Major Extremity Trauma Research Consortium (METRC):

Assessment of Severe Extremity Wound Bioburden at the Time of Definitive Wound Closure or Coverage: Correlation with Subsequent Post-Closure Deep Wound Infection (Bioburden Study)

Sponsored by: DOD OETRP

Contract Number: W81XWH-09-20108

Principal Investigator/Protocol Chair: Michael J. Bosse, MD

Medical Monitor: Marc Swiontkowski, MD

Version 4

August 11, 2011

This template is adapted from the ICH guidance document E6 (Good Clinical Practices), Section 6.

Confidentiality Statement

This document is confidential and is to be distributed for review only to investigators, potential investigators, consultants, study staff, and applicable independent ethics committees or institutional review boards. The contents of this document shall not be disclosed to others without written authorization from METRC (or others, as applicable), unless it is necessary to obtain informed consent from potential study participants.
Signature Page

The signature below constitutes the approval of this protocol and the attachments, and provides the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable U.S. federal regulations and ICH guidelines.

*The Lead Principal Investigator (Protocol Chair) should sign Signature Page 1. A copy of this Signature Page 1 should be filed with the holder of the Regulatory documents and a copy should be maintained at the site.*

Principal Investigator:  _______________________________________________

*Print/Type*

Signed:  ________________________________  Date:  ________________________

*Name/Title*
# CONTENTS

- Signature Page ......................................................................................................................... ii
- **PROTOCOL SUMMARY** ......................................................................................................... 8
- **1. KEY ROLES** ...................................................................................................................... 11
- **2. BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE** ......................... 12
  - 2.1 Background Information .................................................................................................. 12
  - 2.2 Rationale .......................................................................................................................... 14
  - 2.3 Potential Risks and Benefits ............................................................................................ 14
    - 2.3.1 Potential Risks ............................................................................................................ 14
    - 2.3.2 Potential Benefits ....................................................................................................... 14
- **3. STUDY OBJECTIVES** ......................................................................................................... 14
  - 3.1 Primary Objective ............................................................................................................. 14
  - 3.2 Secondary Objectives ....................................................................................................... 15
  - 3.3 Exploratory Objectives ..................................................................................................... 15
- **4. STUDY DESIGN** ............................................................................................................... 15
  - 4.1 Description of the Study Design ...................................................................................... 15
    - 4.2.1 Primary Endpoint ........................................................................................................ 17
    - 4.2.2 Secondary Endpoints ................................................................................................ 17
    - 4.2.3 Exploratory Endpoints .............................................................................................. 18
    - 4.2.4 Substudy Endpoints ................................................................................................... 18
- **5. STUDY POPULATION** ...................................................................................................... 18
  - 5.1 Description of the Study Population ................................................................................. 18
    - 5.1.1 Participant Inclusion Criteria ..................................................................................... 18
    - 5.1.2 Participant Exclusion criteria ..................................................................................... 18
8.2 Enrollment/Baseline .................................................................................................................. 23
8.3 Follow-up .................................................................................................................................. 23
  8.3.1 Retention ................................................................................................................................. 24
8.4 Final Study Visit ......................................................................................................................... 24
8.5 Early Termination Visit ............................................................................................................. 24
8.6 Pregnancy Visit ......................................................................................................................... 24
8.7 Unscheduled Visits .................................................................................................................... 24

9. ASSESSMENT OF SAFETY ....................................................................................................... 24
  9.1 Definitions ................................................................................................................................. 25
    9.1.1 Adverse event ...................................................................................................................... 25
    9.1.2 Unanticipated problem ........................................................................................................ 25
    9.1.3 Serious Adverse Event ........................................................................................................ 25
  9.2 Methods and Timing for Assessing, Recording, and Analyzing, Managing Safety Parameters ......................................................................................................................... 26
    9.2.1 Methods and Timing of Assessment .................................................................................... 26
    9.2.2 AE/SAE Grading and Relationship Assignment ................................................................ 26
    9.2.3 Recoding and Documentation ............................................................................................ 26
    9.2.4 Management of Adverse Events ........................................................................................ 26
  9.3 Adverse Event Reporting Procedures .................................................................................... 26
    9.3.1 Local Reporting Requirements ........................................................................................... 26
    9.3.2 SAE and Unanticipated Problem Reporting Requirements .............................................. 26
    9.3.3 METRC Coordinating Center Reporting Responsibilities .............................................. 27
    9.3.4 Department of Defense Reporting Requirements ............................................................. 27
  9.4 Reporting Pregnancy ................................................................................................................. 28
  9.5 Type and Duration of the Follow-up of Participants After Adverse Events ............................ 28
9.6 Modifications of Study Agent(s)/Intervention(s) for a Participant ........................................ 28
9.7 Halting Rules for the Protocol .................................................................................................. 28
9.8 Stopping Rules for an Individual Participant/Cohort ............................................................... 28
9.9 Premature Withdrawal of a Participant ................................................................................... 28
9.10 Replacement of a Participant Who Discontinues Study Treatment ........................................ 28

10. CLINICAL MONITORING STRUCTURE ................................................................................. 29
10.1 Site Monitoring Plan ............................................................................................................... 29
10.2 Safety Monitoring Plan .......................................................................................................... 29
    10.2.1 Safety Review Plan by the DSMB ................................................................................ 29

11. STATISTICAL CONSIDERATIONS ...................................................................................... 29
11.1 Overview and Study Objectives ............................................................................................ 29
11.2 Sample Size Considerations ................................................................................................. 29
11.3 Randomization ...................................................................................................................... 30
11.4 Missing Data and Measures to Minimize Bias ...................................................................... 30
11.5 Planned Interim Analysis ...................................................................................................... 31
11.6 Analysis Plan ........................................................................................................................ 31

12. QUALITY CONTROL AND QUALITY ASSURANCE ............................................................. 31

13. ETHICS/PROTECTION OF HUMAN SUBJECTS ................................................................. 32
13.1 IRB/Ethics Committee ............................................................................................................ 32
13.2 Informed Consent Process .................................................................................................. 32
    13.2.1 Consent and Enrollment ............................................................................................... 32
    13.2.2 Assessing Capacity to Consent and Consenting a Proxy Respondent ......................... 33
    13.2.3 Consent for Tissue Banking ......................................................................................... 34
    13.2.4 Informed Consent Process or Assent (for a minor) ..................................................... 34
13.2.5 Medical Record Release .......................................................... 35
13.3 Exclusion of Women, Minorities, and Children (Special Populations) ............... 35
13.4 Participant Confidentiality .................................................................. 35
13.5 Study Discontinuation ....................................................................... 35
14. DATA HANDLING AND RECORD KEEPING ..................................... 35
14.1 Data Management Responsibilities .................................................... 35
14.2 Data Capture Methods ..................................................................... 36
14.3 Types of Data .................................................................................. 36
14.4 Source Documents and Access to Source Data/Documents ......................... 37
14.5 Timing/Reports ............................................................................... 37
14.6 Study Records Retention .................................................................. 37
14.7 Protocol Deviations ......................................................................... 37
15. PUBLICATIONS POLICY .................................................................... 37
16. SCIENTIFIC REFERENCES ................................................................. 37
17. APPENDICES ....................................................................................... 40
   APPENDIX A: Study Contact Roster ....................................................... 40
   APPENDIX B: Protocol Committee ........................................................ 41
   APPENDIX C: Data Collection Schedule ............................................... 42
   APPENDIX D: Tissue Sample Collection Procedures .............................. 44
   APPENDIX E: Tissue Sample Procedures ............................................. 47
   APPENDIX F: Consent Template ........................................................... 50
   APPENDIX G: Evaluation to Give Consent ........................................... 62
   APPENDIX H: Patient Baseline Interview Questions ................................ 63
   APPENDIX I: Patient 12 Month Follow up Telephone Interview Questions ....... 71
PROTOCOL SUMMARY

**Title:** Assessment of Severe Extremity Wound Bioburden at the Time of Definitive Wound Closure or Coverage: Correlation with Subsequent Post-Closure Deep Wound Infection (Bioburden Study)

**Sponsor:** DOD OETRP

**Type of study:** Prospective cohort

**Objectives:** The primary objective of this study is to characterize the contemporary extremity wound “bioburden” at the time of definitive wound coverage/closure of severe extremity military and civilian wounds. We will analyze routine tissue samples collected as part of standard of care employing both standard tissue culture microbiology and modern polymerase chain reaction (PCR) technologies. PCR analyses throughout this study will utilize the Ibis T5000 Biosensor System.

Secondary objectives of the study are to determine: (a) the correlation of the identified wound pathogens at the time of wound closure/coverage with subsequent deep wound infections; (b) the correlation of the PCR results with those obtained from standard hospital microbiology; and (c) the efficacy, if any, of antibiotics used in the care of the wound.

**Specific Aim 1:** In a subset of 60 patients, compare the bioburden, as detected by Ibis technology, from each of three sampling techniques (deep tissue; soft tissue composite; composite of tissue from the length and depth of the wound). Samples obtained using the most effective technique identified in this step will be processed using Ibis in subsequent tissue analysis. Effectiveness is defined as the ability to identify key wound infection-causing pathogens.

*Hypothesis 1:* The composite sampling approach will be the most effective technique.

**Specific Aim 2:** Characterize the wound bioburden at the time of definitive wound closure or coverage using the Ibis T5000 Biosensor System PCR technology as compared to standard microbiology techniques.

*Hypothesis 2:* The Ibis technology will detect more species of pathogens than standard microbiology techniques. The percent of patients for whom Ibis will detect all species identified by standard microbiology will be greater than 95%.

**Specific Aim 3:** Characterize the wound bioburden in the patients who develop deep
infection within one year of wound closure, and determine the association between infecting pathogens with initial wound closure bioburden as measured jointly by Ibis and standard microbiology techniques.

**Specific Aim 4:** Document the variability in antibiotic selection and duration, and examine the impact of this selection on subsequent deep infection.

*Hypothesis 4a:* Among patients treated with antibiotic regimens that are appropriate for the pathogens identified by standard microbiology, there will be a lower probability of deep infection than among those patients who received inappropriate antibiotic regimens.

*Hypothesis 4b:* Among patients treated with antibiotic regimens that are appropriate for the pathogens identified by Ibis, there will be a lower probability of deep infection than among those patients who received inappropriate antibiotic regimens.

**Study design:** The Bioburden study is a multi-center, prospective cohort study of wound bacterial bioburden and associated antibiotic care in severe open lower extremity fractures.

**Study duration:** 24 months; 9 month enrollment period, 1 year patient follow up and 3 month data analysis period

**Sample size:** 750 participants

**Number of study sites:** 35 METRC Core sites (24 Civilian Core Clinical Sites and 4 MTF Core Clinical Sites) and approximately 10 Satellite Clinical sites

**Inclusion criteria**
1. All open Grade III tibia fractures (plateau, shaft and pilon) requiring a second procedure following fixation, or traumatic transtibial amputations requiring delayed primary closure, skin grafting and/ or flap coverage.
2. Ages 18 – 64 years inclusive
3. Patients may have risk factors for infection including diabetes, immunosuppression from steroids or other medications, HIV, or other infections.
4. Patients may have a traumatic brain injury.
5. Patients may have other fractures including spine, upper extremity fractures, contralateral lower extremity injuries, ipsilateral pelvis, hip, femur or foot injuries.
6. Patients may be treated initially at an outside institution prior to transfer to the study institution, as long as the definitive fixation was not performed prior to entrance into the study.
7. Patients with bilateral injuries that meet inclusion criteria may be included, but only the limb rated as “more severe” by the treating surgeon will be enrolled in the study.
8. Patients may have co-existing non-tibial infection, with or without antibiotic treatment.
9. Patients may have an existing infection of the surgical wound under treatment at the time of wound closure.
10. Patients may be definitively fixed using any method (nail, plate, ex fix)
11. Patients may have a fasciotomy

**Exclusion criteria**

1. Patient speaks neither English nor Spanish
2. Patient is a prisoner
3. Patient has been diagnosed with a severe psychiatric condition
4. Patient is intellectually challenged without adequate family support
5. Patient lives outside the hospital’s catchment area
6. Patients with planned follow-up at another medical center

**Outcome measures**

The primary study endpoint is the first surgery for treatment of a deep infection (defined below), a non-union, flap failure, or amputation. Surgical re-admissions will be tracked prospectively in order to be able to obtain tissue samples during follow up surgeries, up to the first surgery following the diagnosis of infection.

**Statistical analysis:** Analyses will vary by specific aim. To evaluate our hypotheses, we will use exact and non-parametric hypothesis testing procedures. Confidence intervals (95%) of differences will be computed. Regression analysis will be employed when adjustment between comparison groups is considered necessary.

**Safety monitoring:** The Medical Monitor is responsible for monitoring the accumulated interim data as the study progresses to ensure patient safety.
1. KEY ROLES

Protocol Committee- Responsible for developing a detailed study protocol, provides oversight on study progress and acts to correct deficiencies in the conduct of the study. This committee also drafts the main publications related to the study.

Steering Committee- Steering Committee is the decision making body of the Consortium and makes decisions regarding study design issues, study procedures, allocation of study resources and priorities for meeting competing demands of the Consortium and individual studies. The Steering Committee is composed of Site Investigators from each core METRC clinical center, the Department of Defense Program Officer for METRC, the orthopaedic consultants from the Army, Navy and Air Force, regional representatives of Satellite Clinical Centers, and the Director, Deputy Director, Principal Biostatistician and Principal Economist of the Coordinating Center. The Steering Committee is responsible for approving the protocol.

METRC Coordinating Center- Responsible for maintaining all study documentation, developing and maintaining the master IRB application and consent, circulating any changes to study documents including protocols, case report forms, and IRB materials to each participating center, providing daily oversight and management of study implementation, providing payment to sites for patients enrolled, performing site monitoring, data quality control and analysis of study results.

Core and Satellite Clinical Sites- Responsible for the conduct of clinical studies including patient enrollment, performing study procedures, data collection and conducting study follow-up visits.

Allegheny-Singer Research Institute, Center for Genomic Sciences- Responsible for preparing tissue sample collection kits, processing tissue samples with Ibis and standard tissue culture microbiology and sample storage.

Clinical Outcome Adjudication Committee (COAC)- Responsible for developing the timely medical review and adjudication of trial-specific endpoints utilizing trial-specific definitions; engages other reviewers as needed and in accordance with the COAC Policy; and reports adjudication results to the trial-specific Protocol Committee.

Publication Committee- Responsible for reviewing manuscripts prior to journal submission and reviewing presentations prior to presentation; for mediating and settling disputes and conflicts among study investigators over publication or presentation priorities, authorship, and any other issues related to publications or presentations; for preparing and maintaining a list of concepts for publications and preparing and maintaining a list of approved METRC publications, which shows the status of each manuscript from initiation through publication.

Medical Monitor- Responsible for providing medical guidance and overseeing patient safety for the study. The MM participates in determining the course of action necessary to meet safety goals and objectives. This is achieved through the review of safety reports; resolving safety issues; and interacting with Principal Investigators.
2. BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 Background Information

Infection remains the most common and significant complication following high energy fractures, with rates ranging from 25 – 40%. (Mody et al., 2009; Zalavras et al., 2005; Breidenbach and Trager, 1995; Al-Jabrah et al., 2007; Bosse et al 2002; Burns et al 2010; Johnson et al., 2007; Yun et al., 2008; Murray et al., 2008; Murray, 2009) Up to 15% of recent combat casualties develop osteomyelitis. (Murray et al., 2008). The large traumatic wounds are inoculated with environmental bacteria at the time of injury and are exposed to host and hospital flora colonization during the initial treatment course. In contrast to significant advances made in related extremity trauma care disciplines, the strategies that address the prevention of deep infection following severe open fracture wounds have remained constant for the past 20 years. Wounds are aggressively debrided on the day of injury and as needed thereafter, until a stable wound bed is obtained. All necrotic tissue and organic and inorganic contaminants are removed. Systemic antibiotics are administered. The antibiotic type, dosing, and duration treatment varies with surgeon, hospital and region. The wounds are closed or covered when deemed “ready” by surgeon. At the time of wound closure, antibiotics are typically provided using a broad-spectrum approach. The duration of this therapy varies from 1-14 days. Few surgeons sample the wound bioburden at the time of closure, typically related to the unreliable results from the routine microbiology at predicting subsequent failure due to infection.(Murray et al., 2008; Lee, 1997)

**Prevention and Treatment of Infection**

Evidence supports the initial administration of systemic antibiotics as effective in reducing the infection rate in open fracture care. (Patzakis et al., 1983; Okike and Bhattacharyya, 2006; Zalavras et al., 2004; Zalavras et al., 2005) Many patients with severe extremity wounds are multiple trauma patients, faced with recovery from head, chest and abdominal injuries associated with the extremity injury. These patients are at risk for the development of nosocomial infections and for infection with resistant pathogens. Prolonged or repeated systemic antibiotic exposure is avoided, if possible, in this cohort. The long term impact of systemic antibiotic therapy on severe extremity wounds is currently unknown and may be counter-productive. When antibiotics are administered, a directed therapy approach is preferred. Tissues in the zone of injury are often hypoperfused and the antibiotic concentration in the critical injured tissues is less than the desired therapeutic level. (Hanssen et al., 2005) The wound bacteria and fungi can attach to soft tissues, bone or metal implants and initiate biofilm production.(Costerton 2007) Biofilm colonies are increasingly recognized as the likely source of chronic and implant related infections. (Costerson 2007) Traditional antibiotics are unable to penetrate the biofilm meaning multi-component therapies that prevent biofilm development are needed (Ehrlich et al., 2005).

To address these challenges, many surgeons employ local adjunctive antibiotic therapies in an effort to “sterilize” the wound. Antibiotics are combined with PMMA cement or bio-absorbable ceramics and are placed into the wound area as beads. These implants deliver high concentrations of antibiotics to the local tissues with no appreciable systemic antibiotic exposure. (Zalavras et al., 2004; Zalavras et al., 2005; Wenke et al., 2006) Both animal and clinical
research supports the efficacy of the local antibiotic delivery strategy. (Wenke et al., 2006; Hanssen et al., 2005; Zalavras et al., 2005; Zalavras et al., 2004)

Over the next decade, the treatment of the severe wound is expected to dramatically change. Local wound anti-microbial therapies employing bio-degradable micro or nanospheres, ceramics or chitosan sponges may likely replace systemic therapy. These local antibiotic treatments might be potentiated by combining them with biofilm disbursing agents (bismuth thiols).

The evolution of therapy to the local level requires a better understanding of the bioburden challenge present in our contemporary wounds. At the present time, surgeons treat the severe wounds empirically. Based on the historic deep infection profile at the treating center, antibiotics are selected to prevent infection from that pathogen without actually knowing the pathogen profile of the individual patient’s wound. If a deep infection develops (25-40% of the time), deep cultures are obtained and directed systemic therapy is provided based on the pathogen’s sensitivity profile.

**Identification of infectious agents**

In our current protocols, the surgeon rarely samples the severe wound for pathogen identification and less often, delivers a therapeutic course of antibiotics that are specifically targeted to the dominant bacteria. Currently, we are unable to assess the relationship of a subsequent deep infection to the patient’s bioburden profile at the time of wound closure and are unable to determine the efficacy / impact of the patient’s antibiotic treatment during the hospitalization to the late infection pathogen. In order to change the extremity wound treatment paradigm, we need to better understand the wound bioburden profile, the impact, if any, of systemic and local antibiotics on the prevention of later infection and patient risk factors that might contribute to treatment failure.

Standard microbiological techniques are considered unreliable by most surgeons and, therefore, do not influence patient care decisions (Lee, 1997). Wound surface samples obtained during the course of antibiotic therapy may falsely report “no growth” in cases where the pathogens were suppressed, but not eliminated by the treatment, or report the presence of a readily culturable bacteria that is the principle agent of the later infection. Wenke et al. (2006), Svoboda et al. (2006), and Lallia (2010) have demonstrated that wound surface contamination varies during the course of treatment. Sampling error (selecting the microbiology sample from a clean area of the wound) thus impact the treatment strategy for the large wounds.

Standardizing extremity wound microbiology sampling techniques and the addition of Ibis-based polymerase chain reaction (PCR) techniques to the wound bioburden analysis could enhance our ability to define the magnitude of the wound bioburden and the relationship of subsequent infections to the initial bioburden screen. (Kobayashi et al 2009; Price et al 2009) The Ibis T5000 is a new generation rapid PCR base composition technology ideally suited to the identification of wound bioburden as it provides comprehensive coverage for all eubacterial species without the need to decide a priori what to test for. (Ecker et al., 2008)
2.2 Rationale

This project is designed to determine the microbiology profile of severe extremity trauma bioburden at wound closure, and to compare the PCR determined profile to the bacteria identified by routine microbiology techniques. The results from both identification methods will be compared to the pathogens associated with deep surgical site infections that occur post closure of the wound. The project will leverage the resources of a large extremity trauma consortium and will longitudinally follow patients from admission to one year from injury. The analysis of antibiotics treatment strategies and host risk factors might define opportunities to refine antibiotic selection and treatment duration strategies. The results of this project are expected to form the basis for the design of a PRCT that will evaluate the effect, if any, of local wound therapy on the development of deep infection. PCR analyses throughout this study will utilize the Ibis T5000 Biosensor System, but it is not a goal of this project to develop data to commercialize this technology.

2.3 Potential Risks and Benefits

2.3.1 Potential Risks

While tissue sampling is routinely performed in this setting as part of standard of care, there is always a small risk of injury associated with each tissue removal.

Any time information is collected for a study, there is a small risk of breach of confidentiality. However, this risk is not greater than the risk that already exists in clinical settings when handling medical data.

2.3.2 Potential Benefits

While there are no direct benefits to patients participating in the study, participation may help determine the best treatment for open tibia fractures in the future.

3. STUDY OBJECTIVES

3.1 Primary Objective

The primary objective of this study is to characterize the contemporary extremity wound “bioburden” at the time of definitive wound coverage/closure of severe extremity military and civilian wounds. We will analyze routine tissue samples collected as part of standard of care employing both standard tissue culture microbiology and modern polymerase chain reaction (PCR) technologies. PCR analyses throughout this study will utilize the Ibis T5000 Biosensor System.
3.2 Secondary Objectives

Secondary objectives of the study are to determine: (a) the correlation of the identified wound pathogens at the time of wound closure/coverage with subsequent deep wound infections; (b) the correlation of the PCR results with those obtained from standard hospital microbiology; and (c) the efficacy, if any, of antibiotics used in the care of the wound.

3.3 Exploratory Objectives

N/A

4. STUDY DESIGN

4.1 Description of the Study Design

The Bioburden study is a multi-center, prospective, observational cohort study of wound bacterial bioburden and antibiotic care in open lower extremity fractures. The primary goal of this study is to characterize the contemporary extremity wound “bioburden” at the time of definitive wound coverage / closure employing polymerase chain reaction (PCR) technology. This will be achieved by prospectively collecting wound samples from 750 lower extremity injuries at the time of wound closure. These samples will be shipped to a central laboratory, and analyzed for wound bacterial bioburden using both the Ibis T5000 Biosensor System PCR-ESI-TOF-MS technology and standard tissue culture microbiology techniques. These patients will then be tracked for up to one year to ascertain the onset of surgical site infections; tissue samples at the time of the first follow up surgical procedure will be collected. The follow up surgery tissue samples will also be shipped to the central laboratory for Ibis and tissue culture analyses. These data will be used to assess the roles of baseline wound flora and antibiotic regimens on the later selection and antibiotic resistance profiles of the pathogens in the infected wounds. It should be noted that the first 60 samples will be used to address specific Aim #1. For these first 60 samples Ibis technology will be applied to the three different approaches and results compared. All subsequent Ibis analyses will be done only on the sample obtained using the most effective approach (as defined in Specific Aim #1).

A schematic representation of the Bioburden study can be found below.
Patients with high energy open fractures below the knee (including traumatic amputations), who require either a delayed primary closure, skin grafting and/or local or free tissue transfer for coverage of the injury will be eligible for enrollment. Patients will be recruited from the METRC Core sites, the Military Treatment Facilities, and the METRC Satellite Centers.

Participants will provide informed consent prior to the final wound closure procedure, and asked to provide basic demographic information. At the time of final wound closure, tissue samples will be collected and shipped to the central laboratory for the study. The analysis of these samples will not be available to the surgeon for making treatment decisions. The treating surgeon may, however, independently send a sample to the hospital’s microbiology lab as part of standard assessment and treatment protocols.

Patients will not be asked to participate in follow up visits. The primary study outcome is the first hospital re-admission for surgery for treatment of a deep infection, non union, flap failure, or amputation. Surgical re-admissions will be tracked prospectively in order to be able to obtain tissue samples during follow up surgeries, up to the first surgery for treatment of a deep infection, non union, flap failure, or amputation. At 12 months post injury, a medical record review will be performed for patients who have not come back for surgery during the follow up year to capture missed re-hospitalizations. Patients will receive a brief phone interview at 12
months in order to ensure no missed readmissions. If a patient indicates that he/she had been
readmitted to the hospital, discharge abstracts will be obtained from the treating facility. The
study surgeon will make a determination of whether the surgery qualifies as the first surgery for
treatment of a deep infection, non union, flap failure, or amputation. Microbiology lab reports
will be obtained for that admission.

Following completion of the Ibis analysis, nucleic acids samples will be retained for future
research studies involving host genetics, gene expression and microbial clade analyses. Before
tissues samples are used in a future research study, separate IRB approval will be obtained.
Patients in the study will have the option to decline participation in future genomic studies.

4.2 Study Endpoints

4.2.1 Primary Endpoint

The primary study endpoint is the first surgery for treatment of a deep infection. In addition we
will obtain tissue samples from wounds requiring surgical treatment for a non-union, flap failure,
or amputation because these surgeries may be due to undetected infection. Deep infection is
defined using the CDC criteria (described below). Surgical re-admissions will be tracked
prospectively to obtain tissue samples during follow up surgeries, up to the first surgery
following the diagnosis of infection. The presence or history of infection is routinely assessed at
every clinic visit as well as at every hospital re-admission or outpatient surgical procedure.

**Definition of Infection:** The presence of a deep surgical site infection will be defined by the
criteria of the Centers for Disease Control (Mangram et al., 2008). Deep SSI occurs within 30
days after the operation if no implant is left in place, or within one year if implant is in place and
the infection appears to be related to the operation, and the infection involves deep soft tissues
(e.g., fascial and muscle layers) of the incision, and at least one of the following: (a) Purulent
drainage from the deep incision; (b) A deep incision spontaneously dehisces or is deliberately
opened by a surgeon when the patient has at least one of the following signs or symptoms: fever
(>38°C), localized pain, or tenderness; (c) An abscess or other evidence of infection involving
the deep incision is found on direct examination, during reoperation, or by histopathologic or
radiologic examination; or (d) Diagnosis of a deep SSI by a surgeon or attending physician.

4.2.2 Secondary Endpoints

**Classification of Appropriate Antibiotic Care:** An expert panel consisting of the study PI, two
additional orthopaedic trauma surgeons and at least three infectious disease experts will be
convened to develop a classification grid for the most common and/or expected microbial
species to be found in this study and the related antibiotic treatment regimens used in the initial
care of these patients. For each microbial species, the expert panel will classify a given antibiotic
regimen as “appropriate” and “not appropriate”, based on the best available published data.
Appropriateness will be based on the selection of the antibiotic, the dosage and the duration. As
part of this study, we will collect information on both antibiotic regimens and wound flora.
Using the classification grid and these data, the study Protocol Committee will classify each patient as having received antibiotic care that was “appropriate” or “not appropriate”.

4.2.3 Exploratory Endpoints

N/A

4.2.4 Substudy Endpoints

N/A

5. STUDY POPULATION

5.1 Description of the Study Population

5.1.1 Participant Inclusion Criteria

1. All open Grade III tibia fractures (plateau, shaft, pilon) requiring a second procedure following fixation, or traumatic transtibial amputations requiring delayed primary closure, skin grafting and/or flap coverage.
2. Ages 18 – 64 years inclusive
3. Patients may have risk factors for infection including diabetes, immunosuppression from steroids or other medications, HIV, or other infections.
4. Patients may have a traumatic brain injury.
5. Patients may have other fractures including spine, upper extremity fractures, contralateral lower extremity injuries, ipsilateral pelvis, hip, femur or foot injuries.
6. Patients may be treated initially at an outside institution prior to transfer to the study institution, as long as the definitive fixation was not performed prior to entrance into the study.
7. Patients with bilateral injuries that meet inclusion criteria may be included, but only the limb rated as “more severe” by the treating surgeon will be enrolled in the study.
8. Patients may have co-existing non-tibial infection, with or without antibiotic treatment.
9. Patients may have an existing infection of the surgical wound under treatment at the time of wound closure.
10. Patients may be definitively fixed using any method (nail, plate, ex fix)
11. Patients may have a fasciotomy

5.1.2 Participant Exclusion criteria

1. Patient speaks neither English nor Spanish
2. Patient is a prisoner
3. Patient has been diagnosed with a severe psychiatric condition
4. Patient is intellectually challenged without adequate family support
5. Patient lives outside the hospital’s catchment area
6. Patients with planned follow-up at another medical center

5.1.3 Co-Enrollment Guidelines

If allowed by the local IRB, patients in the Bioburden Study can be enrolled in other METRC and non-METRC studies. The Bioburden Study is a prospective cohort study employing standard wound culture techniques and routine clinical follow-ups. This study will not impact other studies the patient might be enrolled in at the time of recruitment and the recruitment of patients enrolled into the Bioburden Study into other trials should not present a conflict.

5.2 Strategies for Recruitment

5.2.1 Recruitment Overview

Approximately 750 patients will be enrolled from METRC core civilian trauma centers, military treatment facilities and METRC satellite centers over a 9 month period. Core centers are large level I trauma centers with large numbers of severe open fractures and includes sites with a proven track record for successfully recruiting and retaining patients in prospective studies in orthopaedic trauma. Satellite centers are smaller civilian Level 1 trauma centers that elect to participate on an ad hoc basis, as study recruitment needs and center interest intersect. Patients will be recruited during a hospitalization for the treatment of a high energy lower extremity injury. Consenting procedures are described in detail in Sections 8 and 13 of this protocol.

6. STUDY TREATMENTS

6.1 Study Treatment

This is an observational study. As such, there are no study treatments. All patients will be treated as per the participating center’s standard of care.

6.2 Standardization of Care

N/A

6.3 Concomitant Medications and Procedures

N/A

6.4 Precautionary and Prohibited Medications and Procedures

N/A

6.5 Prophylactic Medication and Procedures
6.6 Rescue Medications

N/A

7. STUDY PROCEDURES/EVALUATIONS

7.1 Clinical Evaluation

7.1.1 Medical Record Review

During the index hospitalization, information about the patient’s injury including mechanism, Gustilo grade, OTA classification and wound length in addition to the patient’s medical history and antibiotic treatment will be collected from medical record review.

For patients re-hospitalized during the 12 month study period with a deep infection, or for non-union, flap failure or amputation, information about antibiotics received prior to re-hospitalization will be obtained from the medical record.

At 12 months post injury, a medical record review will be performed for patients who have not come back for surgery during the follow up year to capture missed re-hospitalizations.

7.1.2 Patient Interview

During the index hospitalization, patients will be asked about their race and ethnicity, their smoking history and their general health status before the injury using standard interview questions.

Patients who are not re-admitted to the study site hospital during the 12 month study period will receive a brief telephone interview at the end of the study to ascertain admission(s) to any hospital for deep infection, non-union, flap failure or amputation.

7.2 Laboratory Evaluations

7.2.1 Wound Sampling

During the surgical procedure for final soft tissue closure, the Research Coordinator will work with the study surgeon to collect wound samples that will be shipped to the Center for Genomic Sciences at the Allegheny-Singer Research Institute in Pittsburgh, PA for standard microbiology cultures and Ibis testing. Three tissue samples will be collected for analysis with Ibis and one tissue sample will be collected for standard microbiology analysis. The benefit of Ibis results for
clinical decision making is currently unknown; these results will be used for research purposes only and will not be given to the treating physician.

For patients re-hospitalized during the 12 month study period with a deep infection, for non-union, flap failure or amputation, one tissue sample will be collected for analysis with Ibis and one tissue sample will be collected for standard microbiology analysis. Tissue samples will only be obtained for the first follow-up surgical treatment.

Tissue sampling procedures can be found in the tissue sample collection protocol in Appendix D.

7.2.2 Tissue Sample Shipping Protocol

The wound tissue cultures will be shipped to the Center for Genomic Sciences at the Allegheny-Singer Research Institute in Pittsburgh, PA, via commercial carrier as a UN 3373 Biological Substance Category B. No declaration of dangerous goods is required. A CDC permit is not required. All specimens will be packaged according to IAW IATA packing instruction 650. Detailed shipping instructions are described in Appendix E.

7.2.3 Clinical Site Microbiology

During the index hospitalization, clinical microbiology lab reports will be obtained from the clinical site lab if these analyses are done as part of the standard of care at that center.

Clinical microbiology lab reports will be obtained from the clinical site lab for patients’ first re-admission to the hospital for deep infection, non-union, flap failure or amputation during the 12 month study period.

If a patient indicates that he/she had been readmitted to the hospital, discharge abstracts will be obtained from the treating facility. The study surgeon will make a determination of whether the surgery qualifies as the first surgery for treatment of a deep infection, non union, flap failure, or amputation. Microbiology lab reports will be obtained for that admission.

7.2.4 Microbiology analysis at the Center for Genomic Sciences

Clinical Microbiology tissue samples will be placed in anaerobic transport medium for storage and shipping to Center for Genomic Sciences. The specimens received by the CGS will be processed using only the de-identified METRC study ID. Once at the clinical microbiology lab, the tissue sample will be ground for anaerobic, aerobic, and fungal culture using sterile disposable tissue grinder. Media will be inoculated with the macerated sample and incubated at 35°C at 5% CO2. All aerobic and anaerobic plates and broth will be examined at 24 hours. If there is no growth on the plates or in the broth at 24 hours they will be incubated for an additional 24 hours. At 48 hours if there is no growth on the plates they are discarded. At 48 hours if there is no growth in the broth, it will be kept for an additional 72 hours and checked every 24 hours for signs of growth. At 5 days if the broth culture is negative, it is discarded and a negative report is sent out. Any growth on the plates or in the broth, at any time point during the
5 days, will be identified using current microbiology guidelines and protocols. Fungal plates will be checked at 48 hours and at seven days. The plates will be kept for up to 4 weeks to look for signs of growth. Any growth on the plates will be identified using current microbiology guidelines and protocols. At 4 weeks if no growth is seen, a negative report will be sent out and the plates will be discarded.

7.2.5 *Ibis T5000 analysis at the Center for Genomic Sciences*

The specimens received by the CGS will be processed using only the deidentified METRC study ID. The nucleic acid extraction will be performed using a commercially available kit specified for DNA extraction from tissue (Qiagen chemistry on the Autogen platform). This uses a combination of chemical agents and bead beating validated for many types of tissue. After the nucleic acid extraction, the Ibis assay will be prepared. Each assay kit comes with 10 96-well plates. The instrument itself can accommodate up to 15 plates total. An amplified plate, desalting plate (magnetic bead DNA binding resin), and clean elution plate are included in each run. Each plate has the potential to run 6 samples. The first process is desalting to avoid contamination (using weak anion exchange). Liquid samples are heat-sealed before loading. This protects against amplicon contamination and solvent evaporation. The specific kit used in this study will be the BCAkit (Abbott Laboratories), which uses 16 primers to detect a broad range of bacteria including beta/alpha proteo-bacteria as well as Candida and typical resistant strains of staphylococcal and enterococcal species. The amplicons from the PCRs are analyzed by electron spray ionization time of flight mass spectrometry. The spectral signals can determine the base composition of each amplicon. Able to detect difference in weight of base composition of small quantities of nucleic acid, circumventing the ‘zero-sum’ effect of specific PCR. Weights are mathematically triangulated. The Ibis T5000 is approved for investigational use only. These results will not be used for clinical decision making. The results will not be given to the treating physician.

7.2.6 *Biospecimen Storage*

Following completion of tissue sample analysis, nucleic acids samples will be retained at the Center for Genomic Sciences at Allegheny-Singer Research Institute in Pittsburgh, PA for future host genetics, gene expression and microbial clade analyses. The nucleic acid samples will be documented in the CGS sample storage database and stored in the clinical laboratory freezers at -80 degrees Celsius. Samples will remain de-identified and destroyed after 10 years. Since the samples will be de-identified, no Certificate of Confidentiality will be obtained.

Samples will only be used for the purposes of satisfying the aims of the study as previously described and will not be shared with other investigators outside METRC. The samples will be used for research purposes only and will not be sold. Participants will not be paid for allowing this tissue to be used in research. Future studies involving the analysis of stored tissue samples will require separate IRB approval.
Patients in the study will have the option to decline participation in future genomic studies. If a patient does not wish to have their samples stored for future analysis, they will be destroyed within one year of the end of the study. If a patient decides that they would like to withdraw their consent of participation in the study, they must notify the Research Coordinator in writing. At that time, the METRC Research Coordinator will contact the METRC Coordinating Center and CGS will be notified to destroy the sample immediately. A notice will also be placed in the patient study file acknowledging both the decision to withdraw consent for participation and the date of destruction of the sample.

7.3 Assessment of Participant Compliance with Study

N/A

8. STUDY SCHEDULE

8.1 Screening

Patients will be screened for eligibility in each center by the local Research Coordinator in close coordination with the surgeon investigators. Screening will typically occur within the first day or two after initial debridement and prior to wound closure or coverage. All potentially eligible patients will be entered into REDCap (the METRC distributed data collection system), a study number assigned, and eligibility criteria confirmed. The study PI will be available via pager to answer questions regarding study eligibility. This pager service will be active during regular east and west coast business hours. When the study PI is not available, this pager line will be forwarded to a designated co-investigator. Contact information for the PI and alternate contact is available in Appendix A. In most cases, questions should be resolved at this level. However, an adjudication committee has also been set up for more difficult questions. It will be the responsibility of the Study PI or the designated on-call investigator to refer these questions to the adjudication committee, as well as communicate committee decisions back to the study sites.

8.2 Enrollment/Baseline

Following consent, the patient will be approached by a member of the research team for completion of baseline data Case Report Forms (CRFs). The data to be collected during hospitalization for definitive fixation is outlined in Appendix C.

8.3 Follow-up

Patients will be prospectively monitored for re-hospitalization for deep infection, non-union, flap failure or amputation. A critical aspect for the success of this study will involve prospectively identifying re-hospitalizations in order to collect tissue samples at the time of surgery. Procedures to maximize study retention are outlined in section 8.3.1, below.
8.3.1 Retention

Every effort will be made to retain patients in the study. Patients will be followed until they present with an infection or to 1 year, whichever comes first. Study coordinators at participating sites will utilize a variety of methods to insure that patients are flagged if they are seen in clinic or admitted to the hospital. These methods include, but are not limited to, screening clinic schedules, placing identifying marks on patient charts (i.e., stickers or notes) that indicate research study participation, setting electronic “alarms” that alert study staff when patient records are flagged, etc. Study coordinators will place telephone calls to patients at 1 year if not seen prior for infection. The telephone call will be brief, soliciting information about hospitalization or treatment for infection of the study injury. As part of the site certification process for participation in this study, sites will have to outline the procedures they will use to ensure adequate follow-up of patients and timely identification of surgeries.

Patients enrolled in the study will not receive an honorarium for participation.

8.4 Final Study Visit

At 52 weeks the patient will be interviewed by phone by the site Research Coordinator using a brief CRF designed to elicit information about re-hospitalizations related to the study injury. These interviews will only apply to patients who had not been already re-admitted for surgical treatment for a deep infection, non-union, flap failure and amputation.

8.5 Early Termination Visit

N/A

8.6 Pregnancy Visit

N/A

8.7 Unscheduled Visits

N/A

9. ASSESSMENT OF SAFETY

The study will monitor and report adverse events to ensure patient safety. Definitions and procedures for reporting adverse events are designed to satisfy 45 CFR Part 46, Subpart A; the “Common Rule”, shared by 17 Departments and Agencies as well as 21 CFR 312, the FDA regulation for adverse events. The Common Rule requires written procedures and policies for ensuring reporting of “unanticipated problems” involving risks to participants to IRBs, appropriate institutional officials, and the Department or Agency Head. The medical monitor (MM) is responsible for providing medical guidance and overseeing patient safety for the study. The MM participates in determining the course of action necessary to meet
safety goals and objectives. This is achieved through the review of Serious Adverse Event reports; resolving safety issues; and interacting with Principal Investigators.

Each participating site is responsible for ensuring that all local IRB requirements for reporting adverse events (both internal and external) are met.

9.1 Definitions

9.1.1 Adverse event

Any untoward or unfavorable medical occurrence in a human subject, including abnormal sign (e.g., abnormal physical exam or laboratory finding), symptom or disease temporally associated with the subject’s participation in the study, whether or not considered related to the subject’s participation.

9.1.2 Unanticipated problem

Any incident, experience, or outcome that meets all of the following criteria:
(1) is unexpected, in terms of nature, severity, or frequency, given the research procedures that are described in the protocol and informed consent document and the characteristics of the patients eligible for the study.

(2) is related or possibly related to treatment/procedures under study; possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the study procedures or treatments.

(3) suggests that the participation in the study may place subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

Please note that not all adverse events are unanticipated problems and only some unanticipated problems are in fact adverse events. For instance, if a laptop containing study data is stolen, this is an unanticipated problem but it is not an adverse event since it is not an untoward or unfavorable medical occurrence in a human subject.

9.1.3 Serious Adverse Event

A serious adverse event is defined as:
1. Death
2. Unanticipated events related to tissue sampling
3. Other events that are unexpected AND serious AND related or possibly related to the study
9.2 Methods and Timing for Assessing, Recording, and Analyzing, Managing Safety Parameters

9.2.1 Methods and Timing of Assessment

Adverse events may be discovered during regularly scheduled visits or through unscheduled patient contacts between visits.

Adverse events will assessed during the index hospitalization and re-hospitalizations and recorded on study data forms whether or not they are thought to be associated with the study.

9.2.2 AE/SAE Grading and Relationship Assignment

N/A

9.2.3 Recoding and Documentation

Sites will maintain source documents including but not limited to (laboratory and radiology reports, clinical notes and discharge summaries). After review of initial and final reports by the medical monitor, the events may be reclassified at their discretion.

9.2.4. Management of Adverse Events

Adverse Events and Serious Adverse Events will be managed according to protocol guidelines. If specific guidelines do not exist, AEs/SAEs will be managed according to the medical judgment of the treating physician.

9.3 Adverse Event Reporting Procedures

9.3.1 Local Reporting Requirements.

Study sites must always follow and comply with their own local institution’s adverse event reporting requirements, which may differ from those adopted by the Bioburden study. Depending on the local requirements, a site may report events locally and not report those events to the METRC Coordinating Center. Each participating site is responsible for ensuring that all local IRB requirements for reporting adverse events (both internal and external) are met.

9.3.2 SAE and Unanticipated Problem Reporting Requirements

All Serious Adverse Events must be reported to the METRC Coordinating Center with 72 hours. In addition, unanticipated problems that are not adverse events must also be reported to the METRC Coordinating Center according to the procedures outlined in the METRC Manual of Operations as soon as possible after the event.
9.3.3 METRC Coordinating Center Reporting Responsibilities

The MCC will send a copy of each report received about an event judged reportable to all clinical sites, with instructions for each to forward the report to their IRB. Copies of the report will also be sent to the DoD, the Study PI, and to the Medical Monitor. The MCC will maintain a list of such events for reporting and review at Steering Committee meetings.

9.3.4 Department of Defense Reporting Requirements

The following are reporting requirements and responsibilities of the Principal Investigator to the United States Army Medical Research and Materiel Command’s (USAMRMC) Office of Research Protections (ORP), Human Research Protection Office (HRPO).

1. The protocol will be conducted in accordance with the protocol submitted to and approved by the USAMRMC ORP HRPO and will not be initiated until written notification of approval of the research project is issued by the USAMRMC ORP HRPO.

2. Accurate and complete study records will be maintained and made available to representatives of the U.S. Army Medical Research and Materiel Command as a part of their responsibility to protect human subjects in research. Research records will be stored in a confidential manner so as to protect the confidentiality of subject information.

3. All unanticipated problems involving risk to subjects or others, serious adverse events related to participation in the study and subject deaths related to participation in the study will be promptly reported by phone (301-619-2165), by email (hsrrb@.amedd.army.mil), or by facsimile (301-619-7803) to the USAMRMC, Office of Research Protections, Human Research Protection Office. A complete written report will follow the initial notification. In addition to the methods above, the complete report will be sent to the U.S. Army Medical Research and Materiel Command, ATTN: MCMR-RP, 504 Scott Street, Fort Detrick, Maryland 21702-5012.

4. Suspensions, clinical holds (voluntary or involuntary), or terminations of this research by the IRB, the institution, the Sponsor, or regulatory agencies will be promptly reported to the USAMRMC ORP HRPO.

5. Any deviation to the protocol that may have an adverse effect on the safety or rights of the subject or the integrity of the study will be reported to the USAMRMC ORP HRPO as soon as the deviation is identified.

6. Major modifications to the research protocol and any modifications that could potentially increase risk to subjects will be submitted to the USAMRMC ORP HRPO for approval prior to implementation. All other amendments will be submitted with the continuing review report to the USAMRMC ORP HRPO for acceptance.

7. A copy of the approved continuing review report and the local IRB approval notification will be submitted to the USAMRMC ORP HRPO as soon as these documents become available. A copy of the approved final study report and local IRB
approval notification will be submitted to the USAMRMC ORP HRPO as soon as these documents become available.

(8) The knowledge of any pending compliance inspection/visit by the FDA, OHRP, or other government agency concerning this clinical investigation or research, the issuance of Inspection Reports, FDA Form 483, warning letters or actions taken by any Regulatory Agencies including legal or medical actions and any instances of serious or continuing noncompliance with the regulations or requirements that relate to this clinical investigation or research will be reported immediately to USAMRMC ORP HRPO.

Unanticipated problems involving risk to volunteers or others, serious, unexpected adverse events related to participation in the study and all volunteer deaths related to participation in the study will be promptly reported by phone (301-619-2165), by e-mail (hsrrb@amedd.army.mil), or by facsimile (301-619-7803) to the U.S. Army Medical Research and Materiel Command’s Office of Research Protections, Human Research Protections Office. A complete written report will follow the initial notification. In addition to the methods above, the complete report will be sent to the U.S. Army Medical Research and Materiel Command, ATTN: MCMR-RPH, 504 Scott Street, Fort Detrick, Maryland 21702-5012

9.4 Reporting Pregnancy

N/A

9.5 Type and Duration of the Follow-up of Participants After Adverse Events

N/A

9.6 Modifications of Study Agent(s)/Intervention(s) for a Participant

N/A

9.7 Halting Rules for the Protocol

N/A

9.8 Stopping Rules for an Individual Participant/Cohort

N/A

9.9 Premature Withdrawal of a Participant

N/A

9.10 Replacement of a Participant Who Discontinues Study Treatment
10. CLINICAL MONITORING STRUCTURE

10.1 Site Monitoring Plan

The METRC Coordinating Center will be responsible for site monitoring consistent with ICH/FDA guidelines. Site monitors will visit participating clinical research sites to review the individual subject records, including consent forms, case report forms, supporting data, laboratory specimen records, and medical records (physicians’ progress notes, nurses’ notes, individuals’ hospital charts), to ensure protection of study subjects, compliance with the protocol, and accuracy and completeness of records. The monitors also will inspect sites’ regulatory files to ensure that regulatory requirements are being followed.

The site PI will make study documents (e.g., consent forms, case report forms) and pertinent hospital or clinic records readily available for inspection by the local IRB, the site monitors, the DOD, the Office for Human Research Protections (OHRP), or other regulatory authorities for confirmation of the study data.

10.2 Safety Monitoring Plan

10.2.1 Safety Review Plan by the DSMB

A DSMB is not required for this study. A Medical Monitor has been appointed and will review any SAEs.

11. STATISTICAL CONSIDERATIONS

11.1 Overview and Study Objectives

The primary objective of this study is to characterize the contemporary extremity wound “bioburden” at the time of definitive wound coverage/closure of severe extremity military and civilian wounds employing standard microbiology and new polymerase chain reaction (PCR) technologies.

Secondary objectives of the study are to determine: (a) the correlation of the identified wound pathogens at the time of wound closure/coverage with subsequent deep wound infections; (b) the correlation of the PCR results with those obtained from standard hospital microbiology; and (c) the efficacy, if any, of antibiotics used in the care of the wound.

11.2 Sample Size Considerations
**Specific Aim 1:** While the prevalence of pathogen causing bacteria in the target study population are not known (as this is the primary goal of the proposed study), protocol committee experts predict they may range from 10% for bacteroides fragilis to 50% for methicillin sensitive staph aureus. Using a sample size of 60 patients, we will be able to build a 95% confidence interval range no greater than 25% for the prevalence of these bacteria. We expect that the three tissue samples described in the protocol (deep tissue, soft tissue, and composite tissue) will identify roughly the same rates of these pathogens. Therefore, the decision of which tissue sample to analyze with Ibis for all participants will not be driven by a statistical test but by the clinical judgment of our panel of infectious disease experts and orthopaedic surgeons.

**Specific Aim 2:** For this aim, we will compute the probability of concordance between the Ibis and standard microbiology techniques, where concordance is defined as agreement between the pathogens identified between the two technologies. We will calculate a 95% exact lower confidence limit for the probability of concordance using the Clopper-Pearson method. A simulation study indicates that, with a sample size of 600 and true probability of concordance greater than 97.5% then the proportion of lower confidence limits that are greater than 95% is greater than 90%.

**Specific Aim 3:** We anticipate that 20%, or 120, of enrolled patients will develop a deep infection. We will draw inference about the probability of concordance between the pathogens identified at wound closure and those identified at deep infection. With a sample size of 120, we will be able to construct a 95% confidence interval for the true probability of concordance that is no larger than 17.8%.

**Specific Aim 4:** We anticipate that 50%, or 300, of enrolled patients will be treated by a course of antibiotics that are appropriate for the pathogens identified by standard microbiology. We also assume that 20% of patients will develop deep infection. We will test the null hypothesis of no difference in the probability of deep infection between those who do and do not receive an appropriate course of antibiotics using Fisher's exact test. A simulation study indicates that the study has 80% power to detect an odds ratio for deep infection between these two groups of 1.8. Similarly, we anticipate that 50% of enrolled patients will be treated by a course of antibiotics that are appropriate for the pathogens identified by Ibis. Thus, we will also 80% power to detect an odds ratio for deep infection of 1.8 between those appropriately and inappropriately treated for pathogens identified by Ibis.

### 11.3 Randomization

N/A

### 11.4 Missing Data and Measures to Minimize Bias

Missing data is a serious concern that complicates the interpretation of the study results. We will address this issue from both a study conduct and analysis perspective. Regarding study conduct, we will

1. Limit participant burden and inconvenience in data collection
2. Select high quality investigators
3. Provide pre-study training of investigators as well as on-study reinforcement
4. Reimburse investigators based on follow-ups completed rather than on per-patient basis.
5. Monitor and report missing data rates during the study
6. Emphasize the importance of full participation in the study during the consent process.
7. Collect information on the reasons for missing data.
8. Actively engage participants in the study and educate them about the importance of their engagement.
9. Hold regular Protocol Committee meetings to discuss strategies for enrollment and engagement of participation.
10. Set targets for acceptable rates of missing data and terminating sites that do not meet these targets.

While these efforts will help to minimize missing data, we recognize that missing data is inevitable. With this in mind, we will conduct sensitivity analyses to evaluate the robustness of the study results to various untestable assumptions about the missing data mechanism. In addition to unadjusted analyses, which rely on the missing completely at random assumption (testable), we will also estimate treatment effects (utilizing relevant auxiliary information) under the missing at random assumption. Further, we will explore the effect of departures from the missing at random assumption using pattern-mixture and selection modeling techniques.

11.5 Planned Interim Analysis

N/A

11.6 Analysis Plan

Analyses will vary by specific aim. The specific analyses to be performed for each Specific Aim are outlined in Section 11.2. In general, we will use exact and non-parametric hypothesis testing procedures. Confidence intervals (95%) of differences will be computed. Regression analysis will be employed when adjustment between comparison groups is considered necessary.

In this aim, it may be necessary to adjust for confounding factors that differentiate patients appropriately and inappropriately treated. In this case, we will use logistic regression to adjust for these factors in our analysis.

12. QUALITY CONTROL AND QUALITY ASSURANCE

Quality Control (Q/C) and Quality Assurance (Q/A) procedures that apply to all studies are outlined in the METRC Manual of Operations (MOP). A certification process (also outlined in the MOP) will be used as a basis for training and certification of the study personnel involved in data collection. In addition to consortium wide training and certification procedures, additional requirements may be added based on the nature of the study. Ongoing data edits and audits will
be performed to ensure collection of quality data. The continuous and timely flow of data from the centers to the MCC is an essential prerequisite for maintaining data quality.

Monthly Performance Reports will be distributed to each center summarizing among other things: recruitment, randomization, status of follow-up, data completion, and timelines of data entry.

Allegheny-Singer Research Institute will provide the MCC with regularly scheduled data reports for the purposes of monitoring the quality and integrity of the tissue sample collection. The tissue sample collection and shipping procedures will be modified as necessary based on these data.

13. ETHICS/PROTECTION OF HUMAN SUBJECTS

13.1 IRB/Ethics Committee

IRB approval will be obtained from the MCC at Johns Hopkins Bloomberg School of Public Health, the DoD, and each participating clinical site according to METRC policies and procedures.

Sites must provide the Coordinating Center with a copy of the initial IRB approval notice and subsequent renewals as well as copies of the IRB approved consent statements.

No site can begin work related to this study until the site has been certified in accordance with METRC policies and procedures.

13.2 Informed Consent Process

13.2.1 Consent and Enrollment

A prototype consent has been prepared for the Bioburden study and is attached in Appendix F. Individual sites may add material but may not delete material thought to be necessary for informed consent. Clinical sites may reformat and reword information to conform to their local requirements. The consent form will describe the purpose of the study, the procedures to be followed, and the risks and benefits of participation. Copies of the signed consent forms will be given to the patient, and this fact will be documented in the patient’s record.

Eligible patients will be approached for their consent to participate. Informed consent will be obtained prior to definitive wound closure or coverage.

To encourage a high level of participation from eligible patients, the attending surgeon will be involved in the consent conversation. The conversation will be initiated by the research coordinator and surgeon together. Patients will be informed of the study and intended use of tissue samples obtained intra-operatively and any relevant data that will be collected or analyzed in conjunction with the study. Patients and their families will be provided with a pamphlet.
describing the study, the risks and benefits of participation and what will be expected of them if they choose to participate.

All recruitment materials will be provided in both English and Spanish.

13.2.2 Assessing Capacity to Consent and Consenting a Proxy Respondent

By virtue of the types of injuries studied (resulting from high energy mechanisms such as high speed motor vehicle crashes, high falls, and blast injuries) it is expected that a large proportion (>30%) to have an associated traumatic brain injury which may render them unable to provide consent for the study. Another 10% may remain intubated for some time due to lung issues.

It will be important not to exclude these patients from the study, as it would significantly reduce our ability to produce generalizable knowledge. These patients are at no greater risk of adverse consequences by virtue of their participation in the study, and should be given the same opportunity to participate.

Whenever possible, the patient him or herself (as opposed to a proxy) should be consented. Prior to initiating the consent process, the Research Coordinator will contact the patient’s Physician for confirmation that the patient has the ability to understand the relevant study information and communicate and maintain a choice. If the physician indicates that the patient lacks the capacity to consent the legally authorized representative (LAR) will be contacted.

The research staff will endeavor to answer all questions posed by the patient and his/her family to ensure their understanding of the protocol. A limited number of questions will be asked of all patients after they are introduced to the study and have reviewed the consent form. These questions assess the person’s understanding of the study and what it means to participate, their appreciation of the consequences of participation, and their ability to consider alternatives to participation. A formal comprehension test may be utilized, or comprehension will be assessed by the person(s) obtaining the consent. A template for a comprehension test is provided in Appendix G.

The Research Coordinator will ask the questions and determine the appropriateness of the responses. If the Research Coordinator is at all unsure about the patient’s ability to consent s/he will consult with the study site PI.

A legally authorized representative (LAR) with reasonable knowledge of the potential participant will be approached to consent on the patient’s behalf if one of the following is true:

- The patient is unresponsive or intubated (and likely to remain unresponsive or intubated until the time of definitive fixation).
- The patient cannot adequately answer at least 2 questions and it is determined that the patient’s level of cognition is not likely to change before definitive fixation is necessary.

The choice of LAR will follow standard procedures. The following with be approached in this order of priority:
• Legal guardian
• Proxy (health care agent) named in an advance directive or durable power of attorney for health care;
• Family member or other surrogate identified by the state law on health care decisions.

Guidance will be provided to assist the LAR in making the consent decision. They will advise to base the decision on the participant’s expressed wishes, or, if these are not known, what they believe the participant would have desired under the circumstances of the injury, their beliefs and values. If the LAR does not know what the participant would have wanted, the LAR will advise to base the decision with the participant’s best interest in mind. They will be asked to carefully consider how much leeway the participant would likely give the LAR in making the choice about participation in the study.

Recognizing that consent is an ongoing process, the study team will encourage the participants to ask additional questions that may arise during the course of their participation in the study.

13.2.3 Consent for Tissue Banking

There is a high probability that the individual host immune response plays a significant role in preventing the attachment and proliferation of bacteria (infection) in the open wound. Although not a part of this project, genetic analysis of wound tissue might help to identify host characteristics that modulate the local immune response. Patients will be asked for permission to save a portion of the tissue samples for later genomic evaluation specifically related to the local immune response. If patients agree to the storage and delayed evaluation of the tissues, the samples will be identified and stored at the Center for Genomic Science.

There is a possibility that genetic analyses performed on these samples in the future will meet the criteria for genetic testing. The Guidance on the Genetic Information Nondiscrimination Act states that “among the risks typically associated with genetic research, investigators, IRBs, and research subject advocates, among others, have identified the potential adverse impact on insurability or employability if genetic information about the subject obtained as part of the research was disclosed to, or sought by, insurers or employers.” These risks will be minimized by de-identifying samples prior to storage at CGS. There will be no mechanism to connect genetic tests results to study participants. Furthermore, results from these tests will only be published in aggregate form. It is possible that this donated tissue will help to develop new products in the future. There are no plans to share any potential financial gain with the participants.

13.2.4 Informed Consent Process or Assent (for a minor)

N/A
13.2.5 Medical Record Release

Patients will be asked to sign a medical release form that would allow access to clinical microbiology reports and discharge abstracts from another hospital if he/she is readmitted for treatment of a deep infection during the study period.

13.3 Exclusion of Women, Minorities, and Children (Special Populations)

The proposed study anticipates recruiting a significant proportion of racial/ethnic minorities (African-Americans, Asian-Americans and Hispanics) as well as non-Hispanic white subjects. The study will not include children or prisoners.

13.4 Participant Confidentiality

It is the investigator’s responsibility to conduct the protocol under the current version of Declaration of Helsinki, ICH Guidelines, Good Clinical Practice, and rules of local IRBs. The investigator must ensure that the patient’s anonymity be maintained in their data submission to the Data Coordinating Center.

Patients will be identified only by an identification code but not by their name, SSN, or hospital medical record number. Study Site Investigators will maintain a separate confidential enrollment log which matches identifying codes with the patients’ names and addresses (i.e., available only to local clinic staff).

All study forms, reports, and other records that are part of the study data collection materials will be identified by coded number to maintain patient confidentiality. All paper, records will be kept in locked file cabinets. All electronic records of study data will be identified by coded number. Clinical information will not be released without written permission of the patient, except as necessary for monitoring by the IRB, DOD, or DSMB. Consent procedures and forms, and the communication, transmission and storage of patient data will comply with individual site IRB and DOD requirements for compliance with The Health Insurance Portability and Accountability Act (HIPPA).

13.5 Study Discontinuation

Participants will be informed that they may discontinue the study at any time, for any reason. They will be assured that the medical care which they receive at the participating facility will not be affected should they elect to discontinue participation in the study.

14. DATA HANDLING AND RECORD KEEPING

14.1 Data Management Responsibilities
The research coordinators at each site will obtain the information necessary to complete the case report forms (CRFs) from several sources including but not limited to, the patient's medical record, clinical evaluations and patient interviews. These forms will NOT contain the patient’s name, SSN, or hospital medical record number; they will be identified only by a unique patient-specific study number.

The Site Research Coordinator will enter non-personally identifiable information into a central and secured web-based data management system being implemented for all Consortium studies, known as REDCap. This data management system has incorporated state-of-the-art features for electronic data collection and is configured in accordance with best practices for information technology and research data management.

Hard copy documents containing subject data and patient identifiers (and contact information) will be stored in secure document containers (file cabinets, lockers, drawers, etc.) in accordance with standard document management practices. The data collection forms will be destroyed within five years after study completion, as will the file linking study numbers with personally identifiable information. Paper forms will be shredded and the file containing personally identifiable data at each site will be deleted from local site computers. Each site will provide the Coordinating Center a signed verification that these data have been destroyed.

All research data, in hard copy or electronic form, will be stored and managed in a secure manner following applicable federal regulations and guidelines and according to institutional policies and practices.

At all times only listed key personnel specifically designated and authorized by the Principal Investigator shall have access to any research related documents, including electronic data. All such personnel will be properly trained and supervised regarding the management and handling of confidential materials. The Principal Investigator assumes full responsibility for such training, supervision, and conduct.

14.2 Data Capture Methods

Data will be collected in real time by the investigator or study coordinator directly on paper Case Report Forms (CRFs) which will serve as source documents for the study. Source documents will be signed by PI, other site Investigator, or Research Coordinator including both the CRFs and other medical records (e.g. laboratory & radiology reports, clinical notes and discharge summaries. The Research Coordinator, or an MCC-certified staff member working under the supervision of the research coordinator, will enter the data from the CRFs into the REDCAP database.

14.3 Types of Data

Data will include medical and surgical histories, laboratory reports, adverse events and patient interviews
14.4 Source Documents and Access to Source Data/Documents

Source documents including CRFs, laboratory results, patient surveys, medical records, etc. will be maintained at the site and will be made available to study monitors, and representatives of regulatory agencies including the IRB, FDA and OHRP.

14.5 Timing/Reports

The MCC will send site queries on a weekly basis and site progress reports monthly.

14.6 Study Records Retention

Study records will be maintained in accordance with current ICH guidelines.

14.7 Protocol Deviations

Records of protocol deviations will be noted on the appropriate METRC form with the reason for the deviation recorded, as well as any action taken to mitigate the deviation. These records will be provided to the site’s IRB in accordance with local reporting requirements and be made available to study monitors.

15. PUBLICATIONS POLICY

Publications will be written in accordance with the METRC publication policy (available on the METRC website: www.metrc.org).

16. SCIENTIFIC REFERENCES


17. APPENDICES

APPENDIX A: STUDY CONTACT ROSTER

**Principal Investigator (Protocol Chair)**

Michael Bosse, MD  
Carolinas Medical Center  
PO Box 32816; 1320 Scott Avenue  
Charlotte NC 28232  
Phone: (704) 355-6046  
Pager: 704-355-4088 (#1605)  
Email: Michael.bosse@carolinashealthcare.org

**METRC Coordinating Center Study Principal investigator**

Renan Castillo, PhD  
Johns Hopkins Bloomberg School of Public Health  
Dept. Health Policy and Management  
624 N. Broadway Room 544  
Baltimore MD, 21205  
Phone: 410-614-4024  
Email: rcastill@jhsph.edu

**DOD Program Officer**

Joseph C. Wenke, PhD  
Program Manager  
United States Army Institute of Surgical Research  
3400 Rawley E. Chambers Avenue, Bldg. 3611  
Fort Sam Houston TX, 78234-6315  
Phone: 210-916-3742; Cell: 210-288-2431  
Email: Joseph.Wenke@us.army.mil

**METRC Coordinating Center**  
**Director of Protocol Development**

Lisa Reider, MHS  
Johns Hopkins Bloomberg School of Public Health  
Dept. of Health Policy and Management  
624 N. Broadway Room 355  
Baltimore MD, 21205  
Phone: 410-502-9109  
Email: lsemanic@jhsph.edu

**Medical Monitor**

Marc Swiontkowski, MD (CHAIR)  
Professor of Orthopaedic Surgery  
University of Minnesota  
2512 South 7th Street  
Suite R200  
Minneapolis, MN 55454  
Phone: (612) 273-8000  
Email: swion001@umn.edu

**METRC Coordinating Center**  
**Study Manager**

Marcie Maichle, MS  
Johns Hopkins Bloomberg School of Public Health  
Dept of Health Policy and Management  
624 N. Broadway Room 501  
Baltimore MD, 21205  
Phone: 410-502-8455  
Email: mmaichle@jhsph.edu

**METRC Coordinating Center**  
**Director of data Management**

Anthony Carlini, MS  
Johns Hopkins Bloomberg School of Public Health  
Dept of Health Policy and Management  
624 N. Broadway Room 501  
Baltimore MD, 21205  
Phone: 410-502-8455  
Email: acarlini@jhsph.edu
APPENDIX B: PROTOCOL COMMITTEE

Michael Bosse, MD (Study PI)  
Carolinas Medical Center

Rachel Seymour, PhD  
Carolinas Medical Center

Adam Starr, MD  
University of Texas Southwestern Medical Center

George Russell, MD  
University of Mississippi Medical Center

Theodore Miclau, MD  
University of California at San Francisco

James Toledano, MD  
Naval Medical Center at San Diego

Daniel Scharfstein, PhD  
Johns Hopkins Bloomberg School of Public Health

Renan Castillo, PhD  
Johns Hopkins Bloomberg School of Public Health

Ellen MacKenzie, PhD  
Johns Hopkins Bloomberg School of Public Health

Andrew Pollak, MD  
University of Maryland, Shock Trauma

LCDR Jonathan Forsberg, MD  
Memorial Sloan-Kettering Cancer Center

Joseph Wenke, PhD  
Department of Defense

LTC Clint Murray, MD  
Brooke Army Medical Center

Joseph Carney, MD  
Naval Medical Center at San Diego

Garth Ehrlich, PhD  
Allegheny General Hospital/Allegheny-Singer Research Institute

Rachael Kreft, RN  
Center for Genomic Sciences/Allegheny-Singer Research Institute

Diane Wilson, CTR, CCRP  
Naval Medical Center at San Diego

Lisa Reider, MHS  
Johns Hopkins Bloomberg School of Public Health

Study website: www.metrc.org
## APPENDIX C: DATA COLLECTION SCHEDULE

<table>
<thead>
<tr>
<th>Data Collection Forms</th>
<th>Index Hospitalization</th>
<th>Interim Data Collection</th>
<th>12 Months</th>
<th>Case Report Form #</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Inclusion/Exclusion</strong></td>
<td>X</td>
<td></td>
<td></td>
<td>CRF00</td>
</tr>
<tr>
<td><strong>Patient Information</strong></td>
<td>X</td>
<td></td>
<td></td>
<td>CRF01</td>
</tr>
<tr>
<td><strong>Patient Characteristics</strong></td>
<td>X</td>
<td></td>
<td></td>
<td>CRF02</td>
</tr>
<tr>
<td>Patient Demographics (Gender, age, race, ethnicity)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoking history</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre Injury Health status (one question: General Health Status)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>General Injury Characteristics</strong></td>
<td>X</td>
<td></td>
<td></td>
<td>CRF03</td>
</tr>
<tr>
<td>Date/time of injury</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Circumstances of injury</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AIS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Medical History</strong></td>
<td>X</td>
<td></td>
<td></td>
<td>CRF04</td>
</tr>
<tr>
<td>BMI</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insurance status/type</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medications</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Co-morbidities</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Study Injury Characteristics</strong></td>
<td>X</td>
<td></td>
<td></td>
<td>CRF05</td>
</tr>
<tr>
<td>Mechanism, and type</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gustilo grade</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wound length</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OTA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Index Hospitalization</strong></td>
<td>X</td>
<td></td>
<td></td>
<td>CRF06</td>
</tr>
<tr>
<td>Admit/Discharge dates</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ICU</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Definitive fixation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Complications</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Antibiotic Treatment</strong></td>
<td>X</td>
<td></td>
<td></td>
<td>CRF07</td>
</tr>
<tr>
<td><strong>Medical Record Review</strong></td>
<td>X</td>
<td></td>
<td></td>
<td>CRF08</td>
</tr>
<tr>
<td><strong>Re-hospitalization</strong></td>
<td>X*</td>
<td></td>
<td></td>
<td>CRF09</td>
</tr>
<tr>
<td>only complete for re-hospitalizations for deep infection, non union, flap failure, or amputation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>12 month Follow up Phone Interview</strong></td>
<td>X</td>
<td></td>
<td></td>
<td>CRF10</td>
</tr>
<tr>
<td>Number of readmissions</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Facility type</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Length of Stay</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reason for admission</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Procedures performed</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Presence of infection</td>
<td>Antibiotics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-----------------------</td>
<td>-------------</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Serious Adverse Event Reporting</strong></td>
<td>X*</td>
<td>X*</td>
<td>X*</td>
<td>CRF11</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Tissue Sample Analysis (ASRI)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Ibis workup</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clinical Micro analysis including aerobic, anaerobic, and fungal cultures</td>
<td>X</td>
<td>X (if re-hospitalized for infection)</td>
<td>Results will be provided to the MCC electronically throughout the duration of the study for continued quality control monitoring</td>
</tr>
</tbody>
</table>

*Complete as needed

**Only if obtained as part of Standard of Care
APPENDIX D: 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 recap immediately in order to preserve the sample.

Ibis 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 ronguer 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 ronguer 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.

Figure 1A. Composite Sample  
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 readmitted 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 Ibis 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.
APPENDIX E: TISSUE SAMPLE SHIPPING PROCEDEURES

**Labeling Sample Collection Containers**
The Research Coordinator should use either a ball-point pen or a permanent non-smearing marker to write in the appropriate patient identifier code (provided by REDCap) on the three sample containers (Composite Sample, Deep Tissue Sample and Soft Tissue Sample) as well as the glass Clinical Microbiology Sample. See Example Below:

BIO - ______ - ______

**Recording Sample Collection Kit Number and Type**
Each kit will have a unique identifier located on the back side of the box (see example below):

I-____ ____ ____ (Initial Kit Sample Collection)
R- ___ ______ ___ (Re-Admission Kit Sample Collection)

This number should be recorded on the Index Hospitalization Form.

**Packaging Tissue Samples for Shipping**

Once all the samples have been obtained, be sure each sample container has the correct patient ID written in ball-point pen or a permanent non-smearing marker. Place the Clinical Microbiology glass tube into the Tear Tech Tube Shuttle and seal the shuttle bag. Wrap the tube shuttle with bubble wrap and place the tube shuttle. Next, place all three color coded sample containers into the 95-kPa Specimen Transport Bag and seal the bag. Place the remaining bubble wrap in the bottom of the THERAPAK box and then place the 95-kPa Specimen Transport Bag in and cover with remaining bubble wrap. Prior to sealing the box the Research Coordinator will fill out the THERAPAK List of Contents (blue card) and check the following options:

- Diagnostic Specimens
- Glass and/or Plastic Tubes
- Tube Separator Bag (s)
- Absorbent Pad (s)
- Transport Media or Preservative

Place the List of Contents (blue card) between the outside of the box and the packaging. Seal the box with the one large piece of clear tape provided. Make sure to input the box identification number onto the correct form.
Before closing, fill out the Yellow Sample Card. Fill out the card as accurately as possible.

- Date: MM/DD/YY
- Time: (in Hours and Minutes Military Time)

**Labeling the FedEx Air Bill**

The pre-printed and pre-paid FedEx bill will be located with the Sample Kit. All required information will be pre-printed and site specific. The box will be pre-printed as follows:

Be sure to write in correct date. The date MUST be the same as the day the samples are shipped.

If you are sending a shipment on Friday, be sure to check SATURDAY delivery.
The Sample collection kit should be shipped FedEx Priority Overnight

**NOTE:** If you will be shipping samples on Friday you will need to check Section 6 on the airbill—Special Handling and Delivery Signature Options. “Saturday Delivery”

Once the FedEx US Airbill is Complete based on sample collection day, tear off the first page of the Airbill and place in patient binder.

Next, place the FedEx Airbill inside clear FedEx protective adhesive pouch and attach to the Sample Collection box.

**After Hours Sample Collection Shipping Procedures**

If the sample has been collected during business hours and packaged by the Research Coordinator but cannot be shipped the same day because the FedEx last pick-up has already passed, the Research Coordinator will store the packaged sample in a refrigerated unit until morning when the sample can then be picked up by FedEx. If the sample is collected after business hours or on the weekend and the Research Coordinator is NOT available to package the samples, the surgeon or designated clinician will collect the samples and store them in a refrigerated unit until morning at which time the Research Coordinator certified in shipping UN3373 Category B substances will then package the samples appropriately and ship them at the earliest FedEx pickup available at the site.

**Handling Shipping Returns**

To prevent lost packages and returns, stickers with site contact information and the contact information for the lab at CGS will be adhered to the box in case the airbill comes loose. In the event that a shipment is returned, the Research Coordinator should re-ship to CGS even if the samples may no longer be viable.
APPENDIX F: CONSENT TEMPLATE

JOHNS HOPKINS BLOOMBERG SCHOOL OF PUBLIC HEALTH

INFORMED CONSENT DOCUMENT

Participant Consent Form

Study Title: Assessment of Severe Extremity Wound Bioburden at the Time of Definitive Wound Closure or Coverage: Correlation with Subsequent Post-Closure Deep Wound Infection (Bioburden Study)

Principal Investigator: Renan Castillo, PhD

IRB No.: 00003576

PI Version Date: v.1 5/17/2011

You are being asked to volunteer to be a part of a research study. Please read this form carefully before you sign it. This consent form explains the research study and your part in the study. If you decide not to participate in this study there will be no impact on your medical care. You can choose not to take part and if you join, you may quit at any time. Ask the study doctor or the study staff to explain any words or procedures that you do not clearly understand. Ask as many questions as needed. All of your questions should be answered to your satisfaction before you sign this form.

1. WHAT IS THE PURPOSE OF THIS RESEARCH STUDY?

Wounds from injuries like yours can become infected. Infections, which can be serious and complicate your recovery, are caused by bacteria in the wound. The goal of the Bioburden study is to learn about better ways to identify infection-causing bacteria in wounds like yours. The study will compare two techniques to find out which one does the best job in identifying bacteria that cause future infections. This study will help doctors select the best antibiotics for people with injuries like yours in the future.

Microbiology techniques have been the standard for determining which bacteria may be present in wounds after surgery. Microbiology testing involves collecting samples of tissue and looking at them under a high-powered microscope. The IBIS T5000 was developed to look at those tissue samples using new and more powerful DNA identification technology. However, IBIS technology is approved for research only; the results cannot be used to inform clinical decisions.
This Bioburden study is funded by the Department of Defense (DOD) and is being carried out in more than 25 major trauma centers across the United States, including four military treatment centers that are taking care of service members who are injured in the line of duty.

2. WHY AM I BEING ASKED TO PARTICIPATE?

You are being asked to participate in this study because you are between the ages of 18 to 64 and you have a complex wound from severe extremity trauma. People like you who are being treated at major trauma centers from around the county are being asked to participate. You are one of over 600 patients expected to join the Bioburden study.

3. HOW LONG WILL THE STUDY LAST?

This study will last 1 year following your surgery.

4. HOW DOES THE STUDY WORK?

After you review this informed consent and agree to be part of the study, the following things will happen:

- You will be asked questions about your race, smoking history and your health status before your injury
- Information will be collected about your injury and entered into a database by a member of the research team.
- We will obtain four tissue samples from your wound and will test those samples using microbiology and IBIS techniques.
- Your doctor might also sample the wound surface and send the material to his/her own hospital microbiology laboratory.
- We will ask you to keep a contact card with you and use it to inform the research team if you are re-hospitalized
- If you develop an infection and are re-hospitalized, another tissue sample will be taken. Only two samples will be taken.

After your surgery your care will proceed exactly the same as if you were not in the study. If you do not come back for surgery during the follow up year you will receive a brief phone interview at 12 months to ask you about additional hospitalizations or infections that you have had due to this injury.

5. WHAT ARE THE POTENTIAL RISKS OR DISCOMFORTS?
While tissue sampling is routinely performed during surgery, there is always a small risk of injury associated with each tissue removal.

Any time information is collected for a study there is a small risk of breach of confidentiality. As described below, your research data will be identified by a unique study number rather than your name and all measures allowed by law to protect your confidentiality will be taken by the research staff.

6. **WHAT ARE THE POTENTIAL BENEFITS?**

You will not benefit directly from your participation in this study. However, your participation in the study could help us determine the best treatment for injuries like yours. This information could be very helpful to other people who have this same injury in the future.

7. **DO I GET ANY PAYMENT FOR BEING IN THE STUDY?**

There is no payment for participation in this study.

8. **ARE THERE ANY COSTS INVOLVED IN BEING IN THE STUDY?**

There is no cost to you to participate in this study.

9. **WILL MY INFORMATION BE KEPT PRIVATE?**

The information we collect from you will be kept private to the best of our ability. Your name, birth date, medical record number and any other information that could identify you as an individual will be removed from all study forms. Instead, we will label your forms with a unique study number. The link between your name and your study number will be kept confidential to the greatest extent provided by law. The information collected for the study will be stored in a password protected, HIPPA verified computer database that only authorized members of our research team can use. When we report the results of the study, we will combine the information about you with similar information about hundreds of other people so your individual information will not be identifiable.

All study records will be considered confidential, and your name will not be used in reports or publications.

10. **WILL YOU SHARE MY INFORMATION WITH OTHERS?**
We will use your information only for the purposes of this study. The data from the study may be published. However, you will not be identified by name. People designated from the institutions where the study is being conducted will be allowed to inspect sections of your medical and research records related to the study. This includes people designated by The Johns Hopkins Bloomberg School of Public Health who are overseeing this study. Everyone using study information will work to keep your personal information confidential. Your personal information will not be given out unless required by law.

The Department of Defense is providing funding to sponsor this study. Representatives from the U.S. Army Medical Research and Materiel Command (USAMRMC) Office of Research Protections (ORP) Human Research Protections Office (HRPO) and your local IRB may have access to research records in their role to protect human subjects engaged in research.

11. WHAT ARE MY ALTERNATIVES TO PARTICIPATION?

This is not a treatment study. Your alternative is to not take part. If you choose not to take part, your healthcare will not be affected.

12. WHAT HAPPENS IF I LEAVE THE STUDY EARLY?

Your participation in this study is completely voluntary. You have the right to withdraw from the research study at any time without penalty. Your decision will not affect the medical care you receive. If you decide to stop participating, you should notify the study doctor or the research coordinator at your center.

Your participation in this research study could be ended without your consent. Possible reasons could include our decision to end the study early or other reasons.

13. WHAT HAPPENS IF I AM INJURED OR BECOME ILL BECAUSE I TOOK PART IN THIS STUDY?

If you are injured or become ill because of your participation in this study, you will receive emergency medical care if needed and you will receive assistance in getting other medical care as needed. You or your insurance carrier will be billed for the cost of care, just as you would be billed for any other medical care. If you have any costs that are not covered by insurance, they are your responsibility.
You do not give up any of your legal rights by signing this form. You can seek legal compensation for any injury that may occur to you during the study as a result of an error by a member of the research staff or others.

14. WHO DO I CALL IF I HAVE QUESTIONS OR PROBLEMS?

- <<insert name>>, the study coordinator at your hospital has discussed this information with you and offered to answer any questions you may have. If you have further questions or get sick or injured as a result of being in this study, you can contact <<insert him/her>> at <<telephone number>>. You may also call the Director of the Study at your hospital, <<insert name>>, at <<telephone number>>.

- If you have further questions about your rights as a study participant you can call or contact your local IRB office or the Johns Hopkins Bloomberg School of Public Health IRB Office. The Johns Hopkins Bloomberg School of Public Health is serving as the overall coordinating center for this study that is being conducted in hospitals around the country. Contact the Johns Hopkins IRB if you feel you have not been treated fairly or if you have other concerns. The IRB contact information is:

- <<insert local IRB contact information here>>

  Address: Johns Hopkins Bloomberg School of Public Health  
  615 N. Wolfe Street, Suite E1100  
  Baltimore, MD  21205  
  Telephone: 410-955-3193  
  Toll Free: 1-888-262-3242  
  Fax: 410-502-0584  
  E-mail: irboffice@jhsph.edu

You will receive a copy of this signed consent form.

What does your signature (or thumbprint/mark) on this consent form mean?

Your signature (or thumbprint/mark) on this form means:
• You have been informed about this study’s purpose, procedures, possible benefits and risks.
• You have been given the chance to ask questions before you sign.
• You have voluntarily agreed to be in this study.

____________________________________  ____________________________  
Print name of Adult Participant          Signature of Adult Participant  Date  Time

[ ]

Ask the participant to mark a “left thumb impression” in this box if the participant (or participant’s parent) is unable to provide a signature above.

____________________________________  ____________________________  
Print name of Person Obtaining Consent  Signature of Person Obtaining Consent  Date  Time
Study Title: Assessment of Severe Extremity Wound Bioburden at the Time of Definitive Wound Closure or Coverage: Correlation with Subsequent Post-Closure Deep Wound Infection (Bioburden Study)

Principal Investigator: Renan Castillo, PhD

IRB No.: 00003576

PI Version Date: v.2 7/27/2011

You are being asked for your permission to store tissue samples collected as part of the Bioburden study. Please read this form carefully before you sign it. This consent form explains the research study and your part in the study. If you decide not to participate in this study there will be no impact on your medical care. You can choose not to take part and if you join, you may quit at any time. Ask the study doctor or the study staff to explain any words or procedures that you do not clearly understand. Ask as many questions as needed. All of your questions should be answered to your satisfaction before you sign this form.

1. WHAT IS THE PURPOSE OF THIS RESEARCH STUDY?

Tissue samples from your wound are being collected during surgery to fix your injury because you have agreed to participate in the Bioburden study. If you agree, your samples will be kept in a specimen bank at a central facility. The samples will be de-identified, that is your name and contact information will not be linked to the samples. Your tissue may be used in future research studies to learn more about infection causing bacteria. Your tissue may also be used to look at genetic factors that might be involved in how your immune system responds to infection. These studies will not be done as part of this research.

Your samples will be made available only to researchers whose research studies are approved by an authorized review board or ethics committee. The research on your samples will not have a direct health benefit to you and will not have an effect on your care. Therefore, neither you nor your doctor will receive results of any testing done on your samples and the results will not be included in your medical record. The samples will be used for research purposes only and will not be sold. The research done with your tissue along with that of other patients may help to
develop new products in the future. You will not be paid for allowing this tissue to be used in research. There are no plans to share any potential financial gain with you.

2. WHY AM I BEING ASKED TO PARTICIPATE?

You are being asked for permission to store your tissue because you are between the ages of 18 to 64 and you have a complex wound from severe extremity trauma. People like you who are being treated at major trauma centers from around the county are being asked to participate.

3. HOW LONG WILL THE STUDY LAST?

Your tissue will be stored for up to 10 years.

4. HOW DOES THE STUDY WORK?

After you review this informed consent and agree to have your tissues stored, the following things will happen:

- We will obtain four tissue samples from your wound during surgery to treat your injury.
- If you develop an infection and are re-hospitalized, another tissue sample will be taken. Only two samples will be taken.
- These tissues will be stored at a central research facility.

There is a possibility that studies using these samples in the future will meet the criteria for genetic testing. This means that some of the information from your genes may be found to be related to diseases and other risks. However, your genetic samples will not include your name or any other way to identify they belong to you.

5. WHAT ARE THE POTENTIAL RISKS OR DISCOMFORTS?

While tissue sampling is routinely performed during surgery, there is always a small risk of injury associated with each tissue removal.

Any time information is collected for a study there is a small risk of breach of confidentiality. As described below, your research data will be identified by a unique study number rather than your name and all measures allowed by law to protect your confidentiality will be taken by the research staff.

Risks of genetic testing include the chance that insurers or employers will discriminate against you if the results are made public. However, your genetic samples will not include your name or
any other way to identify they belong to you. There will be no way to connect genetic test results to you.

6. WHAT ARE THE POTENTIAL BENEFITS?

You will not benefit directly from your participation in this study. However, your participation in the study could help us determine the best treatment for injuries like yours. This information could be very helpful to other people who have this same injury in the future.

It is possible that analysis using your tissue will help to develop new products in the future treat injuries like yours. You would not benefit financially from these products.

7. DO I GET ANY PAYMENT FOR BEING IN THE STUDY?

There is no payment for participation in this study.

8. ARE THERE ANY COSTS INVOLVED IN BEING IN THE STUDY?

There is no cost to you to participate in this study.

9. WILL MY INFORMATION BE KEPT PRIVATE?

The information we collect from you will be kept private to the best of our ability. Your name, birth date, medical record number and any other information that could identify you as an individual will be removed from all study forms. Instead, we will label your forms with a unique study number. The link between your name and your study number will be kept confidential to the greatest extent provided by law. The information collected for the study will be stored in a password protected, HIPPA verified computer database that only authorized members of our research team can use. When we report the results of the study, we will combine the information about you with similar information about hundreds of other people so your individual information will not be identifiable.

All study records will be considered confidential, and your name will not be used in reports or publications.

10. WILL YOU SHARE MY INFORMATION WITH OTHERS?

We will use your information only for the purposes of this study. The data from the study may be published. However, you will not be identified by name. People designated from the institutions where the study is being conducted will be allowed to inspect sections of your medical and
research records related to the study. This includes people designated by The Johns Hopkins Bloomberg School of Public Health who are overseeing this study. Everyone using study information will work to keep your personal information confidential. Your personal information will not be given out unless required by law.

The Department of Defense is providing funding to sponsor this study. Representatives from the U.S. Army Medical Research and Materiel Command (USAMRMC) Office of Research Protections (ORP) Human Research Protections Office (HRPO) and your local IRB may have access to research records in their role to protect human subjects engaged in research.

11. WHAT ARE MY ALTERNATIVES TO PARTICIPATION?

Your alternative is to not take part. If you choose not to take part, we will only use your tissue samples for the purposes of the Bioburden study and they will not be stored. Your healthcare will not be affected.

12. WHAT HAPPENS IF I LEAVE THE STUDY EARLY?

Your participation is completely voluntary. You have the right to withdraw from the at any time without penalty. Your decision will not affect the medical care you receive. If you decide that you no longer wish to have your tissue stored, you should notify the study doctor or the research coordinator at your center. Your tissue samples will be destroyed and will not be used for future research studies.

Your participation could be ended without your consent. Possible reasons could include our decision to end the study early or other reasons.

13. WHAT HAPPENS IF I AM INJURED OR BECOME ILL BECAUSE I TOOK PART IN THIS STUDY?

If you are injured or become ill because of your participation in this study, you will receive emergency medical care if needed and you will receive assistance in getting other medical care as needed. You or your insurance carrier will be billed for the cost of care, just as you would be billed for any other medical care. If you have any costs that are not covered by insurance, they are your responsibility.

You do not give up any of your legal rights by signing this form. You can seek legal compensation for any injury that may occur to you during the study as a result of an error by a member of the research staff or others.
14. WHO DO I CALL IF I HAVE QUESTIONS OR PROBLEMS?

- <<insert name>>, the study coordinator at your hospital has discussed this information with you and offered to answer any questions you may have. If you have further questions or get sick or injured as a result of being in this study, you can contact <<insert him/her>> at <<telephone number>>. You may also call the Director of the Study at your hospital, <<insert name>>, at <<telephone number>>.

- If you have further questions about your rights as a study participant you can call or contact your local IRB office or the Johns Hopkins Bloomberg School of Public Health IRB Office. The Johns Hopkins Bloomberg School of Public Health is serving as the overall coordinating center for this study that is being conducted in hospitals around the country. Contact the Johns Hopkins IRB if you feel you have not been treated fairly or if you have other concerns. The IRB contact information is:
- <<Insert local IRB contact information here>>

Address: Johns Hopkins Bloomberg School of Public Health  
615 N. Wolfe Street, Suite E1100  
Baltimore, MD 21205

Telephone: 410-955-3193  
Toll Free: 1-888-262-3242  
Fax: 410-502-0584  
E-mail: irboffice@jhsph.edu

You will receive a copy of this signed consent form.

What does your signature (or thumbprint/mark) on this consent form mean?

Your signature (or thumbprint/mark) on this form means:

- You have been informed about this study’s purpose, procedures, possible benefits and risks.
- You have been given the chance to ask questions before you sign.
- You have voluntarily agreed to be in this study.

________________________  __________________________  ________  ___:___
Print name of Adult Participant  Signature of Adult Participant  Date  Time
Ask the participant to mark a “left thumb impression” in this box if the participant (or participant’s parent) is unable to provide a signature above.

________________________
_____________________________
________________________

Print name of Person Obtaining Consent
Signature of Person Obtaining Consent
Date
Time
APPENDIX G: EVALUATION TO GIVE CONSENT

EVALUATION TO GIVE CONSENT

Procedure: Make a subjective judgment regarding item 1 below. Ask questions 2 through 5. You may select the language to use in asking the questions in order to help the respondent understand them.

1. Is the respondent alert and able to communicate with you?
   Yes ___     No ____  (if condition not likely to change, seek proxy consent)

2. Ask the respondent to name at least one thing that s/he will be asked to do as part of the study.
   Describe __________________________________________________________
   __________________________________________________________

3. Ask the respondent to explain what s/he could do if s/he decided s/he did not want to participate in the study.
   Describe __________________________________________________________
   __________________________________________________________

4. Ask the respondent to explain what s/he would do if s/he were experiencing distress or discomfort at any time during the study.
   Describe __________________________________________________________
   __________________________________________________________

I hereby certify that the above-named respondent is alert, able to communicate, and able to give acceptable answers to items 2, 3, 4, and 5 above.

__________________________________________  _______________________
Research Coordinator                        Date

METRC Bioburden Version 5.0, October 27, 2014  62
# APPENDIX H: PATIENT BASELINE INTERVIEW QUESTIONS

## BIOBURDEN Study:

**Patient Characteristics Form (CRF02)**

| Study ID Number: | **B I O** – ____ ____ – ____ ____ ____ |
| Date Form Completed: | ____ / ____ / ____ |

**Form Completed By:**

**Study Surgeon:**

**REDCap Data Entry By:**

**PURPOSE:** To characterize patient’s demographic characteristics.

**WHEN:** During hospitalization for definitive wound coverage/closure.

**ADMIN BY:** Research Coordinator

**RESPONDENT or DATA SOURCE:** Patient

**INSTRUCTIONS:**

- The RC should complete this form by interviewing the participant during the index hospitalization. While respecting the patient’s right to refuse to answer any specific question, it is important to make every effort to collect complete data. Specifically, if the patient is unsure as to the answer, probe for a “best guess.”
- Do not leave any blanks: if no answer is provided, make sure to mark down “refused” or “don’t know.”
- There are separate Spanish language and Proxy Respondent versions of this form.
- If someone other than the RC is completing this form, the RC should review the completed form for missing responses and resolve any problems before the patient leaves the hospital.
- Consult the BIOBURDEN CRF Guidance Document for detailed instructions about specific items on this form.

Site Research Coordinator

<table>
<thead>
<tr>
<th>Signature</th>
<th>Date</th>
</tr>
</thead>
</table>
PATIENT DEMOGRAPHICS

[Interviewer: Note gender]
- Female
- Male

How old were you on your last birthday? ___ ___

Are you of Latino or Hispanic origin?
- Yes
- No
- Refused
- Don’t Know

What race do you consider yourself to be? Please choose one or more of the following:
  a. White
  b. African American
  c. Asian
  d. American Indian or Alaskan Native
  e. Native Hawaiian or other Pacific Islander
  f. Other
  g. Refused
  h. Don’t know

SMOKING HISTORY

In your lifetime have you ever smoked at least 100 cigarettes, cigars, pipes, or wads/dips of smokeless tobacco (such as chew tobacco, dip, or snuff)?
- Yes
- No = GO TO LAST QUESTION
- Refused = GO TO LAST QUESTION
- Don’t Know = GO TO LAST QUESTION

Did you smoke cigarettes, cigars, or a pipe or use smokeless tobacco during the past 4 weeks?
- Yes
- No = GO TO LAST QUESTION
- Refused = GO TO LAST QUESTION
- Don’t Know = GO TO LAST QUESTION

Was it cigarettes, cigars, a pipe or smokeless tobacco that you used in the last 4 weeks? (select all that apply)
  a. Cigarettes
  b. Cigars
c. Pipe

On average, about how many cigarettes or cigars did you smoke per day?
(1 pack=20 cigarettes)

--- cigarettes/cigars

Units:
(e.g. 20 cigarettes per day)
- per day
- per week
- per month

On average, how often do you use any chewing tobacco, dip, or snuff?

--- wads/dips

Units:
(e.g. 1 wad per day)
- per day
- per week
- per month

On average, how often do you smoke a pipe?

--- pipes

Units:
(e.g. 3 pipes per week)
- per day
- per week
- per month

---

**PRE-INJURY HEALTH STATUS**

Now I would like you to think about your overall health and sense of well being prior to your injury. This question is about your health before you were injured.

In general, would you say your health was… [Read response choices]
- Excellent
- Very good
- Good
- Fair
- Poor
BIOBURDEN Study:
Patient Characteristics Form – PROXY VERSION (CRF02)

Study ID Number: BIO – ___ ___ ___ – ___ ___ ___ ___
Date Form Completed: ___ ___ / ___ ___ / ___ ___

Form Completed By:
Study Surgeon:
REDCap Data Entry By:

PURPOSE: To characterize participant’s demographic characteristics.

WHEN: During hospitalization for definitive wound coverage/closure.

ADMIN BY: Research Coordinator

RESPONDENT or DATA SOURCE: Patient

INSTRUCTIONS:
• The RC should complete this form by interviewing the proxy during the index hospitalization. While respecting the proxy’s right to refuse to answer any specific question, it is important to make every effort to collect complete data. Specifically, if the patient is unsure as to the answer, probe for a “best guess.”
• Do not leave any blanks: if no answer is provided, make sure to mark down “refused” or “don’t know.”
• If someone other than the RC is completing this form, the RC should review the completed form for missing responses and resolve any problems before the patient leaves the hospital.
• Consult the BIOBURDEN CRF Guidance Document for detailed instructions about specific items on this form.

Site Research Coordinator

____________________________________________________________
Signature                                                                                                     Date
PATIENT DEMOGRAPHICS

[Interviewer: Note gender]
- Female
- Male

How old was he/she on his/her last birthday? __ __

Is he/she of Latino or Hispanic origin?
- Yes
- No
- Refused
- Don’t Know

What race does he/she consider himself/herself to be? Please choose one or more of the following:
  i. White
  j. African American
  k. Asian
  l. American Indian or Alaskan Native
  m. Native Hawaiian or other Pacific Islander
  n. Other
  o. Refused
  p. Don’t know

SMOKING HISTORY

In his/her lifetime has he/she ever smoked at least 100 cigarettes, cigars, pipes, or wads/dips of smokeless tobacco (such as chew tobacco, dip, or snuff)?
- Yes
- No = GO TO LAST QUESTION
- Refused = GO TO LAST QUESTION
- Don’t Know = GO TO LAST QUESTION

Did he/she smoke cigarettes, cigars, or a pipe or use smokeless tobacco during the past 4 weeks?
- Yes
- No = GO TO LAST QUESTION
- Refused = GO TO LAST QUESTION
- Don’t Know = GO TO LAST QUESTION

Was it cigarettes, cigars, a pipe or smokeless tobacco that he/she used in the last 4 weeks? (select all that apply)
  a. Cigarettes
b. Cigars
c. Pipe
d. Smokeless Tobacco (i.e., chew tobacco, dip, or snuff)
e. Refused
f. Don’t Know

On average, about how many cigarettes or cigars did he/she smoke per day?
(1 pack=20 cigarettes)

   _ _ _ cigarettes/cigars

Units:
   (e.g. 20 cigarettes per day)
   ▪ per day
   ▪ per week
   ▪ per month

On average, how often does he/she use any chewing tobacco, dip, or snuff?

   _ _ _ wads/dips

Units:
   (e.g. 1 wad per day)
   ▪ per day
   ▪ per week
   ▪ per month

On average, how often does he/she smoke a pipe?

   _ _ _ pipes

Units:
   (e.g. 3 pipes per week)
   ▪ per day
   ▪ per week
   ▪ per month

---------------------------------------------------------------------------------------------------------------------------------------

PRE-INJURY HEALTH STATUS

Now I would like you to think about his/her overall health and sense of well being prior to his/her injury. This question is about his/her health before his/her injury.

In general, would he/she say his/her health was… [Read response choices]
   ▪ Excellent
   ▪ Very good
- Good
- Fair
- Poor
APPENDIX I: PATIENT 12 MONTH FOLLOW UP TELEPHONE INTERVIEW QUESTIONS

**BIOBURDEN Study:**
12 Month Follow-Up Form (CRF10)

Study ID Number: **B I O** – ___ ___ ___ – ___ ___ ___ ___
Date Form Completed: ___ ___ / ___ ___ / ___ ___
Month       Day       Year

Form Completed By:
Study Surgeon:
REDCap Data Entry By:

**PURPOSE:** To collect self-reported hospital admission data from participants who were not identified as having a re-admission for an infection prospectively.

**WHEN:** During a telephone follow-up interview at the end of the 12 month study period

**ADMIN BY:** Research Coordinator

**RESPONDENT or DATA SOURCE:** Patient (or Proxy)

**INSTRUCTIONS:**
- The RC should complete this form by interviewing the participant at 12 months following the index hospitalization. While respecting the patient’s right to refuse to answer any specific question, it is important to make every effort to collect complete data. Specifically, if the patient is unsure as to the answer, probe for a “best guess.”
- Do not leave any blanks: if no answer is provided, make sure to mark down “refused” or “don’t know.”
- There are separate Spanish language and Proxy Respondent versions of this form.
- To the extent possible, adhere to the script provided.
- If someone other than the RC is completing this form, the RC should review the completed form for missing responses and resolve any problems before the patient leaves the hospital.
- Consult the BIOBURDEN CRF Guidance Document for detailed instructions about specific items on this form.

Site Research Coordinator

__________________________________________  ______________________________
Signature                                      Date

METRC Bioburden Version 5.0, October 27, 2014
1. Except for the initial hospitalization for your injury, during the past year, have you stayed at least one night in a hospital or other medical care facility because of your physical health, including problems with your injured limb?
   Yes
   No (STOP – form is complete)
   Refused (STOP – form is complete)
   Refused (STOP – form is complete)
   Don’t Know (STOP – form is complete)

2. How many different times in the past year were you a patient in a hospital overnight? Please give your best estimate.
   — — times
   Refused
   Don’t Know

3. How many of those times were you hospitalized for your leg injury? Please give your best estimate.
   — — times
   Refused
   Don’t Know

For each hospital admission related to the patient’s injured leg identified above in #3, record patient reported information about this admission below and obtain discharge abstract to discuss with the surgeon.

[InterviewerRead] I would like to know more about [the/each] time you stayed in the hospital for your injured leg. [Let’s begin with the first time you were admitted to the hospital...]

4. Can you tell me the name of the facility where you were treated? Interviewer probe: If respondent doesn’t recall, probe by asking, “Do you remember if it was a VA hospital, military hospital, community hospital or someplace else?”

5. In what city is that facility located?

6. In what state is that facility located?

7. During what month and year were you admitted to this facility? ___ ___/20 ___ ___

8. Did you have any surgeries during your hospital stay?
   Yes
9. Do you know what you had surgery for?
   Yes
   No (Go to Question #11)
   Refused (Go to Question #11)
   Don’t Know (Go to Question #11)

10. What was your surgery for?
    Suspected infection
    Problems with bone healing
    Problems with wound healing
    Amputation
    Something else, related to my injured limb

11. Did your surgeon tell you that you had an infection?
    Yes
    No
BIOBURDEND Study:
12 Month Follow-Up Form – PROXY VERSION (CRF10)

Study ID Number: BIO – ___ ___ ___ – ___ ___ ___ ___
Date Form Completed: ___ ___ / ___ ___ / ___ ___

Month Day Year

Form Completed By:
Study Surgeon:
REDCap Data Entry By:

PURPOSE: To collect self-reported hospital admission data from participants who were not identified as having a re-admission for an infection prospectively.

WHEN: During a telephone follow-up interview at the end of the 12 month study period

ADMIN BY: Research Coordinator

RESPONDENT or DATA SOURCE: Patient (or Proxy)

INSTRUCTIONS:
• The RC should complete this form by interviewing the proxy at 12 months following the index hospitalization. While respecting the proxy’s right to refuse to answer any specific question, it is important to make every effort to collect complete data. Specifically, if the proxy is unsure as to the answer, probe for a “best guess.”
• Do not leave any blanks: if no answer is provided, make sure to mark down “refused” or “don’t know.”
• To the extent possible, adhere to the script provided.
• If someone other than the RC is completing this form, the RC should review the completed form for missing responses and resolve any problems before the patient leaves the hospital.
• Consult the BIOBURDEND CRF Guidance Document for detailed instructions about specific items on this form.

Site Research Coordinator

__________________________________________________________________
Signature                                                                                                     Date
1. Except for the initial hospitalization for his/her injury, during the past year, has he/she stayed at least one night in a hospital or other medical care facility because of his/her physical health, including problems with his/her injured limb?
   No (STOP – form is complete)
   Yes
  Refused
   Don’t Know

2. How many different times in the past year has he/she been a patient in a hospital overnight? Please give your best estimate.
   — — times
   Refused
   Don’t Know

3. How many of those times was he/she hospitalized for a complication related to his/her [left/right] leg? Please give your best estimate.
   — — times
   Refused
   Don’t Know

For each hospital admission related to the patient’s injured leg identified above in #3, record proxy reported information about this admission below and obtain discharge abstract to discuss with surgeon.

[InterviewerRead] I would like to know more about [the/each] time he/she stayed in the hospital. [Let’s begin with the first time he/she was admitted to the hospital...]

4. Can you tell me the name of the facility where he/she was treated? Interviewer probe: If respondent doesn’t recall, probe by asking, “Do you remember if it was a VA hospital, military hospital, community hospital or someplace else?”

5. In what city is that facility located?

6. In what state is that facility located?

7. During what month and year was he/she admitted to this facility? ___ ___/20 ___ ____

8. Did he/she have any surgeries during his/her hospital stay?
   Yes
   No (Go to Question #11)
   Refused (Go to Question #11)
   Don’t Know (Go to Question #11)
8. Do you know what he/she had surgery for?
   Yes
   No (Go to Question #11)
   Refused (Go to Question #11)
   Don’t Know (Go to Question #11)

9. What was his/her surgery for?
   Suspected infection
   Problems with bone healing
   Problems with wound healing
   Amputation
   Something else, related to my injured limb
   Something else, not related to my injured limb

10. Did his/her surgeon tell him/her that he/she had an infection?
    Yes
    No

________________________