

SDC Figure 2. Bioburden Tissue Sample Collection Procedures

Tissue Collection during Index Hospitalization

Each site will be provided with tissue sample collection kits each with a unique identifier located on the back side of the box for tracking samples. Each kit will contain:

- THERAPAK 23650G Box for shipping
- Green Labeled Composite Wound Tissue Sample Container
- Blue Labeled Soft Tissue Composite Sample Container
- Yellow Labeled Deep Tissue Sample Container
- Purple Labeled Glass Tube for Clinical Microbiology
- Tear Tech Tube Shuttle
- THERAPAK 95K Transport Bag
- One large piece of clear tape for outside box
- Four small pieces of clear tape for tops of samples containers
- Two pieces of bubble wrap
- List of content (blue index card) for shipping
- Yellow Sample Collection Time and Date card

Standard Microbiology Tissue Collection

A representative surveillance wound tissue sample will be obtained for aerobic, anaerobic bacterial pathogen identification and fungal pathogen detection with sensitivity and specificity. A composite tissue sample will be obtained by a sterile wooden tongue depressor to scrape the regions of the wound that are considered to be at highest risk for infection (**Fig 1A**). This sample will be placed in the PURPLE labeled tube marked Clinical Microbiology Sample. ***The clinical microbiology glass tube cannot be left open for a long period of time; uncap the tube and re-cap immediately in order to preserve the sample.***

PCR Tissue Collection

The following three tissue samples will be obtained:

- A composite wound sample will be obtained by scraping the entire length and depth of the wound using a sterile wooden tongue depressor (**Fig 1A**) and placed in the GREEN labeled container marked Research Tissue 1.
- A deep tissue sample from the deep fracture site will be obtained by a rongeur and/or curette to obtain hematoma and residual debris from the region of the fracture site (**Fig 1B**) and placed in the YELLOW labeled container marked Research Tissue 3.
- A soft tissue composite sample from subcutaneous layers, fascia and muscle) is obtained by using a rongeur or knife blade to remove sample tissue from the areas consider at highest risk for the greatest biobuden yield (**Fig 1B**) and placed in the BLUE labeled container marked Research Tissue 2.

Composite Sample:

From all wound surfaces
and depths

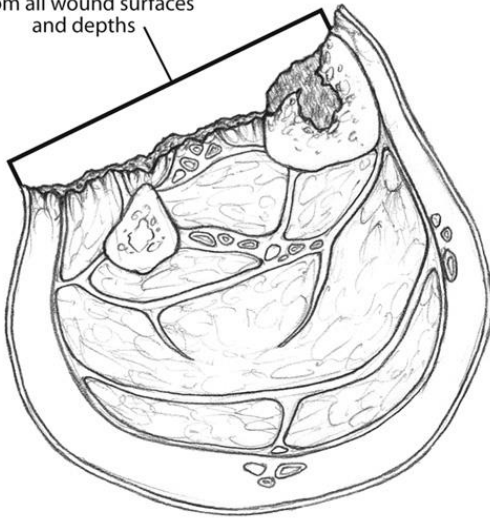


Figure 1A. Composite Sample

Sample:

Deep fracture site

Sample:

Subcutaneous tissue,
fascia, muscle

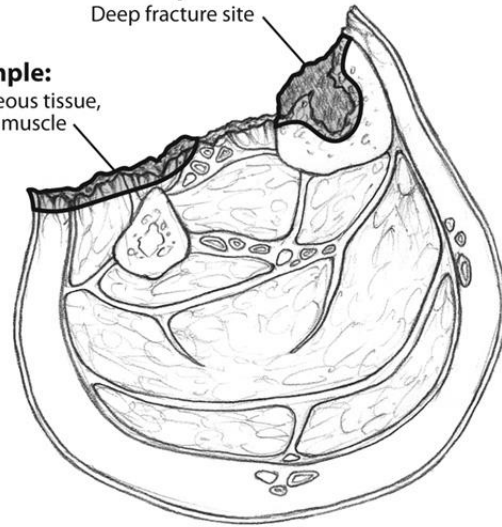


Figure 1B. Wound sampling procedure

Tissue Collection during Re-hospitalization

Each site will be provided with a second set of tissue sample collection kits specifically for tissue collection during re-hospitalization each with a unique identifier located on the back side of the box for tracking samples. Each kit will contain:

- THERAPAK 23650G Box for shipping
- Green Labeled Composite Wound Tissue Sample Container
- Purple Labeled Glass Tube for Clinical Microbiology
- Tear Tech Tube Shuttle
- THERAPAK 95K Transport Bag
- One large piece of clear tape for outside box
- Two small pieces of clear tape for tops of samples containers
- Two pieces of bubble wrap
- List of content (blue index card) for shipping
- Yellow Sample Collection Time and Date card

Tissue samples from patients re-admitted to the hospital during the study period will ONLY be collected if a patient is re-hospitalized for the following reasons:

- a deep surgical site infection,
- non-union,
- flap failure, or
- amputation

During re-hospitalization, two tissue samples will be obtained:

- A representative surveillance wound tissue sample for standard microbiology analysis.
- A representative surveillance wound tissue sample for PCR analysis.

Details regarding when tissue samples should be collected are described below:

Re-hospitalization for a surgical site infection

Tissue samples from the infected surgical site will be collected at the time of the surgical procedure and/or via aspiration of the abscess prior to surgery.

Re-hospitalization for a Non Union or Flap Failure

If a patient is re-admitted for non-union, tissue samples will be collected at the time of the non union surgery from the non-union site.

Re-hospitalization for Flap Failure

If a patient is re-admitted for flap failure, tissue samples will be collected at the time of the debridement flap surgery.

Re-hospitalization for Amputation

If a patient is re-admitted for an amputation, tissue samples will be collected at the time of the amputation or the surgery just prior that established untreatable chronic osteo.