue Practice 15: Programs should have documented policies and procedures for assessment of microorganisms isolated from tissue and whether the tissue is to be discarded or can be released for transplantation purposes.

D. Processing of Musculoskeletal Tissue

Good Tissue Practice 16: Programs should determine the effectiveness of their musculoskeletal mechanical and chemical cleaning, disinfection and sterilization processes on bioburden load; when establishing a process procedure, periodically and when introducing changes to the tissue processing procedures.

Good Tissue Practice 17: Programs using disinfection method for aseptically processed grafts should provide with their tissue, in addition to labeling, educational materials (e.g. package insert) that define "aseptic" and indicate that it does not guarantee or claim sterility as achieved by terminal sterilization.

Good Tissue Practice 18: Programs using sterilization should document the Sterility Assurance Level (SAL) attained.

E. Processing of Cardiac Tissue

Good Tissue Practice 19: If technology moves toward providing decellularized cardiac tissue instead of cryopreserved cardiac tissue, the impact of the decellularization process on bioburden should be assessed.

F. Processing of Skin Tissue

Good Tissue Practice 20: Disinfection procedures for split thickness skin grafts for burn treatment should optimize and maintain an acceptable level of cellular viability to support desired outcomes.

Good Tissue Practice 21: Programs using antibiotic incubation for bioburden reduction should consider scientific evidence when determining dosage, incubation temperature, and duration to optimize disinfection while maintaining cellular viability. Process-specific validation studies should assess, and results support, the dosage, temperature, and incubation duration.

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1.0 INTRODUCTION

Definition: Bioburden is number of contaminating organisms found on a given amount of material.[2] *This concept is important in the area of tissue donation and use.*

The transplantation of human tissue allografts introduces the risk of various complications to the recipient, including the fatal and nonfatal transmission of infectious organisms such as bacteria, fungi, viruses, parasites, and prions.

A review of the international literature by Project NOTIFY[3] demonstrated that disease transmission has occurred and remains a risk in allograft transplantation. Disease transmission has been confirmed following transplantation of tissues that are fresh, frozen, or cryopreserved. Transmitted disease has not been reported for more than 20 years as a result of processed, freeze-dried allografts (except dura) using a validated process that reduces or eliminates microorganisms or that can inactivate viruses. Many of the allografts produced in Canada are fresh, frozen, or cryopreserved and do not undergo extensive processing or sterilization.[4]

Health Canada regulates the processing of tissue grafts and references within those regulations tissue standards published by the Canadian Standards Association (CSA).[5] Health Canada has published guidelines in the document, "*Guidance Document for Cell, Tissue and Organ Establishments - Safety of Human Cells, Tissues and Organs for Transplantation,*"[6] that outlines required information, processes, and tests that must be performed before tissue can be stored at a tissue bank for transplantation. These guidelines provide detailed information and specific requirements relating to the entire spectrum of tissue production covering donor selection; donor screening; microbial and infectious disease testing; tissue recovery; and tissue production, packaging, storage, and distribution.

Health Canada regulations state "An establishment must have documented evidence that demonstrates that the activities, processes and technical procedures that it uses in processing cells, tissues and organs will consistently lead to the expected results". However, no guidance

[2] American Association of Tissue Banks, Definition of Terms. 2013. 13th Edition Standards for Tissue Banking

[3] Project Notify; World Health Organization (WHO). 2011. Notify exploring vigilance notification for organs, tissues and cells. www.notify.library.org

[4] Canadian Blood Services, Environmental Scan of Bioburden Reduction and Control Practices in Tissue Banking. May 2015. Available at https://professionaleducation.blood.ca/en

[5] Canadian Standards Association, Z900.2.2-12 Tissues for Transplantation and Z900.1-12 General Standards for Cells, Tissues and Organs for Transplantation. Available at: http://shop.csa.ca/en/canada/transplantation/cancsa-z9001-12/invt/27017362012

[6] Available at: http://www.hc-sc.gc.ca/dhp-mps/brgtherap/reg-init/cell/cto_gd_ld-eng.php

or information on specific requirements for the disinfection process is provided. Each tissue bank may therefore employ its own method to disinfect tissue.

A Canadian Blood Services survey of US, Canadian, European and Australian tissue bank practices revealed significant variation in the disinfection (bioburden reduction) methods used and significant variation in the quality processes and validations employed to ensure those methodologies are effective[4].

In April 2008, Canada's federal, provincial, and territorial governments gave Canadian Blood Services a mandate for organ and tissue donation and transplantation, including developing leading practices. In 2012, Canadian Blood Services facilitated an eye and tissue banking workshop focused on standardized specifications and practices. At the workshop, the Canadian tissue community directed Canadian Blood Services to facilitate an evidence-based leading practice initiative to develop a national consensus on bioburden reduction and control guidelines for implementation as standard practice within Canadian musculoskeletal, cardiac, and skin banks.[7]

Data on tissue allograft use and procurement in Canada:

- From a Canadian Blood Services 2012 survey of 220 Canadian hospitals 35% reported on utilization of tissue allografts[8].
- In 2015, Canadian eye and tissue banks produced and released 16,105 allografts for transplant.[9]
- In 2015, approximately 20,000 surgical allografts, the vast majority being advanced highly processed grafts, were imported into Canada from tissue banks in the USA.[10] Most were sterilized and therefore the risk of bacterial contamination was highly unlikely.
- Canadian allograft production is limited to more basic grafts, many of which are minimally processed with or without disinfection (as opposed to sterilized)[4], a very low but a remaining risk for transmitting infectious disease.[3]
- In 2015, Canadian tissue banks produced: 7,679 musculoskeletal grafts, 5,563 ocular grafts, 2,371 split thickness skin grafts, 221 cardiac grafts, and 271 amnion grafts.[9]

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[7] Canadian Blood Services. Report - Eye and Tissue Banking in Canada: A Leading Practice Workshop. 2012. Available at: http://www.organsandtissues.ca/s/english-expert/leading-practices-public-awareness-and-education-2

[8] Haun M. Eye and tissue banking in Canada: Where are we headed? 2012 November. Available at: www.lhsc.on.ca/lab/bldbank/assets/LLSGSymposium12/ORBCoN%20London%20Nov%203%202012%20v2.pdf

[9] Canadian Blood Services, Report – Canadian Eye and Tissue Banking Statistics January 1 to December 31, 2015. 2016. Available at https://professionaleducation.blood.ca

[10] Canadian Blood Services. Canadian Imported Surgical Allograft and Acellular Matrix Study. 2013. Available at: http://www.organsandtissues.ca/s/wp-content/uploads/2012/09/CBS-2013-Summary-of-Findings-Costs-of-Importation-of-Musculoskeletal-Allografts-and-Acellular-Dermal-Matrix.pdf

A 2015 survey[9] of 16 Canadian tissue banks in ON (5), AB (3), BC (2), MB (2), SK (1), QC (1), NS (1) and NB (1) revealed the following characteristics of these tissue banks, including eye banks (Table 1): **TABLE 1: Canadian Tissue Bank Characteristics 2013**

Bank Type	Description	# Responses
Multi-tissue banks	Programs that recover two or more distinct tissue types from deceased donors (MS, CV, skin or ocular) - may also recover surgical bone	6
Surgical bone banks	Programs that recover from surgical (living) donors only	1
Tissue banks	Programs that recover one type of tissue from deceased donors excluding ocular (MS, CV or skin) - may also recover surgical bone	5
Eye banks	Programs that only recover ocular tissue	4

2.0 OBJECTIVES

Our aim was to develop, through an evidence-informed consensus process, leading practice guidelines for bioburden reduction and control processes. These guidelines are for implementation as standard practices at Canadian tissue banks in tissue recovery, microbial sampling, and the processing of tissues (musculoskeletal, cardiac, and skin).

2.1 Core Assumptions

- Bioburden reduction as a core component of allograft processing requires the identification of evidence-informed leading practices.
- Collaboration across stakeholder groups is essential to shape a solution that will work for all parties.
- An incremental phased approach is essential to the development and implementation of a standardized approach to bioburden reduction and control.
- The focus is on biomedical considerations (ethical considerations are out of scope).
- The development of standardized recommendations is essential.

2.2 Key Considerations

- Tissue banking is biological manufacturing. The application of biological manufacturing processes, and more specifically validated bioburden reduction methods, within the Canadian tissue community varies.
- Changing current practices and processes to align with recommendations may create challenges for tissue banks.
- Bioburden reduction processes within biological manufacturers may be considered proprietary and evidence on these processes may not be detailed in the public domain.
- Systematic reviews will align with the GRADE process for evidence evaluation and recommendations will align with the AGREE II process for guideline development.

2.3 Scope

Table 2 displays topics that were considered to be in scope and out of scope for this project.

TABLE 2: Topics that were In Scope and Out of Scope for the Project

IN SCOPE	OUT OF SCOPE
• Systematic reviews of the published literature • Analysis of leading Canadian, American, European and Australian commonly practiced bioburden reduction recovery steps and validated tissue processing methodologies • Review of relevant standards and regulations • Review of surveillance reporting of tissue related disease transmissions • Professional education and practice adoption strategy including developing recommendations for amendments to Canadian standards	• Processes associated with ocular tissue recovery, processing, or storage • Surgical bone banking • Transmissible disease testing • Processes associated with acellular dermal matrix recovery or processing • Processes associated with amniotic membranes • Processes associated with environmental monitoring

3.0 PROCESS OVERVIEW AND METHODS

Subject matter experts from Canada and the USA came together as a Bioburden Steering Committee (Table 3). The aim was to develop Canadian guidelines to reduce bioburden in important steps in tissue graft production, including: (a) tissue recovery, (b) microbial sampling, (c) processing of musculoskeletal tissue, (d) processing of cardiac tissue, and (e) processing of skin tissue.[11] Steering Committee members were chosen as subject matter experts in: biological manufacturing; musculoskeletal, cardiac, and/or skin tissue banking; microbiology and infectious diseases; validation methodologies; quality / process improvement leadership; and standards and regulations.

TABLE 3: Bioburden Steering Committee Members

Members	Affiliation
Dr. Jutta Preiksaitis (Chair)	Provincial Laboratory for Public Health; Professor, Division of Infectious Diseases; University of Alberta, Edmonton, AB
Mr. Scott Brubaker	Senior VP of Policy, American Association of Tissue Banks; McLean, VA
Dr. Jeannie Callum	Director of Transfusion Medicine and Tissue Banks; Sunnybrook Health Sciences Centre Blood and Tissue Bank; Toronto; ON
Dr. Graeme Dowling	Medical Director, Comprehensive Tissue Centre, Alberta Health Services
Dr. Ted Eastlund	Eastlund Consulting; Terrero, New Mexico
Dr. Margaret Fearon	Medical Director, Medical Microbiology; CBS, Toronto, ON
Dr. Marc Germain	VP Medical Affairs, Héma-Québec; Saint-Foy, QC
Dr. Michael Gross*	Medical Director, Regional Tissue Bank; Halifax, NS
Mrs. Cynthia Johnson	Quality Leader, Regional Tissue Bank; Halifax, NS
Mr. Ken Lotherington	Senior Manager, Donation and Transplantation, CBS; Halifax, NS
Mr. Ken McTaggart	Associate Director, Product and Process Development, CBS; Ottawa, ON
Mr. Jim Mohr	Senior Advisor, Donation and Transplantation; CBS; Halifax, NS
Dr. Michael Strong	University of Washington, School of Medicine; Seattle, WA
Mr. Martel Winters	Senior Scientist, Nelson Laboratories; Salt Lake City, UT
Ms. Kimberley Young	Director, Donation and Transplantation Program; CBS; Edmonton, AB
Mr. Jie Zhao	Comprehensive Tissue Centre, Alberta Health Services; Edmonton, AB

*Participated in the initial work of the Steering Committee

[11] No conflicts-of-interest were identified on disclosure forms completed by members of the Committees.

3.1 Project Phases

The project included four phases.

Project Phase 1: Committees, Working Groups, and Evidence Base

The Steering Committee was convened and began its deliberations, ensuring that there was a rigorous evidence base for the work. To serve as a foundation, evidence was gathered via:

a) Five systematic reviews of the literature conducted by researchers at McMaster University in Hamilton, Ontario.

b) An environmental scan of current practice (Canada, the USA, the European Union, and Australia) issued by Canadian Blood Services in May 2015.

c) An analysis of related standards and regulations issued by Canadian Blood Services in January 2015.

d) An overview of disease transmission in the transplantation of musculoskeletal, cardiovascular, and skin allografts issued by Canadian Blood Services in January 2015.

For each of the five key topic areas, the Steering Committee formed working groups and appointed additional subject matter experts from Canada and the USA (Appendix 2). Working Group members developed evidence-informed leading practice recommendations, good tissue practice statements, and research priorities that were then presented to the Steering Committee for consideration, discussion, and revision.

Project Phase 2: Consensus Meeting

Phase two convened the Steering Committee for a consensus meeting on May 10, 2016. Members reviewed, refined, and formally adopted the recommendations, good tissue practice statements, and research priorities put forth by the Working Groups as Leading Practice Guidelines. Members identified and approved amendments to Canadian standards, supported by the recommendations, for consideration by the Canadian Standards Association Technical Committees.

Project Phase 3: Community Consultation

Phase three provided the guidelines, final recommendations, good tissue practices, and research priorities to the Canadian tissue community. Community members were asked to identify their support for the recommendations. Members unable to support a recommendation were asked to detail reasons. Community responses were reviewed by the Steering Committee and incorporated in the final report.

Project Phase 4: Professional Education

Phase four will be the execution of the professional education strategy to the Canadian tissue community including: (a) publication of the systematic reviews, (b) publication of the environmental scan, (c) publication of the final recommendations report, (d) submission of recommendations for amendments to the Canadian Standards Association Tissue Standards and (e) submission of abstracts and manuscripts to peer reviewed journals.

3.2 Guideline Review

The recommended guidelines generated by this exercise will be considered to be valid until March 31, 2020; five years following the systematic reviews of the literature. Review and revision of the guidelines is recommended in 2020 to incorporate literature published after March 31, 2015.

It was essential to the development team that evidence and rigorous practices formed the foundation for the deliberations. The guideline development tool "AGREE II" was therefore employed to assess the completeness of the reporting in this project (Appendix 3).

4.0 RECOMMENDATIONS AND GOOD TISSUE PRACTICES

For each of the five topic areas examined, recommendations and good tissue practices[12] were developed (section 4.0), as were research priorities (Section 5.0). Table 4 displays the numbers of recommendations, good practice statements, and research priorities per topic.

TABLE 4: Material presented by topic

Topic	# Research questions	# Recommendations	# Good tissue practices	# Research priorities
Global *	0	5	2	0
Tissue recovery	14	0	8	13
Microbial sampling	11	2	5	7
Processing of musculoskeletal tissue	6	3	3	7
Processing of cardiac tissue	5	1	1	5
Processing of skin tissue	7	2	2	7
System*	0	3	0	0
TOTALS	42	16	21	39

*A number of recommendations and good tissue practices were applicable to multiple topic areas and these were reframed as global recommendations. Discussions also generated recommendations for system improvement.

[12] A recommendation was generated when comparative evidence supported a practice preferred over another practice. A good tissue practice was generated when the evidence informed a practice in the absence of a comparative practice.

4.1 Tissue Recovery

Evidence identified in the systematic reviews was not sufficient to inform specific recommendations. Good tissue practices were developed, often informed by American Association of Tissue Banks (AATB) guidance documents and standards. AATB guidance documents and standards were formed from both consensus opinion and evidence review. For example the American Association of Operating Room Nurses Guidelines has their foundation in evidence and informed the AATB guidelines.

4.1.1 QUESTION #1: Does the evidence identify a maximum ischemic time from asystole to skin prep that should be recommended for adoption or consideration by the Canadian tissue community?

Recommendation

NONE

Good Practice Statement

When determining the maximum ischemic time from asystole to skin prep, programs should reference AATB Guidance Document No. 7 "*Evaluation of Body Cooling*", and follow AATB Standard D5.400

***Rationale*:** There was insufficient evidence to inform a recommendation. The literature was limited and low to very low quality. It was not helpful with respect to determining whether the time interval from asystole to tissue recovery (warm ischemic time) is a predictor of contamination; however, studies have indicated that, in general, a reduction in time from asystole to tissue recovery may be an important factor to decrease contamination. A 2002 study demonstrated that the risk of blood contamination increased each hour following asystole (cessation of heart beating) suggesting that post-mortem time to recovery should be kept to a minimum. Similarly, when reported, the warm ischemic time has been kept to a minimum as some studies have shown that cooling the body may reduce bioburden. At lower temperatures, the growth rate of many bacteria is diminished or stopped completely. AATB Standards provide timelines with respect to performing the skin prep and CSA Cells, Tissues and Organs (CTO) Tissue for Transplant standards require established time and temperature constraints for recovery. In the environmental scan, all Canadian, American, and European tissue banks, plus the Australian tissue bank surveyed, reported performing skin prep within the time limits set by the AATB.

Assessment:

Problem Priority: Low

Benefit/Harm Analysis: No harm was identified

Resource Use and Feasibility: It is reasonable to indicate that the good practice statement is feasible to follow. Research would require funding, personnel, and time

Anticipated Acceptability to the Community: Yes

4.1.2 QUESTION #2: Does the evidence identify a preferred time frame for body cooling?

Recommendation

NONE

Good Practice Statement

When developing standards of practice related to time requirements for body cooling, programs should reference AATB Guidance Document No. 7 "*Evaluation of Body Cooling",* and follow AATB Standard D5.400.

Rationale: There was insufficient evidence identified to inform a recommendation. The limited evidence available was specific to cardiac tissue and there were no relevant survey questions in the environmental scan. The AATB has relevant standards and a guidance document that informed discussion. When reported, some studies have shown that cooling the body may reduce bioburden. At lower temperatures, the growth rate of many bacteria is diminished or stopped. Ideally, recovery should begin as soon as possible post-asystole; however, many factors play a role in delays that occur before tissue recovery can begin. Untoward delay, especially in the absence of body cooling, has led to system failures and resulted in transmission of bacterial infections (*Clostridium sordellii*) and the death of a tissue allograft recipient. In this later case, the donor body was not cooled for 19 hours after death (asystole), subjected to a short period of cooling (4 hours), and tissue recovery began about 23.5 hours after death.

Assessment:

Problem Priority: Low

Benefit/Harm Analysis: No harm was identified; however there is a potential risk/harm of not adopting the good practice statement.

Resource Use and Feasibility: None

Anticipated Acceptability to the Community: Yes

4.1.3 QUESTION #3: Does the evidence identify a preferred length of recovery for controlling bioburden?

Recommendation

NONE

Good Practice Statement

NONE

Rationale: There was insufficient evidence to inform a recommendation. Ideally, recovery should be done methodically, and with sufficient staff, to ensure appropriate surgical technique without unduly extending the recovery time.

4.1.4 QUESTION #4: How do any of the variables (1 to 3 above) correlate to bioburden, e.g., donor skin condition (cuts or abrasions); presence of medical interventions; cleanliness of skin; trauma; and compound fractures?

Recommendation

NONE

Good Practice Statement

With respect to donor skin condition, programs should reference AATB Guidance Document No. 2 *"Prevention of Contamination and Cross-contamination at Recovery: Practices & Culture Results Requirement"* and follow AATB Standard D5.500 IV.

Rationale: There was insufficient evidence to inform a recommendation; however, AATB Standards D5.500 and "*Appendix IV Prevention of Contamination and Cross-Contamination at Recovery: Practices & Culture Results, AATB Guidance Document No 2 Requirement*" provide direction to reduce contamination at tissue recovery.

Assessment:

Problem Priority: None

Benefit/Harm Analysis: None

Resource Use and Feasibility: None

Anticipated Acceptability to the Community: Yes

4.1.5 **QUESTION #5**: Does the evidence identify a preferred skin prep procedure?

Recommendation

NONE

Good Practice Statement

When developing protocols for skin preparation, programs should reference AATB Guidance Document No. 2 *"Prevention of Contamination and Cross-contamination at Recovery: Practices & Culture Results Requirement"* and follow AATB Standard D5.500.

***Rationale**:* There was insufficient evidence to inform a recommendation. There were three clinical studies of low and very low quality evidence. According to the environmental scan, Canadian and American tissue banks report similar types of skin disinfectants applied prior to tissue recovery. Chlorhexidine is the most commonly used skin disinfectant for skin recoveries; however, Canadian programs use it less often than do American programs for bone, connective tissue, and cardiovascular recoveries. It's common that alcohol is used in Canada and the USA to prep the skin prior to recovery of all tissue types. AATB Standards D5.500 and "*Appendix IV Prevention of Contamination and Cross-contamination at Recovery: Practices & Culture Results Requirement*" provide direction regarding skin preparation.

Assessment:

Problem Priority: Low

Benefit/Harm Analysis: None

Resource Use and Feasibility: Adoption of a safe alternative skin prep solution would not have significant impact on Canadian tissue banks; any research would require funding, personnel, and time

Anticipated Acceptability to the Community: Yes

4.1.6 QUESTION #6: What are the most effective physical barriers that can be used to reduce contaminating tissue at recovery?

Recommendation

NONE

Good Practice Statement

In consideration of protective barriers to use at tissue recovery programs should reference AATB Guidance Document No. 2 *"Prevention of Contamination and Cross-contamination at Recovery: Practices & Culture Results Requirement"* and follow AATB Standard D5.500.

***Rationale**:* There was insufficient evidence to inform a recommendation. A contributing factor to contamination during recovery is bioburden introduced by staff. In the environmental scan, common practices were reported by all Canadian, American, and European recovery services and tissue banks. Most programs require recovery room staff to wear the same protective and barrier attire worn by hospital operating room (OR) staff and also require double gloving.

Assessment:

Problem Priority: Low

Benefit/Harm Analysis: None

Resource Use and Feasibility: Tissue banks are currently following general OR standards; any research would require funding, personnel, and time.

Anticipated Acceptability to the Community: Yes

4.1.7 QUESTION #7: What is the most effective process (i.e., order of steps) to reduce contamination when prepping the skin of a donor?

Recommendation

NONE

Good Practice Statement

NONE

Rationale: There was insufficient evidence to inform a recommendation.

4.1.8 QUESTION #8: What are the most effective tissue excision techniques to reduce contamination?

Recommendation

NONE

Good Practice Statement

With respect to tissue excision techniques to control contamination and cross-contamination, programs should reference AATB Guidance Document No. 2 *"Prevention of Contamination and Cross-contamination at Recovery: Practices & Culture Results Requirement"* as well as follow AATB Standards D5.300/D5.310.

***Rationale**:* There was insufficient evidence to inform a recommendation. However, there is relevant information contained in AATB Standards D5.300 and D5.310 as well as in *"Appendix IV Prevention of Contamination and Cross-contamination at Recovery: Practices & Culture Results Requirement*."

Assessment:

Problem Priority: Low

Benefit/Harm Analysis: None

Resource Use and Feasibility: Tissue banks are currently following general OR standards; any research would require funding, personnel, and time

Anticipated Acceptability to the Community: Yes

4.1.9 QUESTION #9: What is the most effective storage condition for preventing contamination?

Recommendation

NONE

Good Practice Statement

NONE

***Rationale**:* There was insufficient evidence to inform a recommendation. The environmental scan revealed that tissue banks and tissue recovery services in Canada, the USA, and Europe ship the recovered whole heart to the heart valve processor on wet ice to keep the heart cold; however, there is variation in the transport medium used. In practice, other tissue types are shipped in a similar manner.

4.1.10 QUESTION #10: What are the most effective tissue handling practices to reduce contamination?

Recommendation

NONE

Good Practice Statement

NONE

***Rationale**:* There was insufficient evidence to inform a recommendation.

4.1.11 QUESTION #11: Does preceding tissue donation with an autopsy or organ donation increase contamination?

Recommendation

NONE

Good Practice Statement

NONE

***Rationale**:* There was insufficient evidence to inform a recommendation. Some USA tissue banks collect this information and data sharing would be valuable.

4.1.12 QUESTION #12: Can the personal hygiene or cleanliness of the recovery staff affect tissue bioburden?

Recommendation

NONE

Good Practice Statement

NONE

***Rationale**:* There was insufficient evidence to inform a recommendation.

4.1.13 QUESTION #13: Can an acute illness such as exhibiting flu-like symptoms (e.g., upper respiratory, lower gastrointestinal, a fever that causes sweating, or an illness that can affect clear thinking) affect tissue bioburden?

Recommendation

NONE

Good Practice Statement

With respect to personnel that may have serious medical illness, programs should follow AATB standard J3.720

***Rationale**:* There was insufficient evidence to inform a recommendation. There is a theoretical risk that contaminants could be introduced into the field and/or onto the tissue. The adoption of consistent program-driven standards related to recovery staff illness is a reasonable approach to reducing the risk while meeting the corporate human resource policies of a tissue program.

Assessment:

Problem Priority: Low

Benefit/Harm Analysis: There is no risk in this statement

Resource Use and Feasibility: None

Anticipated Acceptability to the Community: Yes

4.1.14 QUESTION #14: Can the presence of open lesions on recovery personnel affect tissue bioburden?

Recommendation

NONE

Good Practice Statement

With respect to personnel that have open lesions, programs should follow AATB standard J3.720.

***Rationale**:* There was insufficient evidence to inform a recommendation. There is a theoretical risk that contaminants could be introduced into the field and/or onto the tissue or personnel could become infected from microorganisms on the tissue.

Assessment:

Problem Priority: Low

Benefit/Harm Analysis: There is no risk in this statement

Resource Use and Feasibility: None

Anticipated Acceptability to the Community: Yes

4.2 Microbial Sampling

4.2.1 QUESTION #1: Do post-mortem blood cultures provide relevant evidence of the bioburden contamination of donor tissue? Sub questions: (a) Under what circumstances is the collection of post-mortem blood cultures clinically relevant or necessary? (b) What is the optimal time post-mortem for collection? (c) What is the optimal method to prepare and draw post-mortem blood? (d) What is the optimal donor site to obtain blood cultures? and (e) Is there evidence that positively correlates positive blood cultures with positive preprocessing cultures, and do the quantitative culture results impact the result of preprocessing culture results?

Recommendation

NONE

Good Practice Statement

Microbial testing by sampling and culturing of the tissue, pre- and post-processing, is the most direct measure of microbial contamination and should be performed. Evidence is lacking to support incremental tissue safety associated with the use of post-mortem blood cultures as an additional screening test. Post-mortem donor blood cultures are not required or recommended.

***Rationale**:* Donor post-mortem blood cultures may detect: (a) occult ante-mortem bacteremia, (b) translocation of organisms from mucosal surfaces post-mortem, or (c) contamination of the blood sample during the collection of the sample. Very limited, low-to-moderate quality evidence suggests that post-mortem blood cultures have both poor positive and poor negative predictive values for determining tissue bioburden when direct assessment of bioburden by swabbing is the gold standard. Moreover, only anecdotal case reports have presented data to suggest that post-mortem blood cultures detect clinically relevant bacteremia and potential tissue contamination not detected by direct culture of tissue. Quantitative measurements are not directly available from the published literature but available reports suggest that post-mortem blood cultures (versus direct tissue culturing alone) would result in additional tissue loss through discard or need for additional processing such as irradiation. In summary, evidence is lacking to support incremental tissue safety associated with the use of post-mortem blood cultures as an additional screening test.

Assessment:

Problem Priority: Moderate

Benefit/Harm Analysis: No harms were identified

Resource Use and Feasibility: Improve program resources, less testing, and risk of discarding

suitable tissue due to false negative tests.

Anticipated Acceptability to the Community: Yes

4.2.2 QUESTION #2: Does the evidence identify a preferred sample collection method for culture testing, process, or parameters that should be recommended for adoption or consideration by the Canadian tissue community?

Recommendation

Programs should validate the sensitivity of sampling methods and specificity of testing methods used to obtain or test specimens for microbial culture to assess tissue bioburden.

Good Practice Statement

The use of elution and swabbing methods to obtain samples for microbial testing avoids destructive testing and should be considered for most tissue applications.

***Rationale**:* Each sampling method has strengths and weaknesses. To determine preferred sampling methods, direct comparisons of methods and a "gold standard" or "true value" is required. Spiked cultures using appropriate organisms, sample sizes, and controls are just a few examples of the considerations necessary when validating sampling methods. In clinical or laboratory cohort studies this gold standard is lacking, making sensitivity, specificity , positive and negative predictive values, accuracy, and precision of the sampling plan and culture methods difficult to determine. While some methods are better documented than others, the evidence does not support a preferred plan and method for any of the tissue types. Even when tissue was artificially contaminated with microbes, the results varied amongst contaminating pathogens and methods. Another variable that was not consistently evaluated or considered in most studies was the influence that microbial culturing technique has on the specificity, detectability of microbes, regardless of the sampling method employed. Multiple combinations of sampling methods and microbial culturing techniques make the isolation of the sample type for comparison difficult. Inhibition could also play a role in the variability of study results, leading to false negatives. A number of studies pointed out false negatives but only two calculated negative predictive values for their methodologies. Comparative analyses of culture testing methodologies with standardized quantitative assessment are not available and therefore no recommendation as to a preferred culturing methodology could be made for any tissue type.

Assessment:

Problem Priority: Moderate to high

Benefit/Harm Analysis: The benefits outweigh the resource challenges

Resource Use and Feasibility: These recommendations may pose a resource challenge

to smaller programs in relation to the performance of sampling validation, but they are appropriate for implementation using internal resources or contracting with experts

Anticipated Acceptability to the Community: Yes

4.2.3 QUESTION #3: Does the time of sample collection impact bioburden risk?

Recommendation

NONE

Good Practice Statement

NONE

Rationale: There was insufficient evidence to inform a recommendation. The literature review did not identify any evidence related to the time of sampling and the impact on culture test results. Culture testing is required at any point where the tissue is exposed to a risk of contamination. In addition, appropriate monitoring and validations must be conducted in all processing and packaging processes.

4.2.4 QUESTION #4: How does the location of sampling site impact culture results?

Recommendation

SEE THE RECOMMENDATION IN 4.2.3

Good Practice Statement

NONE

Rationale: There was insufficient evidence to inform a recommendation. The literature review did not identify any evidence (as related to the impact on bioburden assessment and patient outcome risk) related to: (a) time of sampling; (b) location of biopsy sites, i.e., tissue sampling; (c) location(s)/surfaces of the swab sampling collected for microbial testing; or (d) sample size when only a portion of the tissue was selected for culture testing, i.e., tissue sampling and swabbing. Previous recommendations in this report support the need to validate a program's sampling methodology.

4.2.5 QUESTION #5: What evidence supports or negates the presence of bacterial contamination?

Recommendation

Programs should validate the sensitivity of sampling methods used to obtain specimens for microbial culture to assess tissue bioburden.

Good Practice Statement

NONE

***Rationale**:* Most literature did not consider the sensitivity of the culturing techniques used although there was mention of factors that could affect culture results, e.g., type of media, incubation time, and plating method. The research priorities are intended to provide evidence that supports appropriate and effective methods for each tissue type.

Assessment:

Problem Priority: Moderate to High

Benefit/Harm Analysis: Benefits outweigh the resource challenge

Resource Use and Feasibility: Implementation is feasible either internally or from contracted experts.

Anticipated Acceptability to the Community: Yes

4.2.6 QUESTION #6: What evidence supports or negates qualitative versus quantitative culture analysis and are there advantages of performing quantitative bacterial and fungal bioburden testing over qualitative testing?

Recommendation

None

Good Practice Statement

Microbial identification testing should be performed on all positive cultures to determine the genus and species present during tissue recovery, processing, environmental monitoring and final sterility testing as part of a program's quality management monitoring system.

***Rationale**:* Very few studies quantified the identified microorganisms. Most provided a contamination rate along with species determination. The literature did not identify the advantages of quantitative versus qualitative measurements of microorganisms; however, the qualitative identification of microorganisms is necessary to meet regulations for some tissues. Furthermore, both qualitative and quantitative assessment of microorganisms is necessary if a program is attempting to determine their sources of contamination, effectiveness of their procedures, monitoring requirements of their quality assurance system, and the sensitivity of their culturing methods. Although each program should develop quantitative and qualitative data for its own processes and methods, a national study could provide a useful reference for smaller programs that do not have the necessary volume or diversity of tissues to determine a suitable microbial culturing method. Also, sampling methods affect the qualitative accuracy of bioburden measurement and therefore should be selected based on a validated study specific to the program.

Assessment:

Problem Priority: Moderate to High

Benefit/Harm Analysis: Benefits outweigh the resource challenge

Resource Use and Feasibility: This would require national coordination and participation of multiple programs

Anticipated Acceptability to the Community: Yes

4.2.7 QUESTION #7: Are there quantitative limits of bacterial and fungal bioburden levels found during tissue recovery that should preclude further tissue processing?

Recommendation
NONE

Good Practice Statement
NONE

***Rationale**:* There was insufficient evidence to inform a recommendation.

4.2.8 QUESTION #8: What testing method most accurately determines the quantification of bacterial and fungal load?

Recommendation

NONE

Good Practice Statement

NONE

Rationale: There was insufficient evidence to inform a recommendation. Only two papers in the literature review tested bioburden load (quantified microorganisms) and neither suggested a superior testing method. The reported range of microorganisms suggests that the variance among tissue samples could be significant, which could translate into mixed clinical outcomes.

4.2.9 QUESTION #9: Which method determines the origin of contamination?

Recommendation

NONE

Good Practice Statement

NONE

Rationale: There was insufficient evidence to inform a recommendation.

4.2.10 QUESTION #10: How are testing results categorized (true positives vs. false positives vs. false negatives)?

Recommendation

Testing laboratories should determine the specificity of their final culture testing methods and quantify the negative predictive value, i.e., the probability of a true negative culture result.

Good Practice Statement

NONE

Rationale: Although programs may elect to determine the positive predictive value (probability of a true positive culture result) in order to avoid the unnecessary discard of suitable tissue, it is a greater priority to risk the release of tissue that tests negative for microorganisms when it is actually positive. It is unlikely that a program will have 100% negative predictive values; however, such an analysis will allow programs to understand the sensitivity and limitation of their testing and the inherent risk to the program and the recipient. (In the literature review, two papers examined the negative predictive values of their culture methods.)

Assessment:

Problem Priority: Moderate to high

Benefit/Harm Analysis: Benefits outweigh the resource challenge.

Resource Use and Feasibility: Implementation is feasible either internally or from contracted experts.

Anticipated Acceptability to the Community: Yes

4.2.11 QUESTION #11: Which pathogens are considered significant, precluding tissue from further decontamination steps? Which pathogens found during (a) tissue recovery or (b) tissue processing should preclude any further tissue processing or product release?

Global Recommendation

For each tissue type, programs should consult microbiology experts and other industry experts to identify a comprehensive list of microbes that necessitate tissue discard when identified in the transport solution or at any processing stage. The list shall include, but be not limited to, *Clostridium* spp, *Streptococcus pyogenes*, *Staphylococcus aureus* and fungi. Pathogens that render tissue unacceptable for transplant should be documented in policies and procedures.

Good Practice Statements

- Programs should have documented policies and procedures for assessment of microorganisms isolated from tissue and whether the tissue is to be discarded or can be released for transplantation purposes.
- Any pathogen found that cannot be eliminated during processing makes the tissue unacceptable for transplant and the pathogens should be documented in policies and procedures.

***Rationale**:* Although some standards and regulations specify lists of microorganisms that are acceptable for release without disinfection, or that mandate tissue discard prior to processing, these lists of organisms appear to be largely generated by consensus opinion rather than being evidence-based. However, a number of pathogens are associated with severe morbidity and mortality and it is unlikely that additional data will be generated with respect to their pathogenicity classification in the tissue transplant setting. Standardizing lists of such pathogens that would require tissue discard within Canadian programs (by tissue type) would be a reasonable goal. The number of proven cases of tissue-transmitted infection by pathogens within the scope of this project is small. Ongoing surveillance initiatives such as project NOTIFY, combined with clear definitions of possible, probable, and proven tissue-transmitted infections, would be important to inform updates to these lists.

Assessment:

Problem Priority: Low

Benefit/Harm Analysis: Benefits outweigh the resource challenge.

Resource Use and Feasibility: Implementation is feasible either internally or from contracted experts.

Anticipated Acceptability to the Community: Yes

4.3 Processing of Musculoskeletal Tissue

4.3.1 QUESTION #1: Does the evidence identify a cleaning method or methodology for processing of musculoskeletal tissue that should be recommended for adoption or consideration by the Canadian tissue community?

Recommendation

NONE

Good Practice Statement

Programs should determine the effectiveness of their musculoskeletal mechanical and chemical cleaning, disinfection and sterilization processes on bioburden load when establishing a process procedure, periodically and when introducing changes to the tissue processing procedures.

Rationale: There was insufficient evidence to inform a recommendation. Following tissue recovery, most programs, will clean the allograft to remove extraneous tissues. This improves the aesthetic of the allograft, reduces operating time that would have been spent preparing the tissue at the time of surgery, and reduces immunogenicity. While a number of studies reported on cleaning processes, the impact of the cleaning processes on bioburden and subsequent bioburden reduction processes was not assessed. An international environmental scan of tissue banks detailed a variety of cleaning methodologies including mechanical and chemical processes and the use of alcohol, hydrogen peroxide, and detergents. The direct impact of cleaning processes on bioburden is unknown. The impact of residual chemicals from cleaning solutions on subsequent bioburden processes is unknown; residual solutions could potentially have a deleterious effect. Understanding the impact of cleaning processes could inform more effective bioburden reduction practice.

Assessment:

Problem Priority: Low to moderate

Benefit/Harm Analysis: No harms were identified

Resource Use and Feasibility: This statement may pose a resource challenge to smaller programs but it is appropriate as a leading practice using either internal resources or contracting with experts

Anticipated Acceptability to the Community: Yes

4.3.2 Question #2: Does the evidence identify a preferred decontamination method, process, or parameter for musculoskeletal tissue that should be recommended for adoption or consideration by the Canadian tissue community?

Global Recommendation

Disinfection procedures for musculoskeletal and cardiac tissue must be validated with quantification of log reduction, using challenge organisms. Qualitative analysis, such as calculation of discard and/or contamination rates, is acceptable for process verification but should not be used as a surrogate for the quantitative validation of log reduction.

Good Practice Statement

Programs using a disinfection method for aseptically processed grafts should provide with their product, in addition to labeling, educational materials (e.g. package insert) that define "aseptic" and indicate that it does not guarantee or claim sterility as achieved by terminal sterilization.

***Rationale*:** The literature shows significant variation in disinfection procedures and validation methods, including quantitative bioburden log reduction, qualitative analysis of changes in discard rates, and/or changes in contamination rates. Comparative studies are uncommon and, as validation metrics are not standardized, comparative analysis of reports and identification of a preferred method are not possible. Studies did not compare disease transmission risks in aseptically processed grafts with terminally sterilized grafts. This research may not actually be feasible given the technical challenges and significant resource implication of such a study. Surgeons must be able to identify the balance between safety (e.g. sterility) and clinical utility. They may incorrectly assume that aseptically processed grafts are sterile. Education of end users (including surgeons) about the difference between aseptic processing and terminal sterilization would be useful. Bioburden reduction processes validated using qualitative metrics such as change in contamination rates only provide general insight into efficacy. Validation that quantifies the log reduction of an established load of known organisms provides more specific efficacy information quantifying microorganism kill rates.

Assessment:

Problem Priority: High

Benefit/Harm Analysis: Benefits outweigh any resource challenges

Resource Use and Feasibility: This statement may pose a resource challenge to smaller programs but it is an appropriate recommendation as a leading practice using either internal resources or contracting with experts.

Anticipated Acceptability to the Community: Yes

4.3.3 **Question #3**: Does the evidence identify a preferred terminal sterilization method, process, or parameter that should be recommended for adoption or consideration by the Canadian tissue community?

Recommendations

- Irradiation sterility testing should comply with the Radiation Sterilization Standards ANSI/AAMI/ISO 11137 and AAMI TIR 33 (soon to be ISO 13004). Irradiation is the preferred method for the terminal sterilization of nonviable musculoskeletal allografts.
- The Sterility Assurance Level (SAL) that should be demonstrated following the sterilization of musculoskeletal allografts is 10^{-6}. Alternative SAL values can be considered for other tissue allografts based on evidence-based risk assessment.

Good Practice Statement

Programs using sterilization should document the Sterility Assurance Level (SAL) attained.

Rationale: The most robust data (18 studies) on the use of terminal sterilization methods to reduce bioburden assess irradiation. Both gamma and electron beam irradiation showed similar capacities in bioburden reduction and maintenance of tissue following treatment. The greatest logarithmic reduction in bioburden (> 8.2 fold) was observed when samples were exposed to 50 kGy of irradiation. Peracetic acid-ethanol was used in one study to sterilize allografts, and post-transplantation assays revealed good clinical outcomes for all recipients. The sterility of any product is defined by the probability of a viable microorganism on the product after sterilization (SAL). This measurement informs surgeons about the efficacy of the sterilization process and risk of contamination in the products they are using. SAL is expressed as a quantitative value 10^{-n} to assure sterility, with 10^{-6} being used most frequently. There is no regulatory requirement for a minimal SAL in the sterilization of allografts and SAL values were not always provided in the literature reviewed.

Assessment:

Problem Priority: Moderate to high

Benefit/Harm Analysis: Benefits outweigh the resource challenge

Resource Use and Feasibility: This recommendation and statement may pose a resource challenge to smaller programs but they are appropriate for implementation using internal resources or contracting with experts

Anticipated Acceptability to the Community: Yes

4.3.4 Question #4: Does the evidence of the impact of terminal sterilization on the functionality of bone negate or support a recommendation for a preferred terminal sterilization method for consideration by the Canadian tissue community?

Recommendation

Programs employing the irradiation of musculoskeletal tissue should consider the use of lower dosage, e.g., 12-17 kGy, and low temperature (dry ice conditions) in order to reduce potential negative biomechanical changes and the clinical impact of terminal sterilization of musculoskeletal tissue by high dose irradiation, e.g. doses of >20kGy.

Good Practice Statement

NONE

***Rationale*:** Nine laboratory studies reported that irradiation with dosages between 25 and 50 kGy caused increases in resilience and elastic limit, and decreases in failure load and deformation energy in musculoskeletal tissue. All of these material and mechanical attributes of musculoskeletal tissues are important to the viability of the allograft, successful clinical application, and patient treatment. Most studies that reported effective reduction of bioburden with minimal effect on the allograft viability used dosages ranging from 18 to 35 kGy. Ten studies reported that irradiation with dosage ≤25 kGy caused increases in lipid peroxidation, and no difference in ultimate stress, Young's modulus, yield strain, yield stress, residual strain, micro-crack density, diffuse damage, trabecular micro-fracture, cyclic elongation response or failure load. Overall, the evidence indicates the higher the dose and the higher the temperature, the greater the potential negative impact on the structure and function of the tissue for its intended use. However, given a lack of comparative evidence, we are unable to provide a recommendation for a specific dose and temperature to be used.

Assessment:

Problem Priority: Moderate to high

Benefit/Harm Analysis: No harm

Resource Use and Feasibility: No resource issue regarding the reduction of dose or temperature

Anticipated Acceptability to the Community: Yes

4.3.5 Question #5: Does the evidence of the impact of terminal sterilization on the functionality of tendons negate or support a recommendation for a preferred terminal sterilization method for adoption or consideration by the Canadian tissue community?

Recommendation

See 4.3.4

Good Practice Statement

NONE

***Rationale*:** The evidence supports concerns regarding the impact of irradiation on tendons. While irradiation is as efficacious in reducing bioburden in tendons as it is in bone, the negative impact on the clinical efficacy and quality of the tissue is greater. The evidence indicates that the use of irradiation for tendons can achieve adequate bioburden reduction (SAL 10^{-6}) using a low dose irradiation regime (e.g., 13 to 17kGy) at low temperature (dry ice conditions) for tendons with initial low bioburden. However, more research is needed.

Assessment:

Problem Priority: Moderate to high

Benefit/Harm Analysis: No harm

Resource Use and Feasibility: No resource issue

Anticipated Acceptability to the Community: Yes

4.3.6 Question #6: What are the most effective storage parameters for preventing and inhibiting microbial growth?

Recommendations

NONE

Good Practice Statement

NONE

***Rationale*:** There was insufficient evidence to inform a recommendation.

4.4 Processing of Cardiac Tissue

4.4.1. **QUESTION #1**: Does the evidence support a leading practice recommendation for a post-recovery pre-processing storage process that will reduce bioburden in cardiac tissue?

Recommendation

NONE

Good Practice Statement

If technology moves towards providing decellularized cardiac tissue instead of cryopreserved viable cardiac tissue, the impact of the decellularization process on bioburden should be assessed.

***Rationale**:* There was insufficient evidence to inform a recommendation. Pre-processing storage and transportation processes vary among programs, including the use of different transport solutions. Some programs cut the apex of heart prior to immersion in transport solution to improve the distribution of solution within the heart. There were no comparative analyses to assess the impact of varying processes on preservation and bioburden. Current storage and transportation practices focus on minimizing cellular damage and maintaining viability and would theoretically maintain the viability of microbes as well as tissue. The literature does not address the importance of cellular viability for clinical efficacy in cardiac valves. If cellular viability is not required, more aggressive bioburden reduction processes could be employed. Some manufacturers provide decellularized cardiac tissue; this implies that viability is not required as most of the cellular material is removed from the tissue. Some decellularization processes are anti-microbial and reduce bioburden.

Assessment:

Problem Priority: Low

Benefit/Harm Analysis: No harm was identified

Resource Use and Feasibility: It is reasonable to indicate that the good practice statement is feasible to follow. Any research would require funding, personnel and time

Anticipated Acceptability to the Community: Yes

4.4.2 **QUESTION #2**: Does the evidence support a leading practice recommendation for a cleaning and rinsing process to reduce bioburden in cardiac tissue?

Recommendation

NONE

Good Practice Statement

NONE

***Rationale**:* There was insufficient evidence to inform a recommendation.

4.4.3. **QUESTION #3:** Does the evidence support a leading practice recommendation for specific antibiotics or antifungal cocktails to reduce bioburden?

Recommendations

- Disinfection procedures for musculoskeletal and cardiac tissue must be validated with quantification of log reduction, using challenge organisms. Qualitative analysis, such as calculation of discard and/or contamination rates, is acceptable for process verification but should not be used as a surrogate for the quantitative validation of log reduction.
- Programs that process tissue with antibiotics should use broad spectrum antibiotics active against common contaminants and in a concentration and temperature effective to eliminate virulent or otherwise unacceptable microorganisms.
- Programs that process with antibiotics or antifungals or both should validate rinsing methods to be sure antimicrobial residuals do not inhibit detection of microorganisms.

Good Practice Statements

Global

- Programs should track tissue recovery, in-processing tissue sampling, tissue processing environmental culture results, final sterility test results and contamination rates as well as the type of organisms identified, thus monitoring trends and conducting root cause analysis to inform practice change, as required.
- Programs should evaluate and monitor their bioburden reduction processes periodically and re-evaluate them after significant changes to practice.

***Rationale**:* The literature reports antibiotic, and in some instances antifungal, incubation as the preferred cardiac disinfection methodology. Past chemical methods have led to reduced viability of heart valves. Some incubation methods are better documented than others but this does not necessarily mean they are better. The literature reports significant variation in antibiotics used, dosage, incubation time and temperature, and validation metrics. No recommendations about preferred methodologies are possible as there are no comparative analyses of methodologies using standardized metrics.

As cardiac valves are not sterilized, there is a potential for microbes to survive disinfection. It is of critical importance that microbial sampling and culture techniques capture and identify any contaminants that may have survived disinfection. The literature reports cases where contamination was masked by antimicrobial residuals and disease transmission occurred so programs must validate their sampling and culture techniques to ensure they capture and identify potential contaminants and ensure antibiotic residuals have no impact on this identification. Bioburden reduction processes validated using qualitative metrics such as change in contamination rates only provide general insight into efficacy. Validation that quantifies the log reduction of an established load of known organisms provides more specific efficacy information quantifying microorganism kill rates.

In summary, there are numerous disinfection methods (e.g., a variety of antibiotics or antifungals, incubation times, and incubation temperatures), therefore the sampling and culture methods used must be properly validated and account for inhibition.

Assessment:

Problem Priority: High

Benefit/Harm Analysis: No harm was identified

Resource Use and Feasibility: These recommendations and statements may pose a resource challenge to smaller programs but they are appropriate for implementation using internal resources or contracting with experts.

Anticipated Acceptability to the Community: Yes

4.4.4 Question #4: Does the evidence support a leading practice recommendation for specific antibiotic and antifungal incubation parameters such as temperature?

Recommendation

To reduce bioburden optimally, the temperature used during cardiac antimicrobial incubation should be 37°C. While antimicrobials may have some activity at lower temperatures, they are not as effective at lower temperatures and have a lower rate of microorganism kill.

Good Practice Statement

NONE

Rationale: The literature reports that the temperature and duration of antibiotic incubation varies among programs. Historically, incubation at 4°C was standard practice to minimize cellular damage but some programs have now transitioned to 37°C. Most studies reported that cardiovascular allografts were incubated in an antibiotic-containing solution for 6 to 24 hours at 4° to allow the antibiotic to function while maintaining tissue integrity. One study showed a greater reduction in the bioburden load at 37°C vs the same treatment at 4°C and two other studies showed the greatest logarithmic bioburden load reduction at 37°C vs 4°C and 22°C.

Assessment:

Problem Priority: Moderate

Benefit/Harm Analysis: No harm was identified

Resource Use and Feasibility: The recommendation is appropriate for implementation and not resource intensive.

Anticipated Acceptability to the Community: Yes

4.4.5 QUESTION #5: Is there evidence to support a leading practice recommendation for specific processing methods for human cardiac valve allografts (HCVA) to increase implant survival or reduce co-morbidities?

Recommendation

NONE

Good Practice Statement

NONE

Rationale: There was insufficient evidence to inform a recommendation.

4.5 Processing of Skin Tissue

4.5.1 QUESTION #1: Does the evidence identify a preferred post-recovery pre-processing storage method or parameter that should be recommended for adoption or consideration by the Canadian tissue community in the processing of skin tissue?

Recommendation

NONE

Good Practice Statement

NONE

Rationale: There was insufficient evidence to inform a recommendation. In three studies, storage preservation with glycerol (70% to 85%) with penicillin (100 U/ml) and streptomycin (100 µg/ml) reduced the contamination rate to an average of 0.4% (range: 0% to 1.24%). The literature indicates glycerol has antimicrobial activities and is a common ingredient in storage and decontamination processes in Europe. In North America, low dose glycerol is commonly used as a cryoprotectant in the preservation of skin grafts but is not used in storage media. Its impact on bioburden and cellular viability as a component of a storage solution component is unknown.

4.5.2 QUESTION #2: Does the evidence identify a cleaning or rinsing process that should be recommended for adoption or consideration by the Canadian tissue community?

Recommendation

NONE

Good Practice Statement

NONE

Rationale: There was insufficient evidence to inform a recommendation.

4.5.3 QUESTION #3: Does the evidence identify a preferred antibiotic, antifungal, or combination that should be recommended for adoption or consideration by the Canadian tissue community?

Recommendation

Skin antibiotic disinfection processes should be validated. Quantitative validation of the bioburden log reduction using challenge organisms, which is accepted as an industry standard, is the preferred validation process.

Global Recommendation

Programs considering the use of antifungals on tissue where cellular viability is required should carefully assess and consider the risks of their use. Many antifungals are cytotoxic and will reduce cellular viability.

Good Practice Statements

- Disinfection procedures for split thickness skin grafts for burn treatment should optimize and maintain an acceptable level of cellular viability to support desired outcomes.
- Programs using antibiotic incubation for bioburden reduction should consider scientific evidence when determining dosage, incubation temperature, and duration to optimize disinfection while maintaining cellular viability. Process-specific validation studies should assess, and results support, the dosage, temperature, and incubation duration.

***Rationale**:* The evidence describes various combinations of antibiotics, antifungals, dosages, incubation times, and temperatures, with variation in the validation metrics. A lack of standardization prevents comparative analysis and identification of a preferred disinfection method. The most common disinfection strategy in the literature was a combination of broad spectrum antibiotics. An international environmental scan identified widespread use of a variety of antibiotics and antibiotic combinations for decontamination. Cellular viability is required for the treatment of burns; as many antifungal are cytotoxic their use is limited in skin disinfection (2 of 12 skin banks). Studies differed in the concentration of antibiotics used, the incubation period, and the temperature at which disinfection occurred. Minimal differences in bioburden reduction were observed with varying concentrations of antibiotics. One study found that incubation of the tissue with antibiotics at a temperature of 37°C for only 3 hours was effective in reducing the number of positive cultures to between 0% and 27%. Most studies used a lower temperature, equivalent to refrigeration, for an extended period of time (up to 4 weeks) to inhibit bacterial growth.

Assessment:

Problem Priority: Moderate to high

Benefit/Harm Analysis: No harms identified

Resource Use and Feasibility: The recommendations and good practice statements may pose a resource challenge to smaller programs in relation to advancing the sophistication of their validation practices; implementation is feasible either internally or from contracted experts

Anticipated Acceptability to the Community: Yes

4.5.4 QUESTION #4: Does the evidence identify preferred antibiotic and antifungal incubation parameters that should be recommended for adoption or consideration by the Canadian tissue community?

Recommendation

See recommendation for Question 3 above

Good Practice Statement

NONE

Rationale: See the material presented for Question 3.

4.5.5 QUESTION #5: Does the evidence identify a preferred sterilization method, process, or parameters for the processing of skin tissue that should be recommended for adoption or consideration by the Canadian tissue community?

Recommendation

To maintain cellular viability, terminal sterilization using processes such as irradiation or peracetic acid should not be employed on split thickness skin grafts used in burn treatment.

Good Practice Statement

NONE

Rationale: The literature provides some evidence identifying sterilization methods for skin

tissue. In two studies, an irradiation dose of 25 kGy was sufficient for disinfection of skin allografts. In other studies, irradiation was shown to reduce tissue integrity. Irradiation of skin tissue stored in 20% glycerol solution reduced the incidence of tissue damage. Chemical disinfection of skin is another method discussed in the literature. One study demonstrated that treatment with 0.1% peracetic acid for 90 minutes reduced the contamination rate to 0%. Higher concentrations of 0.35% peracetic acid have been reported to reduce tissue integrity. However, there is an opposing clinical option for the sterilization of split thickness grafts. Tissue integrity and cellular viability of the skin graft assist in the healing of a burn so sacrificing these in order to achieve sterility is not desirable. Instead, skin allografts processed without detectable microbes or with acceptable microbes, as identified by microbiology or professional experts, are considered acceptable for transplant for burn treatment.

Assessment:

Problem Priority: Low

Benefit/Harm Analysis: No harms identified

Resource Use and Feasibility: None (no Canadian programs are currently sterilizing (irradiating or peracetic acid) skin grafts

Anticipated Acceptability to the Community: Yes

4.5.6 QUESTION #6: Does the evidence identify a preferred preservation method, process or parameters that should be recommended for the processing of skin tissue for adoption or consideration by the Canadian tissue community?

Recommendation

NONE

Good Practice Statement

NONE

Rationale: There was insufficient evidence to inform a recommendation.

4.5.7 QUESTION #7: Does the evidence identify a specific bioburden reduction method, process or parameter for the processing of skin tissue that increases implant survival or reduces patient morbidity?

Recommendation
NONE

Good Practice Statement
NONE

***Rationale**:* There was insufficient evidence to inform a recommendation.

5.0 IDENTIFIED EVIDENCE GAPS

Five working groups of experts reviewed (a) systematic reviews of the published literature, (b) international environmental scans, (c) analysis of regulatory documents, and (d) a review of documented disease transmissions to identify leading practice recommendations. A consistent finding among all groups was the lack of scientific publications identified by the systematic reviews and the lack of comparative analyses within the existing publications. While programs undertake data analysis in many target areas, and data are referenced in conference abstracts, presentations, and discussion, little is published in the peer reviewed literature.

The lack of published literature meant the Working Groups were challenged in developing recommendations. However, the experts identified key evidence gaps and corresponding research projects that could provide the evidence required to inform leading practices. A number of themes evolved in relation to required research:

- *Sensitivity of microbial sampling and specificity of testing*: Comparative analysis and validation of sampling and culture techniques, for each tissue type, to determine the capability and sensitivity in capturing and specificity in identifying the range of microorganisms that could potentially contaminate tissue to inform recommendations of preferred methodologies.
- *Disinfection procedures:* Comparative analysis and validation of disinfection procedures, including antibiotic incubation and irradiation to inform recommendations of preferred methodologies, identifying dosage, time, and temperature.
- *Validations*: The identification of preferred validation methodologies and metrics to ensure a standardized process that supports safety and comparative analysis of procedures.
- *Clinical Outcomes:* Analysis of the impact of disinfection procedures on clinical outcomes to inform recommendations of preferred methodologies.
- *Impact of Bioburden:* Analysis of the impact of bioburden and disease transmission on clinical outcomes to provide insight into the actual risk of disease transmission in relation to specific decontamination and processing methodologies, and also to provide insight into any potential benefits or impacts of normal flora in relation to skin allografts.

The Working Groups detailed 39 research projects that would help inform leading practice. It was noted that there are two categories of research projects: (1) new research, and (2)

collecting, compiling, and analyzing existing data. Each Working Group identified the high priority research projects that would have the most impact on practice.

5.1 Tissue Recovery

- **R1 (Priority):** To evaluate bioburden at tissue recovery, select a period of time and conduct a retrospective, comparative analysis of culture results for skin, musculoskeletal, and cardiac tissue with respect to:

  1) traumatic versus non-traumatic deaths (needs a definition);

  2) maximum ischemic time from asystole to skin prep;

  3) culture method used (i.e., swab, elution);

  4) time from beginning of recovery to the end;

  5) number of recovery personnel;

  6) recovery environment (site);

  7) types of skin disinfectant(s) used;

  8) pre- and post-autopsy recovery; and

  9) pre- and post-organ donation.

  The use of impregnated draping, removal of first scalpel, segregated packaging tables, changing gloves between tissues recovered, order of tissue recovery, transport media, and transport/storage temperature could also be assessed.

- **R2 (Priority):** Conduct a comparative analysis of traumatic versus non-traumatic deaths with respect to the maximum ischemic time from asystole to skin prep.

- **R3 (Priority)**: Conduct a comparative analysis of the relation between recovery time, recovery environment, and number of recovery personnel on bioburden, and specifically pre-processing cultures (as tissue banks collect this information they could be surveyed and a multivariate analysis undertaken).

- **R4 (Priority):** Evaluate the ability of alternative skin disinfectants to reduce bioburden while maintaining skin tissue quality.

- **R5:** Determine the impact of impregnated draping on contamination rate during tissue recovery.

- **R6:** Determine the impact on cross contamination of removing the scalpel used for first skin cut.
- **R7:** Determine the impact on bioburden of segregation of packaging at recovery stations.
- **R8:** Determine the impact on cross contamination of changing of gloves between recovered tissues.
- **R9:** Determine the impact on bioburden of the order of tissue recovery.
- **R10:** Determine the impact of transport media on bioburden.
- **RP11:** Determine the impact of transport storage temperatures on bioburden.
- **RP12:** Compare bioburden for skin donors pre- and post-autopsy.
- **RP13:** Compare bioburden for skin donor's pre- and post-organ donation.

5.2 Microbial Sampling

- **R14 (Priority):** Review the methods and techniques used to validate sampling practices, based on regulatory and literature references. Then, using selected methods from the review, compare the three sampling methods for each tissue type (musculoskeletal, cardiac, and skin): swab, tissue sample, and immersion, spiked with appropriate panels and concentrations of pathogens combined with standardized microbial culturing techniques. This research will inform programs about the preferred sample collection methods for each tissue type.
- **R15 (Priority)**: Determine highly virulent pathogens by tissue type (musculoskeletal, cardiac, skin) and establish a Canadian consensus document listing highly virulent pathogens for each tissue type that require tissue discard.
- **R16 (Priority)**: Facilitate the exchange, collation, and analysis of existing bioburden data from Canadian tissue programs to inform practice.
- **R17:**
  - **R17A**: Compile and publish a report listing microbial testing methods used to assess bioburden in all tissue types used by Canadian and International tissue programs, along with validation processes and the results of validation.
  - **R17B**: Compile the validated microbial testing methods used by transfusion medicine and transplantation programs for blood and stem cell components in Canada.

Compare these methods with the results from R17A and publish a report with recommendations for validating microbial testing methods and specificity.

- **R18:** Conduct a multi-center study that provides qualitative and quantitative data (if available) regarding microorganisms present in recovered tissues. A general determination of contamination rates and organisms present in recovered tissues by tissue type would provide a benchmark for Canadian programs to compare bioburden measurement methods. Also, this research would provide data regarding organisms of higher versus lower pathogenicity that are present in various tissues, and the corresponding discard rates.

- **R19**: In the research undertaken to address R18, skin tissue organisms could be reviewed and a list of acceptable micro-organisms developed. Plan additional studies to determine whether quantitative measurement of these microorganisms is useful to determine limits of bioburden that might be acceptable for transplant.

- **R20:** Arrange for ongoing surveillance of tissue-transmitted infection in Canada using internationally defined case definitions of possible, probable, and proven infections, with analysis and data sharing to inform practice.

5.3 Processing of Musculoskeletal Tissue

- **R21 (Priority)**: Determine the actual risk of bacterial disease transmission, and more specifically bacterial transmission, from aseptically processed grafts versus terminally sterilized grafts.

- **R22 (Priority)**: Determine the minimal requirements, components, and metrics for the validation of a bioburden reduction process.

- **R23**: Conduct a comparative analysis of the efficacy of musculoskeletal disinfection methodologies, e.g., antibiotic incubation assessing variations in antibiotic type, dosage, incubation temperature and duration against known common contaminants and high risk organisms.

- **R24:** Identify an optimal irradiation methodology and analyze irradiation sterilization methodologies comparing dosage, temperatures, and duration, with a link to the impact on tissue quality and clinical effectiveness.

- **R25**: Undertake research on disinfection and sterilization methodologies using peracetic acid, with an analysis of the impact on tissue quality and clinical effectiveness.

- **R26**: Determine the impact of varying irradiation dosage, duration, and temperature on both bioburden reduction and the clinical functionality of tendons.

- **R27**: Explore the need to irradiate/ sterilize tendons, whether aseptic processing is a preferred method for bioburden reduction and control for tendons, and the impact of aseptic processing on the safety (disease transmission risk) and clinical functionality of tendons

5.4 Processing of Cardiac Tissue

R28 (Priority): Undertake a comparative analysis to identify and validate (a) the antibiotic and antifungal combinations and concentrations, and (b) the incubation temperatures and duration, that are most effective in eliminating common contaminants, pathogens, and resistant organisms while maintaining cellular viability.

R29: Compare the impact of different pre-processing storage parameters on bioburden.

R30: Compare the impact of different cleaning and rinsing practices on bioburden.

R31: Define standard criteria and processes to validate cardiac bioburden disinfection processes, e.g., identify the log reduction required to achieve an appropriate level of decontamination for cardiac valves.

R32: Compare the effect of incubation temperature on bioburden reduction and clinical efficacy.

5.5 Processing of Skin Tissue

R33 (Priority): Determine whether selective disinfection improves clinical outcomes in skin grafting, e.g., does the presence of normal flora (low virulence organisms) on skin grafts inhibit colonization by pathogens and improve clinical outcomes?

R34 (Priority): Undertake comparative analyses to identify and validate: (a) antibiotic and antifungal combination(s), and (b) dosage incubation temperature and duration, that would be most effective in eliminating pathogens and resistant organisms while maintaining cellular viability.

R35: Via a randomized study, assess the impact of storage duration between recovery and cryopreservation on bioburden and cellular viability.

R36: Assess the impact of storage temperature and duration on bioburden and cellular viability with an analysis of the impact of high dose glycerol as a component of the storage solution.

R37: Explore (a) whether low dose irradiation can be used as a bioburden reduction method for split thickness skin allografts (while still maintaining cellular viability); and (b) the most

effective parameters (dose, temperature and duration) to accomplish optimal bioburden reduction while maintaining viability.

R38: Explore (a) whether low dose peracetic acid can be effective in reducing bioburden while maintaining cellular viability and integrity; and (b) the most effective parameters (dose, temperature and duration) to optimize bioburden reduction while maintaining cellular viability.

R39: Compare the effect of DMSO and glycerol on cellular viability and bioburden reduction in cryopreservation.

5.6 System Recommendations

As indicated by the evidence gaps, identified practices currently employed by Canadian tissue banks do not appear to be evidence-based and many processes do not appear to be validated through an appropriate scientific methodology. Barriers and challenges to evidence generation and the application of scientific methodology within the Canadian tissue community include:

a) the lack of research scientists, research funding and academic culture within tissue banking,

b) limited technical expertise and experience with manufacturing validations and corresponding standards (e.g., ISO, ANSI/AAMI, USP),

c) proprietary, business, and competiveness constraints which inhibit publishing and information/data sharing,

d) the lack of comprehensive data analysis and information sharing between Canadian surveillance programs and Canadian allograft manufacturers,

e) the location of many tissue programs within hospital environments is not necessarily conducive or supportive of manufacturing endeavors, and

f) lack of resource allocation to support an evidence-based approach to manufacturing.

While acknowledging these challenges and barriers, evidence-based processes, validated using scientific methodology, are essential for the manufacturer of allograft tissue and should be required for Canadian tissue manufacturers.

Recommendations

The focus of Canadian tissue banks is the provision of safe effective quality tissue allografts in adequate supplies; the provision of which requires research, publication and data sharing.

The Steering Committee validated the critical importance of surveillance and adverse event data in informing practice.

Recommendations

Surveillance programs such as the Cells Tissues and Organs Surveillance System (CTOSS) should provide to Canadian programs greater analysis and insight into their data to inform practice.

The Steering Committee identified a need for greater exchange and analysis of existing evidence and data and a need to facilitate a process for the collection of new knowledge.

Recommendations

Canadian Blood Services and Héma-Québec, as established biologic manufacturers with infrastructure and core expertise supporting evidence-based scientific methodologies in the manufacture of biologics should undertake and collaborate in an initiative to:

- Explore the development of a national tissue committee to support collaborations within the tissue community to maintain leading practices.
- Encourage the collection, analysis and exchange of existing data on bioburden reduction and control.
- Develop analytics to inform quality improvement.
- Identify opportunities for collaborative and or consolidated approaches to support of the implementation of standardized leading practices.
- Advocate for research funding, including targeting the Canadian Institutes for Health Research (CIHR), to advance the scientific rigor of tissue manufacturing in Canada, linking to local vigilance and surveillance to advance evidence and improve practice.

6.0 CANADIAN STANDARDS ALIGNMENT

The manufacture of tissue allografts in Canada is regulated by Health Canada. Health Canada regulations reference requirements in relation to Canadian Standards Association standards, specifically:

- CAN/CSA-Z900.1-12 Cells, tissues, and organs for transplantation: General requirements
- CAN/CSA-Z900.2.2-12 Tissues for transplantation

These standards were reviewed in relation to the guideline recommendations and good tissue practices. Recommendations for amendments to Z900.2.2-12, and the addition of new standards, were drafted to align these standards with the recommendations and good tissue practice statements.

The Steering Committee reviewed, discussed, revised, and approved the proposed amendments for submission to the Canadian Standards Association for consideration in the current standards review cycle.

- The proposed amendments fall under six main themes: Inhibition; sensitivity of sampling and testing; bioburden reduction: incubation; sterilization methods; and unacceptable microbes.
- Steering Committee members recommended that all Good Practice Statements be proposed as "notes" for inclusion in the Canadian standards. (Notes are not specific requirements but are provided as advice and direction to standards users.)
- Steering Committee members recommended that CSA consider the AATB Tissue Guidance document "Microbiological Process Validation and Surveillance Program" (released April 2016) as a key reference document which will provide additional rationale and strength to the proposed amendments.

The proposed amendments were detailed in document titled *"Alignment of Bioburden Leading Practice Guidelines with Canadian Standards Association Cell, Tissue and Organ (CTO) Standards May 2016"* and submitted to the Canadian Standards Association for consideration in the current standards review cycle. The Canadian Standards Association Technical Committee for the Safety of Tissues reviewed and adopted many of the proposed amendments at a June 2016 meeting. The proposed amendments to standards will go to public consultation as part of the established review process. The Technical Committee will consider community feedback, adjust as they believe appropriate, and finalize the updated standards for the 2017 release.

7.0 COMMUNITY CONSULTATION

A preliminary Bioburden Reduction and Control Leading Practice Guidelines report was distributed to all Canadian musculoskeletal, cardiac, skin and eye banks on July 18, 2016. While the guidelines are not applicable to ocular tissue the report was provided to all Canadian eye banks for their insight. The five systematic reviews of the literature were provided to all banks to inform their review of the guidelines and to provide an evidence base to inform ongoing practice. Consultation with the community was solicited by an online survey link in the July 18th distribution (see Appendix 4). The following message was communicated with a request for responses by August 31, 2016.

"We are consulting you, as a tissue bank and member of the community, to ascertain your programs support for the guidelines and their implementation. We are interested in your feedback, and specifically,

- *Your support for the guidelines, and*
- *Any significant challenges or barriers to the implementation of the guidelines"*

Two reminder communications were sent and the deadline for response was extended to September 19, 2016. No eye banks responded. 60% (n=6) of the ten Canadian banks producing cardiac, skin or musculoskeletal grafts responded.

Five of six respondents indicated their support of the guidelines. One supporting program provided detailed feedback on specific recommendations and good practice statements which was presented to the Steering Committee and incorporated into the final draft. Of the five programs supporting the guidelines three indicated resource challenges would hinder the implementation of the recommendations. One program did not support the guidelines; stating that while they do not disagree with the recommendations they do not have the resources to implement or maintain the leading practices and therefore could not support the recommendations. One respondent suggested the development of a collaborative plan to centralize, at the national level, as much implementation work as possible, with the remaining to be resourced at the local/provincial level.

8.0 CONCLUSIONS

Through evidence-based materials and consensus, the Steering Committee developed leading practice recommendations and good tissue practices, presented as guidelines for the Canadian tissue community. These guidelines aim to standardize practice and improve the safety of tissue transplantation. Participants in our process valued (a) the collection and collation of evidence to inform "passionate, evidence-based conversations," and (b) the opportunity to contribute to national leading practice guidelines designed to improve the safety of tissue transplantation through bioburden reduction and control.

The systematic reviews of the literature, the environmental scan and the overview of reported disease transmissions are important resources providing an evidence base to Canadian banks to inform practice. Amendments aligning the Canadian Tissue Standards with these guidelines will incorporate evidence-based practice within standards and regulations, and will support adoption of these practices by the tissue community.

A key finding was the lack of published literature or scientific evidence to inform practice. It appears many of the standards and practices now in place were developed using consensus opinion rather than published evidence. A meaningful outcome of our process was the identification of evidence gaps to inform research opportunities and priorities. These priorities will point researchers to areas where evidence can inform key practice improvements. Canadian Blood Services and Héma-Québec are established biological manufacturers with the infrastructure and expertise to facilitate evidence gathering and new research to continue to inform and improve leading practices in the manufacture of tissue allografts.

The resource implications of our recommendations were considered. While detailed cost analysis was not undertaken, it was felt that the resource implications are generally manageable and appropriate for organizations producing human biologics for transplantation. Resource requirements may be decreased through collaborative implementation strategies.

We recommend that tissue programs, researchers, and other stakeholders identify collaborative opportunities and a consolidated approach to the implementation of these guidelines and to the generation of additional evidence required to improve the practice and safety of tissue transplantation. It is important for regulatory and standards organizations to consider these guidelines and to support research opportunities in key priority areas where evidence could inform practice. The lack of technical and scientific evidence supporting bioburden related practices should encourage programs to reevaluate their risk mitigation strategies, including review of these recommendations. Biologic manufacturing requires strong scientifically valid processes and procedural evidence; resources should be allocated to support the implementations of these recommendations.

APPENDIX 1: WORKING GROUP MEMBERS

WORKING GROUP	LEAD	ADDITIONAL MEMBERS	CANADIAN BLOOD SERVICES PROJECT MANAGER
Tissue Recovery	Scott Brubaker	Brian Hamilton	Ken Lotherington
		Gary Rockl	
		Jie Zhao	
Musculoskeletal Processing and Validation	Dr. Marc Germain	Karl Shaver	Jim Mohr
		Jacynthe Tremblay	
		Louis Thibault	
		Gary Rockl	
		Martell Winters	
Cardiac Processing and Validation	Dr. Marc Germain	Dr. Graeme Dowling	Jim Mohr
		Sonny Lazaro	
		Dr. Michael Strong	
		Jacynthe Tremblay	
Skin Processing and Validation	Cynthia Johnston	Dr. Jeannie Callum	Jim Mohr
		Dr. Robert Carlotto	
		Dr. Ian Davis	
		Dr. Ted Eastlund	
		Dr. Paul Gratzer	
		Lisa Merkley	
Microbial Sampling	Dr. Jutta Preiksaitis & Dr. Margaret Fearon	Dr. Jelena Holovati	Ken Lotherington
		Dr. Sandra Ramirez	
		Sean Margueratt (consultant)	

APPENDIX 2: EVIDENCE COLLATION METHODOLOGY

A. Systematic reviews of the literature

The systematic reviews of the published literature were the evidence foundation documents that informed the development of evidence-based guidelines.

Experts at McMaster University were retained to complete the five systematic reviews of the literature. The conduct of the systematic reviews adhered to rigorous methodology. In brief, search strategies were developed based on PICO (**P**roblem, **I**ntervention, **C**omparison, **O**utcome) questions posed by each of 5 five working groups. The search strategy was applied to the MEDLINE, EMBASE, and PubMed databases in searches spanning 30 to 40 years up to spring 2015. Searches included publications in English, excluding animal studies, case reports, and conference abstracts. Citations were screened in duplicate using eligibility criteria. Clinical studies that met the criteria were evaluated for quality using the Grades of Recommendation, Assessment, Development, and Evaluation (GRADE) assessment.[13] Data abstraction forms and evidence tables were guided by the questions in the analytic framework and approved and finalized by the working groups. Reviewers independently abstracted data and all data abstraction was checked by the senior reviewers. Senior reviewers detailed the systematic review in collaboration with subject matter experts from the working groups. Meta-analyses were not performed due to heterogeneity among clinical studies. The five systematic reviews:

1. Disinfection of Human Cardiac Allografts in Tissue Banking: A Systematic Review Report. Cardiac Tissue Processing and Validation Subgroup, Lead: Germain M. (2016).[14]
2. Disinfection of Human Musculoskeletal Allografts in Tissue Banking: A Systematic Review Report. Musculoskeletal Tissue Processing and Validation Subgroup, Lead: Mohr J. (2016).[15]
3. Disinfection of Human Skin Allografts in Tissue Banking: A Systematic Review Report. Skin Tissue Processing and Validation Subgroup, Lead: Johnston C. (2016).[16]

[13] GRADE assessment analyzes a study's limitations, inconsistency of results, indirectness of evidence, imprecision, and reporting bias, and evaluates the quality of its evidence, thus allowing for informed recommendations. There is no validated quality assessment tool for laboratory-based studies because basic science research is inherently considered level IV, or low quality evidence.

[14] Germain M., et al. 2016. Cell and Tissue Banking. http://link.springer.com/article/10.1007/s10561-016-9570-9?wt_mc=Internal.Event.1.SEM.ArticleAuthorOnlineFirst

[15] Mohr J., et al. 2016. Cell and Tissue Banking. http://link.springer.com/article/10.1007/s10561-016-9584-3?wt_mc=Internal.Event.1.SEM.ArticleAuthorOnlineFirst

[16] Johnson C., et al. 2016 Cell and Tissue Banking. http://link.springer.com/article/10.1007/s10561-016-9584-3?wt_mc=Internal.Event.1.SEM.ArticleAuthorOnlineFirst

4. Microbial Sampling of Human Tissue Allografts in Tissue Banking: A Systematic Review Report. Microbial Sampling Subgroup, Co-Leads: Preiksaitis J. and Fearon M. (2016).
5. Tissue Recovery Practices and Bioburden: A Systematic Review Report. Tissue Recovery Subgroup, Lead: Brubaker S. (2016).[17]

B. Environmental scan of current practice

Using SurveyMonkey software, tissue banks in Canada, the USA, Europe, and Australia were electronically surveyed to determine the bioburden reduction and control practices they employ. Five surveys were developed:

- Environmental Monitoring, Clean Rooms, and Sterilizers (37 questions)
- Tissue Recovery (39 questions)
- Bone Processing and Validation (48 questions)
- Skin Processing and Validation (79 questions)
- Cardiovascular Tissue Processing and Validation (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. The results were then collated and presented in chart form, providing a comparison of practices and the frequency of practices within the four jurisdictions.

C. Related standards and regulations

In collaboration with subject matter experts, the Canadian Blood Services project core team identified 19 sets of regulations, standards, and guidance documents with sections relevant to bioburden reduction and control. The sources included the American Association of Tissue Banks (AATB) Standards, 13th Edition; the Association for the Advancement of Medical Instrumentation (AAMI); the American National Standards Institute (ANSI); the European Union; Health Canada / Canadian Standards Association; and the USA Code of Federal Regulations (CFR).

D. Overview of disease transmission in musculoskeletal, cardiovascular, and skin

To inform expert discussions, an overview of national and international surveillance systems was prepared that reported on the incidence of disease transmission in musculoskeletal, cardiac, and skin transplantation. Health Canada and the Public Health Agency of Canada

[17] Brubaker S. et al, 2016 Cell and Tissue Banking. http://link.springer.com/article/10.1007/s10561-016-9590-5?wt_mc=Internal.Event.1.SEM.ArticleAuthorOnlineFirst

provided summaries of surveillance data. Additional evidence was gathered from published reports:

- Project Notify: World Health Organization
- European Commission: Health and Consumers Directorate-General
- USA Food and Drug Administration MedWatch

APPENDIX 3: COMPLIANCE WITH THE AGREE II INSTRUMENT

In 2003, the international AGREE Collaboration (**A**ppraisal of **G**uidelines, **R**esearch and **E**valuation), created the AGREE instrument using rigorous methodologies; this was updated in 2010[18] and 2013[19] as AGREE II. Its purpose is to assess the process of clinical practice guideline development. The table below illustrates how our processes comply with AGREE II, as adapted for the laboratory / biological manufacturing audience to whom the recommendations and good practice statements are aimed.

AGREE II Item (23 items under 6 domains)	Compliance with this item
Domain #1: Scope and purpose	
1. Overall objectives (of the project) are specifically described	Yes – described under Objectives
2. Health questions covered (by the project) are specifically described	Yes – the research questions are posed under each topic
3. Population to whom the guidance is meant to apply is specifically described	Yes – described under Objectives
Domain #2: Stakeholder involvement	
4. Development group includes individuals from all relevant professional groups	Yes – described in the Process Overview/Methods
5. Views and preferences of target population have been sought	Yes – covered by the membership of the Steering Committee and Working Groups
6. Target users are clearly defined	Yes – described under Objectives
Domain #3: Rigor of development	
7. Systematic methods were used to search for evidence	Yes – described in the Process Overview/Methods
8. Criteria for selecting the evidence are clearly described	Yes – described in the Process Overview/Methods
9. Strengths and limitations of evidence are clearly described	Yes – described in each systematic review
10. Methods for formulating the recommendations are clearly described	Yes – described in the Process Overview/Methods
11. Health benefits, side effects, and risks were considered in	Only risks were considered relevant –

[18] Brouwers MC, Kho ME, Browman GP, et al. AGREE II: advancing guideline development, reporting and evaluation in health care. CMAJ. 2010 Dec 14;182(18):E839-42. Available at: http://www.agreetrust.org/wp-content/uploads/2013/10/AGREE-II-Users-Manual-and-23-item-Instrument_2009_UPDATE_2013.pdf

[19] Welcome to the AGREE Enterprise website. 2016. Available at: http://www.agreetrust.org/

formulating recommendations	described under Process/ Methods

Domain #3: Rigor of development (CONTINUED)	
12. Explicit link between recommendations and supporting evidence	Yes – contained in the rationale for each research question
13. Materials externally reviewed by experts	Yes – membership of the Steering Committee and an expert consultant
14. Procedure for updating the guideline is provided	Yes - guidelines to be reviewed in 2020
Domain #4: Clarity of presentation	
15. Recommendations are specific and unambiguous	Yes
16. Different options for management of the condition or health issue are clearly presented	Not applicable to this project
17. Key recommendations are easily identifiable	Yes
Domain #5: Applicability	
18. Describes facilitators and barriers to application	Yes – detailed in each recommendation
19. Provides advice and/or tools on how the recommendations can be put into practice	Yes – in Conclusions
20. Potential resource implications of applying the recommendations have been considered	Yes – in Conclusions
21. Presents monitoring and/or auditing criteria	Programs' bioburden practices are audited by regulators
Domain #6: Editorial independence	
22. Views of the funding body have not influenced the content of the guideline	Yes – in in Canadian Blood Services front page disclaimer
23. Competing interests of development group members have been recorded and addressed	Yes – described under Process/Methods

APPENDIX 4: COMMUNITY SURVEY

Bioburden Leading Practice Guidelines: Preliminary Report

The following questions pertain to the Bioburden Reduction and Control Leading Practice Guidelines: Preliminary Report, June 2016 which has been provided for your review by email. The questions relate specifically to the 16 recommendations and 21 good practices statements. We are consulting you, as a tissue bank and community member, to ascertain your programs support for the guidelines and their implementation. We are interested in your feedback and specifically: does your program support of the guidelines? and, does your program see any significant challenges or barriers to the implementation of the guidelines? Please review and answer the following questions. Your responses will be reviewed by the Steering Committee and a summary will be included in the final guidelines report which will be published this fall. Thank you for your participation, we value your feedback.

Program Responding (Name of Tissue Bank) :

Type here

Name of Respondent:

Type here

Contact Email:

Type here

Contact Number:

Type here

Recommendations

Support is defined as: "My program agrees with the statement, sees value in this leading practice and will consider implementation".

- [ ] Our program supports the 16 guideline recommendations and has no significant challenges with the recommendations.
- [ ] Our program supports the 16 guideline recommendations but have challenges with specific recommendations. Please specify the specific recommendation number and your concerns: Type here
- [ ] Our program does not support the 16 guidelines recommendations. Please explain: Type here

Good Practice Statements

Support is defined as: "My program agrees with the statement, sees value in this leading practice and will consider implementation".

- ○ Our program supports the 21 good practice statements and has no significant challenges with the good practice statements.
- ○ Our program supports the 21 good practice statements but have challenges with specific good practice statements . Please specify the good practice Type here

statement number and your concerns:

○ Our program does not support the 21 good practice statements. Please explain:

Type here

Please provide any additional comments or feedback you would like to share:

Type here

Submit

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Administrator

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