APPENDIX B: ANALYTICAL FRAMEWORK

Cleaning and Disinfection of Frozen Human Musculoskeletal Allografts
(i.e. bone, soft tissue, connective tissue, cartilage and osteoarticular tissue)

1. Does the method for manual cleaning of tissues impact bioburden load?
   a. What method(s) for cleaning and removing extraneous tissue, cells and lipids from musculoskeletal tissue results in the lowest bioburden load?

2. Do different decontamination methods result in different bioburden reduction loads (both qualitatively and quantitatively)?
   a. What decontamination method is most effective in reducing the bioburden content (load) during tissue processing? **Effective** is defined as producing the highest reduction in bioburden both quantitatively and qualitatively. Variables to compare:
      i. Mechanical cleaning of tissue
      ii. Chemical treatments
      iii. Rinse agents
      iv. Antibiotics
      v. Sonification
      vi. Centrifugation
      vii. Pressure cycles
   b. What are the most effective parameters for decontaminating bone during tissue processing in reducing bacterial content (load)? **Effective** is defined as producing the highest reduction in bioburden both quantitatively and qualitatively. Variables to compare:
      i. Concentrations
      ii. Combinations
      iii. Incubation temperature
      iv. Incubation duration
      v. Exposure times
      vi. Sequence of application

Final Sterilization of Musculoskeletal Tissue

3. Do different terminal sterilization methods result in different sterility assurance levels?
   a. What are the most effective methods for final (terminal) sterilization? **Effective** is defined as achieving sterilization (a 6 log reduction in bioburden). Variables to compare:
      i. Dry heat
      ii. Electron beam radiation
      iii. Gamma radiation
      iv. Ethylene oxide gas
      v. Moist heat
      vi. Supercritical CO₂
b. What are the most effective parameters for final (terminal) sterilization to achieve a 6 log reduction? Effective is defined as achieving sterilization (a 6 log reduction in bioburden). Variables to compare:
   i. Radiation dose
   ii. Chemical concentration
   iii. Exposure time

d. Does the method of terminal sterilization impact the functionality of bone tissue?
   a. What are patient outcomes related to irradiated bone?

e. Does method of terminal sterilization impact the functionality of tendons?
   a. What are patient outcomes related to irradiated tendons?

Storage and Transportation

f. Do temporary storage parameters effect bioburden load?
   a. What are the most effective storage parameters for preventing and inhibiting microbial growth? Effective is defined preventing proliferation of microbes. Variables to compare:
      i. Storage method
      ii. Storage temperature
      iii. Transportation duration