The BioCleanse® Tissue Sterilization Process: A Proven Standard for Tissue Safety
Who is RTI Biologics?

• RTI Biologics, Inc. (RTI) is the leading provider of sterile biological implants for surgeries around the world with a commitment to advancing science, safety and innovation.
• RTI prepares human donated tissue and bovine tissue for transplantation through extensive testing and screening, precision shaping and proprietary, validated sterilization processes.
• RTI’s allograft and xenograft implants are used in sports medicine, orthopedic, dental, hernia and other specialty surgeries.
• RTI was the first company to offer precision-tooled bone implants and assembly technology to maximize each gift of donation.
• RTI’s 65,000 square foot state-of-the-art processing facility is located in Alachua, FL.

“One tissue bank has developed and implemented a low-temperature chemical-sterilization approach (BioCleanse) that kills spores but preserves the biomechanical integrity and function of some allografts.”

—New England Journal of Medicine
What is the BioCleanse® process?

- A patented, validated sterilization process
- A complex, proprietary combination of mechanical and chemical processes, working in conjunction with each other
- An automated, pharmaceutical grade tissue sterilization process
- A clinically successful sterilization process used on grafts that provide a natural biologic scaffold in orthopedic, spine and sports medicine procedures

Why was the BioCleanse® process developed?

Although the risk of disease transmission is low, the possibility still exists that it could occur, even with accepted donor screening and testing—unless the tissue is sterilized through an effective and validated process.

RTI’s commitment to advancing science, safety, and innovation led the company to develop the BioCleanse® Tissue Sterilization Process. The BioCleanse process sterilizes tissue, is scientifically proven to address donor to recipient disease transmission, and retains tissue’s biomechanical integrity.

Even if other safeguards fail, RTI’s BioCleanse technology sterilizes tissue.

Why is the BioCleanse® process important?

- Sterilizes tissue through an effective and validated process
- Scientifically proven to address the risk of donor to recipient disease transmission
- Thoroughly penetrates tissue
- Preserves biomechanical and structural integrity
- Preserves biocompatibility
- Inactivates or removes bacteria, fungi & spores
- Redundant safeguards such as computer monitoring have been designed into the process to ensure tissue safety

More than a million implants. Zero incidence of allograft-associated infection. It’s not just our goal. It’s our track record.

How does the BioCleanse® process work?

The BioCleanse® system sterilizes tissue using a complex, proprietary combination of mechanical and chemical processes, working in conjunction with each other.

The mechanical component applies oscillating positive and negative pressure in the presence of the chemical agents (including detergents and sterilants), which gently perfuse the tissue. This combination removes blood and lipids, and inactivates or removes pathogenic microorganisms.

Repeated rinses throughout the process remove debris, and final rinses remove residual chemicals, leaving the tissue biocompatible. Bone grafts are terminally sterilized using a validated method to achieve a $10^{-6}$ sterility level.

“Low-temperature chemical sterilization technologies that kill spores but preserve the biomechanical integrity and function of some allografts are being evaluated.”

(Centers for Disease Control and Prevention, Morbidity and Mortality Weekly Report, March 14, 2002)
Redefining “sterile”

“At the core of RTI’s processing facility is the BioCleanse® system, featuring fully automated equipment and technology.”

“Sterile” means the state of being free from all living organisms.
- Sterilization is a process intended to inactivate or remove viable forms of microbial life, including bacterial spores, to achieve an acceptable sterility assurance level.
- Sterility Assurance Level (SAL): the probability of survival of microorganisms after a sterilization process and a predictor of the efficacy of the process.

“The path from recovery to implantation”


Why is sterilization important?
- In order for a graft to be sterile and non-immunogenic, viruses, bacteria, and spores must be inactivated or removed through a validated sterilization process. Aseptic processing and other methods do not inactivate or remove these contaminants.
- To achieve sterilization, individual processes should be validated by tissue type.
  - Validation should include:
    - Tissue penetration
    - Viral inactivation
    - Removal of a wide-range of organisms using worst-case testing
    - Retention of biocompatibility and tissue functionality
- Because of window periods, false negative serological test results have been documented by tissue processors, which may risk transmission of disease to patients if the tissue has not been sterilized through a validated sterilization process.

Sterilization at RTI
- Where possible, RTI has advanced beyond the use of aseptic processing.
- Implants processed at RTI are sterilized through one of three processes:
  - The BioCleanse® process sterilizes bone and sports medicine soft tissue grafts.
  - To remove any surface bacteria, bone grafts are terminally sterilized using a validated method to achieve a 106 sterility level.
  - RTI’s soft tissue grafts undergo a final post-processing 14-day sterility culture prior to release to achieve sterility without the use of irradiation.

The path from recovery to implantation

- Bone and sports medicine soft tissue
- Low temperature chemical process

BioCleanse® Tissue Sterilization Process

- BioCleanse label signifies tissue has been sterilized

Aseptic Processing

- Aseptic processing (no validated viral inactivation)

Tissue implanted into patient

BioCleanse® Tissue Sterilization Process Validation

- Penetration
- Viral inactivation
- Sterilization Efficiency
- Biocompatibility
- Tissue Functionality
- Tissue Specific Processing
- Worst Case Testing
- Automation

“Aseptic processing does not eradicate contamination with organisms, and antibiotic/antifungal solutions will not eliminate spores or organisms such as Clostridium spp.”
The BioCleanse® Tissue Sterilization Process:

A. Removes blood, lipids, marrow

Studies show that the BioCleanse® chemicals and processing method effectively sterilize tissue-based products with complex matrices.

- Processed aseptically: Cellular elements in the Haversian Canal
- Processed through the BioCleanse process: No cellular elements present

B. Thoroughly penetrates tissue

Studies found that after 5 minutes contact time (normal cycle: 218 minutes), the BioCleanse® process completely perfused inner matrices of cortical and cancellous bone with sterilants.

- Untreated cortical bone matrix
- Treatment specimen demonstrating complete endothelial perfusion, including treatment of lacunae

C. Inactivates or removes HIV, hepatitis, fungi, spores – the complete antimicrobial spectrum

Sterilization studies show that the BioCleanse® process inactivates or removes the most resistant organisms.

- Relevant and Model Viruses
  - Human Immunodeficiency Virus (HIV)
  - HCV Model (IVD)
  - Herpes Virus Model (PV)
  - Hepatitis A Virus (HAV)
  - Parvovirus (PPv)
- Spores
  - Bacillus subtilis

Validated to Inactivate:

- Vegetative Bacteria and Fungi
  - Staphylococcus aureus
  - Escherichia coli
  - Pseudomonas aeruginosa
  - Candida albicans
  - Staphylococcus epidermidis
  - Enterococcus
  - Enterobacter cloacae
  - Citrobacter freundii
  - Proteus vulgaris
  - Acinetobacter calcoaceticus

D. Preserves biomechanical and structural integrity

Studies show the BioCleanse® process inactivates or removes contaminants while preserving tissue strength and biocompatibility.

- Axial Compression Strength (MPa)

E. Sterilizes bone and soft tissue

Studies show that after the BioCleanse® process, cellular material is removed, providing a clean scaffold that allows for remodeling and collagen that retains its crimp pattern under scanning electron microscopy (SEM).

F. Renders bovine bone grafts safe and biocompatible

Immunogenic Response: Old World Primate Model

The processing of xenograft bone rendered it sufficiently non-antigenic such that it did not elicit hyperacute rejection in an old world primate model. At a three-month timepoint, the immunological and histological evaluations demonstrated the potential for long-term viability of BioCleanse-processed xenograft bone implants in the most extreme situation of a model with preformed non-active antibodies.

- Brain Cancellous Bone Control
- BioCleanse® Bone Cancellous Bone

- Physiologic
- BioCleanse®

- Histology: appearance of tendon

- *This example is one study of many performed on the effect of the BioCleanse process on xenograft tissue.

---

1. *Studies A, B & D were performed on allograft tissue. Study E was performed on soft tissue. Study F was performed on xenograft tissue.*
RTI's primary goal is to ensure patient safety. To fulfill this goal, RTI employs stringent donor screening, laboratory testing and tissue preparation validated to inactivate or remove pathogens. These redundant safeguards provide the highest level of confidence that patients will receive safe, high quality tissue.

Before processing tissue, a risk assessment is performed on every potential donor. Family members are interviewed, the donor's medical records are evaluated, and if necessary, the donor's physician is consulted. Blood samples from donors are tested for the presence of infectious diseases, including but not limited to:

- HIV-1
- HBV
- HCV
- HTLV
- Syphilis
- AIDS
- Approved Mycobacteria
- MRSA
- Pseudomonas
- Staph
- Streptococcus
- E coli
- Neisseria gonorrhoeae
- Hepatitis B & C

The final determination of donor eligibility is made by RTI’s medical director – a licensed physician – utilizing all available, relevant information. After donor eligibility has been determined, tissue from a single donor is processed into final shapes, placed into an individual BioCleanse® chamber and sterilized.

RTI uses many different review processes and tests, including but not limited to:

- Family/Next of Kin interview
- Medical/hospital record review
- Behavioral/lifestyle risk assessment
- Medical examiner/coroner’s report (autopsy report, when available)
- Laboratory, pathology and radiology reports

SeroLogic Testing

- HCV Antibody
- HBV Surface Antigen
- HIV 1 & 2 Antibody
- HIV Total Core
- HTLV 1 & 2 Antibody
- Syphilis
- HIV-1 NAT
- HCV/NAT

Microbial Testing

- Pre-processing; culturing. Performed before processing begins, removes potentially unsuitable tissue from process
- Sterility confirmation: Performed at packaging for products that are not terminally sterilized
- Environmental controls: Monitors cleanliness of processing environment

RTI’s 65,000 square foot, state-of-the-art processing facility features the latest equipment and technology, such as a pharmaceutical-grade water sterilization system and computer safeguards.

Related Articles


New England Journal of Medicine, Vol. 350, No. 25; June 17, 2004


C. Randal Mills, PhD, CTBS; Michael R. Roberts, MD, CTBS; John R. Bianchi, PhD, “Method to Determine Germicidal Inactivation in Allograft Processing.” Presented at 2005 American Society for Testing and Materials meeting.

Michael R. Roberts, MA, CTBS; C. Randal Mills, PhD, CTBS; Jerry Chang, BS, Jeffrey Maxy, MD; “Osteo Perfusion into Cadaveric Human Bone as an Indication of Sterilant Penetration.” Presented at 2005 American Association of Tissue Banks annual meeting.


Donna M. Squillace, MS, Matthew C. Summerlin, MS, Gina Scari, BS, John R. Bianchi, PhD, “Biomechanical Integrity of Human Allograft Bone After Sterilization.” Presented at 2005 Society for Biomaterials meeting.