VIRAL AND BACTERIAL EFFECTIVENESS OF ALLOWASH XG®

How has LifeNet Health demonstrated the effectiveness of Allowash XG® to remove viral contamination?

LifeNet Health's Allowash XG® technology encompasses a comprehensive patented and validated process that has been shown to:

- remove greater than 99% of bone marrow elements from the internal bone matrix
- render tissue sterile to a Sterility Assurance Level (SAL) of 10^{-6} through chemical treatment and sterilization steps
- inactivate enveloped and non-enveloped viruses
- not compromise the biomechanical or biochemical properties of the tissue as needed for its intended surgical application

Studies were performed to demonstrate the effectiveness of viral and bacterial inactivation by the disinfection and low-dose gamma irradiation steps used during the Allowash XG process. The results (see Tables below) indicate these steps are effective in inactivating viral particles that may be found in human allograft bone, thus ensuring safety from disease transmission.

Virus	Nucleic acid	Virus Type	**Log Inactivation-Disinfection steps of Allowash XG[1]**	**Log Inactivation-Gamma Irradiation step of Allowash XG[1]**	Log Inactivation-Disinfection and Irradiation steps of Allowash XG[1]
Human Immunodeficiency Virus (HIV)	RNA	Enveloped	**9.2**	**>3.2**	**>12.4**
Porcine Parvovirus (PPV)	DNA	Non-enveloped	**2.2**	**1.6**	**3.8**
Pseudorabies Virus (PrV)	DNA	Enveloped	**8.3**	**3.8**	**12.1**
Bovine Viral Diarrhea Virus (BVDV)	RNA	Enveloped	**5.8**	**4.6**	**10.4**
Hepatitis A Virus (HAV)	RNA	Non-enveloped	**3.2**	**2.5**	**5.7**

[1]Moore, MA, "Inactivation of Enveloped and Non-Enveloped Viruses on Seeded Human Tissues by Validated Low Dose Gamma Irradiation Sterilization Process" American Association of Tissue Banks Annual Meeting, Las Vegas, September 14-17, 2009

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How has LifeNet Health demonstrated the effectiveness of Allowash XG to remove bacterial and fungal contamination?

Organism	Allowash	3% H_2O_2	Antibiotics	70% IPA	Additive Log Kill
C.sordelli	**2.3**	**5.87**	**1.14**	**1.96**	**11.27**
E.faecalis	**0.14**	**4.98**	**1.15**	**5.9**	**12.17**
E. coli	**0.32**	**6.78**	**6.23**	**5.96**	**19.29**
P.acnes	**0.56**	**6.66**	**5.78**	**5.24**	**18.24**
A. niger	**0.03**	**6.49**	**0.28**	**5.59**	**12.39**
S.aureus	**0.44**	**4.09**	**0.94**	**6.45**	**11.92**
P.aeruginosa	**0.77**	**6.18**	**6.78**	**6.78**	**20.51**
B.subtilis	**3.48**	**2.68**	**2**	**1.34**	**9.5**
C.albicans	**0.96**	**5.57**	**4.43**	**5.56**	**16.52**

Two studies were performed to characterize the bioburden reduction capabilities of the solutions used during the Allowash XG process on bacterial and fungal contamination. In one study, the protocol used for the processing of cut (bone) grafts was assessed; in the other, the protocol for the processing of soft tissue was tested.

Representative bacteria and fungi were seeded into a known volume of bone marrow, which was then applied to a cancellous cube model graft. The seeded cancellous cubes were subjected to Allowash XG steps 4 and 5. The challenge microorganisms were selected based on the guidelines of the United States Pharmacopeia (USP):

- *Aspergillus niger*
- *Bacillus licheniformis*
- *Bacillus subtilis*
- *Candida albicans*
- *Clostridium sporogenes*
- *Pseudomonas aeruginosa*
- *Staphylococcus aureus*
- *Staphylococcus epidermis*

All materials tested passed a 14-day sterility test as specified by USP <26> standards. Culture media passed a growth promotion test as specified by USP <26> standards and bacteriostasis/fungistasis testing of the samples cubes and positive controls demonstrated that no residual processing chemicals remained that would prevent the growth of microorganisms in the assay.

The studies indicate that the chemical solutions used as part of steps 4 and 5 of the Allowash XG (see table on next page) process are effective in substantially reducing bacterial and fungal bioburden. Coupled with removal of over

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99 percent of lipids, bone marrow, and blood elements and terminal sterilization, Allowash XG is effective in eliminating bacteria and fungi that may be found in human tissue.

The following table provides an overview of the six Allowash XG steps.

Steps to Sterilization	Description Summary
1. Bioburden Control	Rigorous screening routine based on FDA and AATB guidelines with strict donor exclusion criteria and donor tissue recovery under aseptic conditions
2. Bioburden Assessment	Extensive serologic testing for microbiological contamination that includes bacteria, fungi, and infectious diseases
3. Minimizing Contamination	State-of-the-art processing facilities to maintain cleanliness levels designed to eliminate the possibility of cross-contamination by exceeding regulatory standards
4. Rigorous Cleaning, Blood and Marrow Removal	Flushing, centrifugation, hypotonic processes, and ultrasonication to solubilize and remove blood elements, including marrow and lipids
5. Disinfection and Rinsing Regimen	Intensive decontamination, disinfection, and cleaning regimen designed to remove and eliminate viruses and bacteria, followed by centrifugation and/or microabsorption to remove residual water
6. Terminal Sterilization	Controlled dose gamma irradiation at low temperatures resulting in sterile allograft tissue with an SAL of 10^{-6}

LifeNet Health's Allowash XG® technology encompasses a comprehensive patented 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 subsequent chemical treatment and sterilization steps have been shown to render tissue sterile to a Sterility Assurance Level (SAL) of 10^{-6} and to inactivate enveloped and non-enveloped viruses without compromising the biomechanical or biochemical properties of the tissue as needed for its intended surgical application.

Since 1995, over 3 million bio-implants processed using Allowash Technology have been distributed by LifeNet Health, with no disease transmission.

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