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Ensuring the Safety of Allograft Tissue

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The risk of bacterial infection and disease transmission through tissue transplantation continues to cause significant concern among transplant recipients and implanting surgeons. Despite these concerns, musculoskeletal allograft usage has increased markedly in the past decade. The American Association of Tissue Banks (AATB) reports that in 2005 more than 1,300,000 musculoskeletal allografts were distributed in the United States. This is twice the number of allografts that were distributed in 1999. Overall, more than six million musculoskeletal allografts have been safely transplanted in the United States in the past decade.¹

Preparation methods used by tissue processing facilities, which include screening for disease, microbiological testing, and aseptic processing, substantially reduce risk but do not completely eliminate the possibility of infections associated with allograft implantation. Sterilization has been adopted by several allograft processors as a method for eliminating microorganisms without adversely affecting the biomechanical and biochemical characteristics of allograft tissue. This article

examines the current state of allograft safety and steps that are being taken by the tissue banking industry to minimize the risk of disease transmission through allograft tissue.

THE RISK OF DISEASE TRANSMISSION

Hepatitis B virus (HBV), hepatitis C virus (HCV), human immunodeficiency virus (HIV), and human T-lymphotropic virus (HTLV) have all been reported to have been transmitted by tissue transplantation.^{1,2,3} According to a recent study that looked at data from the various review and testing procedures utilized by tissue banking organizations in the United States, the estimated incidence of viremia at the time of donation is as follows: 1 in 55,000 for HBV; 1 in 34,000 for HCV; 1 in 42,000 for HIV; and 1 in 128,000 for HTLV.⁴ The study concludes that the prevalence rates of HBV, HCV, HIV, and HTLV infections are lower among tissue donors than in the general population. The authors estimate that the prevalence ratios for tissue donors relative to those in the general population are 0.54 for HBV, 0.61 for HCV, and 0.46 for HIV. This lower finding is not surprising, as tissue donors are carefully selected

Table I. Window period and estimated risk of prevalence

	Virus	
	HIV	HCV
Window Period using FDA Licensed Tests	HIV antibody – 22 days NAT* – 7 days	HCV antibody – 70 days NAT* – 7 days
Blood Donor Estimated Risk (repeat donor) ^a	with NAT* – 1:2 million	with NAT* – 1:2 million
Tissue Donor Estimated Risk ^{b**}	without NAT* – 1:55,000 with NAT* – 1:173,000	without NAT* – 1:42,000 with NAT* – 1:421,000
<small>*NAT: Nucleic Acid Testing Source: (a) Stramer et al, 2004⁵ (b) Zou et al, 2004⁴ **This is difficult to estimate for tissue donors because of increased prevalence and smaller donor pool.</small>		

Adapted from Joyce et al, 2006¹

based on medical history, physical examination, and interviews with next of kin.

Furthermore, blood samples from each tissue donor are tested for infectious diseases as required by the U.S. Food and Drug Administration (FDA) and American Association of Tissue Banks (AATB). Nonetheless, the concern of testing being performed during the so-called viremic window period, the period of time from infection until the virus can be detected by laboratory assays, is legitimate. Improved screening tests such as the recently introduced Nucleic Acid Testing (NAT) are implemented by many tissue banks as they become available and approved for use in donor tissue screening by the FDA. Table I provides an overview of the effect of NAT testing in the detection of HIV and HCV infection.

COMBATING LIMITATIONS IN TISSUE SAFETY

While the goal of allograft tissue processing is to provide the safest possible products to the surgical community while preserving the inherent tissue characteristics of the graft, **even with adequate donor screening there remains a risk of allograft contamination.** Oversight of tissue-banking practices has, however, become increasingly stringent to include monitoring by the FDA, the AATB, and individual state agencies.

The FDA requires preparation, validation, and written procedures to reduce the probability of contamination during processing. The requirements under the Current Good Tissue Practices (CGTP) for human cells, tissues, and cellular and tissue-based products cover procedures, facilities, personnel, equipment, supplies, reagents, process and labeling controls, process changes and validation, storage, receipt and distribution, records, tracking, as well as handling of complaints.^{6,7,8} The AATB has established quality standards for procuring and processing tissue including the time limits for retrieval and for screening donors. The AATB also publishes recommendations for preservation, sterilization, preparation, evaluation, and labeling of tissues.⁹ Individual tissue banks can apply for voluntary accreditation by meeting AATB standards, which

include use of aseptic techniques, microbiological testing (i.e., aerobic, anaerobic, and fungal pre- and post-processing cultures, as appropriate), and adverse outcomes reporting.

Despite these recognized guidelines, procedures for the preparation of allografts could further be enhanced for safety. **Not all tissue banks, for instance, apply for AATB accreditation;** in 2002, approximately 10% of musculoskeletal allografts were processed by non-accredited tissue banks.¹⁰ Kainer and colleagues¹¹ demonstrated in a recent investigation that infections acquired through bacterial contamination of allografts have the potential to result in substantial complications or death. The study recommends that current regulations and standards for processing and testing allograft tissue need to be enhanced to prevent such life-threatening allograft-associated infections.

ABBREVIATIONS

AAMI	Association for the Advancement of Medical Instrumentation
AATB	American Association of Tissue Banks
ANSI	American National Standards Institute
CGMP	Current Good Manufacturing Practices
CGTP	Current Good Tissue Practice
EO	Ethylene Oxide
FDA	Food and Drug Administration
GMP	Good Manufacturing Practices
HBV	hepatitis B virus
HCV	hepatitis C virus
HIV	human immunodeficiency virus
HTLV	human T-lymphotropic virus
ISO	International Organization for Standardization
NAT	Nucleic Acid Testing
SAL	Sterility Assurance Level
USP	United States Pharmacopeia

DEFINING STERILITY

Strictly speaking, a product should only be considered sterile when there is a complete absence of viable microorganisms; however, due to limitations in processing technology and environmental monitoring, no aseptic environment or aseptically produced product is provably sterile.^{12,13}

The United States Pharmacopeia (USP) establishes in their standards that a sterility assurance level (SAL) of 10^{-3} is comparable to the microbial survivor probability of aseptically produced products and is a level similar to the overall efficiency of an aseptic operation. A SAL of 10^{-3} is sometimes equated to culture negativity in microbiological testing. In contrast, physical sterilization technologies result in an SAL of 10^{-6} or lower, that is, whereas a 10^{-3} SAL provides a probability of one viable microorganism in a thousand units, products with a 10^{-6} SAL will have no more than a single viable particle in a million units.^{12,13} Consequently, **the lower the SAL, the lower the chance of contamination by microorganisms and the greater the assurance of sterility.**

In guidelines set forth by the Association for the Advancement of Medical Instrumentation (AAMI), the recommended SAL varies according to the intended use of the product.¹⁴ Sterilized medical devices that

are not intended to be in contact with breached skin or compromised tissues are generally thought to be safe for use with an SAL of 10^{-3} ; **invasive and surgically implanted devices should have an SAL of at least 10^{-6} .**

Current regulations do not require tissue banks to eliminate bacteria present on tissues at the time of recovery or to use processing methods that guarantee tissue sterility.¹¹ Most tissue banks process allografts under aseptic conditions by treating the tissue with various chemical, mechanical, and detergent steps, using methods that prevent, restrict, or minimize the contamination with microorganisms from the environment, processing personnel, or equipment.⁹

Aseptic processing alone does not reduce the inherent microbial bioburden present in donor tissue but only minimizes the risk of additional contamination. Due to the limitations of processing technology and environmental monitoring, aseptic processing does not eradicate microorganisms and spores, especially in tissue that is heavily contaminated at the time of recovery.^{13,15} Reduction of the microbial burden can only be accomplished through understanding of the bioburden of the pre-sterilized product, aseptic processing, use of a validated cleaning and disinfection process, a validated terminal sterilization process, and the correct interpretation of test results.^{12,16}

Q: What do clean room classes signify?

A: According to Federal Standard 209E,¹ clean rooms are classified by the maximum number of particles that are 0.5 micron (μm) or greater in size per cubic foot of air in the room; for example, up to 100 particles of this size can be counted per cubic foot of air in a Class 100 clean room. In 1999, Federal Standard 209E was superseded by ANSI/IES/ISO 14644-1 Standard,² and the newer nomenclature is now universally utilized. The following table provides an overview of clean room classes and their typical uses.

Clean Room Classifications		Maximum Number of Particles per Cubic Foot of Air of Diameter $\geq 0.5\mu\text{m}$	Typical Uses
ANSI/IES/ISO 14644-1	US Federal Standard 209E		
ISO Class 4	Class 10	10	• integrated circuit manufacturing
ISO Class 5	Class 100	100	• medical implant manufacturing • hard drive manufacturing
ISO Class 6	Class 1,000	1,000	• pharmaceutical manufacturing
ISO Class 7	Class 10,000	10,000	• hospital operating rooms

¹ Federal Standard 209E. Airborne particulate cleanliness classes in cleanrooms and clean zones. Mount Prospect, IL: Institute of Environmental Sciences; September 11, 1992.

² ANSI/IES/ISO 14644-1:1999. Cleanrooms and associated controlled environments—Part 1: Classification of air cleanliness. May 1, 1999



TISSUE STERILIZATION TECHNIQUES

Several tissue banks have developed methods for tissue sterilization with the goal of ensuring the maximum safety of allograft tissue. Sterilization of allograft tissue has associated challenges, however:¹¹

- Not all sterilants such as gases and liquids have adequate tissue penetration
- Musculoskeletal tissue may have a high incoming bioburden
- Tissue is an organic material that can serve to protect microorganisms, leading to a failure in the sterilization process
- The biomechanical and biochemical properties of tissue can be adversely affected

Numerous sterilants and sterilant combinations are used to eradicate microorganisms on allograft tissues. These include chemical sterilants, gas plasma, ethylene oxide (EO), gamma radiation, and e-beam radiation as well as sterilization systems such as those developed by several allograft tissue processors.

Ethylene oxide gas treatment and gamma irradiation are two sterilization methods that are typically employed by tissue banks and have known bactericidal and virucidal effects. Even so, both methods have the potential to create technical problems with tissue. Ethylene oxide has a limited capacity to penetrate tissue and has been associated with adverse patient outcomes such as chronic synovitis¹⁷, therefore it has been largely abandoned as a sterilizing agent for tissue. High doses of unprotected gamma radiation that are effective against viruses have been shown to adversely affect the biomechanical properties of allografts.^{18,19} Gamma irradiation is, nevertheless, the most popular option for the sterilization of allograft tissue.

To overcome the potential issues associated with gamma irradiation, several tissue banks have now developed controlled-dose, low-temperature sterilization approaches to eradicate vegetative microorganisms and spores while preserving

biomechanical integrity and function of allograft tissue necessary for surgical applications. **The Association for the Advancement of Medical Instrumentation (AAMI) has instituted standards and recommended practices for the radiation sterilization of health care products that have been adopted by the tissue banking industry.**^{14,20} Based on the ANSI/AAMI/ISO 11137 Method 2B guidelines, Moore and colleagues at LifeNet Health undertook a study to validate sterilization of musculoskeletal grafts, both soft tissue and bone grafts, using gamma irradiation.²¹ The sterilization method determines the minimum absorbed dose of radiation necessary to achieve a SAL of 10^{-6} for products with consistently low levels of microbial bioburden. Using LifeNet Health's patented Allowash XG[®] technology, the investigators demonstrated that Method 2B terminal sterilization validation can readily be transferred from the medical device industry to tissue banking by appropriately modifying the microbiological assessment methods to include testing for both aerobic and anaerobic microorganisms. Valid and reliable results are produced when appropriate considerations are taken into account.

It should be noted that the destruction of microbiological contaminants by physical or chemical agents follows an exponential law. The probability that a microorganism can survive is a function of a number of different factors; these include the number and types of contaminants on the product, the lethality of the sterilization method, and, under specific circumstances, the environment in which the organisms are situated during the sterilization process. Consequently, the sterility of a particular unit of product cannot be assured unconditionally.²⁰ Furthermore, the efficacy of the sterilization process cannot be verified by inspection or testing of the product itself. The sterilization process, even if it is validated and controlled, is not the only factor that assures that an allograft is sterile and suitable for implantation. The incoming bioburden of the donor tissue, a controlled environment in which the tissue is processed, packaged, and stored as well as the integrity and barrier properties of the packaging all contribute to the safety of the final product.²⁰

ALLOWASH XG® – A NEW STANDARD FOR ALLOGRAFT SAFETY

With the launch of Allowash® in 1995, LifeNet Health pioneered a novel allograft cleaning and disinfection process. This process was further refined through the introduction of Allowash XG® in 2005. By removing microorganisms through a controlled and patented system, **LifeNet Health’s proprietary validated and patented Allowash XG® process results in sterile allograft tissue with a 10⁻⁶ SAL.** Unlike other sterilization processes, Allowash XG® provides product sterility without compromising the biomechanical or biochemical properties of allograft tissue for clinical use. The six steps of the Allowash XG® process are outlined below:

Step One—Bioburden Control: All donors accepted for tissue recovery are subjected to a meticulous and rigorous screening routine that exceeds the requirements set forth by the FDA and AATB. Donors are then recovered under strict aseptic conditions. This first step allows for a stringent control of bioburden on incoming tissue even before it enters LifeNet Health’s processing facilities.

Step Two—Bioburden Assessment: All recovered tissue is sampled for microbiological contamination at the time of recovery. Standard microbiological methods utilizing both aerobic and anaerobic media are employed to culture and identify bacteria and fungi. Donor blood samples are also tested for infectious diseases as required and evaluated for potential hemodilution that may affect the reliability of

serology test results and thus donor acceptability. This extensive serological testing exceeds industry standards and utilizes the latest NAT testing techniques, allowing LifeNet Health to further control and rule out incoming bioburden.

Step Three—Minimized Contamination: LifeNet Health’s state-of-the-art processing facilities contribute to maintaining a low bioburden on tissues. Designed specifically for the processing and preservation of musculoskeletal and cardiovascular tissue allograft, all processing areas have been designed to allow compliance with FDA, state, and federal requirements, including Current Good Manufacturing Practices (CGMP) for medical devices. Our processing facilities maintain cleanliness levels that minimize or eliminate environmentally induced graft contamination.

Step Four—Rigorous Cleaning: Through treatment with hypotonic solutions and antimicrobial reagents and/or use of processes such as ultrasonication and centrifugation, blood elements as well as bone marrow and lipids are solubilized and removed from the tissue. Key solutions that contain a combination of detergents are forced into and through the bone matrix and then directed to waste, resulting in the lysis of cells and cleaning of the tissues.

Step Five—Disinfection and Rinsing: The tissue, freed from over 99% of marrow and lipids, is subjected to an intensive decontamination, disinfection, and cleaning regimen designed to remove and eliminate viruses, bacteria, and fungi. Tissue then undergoes water soak mediation to remove processing reagents, followed by centrifugation and/or micro-absorption to

Q: What is Sterility Assurance Level?

A: Sterility Assurance Level (or SAL) is the probability of a single viable microorganism occurring on a product after sterilization. For example, products with a 10⁻⁶ SAL will have no more than a single viable particle in a million units.

Q: What is the difference between SAL and log kill?

A: Log kill defines the order of magnitude by which a population of microorganisms is reduced. As an example, a six-log kill (also known as 1 X 10⁶ kill rate)

leads to a reduction of the number of live organisms in a population by six orders of magnitude. The resulting number of live organisms depends on the starting population; for example, if the starting population is 10⁸, the number of viable particles that remain as contaminants after a six-log kill is 10². SAL, on the other hand, is an absolute number and does not depend on the number of live organisms in the starting population. An SAL of 10⁻⁶ ensures that no more than a single viable particle in a million units remains after treatment. .



remove excess water and processing residuals. *Step Six—Terminal Sterilization:* The Allowash XG® process concludes with a validated controlled level dose of gamma irradiation administered at low temperatures after the tissue has been packaged.

This final step results in tissue with a Sterilization Assurance Level (SAL) of 10^{-6} without compromising the biomechanical or biochemical properties of the tissue needed for its intended surgical application.²¹

CONCLUSION

Using a validated methodology, controlled dose, low-temperature gamma irradiation can be used to obtain sterile allografts. Peer-reviewed literature, pre-clinical testing, and clinical outcome data all indicate that allografts processed using Allowash XG® exhibit no measurable detrimental effects in regard to the properties of the tissues needed for surgical applications. Whereas other tissue banks might claim sterility at a SAL of 10^{-3} , the Allowash XG® system delivers sterile allograft tissue to a SAL of 10^{-6} .

When making their choice among tissue suppliers, clinicians seek to find a balance between utmost tissue safety and greatest tissue efficacy in order to achieve the best patient outcome possible. With allograft tissue processed with Allowash XG® technology, LifeNet Health is able to satisfy both needs. Today, it is more critical than ever that physicians and hospital administrators rely on sterile tissue provided by well-known, accredited tissue banks such as LifeNet Health. With Allowash XG®, LifeNet Health takes tissue safety to the next level.

LifeNet Health's Allowash XG® technology encompasses a comprehensive and validated process during which greater than 99% of bone marrow and blood elements are removed from the internal bone matrix. This step, along with a subsequent chemical sterilant treatment, has been shown to substantially reduce the bacterial and fungal bioburden and inactivate viruses. The Allowash XG® process concludes with a controlled terminal sterilization step that results in a SAL of 10^{-6} without compromising the biomechanical or biochemical properties of the tissue as needed for its intended surgical application.

Since 1995, over 1.5 million allografts have been delivered to the medical industry and no incident of disease transmission has been directly linked to tissue screened and processed by LifeNet Health.

Q: What are the tissue banking industry's standards for the irradiation of allograft tissue?

A: While there is currently no standard for the sterilization of allograft tissue in place for the tissue banking industry, several tissue banks have developed methods for tissue sterilization with the goal of ensuring the complete safety of allograft tissue. Numerous sterilants and sterilant combinations are used to eradicate microorganisms on allograft tissues including chemical sterilants, gas plasma, ethylene oxide (EO), gamma radiation, and e-beam radiation as well as sterilization systems.

The United States Pharmacopeia (USP) establishes in their standards that a sterility assurance level (SAL) of 10^{-3} is comparable to the microbial survivor probability of aseptically produced products and is a level similar to the overall efficiency of an aseptic operation. In contrast, physical sterilization technologies such as irradiation result in an SAL of 10^{-6} or lower, that is, whereas a 10^{-3} SAL provides a probability of one viable microorganism in a thousand units, products with a 10^{-6} SAL will have no more than a single viable particle in a million units. Consequently, the lower the SAL, the greater is the assurance of sterility.

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