

Assessing Microbiome in Primary Anterior Cervical Fusion Patients

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Study Site:

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REVISION HISTORY

Version	Date	Summary of Changes
1.0	20-JAN-2026	Original Version
2.0	15-AUG-2026	Added explicit language that SOC CT imaging at 6 and 12 months will be used to evaluate fusion and screw lucency/radiolucency, and updated the relevant follow-up text and procedure table accordingly.

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LIST OF ABBREVIATIONS

ACDF	Anterior Cervical Discectomy and Fusion
AE	Adverse Event
BMI	Body Mass Index
CRP	C-Reactive Protein
CSF	Cerebrospinal Fluid
DEXA	Dual-Energy X-ray Absorptiometry
EMR	Electronic Medical Record
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act
HSS	Hospital for Special Surgery
IRB	Institutional Review Board
LAT	Lateral (radiographic view)
mNGS	Metagenomic Next-Generation Sequencing
MRI	Magnetic Resonance Imaging
NDI	Neck Disability Index
OR	Operating Room
PHI	Protected Health Information
PI	Principal Investigator
SAE	Serious Adverse Event
SF-12	Short Form-12 Health Survey
UPIRSO	Unanticipated Problem Involving Risks to Subjects or Others
VAS	Visual Analog Scale
WBC	White Blood Cell Count

1. STUDY SUMMARY

Study Title	Assessing Microbiome in Primary Anterior Cervical Fusion Patients
Study Site	Hospital for Special Surgery 535 East 70th Street New York, NY 10021
Study Number	2023-1514
Protocol Date	15-AUG-2026
STUDY OVERVIEW	
Study Design	A single-center, prospective, observational cohort study
Purpose	To evaluate the presence and composition of bacterial communities in patients undergoing primary anterior cervical discectomy and fusion (ACDF) by comparing standard microbiologic culture techniques with advanced metagenomic sequencing methods, and to assess the association of bacterial findings with clinical and radiographic outcomes.
Expected Study Duration	Approximately 3 years
Evaluation Schedule	Subjects will be evaluated preoperatively and intraoperatively at the time of index surgery. Postoperative clinical and radiographic assessments will occur as part of standard of care follow-up at approximately 6 weeks, 6 months, 12 months, and annually thereafter up to 24 months.
ELIGIBILITY CRITERIA	
Intended Subject Population	A total of up to 76 subjects undergoing primary anterior cervical discectomy and fusion with instrumentation at Hospital for Special Surgery will be enrolled.
Inclusion Criteria	In order to be eligible to participate in this study, subjects must meet all of the following criteria: 1.Age between 18 and 80 years. 2.Indicated for primary anterior cervical discectomy and fusion involving one to three contiguous cervical levels. 3.Physically and mentally able and willing to comply with the Protocol, including the ability to read and complete the required forms and willing and able to adhere to the scheduled follow-up visits and requirements of the Protocol. 4.Medically cleared for surgery. 5.Able to understand the study requirements and provide written informed consent.

Exclusion Criteria	Subjects who meet any of the following criteria will be excluded from participating in this study: 1. Have an active systemic infection or evidence of infection at the operative site. 2. Have a history of or anticipated treatment for active systemic infection, including HIV or hepatitis C. 3. Have congenital bony or spinal cord abnormalities that affect cervical spine stability, including but not limited to congenital fusion anomalies. 4. Have prior trauma to the cervical spine (C3–C7) resulting in significant bony, ligamentous, or spinal cord injury. 5. Have had any prior cervical spine surgery at the operative level. 6. Have had a prior cervical fusion procedure at any level. 7. Have a significantly increased risk of osteoporosis. Screening may include clinical assessment or prior bone mineral density testing. Subjects with a documented DEXA T-score ≤ -1.5 within 24 months prior to surgery may be excluded at the discretion of the investigator. 8. Have active malignancy or a history of invasive malignancy, except for non-melanoma skin cancer. 9. Have known allergies to cobalt, chromium, molybdenum, nickel, or polyethylene. 10. Are pregnant or breastfeeding at the time of enrollment, or plan to become pregnant within three years following surgery. 11. Have rheumatoid arthritis, lupus, or other autoimmune diseases affecting the musculoskeletal system. 12. Have a Body Mass Index (BMI) greater than 40 kg/m². 13. Have medical or neurologic conditions that would preclude accurate clinical evaluation or compliance with follow-up (e.g., neuromuscular disorders). 14. Require chronic daily high-dose oral or inhaled corticosteroids or other immunosuppressive therapies. 15. Have a current or recent history (within one year prior to screening) of substance abuse, including alcoholism or narcotic addiction, requiring intervention. 16. Have used another investigational drug or medical device within 30 days prior to surgery.
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17. Are taking medications known to interfere with bone or soft-tissue healing, including high-dose corticosteroids, immunosuppressive agents, or chemotherapeutic drugs.
18. Have pending personal litigation related to spinal injury (worker's compensation is not an exclusion).
19. Anticipate relocation greater than 50 miles that may interfere with completion of required follow-up visits.
20. Have mental illness or belong to a vulnerable population (e.g., prisoner or developmentally disabled) that would compromise the ability to provide informed consent or comply with study requirements.
21. Have an uncontrolled seizure disorder.
22. Have a history or evidence of psychosocial disorders that could interfere with accurate completion of self-reported outcome measures or adherence to the protocol.
23. Insulin-dependent diabetes mellitus or other medical conditions that significantly increase surgical risk or impair healing.

STUDY ENDPOINTS	
Primary Endpoint	The Primary Endpoint is the detection and characterization of bacterial organisms at the time of primary anterior cervical discectomy and fusion, as defined by: 1. Presence or absence of bacterial organisms identified from intraoperative specimens using standard microbiologic culture. 2. Presence, taxonomic composition, and relative abundance of bacterial organisms identified using metagenomic next-generation sequencing (mNGS). 3. Concordance and discordance between organisms identified by standard culture and metagenomic sequencing methods. Safety evaluations will include endpoints based on adverse event (AE) reporting, including postoperative infection.
Secondary Endpoints	The Secondary Endpoints will be based on data collected through 24 months of postoperative follow-up. Secondary endpoints include assessment of associations between microbiologic findings and clinical and radiographic outcomes, including: • Change in Neck Disability Index (NDI) score from baseline at each follow-up time point and at 24 months. • Neck pain as measured on a 100 mm Visual Analog Scale (VAS) at baseline and each follow-up visit. • Left and right arm pain measured separately on a 100 mm VAS at baseline and each follow-up visit. • Health-related quality of life as measured by the Short Form-12 (SF-12) at baseline and each follow-up visit. • Radiographic evidence of fusion, implant stability, or loosening at operative levels as assessed on standard-of-care imaging. • Incidence of postoperative surgical site infection or need for antimicrobial therapy. • Need for secondary surgical intervention at the index level(s). • Treatment satisfaction at final follow-up. • Strain level comparison between different levels.



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SCHEDULE OF PROCEDURES

Evaluation							3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29
	Baseline (within 90 days of surgery)	Perioperative prior to discharge	6 weeks ± 14 days	6 months ± 30 days	12 months ± 60 days	24 months ± 60 days	
History & Physical	X						
Informed Consent	X						
SCORE Questionnaire	X						
MRI	X						
X-Rays: FLEX/EXT	X						
X-Rays: Neutral LAT	X		X	X	X	X	
Assessment of Culture Swabs		X					
VAS Neck /Arm Pain	X		X	X	X	X	
VAS Satisfaction			X	X	X	X	
NDI	X		X	X	X	X	
SF-12	X		X	X	X	X	

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2. INTRODUCTION

2.1 Background

2.1.1 Subclinical Bacterial Biofilms and Persistent Neck Pain Following ACDF

A significant percentage of patients continue to suffer from severe and chronic neck pain even after surgical intervention. Despite technological advancements in surgery, the incidence of such cases remains high, potentially affecting 15-40 percent of patients [1]. This persistent pain condition significantly impacts both patients and the healthcare system. The exact cause of this pain is not well understood, with bacterial contamination around implants being one potential contribution. Diagnosing bacterial contamination around cervical implants is complex, as clinical symptoms and standard investigations frequently fail to specifically or sensitively detect the responsible microorganisms. This study aims to understand the characteristics of bacterial contamination of the cervical spine.

Biofilm formation post-spinal surgery presents a substantial clinical challenge. Diagnosis is difficult because the pain experienced by patients is disproportionate to objective findings, fever is often absent or mild, and inflammatory markers and white blood cell counts typically remain within normal ranges [2]. Concern is escalating as the frequency of spinal fusion surgeries, such as anterior cervical discectomy and fusion (ACDF)—the most common operation on the cervical spine—increases. Between 2010 and 2021, over 181,000 single-level ACDF procedures were performed each year [3]. The implication is that with the rise in spinal fusion surgeries, the incidence of bacterial contamination around implants might also increase. Currently, no clinical test can accurately diagnose bacterial contamination around implants when clinical, laboratory, culture, and radiological assessments are normal. This study intends to address this gap by identifying occult infections and investigating why some patients undergoing cervical spine surgery continue to experience persistent, disabling neck pain. Occult infections are identified through meticulous cultures taken under sterile conditions, typically in the operating room before administering antibiotics, from the surgical wound and hardware site.

Traditionally, these cultures are incubated for aerobic and anaerobic bacteria for 2-3 weeks in a microbiology laboratory. The introduction of metagenomics represents a significant advancement in this field. The rationale behind this research question stems from the limitation of swab methods, which are only known to capture approximately 30% of the biofilm's flora [4]. By employing metagenomic techniques, we can gain a comprehensive understanding of the flora present and correlate it with surgical outcomes and aseptic loosening. A key objective is to identify the mechanism of action of aseptic loosening and potentially link it to specific bacteria, which could enhance the approach to managing these postoperative complications.

- [1] Klessinger S. Interventional pain therapy in cervical post-surgery syndrome. *World J Anesthesiol* 2016; 5(2): 38-43
- [2] Callanan TC, Abjornson C, DiCarlo E, et al. Prevalence of Occult Infections in Posterior Instrumented Spinal Fusion. *Clin Spine Surg*. 2021;34(1):25-31. doi:10.1097/BSD.0000000000001014
- [3] Ratnasamy PP, Rudisill KE, Maloy GC, Grauer JN. Cervical Disc Arthroplasty Usage Has Levelled Out From 2010 to 2021. *Spine (Phila Pa 1976)*. 2023 Oct 15;48(20):E342-E348. doi: 10.1097/BRS.0000000000004560. Epub 2022 Dec 28. PMID: 36728785; PMCID: PMC10300227
- [4] Branck TA, Hurley MJ, Prata GN, Crivello CA, Marek PJ. Efficacy of a Sonicating Swab for Removal and Capture of *Listeria monocytogenes* in Biofilms on Stainless Steel. *Appl Environ Microbiol*. 2017;83(11):e00109-17. Published 2017 May 17. doi:10.1128/AEM.00109-17

2.1.2 Limitations of Culture-Based Detection and Rationale for Metagenomic Analysis

This current study builds upon our team's prior research, particularly focusing on the prevalence and types of bacteria present in patients undergoing primary posterior instrumented lumbar spine surgery. In our previous study, we enrolled 54 patients (29 females and 25 males, average age 61.4±12.9 years, average BMI 28.2±4.4 kg/m²), undergoing surgeries in the L3-S1 range of the lumbar spine, with varying numbers of levels operated upon. A key strength of this study was the detailed collection and analysis of 525 culture samples, approximately 5 swabs per patient, revealing a 33.3% rate of positive cultures. This study allowed for a nuanced understanding of bacterial prevalence in surgical sites, particularly highlighting the predominance of *C. acnes*. [1]

However, a limitation of our prior study was the potential underestimation of bacterial diversity in the cervical spine, as the methods employed may have missed certain bacteria. This gap is crucial, considering the diverse bacterial flora possibly present in surgical sites, as indicated by other studies like Fang et al. (2005) [2] and Heckmann et al. (2015) [3], which emphasized the risk factors for infection after spinal surgery and the specific role of *Propionibacterium acnes* in native hip infections. Additionally, the systematic review by Urquhart et al. (2015) [4] suggests a potential link between low-grade bacterial infections and back pain, further underlining the importance of accurately identifying all bacterial presence.

Our current study aims to address this deficiency by incorporating metagenomic analysis. This approach represents a significant advancement over traditional culture methods, which typically capture only 20-30% of the biofilm's flora. By using metagenomics, we can more comprehensively identify the bacterial flora present at surgical sites and correlate this with surgical outcomes and phenomena like aseptic loosening. The potential to tie specific bacteria to the mechanism of action of aseptic loosening could be key in managing postoperative complications and aligns with the growing body of evidence suggesting the importance of understanding microbial environments in surgical sites.

In the study by Wang et al., the objective was to identify pathogens possibly contributing to spinal infections through metagenomic next-generation sequencing (mNGS). Among 25 patients with suspected bacterial infections, clinical assessments were normal. Samples were obtained for histopathological examination and compared with mNGS to detect bacterial presence. The sensitivity of mNGS was significantly higher than that of bacterial cultures and provided evidence of low bioburden cases that would not be detectable by traditional culture methods. Follow-up showed normal CRP and WBC levels when patients were treated for these low-burden bacterial infections. [5]

In summary, while our previous study laid a strong foundation for understanding the bacterial landscape in spinal surgeries, its primary limitation was the potential underestimation of bacterial diversity. Our current research aims to fill this gap, potentially transforming our understanding and management of postoperative infections and complications.

[1] Callanan TC, Abjornson C, DiCarlo E, et al. Prevalence of Occult Infections in Posterior Instrumented Spinal Fusion. *Clin Spine Surg.* 2021;34(1):25-31. doi:10.1097/BSD.0000000000001014

[2] Fang A, Hu SS, Endres N, Bradford DS: Risk Factors for Infection After Spinal Surgery. *Spine (Phila Pa 1976)* 2005;30:1460–1465.

[3] Heckmann N, Sivasundaram L, Lieberman JR: *Propionibacterium acnes* infection in the native hip. *JBJS Case Connect* 2015;5:

[4] Urquhart DM, Zheng Y, Cheng AC, et al: Could low grade bacterial infection contribute to low back pain? A systematic review. *BMC Med* 2015;13:13.

[5] Wang G, Long J, Zhuang Y, et al. Application of metagenomic next-generation sequencing in the detection of pathogens in spinal infections. *Spine J.* 2023;23(6):859-867. doi:10.1016/j.spinee.2023.02.001

2.2 Defining the Role of Occult Bacterial Burden in Aseptic Loosening After ACDF

The primary objective of this study is to bridge gaps in our current understanding of the role of bacteria in cervical spine surgery. Despite advancements in surgical techniques, the phenomenon of aseptic loosening, particularly in spinal surgery, remains poorly understood. As indicated in this study, the significant incidence of aseptic loosening in spine surgeries highlights the need for a deeper investigation into the microbiological factors that may contribute to this phenomenon. By focusing on assessing, characterizing, and evaluating the impact of bacteria in the spine, this study seeks to provide insights that could significantly enhance our understanding and management of such postoperative complications.

2.3 Clinical Study Rationale

Addressing these research questions will fundamentally improve clinical practice, reshape concepts about postoperative complications in cervical spine surgery, and corroborate the findings of other investigators in this field. By delving into the study of bacteria in the spine cervical surgery, this research moves beyond identifying bacterial presence. Instead, it aims to elucidate the potential role of these bacteria in postoperative complications, thereby providing a clearer understanding of why some patients experience persistent symptoms after surgery.

Building on our previous work, which uncovered a 33% rate of occult infections in presumed aseptic patients undergoing surgery for posterior instrumented lumbar spinal fusion, this study extends the inquiry into the cervical spine domain. Our prospective observational study revealed the significant presence of *Cutibacterium acnes* and other bacteria, challenging the traditional belief that the absence of overt infection signs equates to a lack of significant bacterial presence at the operative site. This insight into the prevalence and nature of occult infections in spine surgery patients underscores a potentially unrecognized factor contributing to postoperative complications. By investigating these hidden infections in the context of cervical spine surgery, our study aims to uncover similar patterns and relationships between occult bacterial presence and adverse postoperative outcomes. This knowledge is crucial for developing strategies to identify and manage these risks preemptively.

This understanding will be pivotal in identifying high-risk patients for surgery and the influence of implementation of antimicrobial technologies in surgical procedures, which would be a significant advancement in reducing the risk of postoperative complications related to bacterial presence.

The outcomes of this study will not only change the way clinicians approach cervical spine surgeries but will also enhance the overall patient care strategy by providing solutions to manage ongoing symptoms more effectively. This research stands to confirm and build upon the work of other investigators, solidifying a more comprehensive understanding of the role of bacteria in primary spine surgery and potentially setting new standards in spinal surgery practices.

2.4 Integration With Related Institutional Protocols

2.4.1 Microbiology Culture Protocol

2.4.2 Infectious Disease Clinical Framework and Oversight

3. STUDY DESIGN

3.1 Study Overview

This investigation is a prospective, observational clinical study designed to characterize the bacterial microbiome associated with primary anterior cervical discectomy and fusion (ACDF) procedures and to compare traditional culture-based microbiology with metagenomic next-generation sequencing (mNGS).

The study will enroll adult patients aged 18-80 undergoing primary ACDF with anterior plate and screw instrumentation at a single academic tertiary care center.

This study is non-interventional with respect to clinical care. All surgical procedures, perioperative management, and

postoperative follow-up will be performed according to standard of care. Study-related activities consist solely of intraoperative specimen collection and longitudinal collection of clinical and radiographic data obtained during routine clinical follow-up.

3.2 Study Duration

The anticipated duration of the study is approximately 36 months, consisting of:

- Enrollment period: Approximately 12 months
- Follow-up period: Up to 24 months postoperatively after surgery for each enrolled subject.

Primary microbiologic analyses will be performed at the time of surgery, while clinical and radiographic outcomes will be assessed longitudinally through standard of care postoperative follow up.

3.3 Study Population

Eligible subjects are adults aged 18 to 80 years scheduled to undergo primary ACDF involving one to three cervical levels between C3 and T1 with anterior plate and screw instrumentation, as clinically indicated by the treating surgeon.

3.3.1 Inclusion Criteria

Subjects must meet all of the following criteria to be eligible for participation in this study:

1. Age between 18 and 80 years at the time of enrollment.
2. Indicated for primary anterior cervical discectomy and fusion (ACDF) involving one to three contiguous cervical levels, as determined by the treating surgeon.
3. Physically and mentally able and willing to comply with the protocol, including the ability to read and complete required forms and adhere to scheduled follow-up visits.
4. Medically cleared for surgery according to standard institutional preoperative evaluation procedures.
5. Able to understand the study requirements and provide written informed consent prior to participation in any study-related activities.

3.3.2 Exclusion Criteria

Subjects who meet any of the following criteria will be excluded from participation in this study:

1. Have an active systemic infection or evidence of infection at the operative site.
2. Have a history of or anticipated treatment for active systemic infection, including HIV or hepatitis C.
3. Have congenital bony or spinal cord abnormalities that affect cervical spine stability, including but not limited to congenital fusion anomalies.
4. Have prior trauma to the cervical spine (C3–C7) resulting in significant bony, ligamentous, or spinal cord injury.
5. Have had any prior cervical spine surgery at the operative level.
6. Have had a prior cervical fusion procedure at any level.
7. Have a significantly increased risk of osteoporosis. Screening may include clinical assessment or prior bone mineral density testing. Subjects with a documented DEXA T-score ≤ -1.5 within 24 months prior to surgery may be excluded at the discretion of the investigator.
8. Have active malignancy or a history of invasive malignancy, except for non-melanoma skin cancer.

9. Have known allergies to cobalt, chromium, molybdenum, nickel, or polyethylene.
10. Are pregnant or breastfeeding at the time of enrollment, or plan to become pregnant within three years following surgery.
11. Have rheumatoid arthritis, lupus, or other autoimmune diseases affecting the musculoskeletal system.
12. Have a Body Mass Index (BMI) greater than 40 kg/m².
13. Have medical or neurologic conditions that would preclude accurate clinical evaluation or compliance with follow-up (e.g., neuromuscular disorders).
14. Require chronic daily high-dose oral or inhaled corticosteroids or other immunosuppressive therapies.
15. Have a current or recent history (within one year prior to screening) of substance abuse, including alcoholism or narcotic addiction, requiring intervention.
16. Have used another investigational drug or medical device within 30 days prior to surgery.
17. Are taking medications known to interfere with bone or soft-tissue healing, including high-dose corticosteroids, immunosuppressive agents, or chemotherapeutic drugs.
18. Have pending personal litigation related to spinal injury (worker's compensation is not an exclusion).
19. Anticipate relocation greater than 50 miles that may interfere with completion of required follow-up visits.
20. Have mental illness or belong to a vulnerable population (e.g., prisoner or developmentally disabled) that would compromise the ability to provide informed consent or comply with study requirements.
21. Have an uncontrolled seizure disorder.
22. Have a history or evidence of psychosocial disorders that could interfere with accurate completion of self-reported outcome measures or adherence to the protocol.
23. Insulin-dependent diabetes mellitus or other medical conditions that significantly increase surgical risk or impair healing.

3.4 Informed Consent Procedures

Each potential participant will be provided with an IRB-approved informed consent form and given ample opportunity to review the information provided and ask questions. Participation in this study is entirely voluntary.

Only patients who meet all inclusion criteria and none of the exclusion criteria will be considered eligible for enrollment. Prior to any study-related activities, including collection of research biospecimens or entry of research data into study databases, written informed consent will be obtained.

Informed consent will be obtained by the Principal Investigator or a qualified study team member with documented delegated authority. The individual obtaining consent will conduct a discussion with the patient regarding the study and will also sign the consent form. A copy of the signed informed consent form will be provided to the participant, with copies retained in the medical record and Investigator Site File in accordance with institutional policy. Consent to participate in the study will be clearly documented in the patient's medical record.

If more than 60 days have elapsed between the time of informed consent and the index surgical procedure, the informed consent will be reviewed with the participant and re-signed prior to enrollment.

3.4.1 Identification of Eligible Patients

Potentially eligible participants will be identified through routine clinical review of surgical schedules, outpatient clinic visits, and preoperative evaluations for patients scheduled to undergo primary anterior cervical discectomy and fusion at Hospital for Special Surgery.

Eligibility will be confirmed by review of the medical record to ensure that all inclusion criteria are met and no exclusion criteria apply. Identification and eligibility confirmation will occur prior to surgery and before initiation of any study-related procedures.

3.4.2 Consent Approach and Timing

Eligible patients will be approached for participation during a preoperative clinic visit or during the preoperative evaluation period, at a time deemed appropriate by the treating surgeon and study team.

Written informed consent must be obtained prior to any study-related activity, including intraoperative specimen collection. No research procedures will be performed until informed consent has been obtained and documented.

3.4.3 Documentation of Consent

Written informed consent will be documented by the participant's signature on the IRB-approved consent form. The study team member obtaining consent will also sign and date the form.

A copy of the signed consent form will be provided to the participant. Copies will be maintained in the participant's medical record and the Investigator Site File. Consent to participate in the study will be clearly documented in the medical record in accordance with institutional requirements.

3.4.4 Consent for Biospecimen Collection and Future Use

As part of the informed consent process, participants will be informed of and asked to consent to the collection of intraoperative swab specimens for microbiologic culture and metagenomic sequencing as described in this protocol.

Participants will also be asked to consent to the storage and future research use of collected biospecimens and associated de-identified data. Biospecimens will be labeled using study identifiers only, and no direct patient identifiers will be included. Any future use of biospecimens will be limited to research purposes consistent with IRB approval.

Participants may decline consent for future use of biospecimens while still participating in the primary study, as permitted by the approved consent form.

3.5 Enrollment and Assignment of Study ID

A participant will be considered enrolled in the study once written informed consent has been obtained and eligibility has been confirmed.

Upon enrollment, each participant will be assigned a unique study identification number. This study ID will be used to label all study-related data and biospecimens and will serve as the primary identifier throughout the study.

The linkage between study identification numbers and patient identifiers will be maintained in a secure enrollment log accessible only to IRB-approved study personnel. This linkage file will be stored separately from study data and biospecimens to protect participant confidentiality.

3.6 Withdrawal Prior to Surgery

Participation in this study is voluntary, and participants may withdraw from the study at any time without penalty or impact on future medical care.

Participants who withdraw prior to undergoing the index surgical procedure will not have intraoperative research biospecimens collected. Any study-related data collected prior to withdrawal may be retained and used in a de-identified manner unless the participant requests otherwise, to the extent permitted by law and institutional policy.

The reason for withdrawal, if provided, will be documented in the enrollment log and study records.

3.7 Specimen Collection and Study Groups

3.7.1 General Principles

All intraoperative swab specimens will be collected using a standardized technique to minimize sampling variability and reduce technical error, particularly for metagenomic analyses.

For each designated anatomic site, the swab tip will be placed in direct contact with the target surface and rotated with gentle pressure for a minimum of 30 seconds.

All sampling will be performed under sterile conditions and in accordance with the predefined sampling order to limit cross-contamination.

Each sampling event consists of two swabs from the same site:

1. One swab for conventional aerobic and anaerobic culture.
2. One swab for metagenomic sequencing.

Swabs will be removed from packaging immediately prior to use, applied to the target site to collect surface fluid and microbial material, and placed immediately into separate, pre labeled sterile transport tubes. No tissue biopsies are taken for research purposes. Only swab-based specimens are collected.

3.7.2 Defined Specimen Collection Sites

The following sites will be collected for every enrolled subject unless an intraoperative safety or anatomic limitation prevents safe sampling. If a site is not collected, the reason will be recorded.

1. Oral cavity. Buccal swab obtained prior to surgical skin preparation and draping.
2. Air control. Sterile swab exposed to ambient operating room air over the planned incision field after surgical prep and draping, prior to skin incision, without contacting skin or drapes.
3. Suction control. Sterile swab of each suction tip used in the surgical field. Suction may be briefly activated while swabbing to sample internal lumen exposure.
4. Skin. Post prep and pre incision swab obtained adjacent to the marked incision, sampling the prepared skin surface without contacting non prepared areas.
5. Muscle. Swab of vascular muscular tissue encountered during the approach, including strap muscles or longus colli.
6. Paraesophageal adventitia. Swab of paraesophageal adventitial surface encountered during the anterior approach, avoiding contact with instruments or non-target surfaces.
7. Prevertebral fascia. Swab of the prevertebral fascia overlying the target level, obtained prior to localization needle

placement if feasible.

8. Intervertebral disc superficial. Swab of the annulus surface after exposure and level confirmation, prior to disc removal.

9. Intervertebral disc deep. Swab of disc space material during discectomy, sampling deeper disc space contents.

10. Cortical bone or endplate surface. Swab of exposed vertebral body rim or prepared endplate surface after disc removal and endplate preparation, prior to interbody placement and plate application.

11. Plasma. Metagenomics only specimen. Blood collected from an existing arterial line. Plasma will be processed in the operating room per protocol and used for mNGS analyses.

3.7.3 Labeling and Chain of Custody

All specimen containers will be labeled with:

1. Study ID
2. Specimen site code corresponding to the defined anatomic sampling list
3. Date and time of collection
4. Operating surgeon
5. Abjornson Laboratory designation

No patient identifiers will be placed on specimen containers. The linkage between Study ID and patient identifiers will be maintained in a separate, secure key file accessible only to IRB-approved study personnel.

A specimen log will document:

1. Each specimen collected
2. Specimen site and site code
3. Time of collection
4. Collector initials
5. Receiving laboratory confirmation

3.7.4 Laboratory Pathways

Each anatomic sampling site yields two swabs that follow separate laboratory pathways: conventional microbiologic culture and metagenomic next-generation sequencing (mNGS).

Pathway A, Conventional Microbiology:

1. Swabs designated for conventional microbiologic analysis will be delivered directly from the operating room to the institutional microbiology laboratory.
2. Specimens will undergo aerobic and anaerobic culture using institutional standard operating procedures, including extended long-hold incubation consistent with the laboratory's spine culture protocol.
3. Cultures will be monitored throughout the incubation period. When growth is detected, organism identification and antimicrobial susceptibility testing will be performed using standard clinically validated laboratory methods.

Results generated through Pathway A constitute standard-of-care diagnostic testing and will be reported in the electronic medical record for review by the treating clinical team.

In addition to pathways, a specific ordering method will be done for all swabs in pathway A (microbiology):

- 3 swabs (air, oral, suction tip) are non standard of care and they will be ordered under a newly created CDM code under CPT code 87070.
- Remaining 7 swabs will be ordered as standard of care in Epic system to microbiology.

Pathway B, Metagenomic Sequencing:

Swabs designated for metagenomic analysis will be processed under a research-only protocol in collaboration with the Weill Cornell Medicine Microbiome Core. Strain-level comparisons will be conducted to determine site-specific origin of bacteria.

Are all tests standard of care? NO

- The metagenomics testing is not standard of care. This will be paid for by internal funds. Additionally, the research swabs (air, oral, suction tip) from microbiology are not standard of care and are research swabs.

Sample Storage and Batch Submission:

1. Following intraoperative collection, swabs for mNGS will be placed into approved storage solution and stored at -80°C at Hospital for Special Surgery.
 - o Short-term storage at -20°C for up to 48 hours is permitted if immediate transfer to -80°C is not feasible.
2. Samples will remain frozen until a complete batch is assembled (anticipated batch size approximately 48 samples) to minimize freeze-thaw cycles and optimize sequencing efficiency.
3. Prior to batch submission, a sequencing request will be generated through the Weill Cornell Medicine Core Facilities iLab system.
4. The Microbiome Core will provide pre-labeled extraction tubes and custom identifiers following submission of the iLab request.
5. All samples will be labeled using study identifiers and specimen site codes only. No patient identifiers or protected health information will accompany samples.

Swab Processing and Bacterial Pelleting:

To maximize bacterial DNA recovery from low-biomass swab-based specimens, samples will undergo a pelleting-based concentration protocol prior to DNA extraction, as recommended by the receiving laboratory.

All processing will be performed using appropriate personal protective equipment and sterile technique to minimize contamination.

Materials required include:

- Swabs in storage solution
- Sterile 1.5 mL microcentrifuge (Eppendorf) tubes
- Microcentrifuge capable of $\geq 14,000$ RPM
- Vortex mixer
- Sterile elution buffer (50 μL per sample)
- Sterile pipette tips and calibrated micropipettes
- -80°C freezer
- Labeled extraction tubes provided by the Weill Cornell Medicine Microbiome Core

Processing steps are as follows:

1. Each swab will be vortexed in its storage solution tube for approximately 30 seconds to release bacterial material into suspension.
2. Using a sterile pipette, the entire volume of swab liquid (approximately 1 mL) will be transferred into a sterile 1.5 mL microcentrifuge tube.
3. The tube will be centrifuged at maximum speed ($\geq 3,260 \times g$) for 30 minutes at 4°C . If a refrigerated centrifuge is not available, centrifugation at room temperature is acceptable.
4. Following centrifugation, the supernatant will be carefully aspirated and discarded without disturbing the bacterial pellet at the bottom of the tube.

5. 50 μ L of elution buffer will be added to the pellet.
6. The pellet will be fully resuspended by brief vortexing or gentle pipetting.
7. The entire 50 μ L resuspended sample will be transferred into the appropriately labeled extraction tube provided by the Weill Cornell Medicine Microbiome Core.
8. Extraction tubes will be immediately stored at -80°C until batch shipment.

This pelleting-based approach minimizes input volume and maximizes the proportion of bacterial material entering the extraction process compared with direct liquid transfer alone, thereby improving DNA yield from swab-derived specimens.

DNA Extraction, Sequencing, and Bioinformatics:

DNA extraction, library preparation, sequencing, and bioinformatic processing will be performed by the Weill Cornell Medicine Microbiome Core using standardized, validated protocols, including:

- Mechanical and chemical lysis using bead-based disruption
- Automated DNA extraction (Promega Maxwell platform)
- DNA quantification using high-sensitivity fluorometric assays
- Shotgun metagenomic library preparation (Illumina DNA Prep)
- Sequencing on the Illumina NovaSeq X platform (paired-end 150 bp, 5% PhiX)
- Bioinformatic processing including quality control, host read removal, taxonomic profiling (MetaPhlAn4), and functional profiling (HUMAN3)

3.7.5 Blinding and Release of Research-Related Results

Conventional microbiology specimens collected under Pathway A are obtained and processed as part of standard-of-care clinical management. However, patient-facing release of microbiology results associated with this study will be restricted during the active study period.

Research-related orders will be linked to the approved research study and designated research diagnosis within the electronic medical record. In accordance with institutional policy, these orders will be configured as manual release only, and microbiology results will not be automatically released to patients through the patient portal while the study is ongoing. Standard of care swabs will follow standard practices and be released to patients in their patient portal as well as being visible in EPIC to medical staff. All research swabs will be blinded to patients, physicians, and surgeons.

Standard of care microbiology results will remain accessible to the treating clinical team for standard clinical interpretation and patient management. Restriction of patient-facing release applies only to automated or portal-based result disclosure and does not alter clinical care.

Metagenomic next-generation sequencing (mNGS) results generated under Pathway B are research-only and will not be released to patients or used to guide clinical decision-making unless independently confirmed through clinically validated diagnostic testing and supported by clinical assessment.

Release of research-related results to subjects, if applicable, will occur only after study completion and in accordance with institutional policy and IRB approval.

3.8 Schedule of Procedures

All study-related data collection occurs in conjunction with routine clinical care. Participation in this study does not require additional clinical visits, imaging studies, laboratory tests, or procedures beyond standard perioperative and postoperative management for patients undergoing primary anterior cervical discectomy and fusion (ACDF).

The schedule of procedures is designed to capture microbiologic, clinical, and radiographic data at clinically relevant time points while minimizing participant burden.

3.8.1 Baseline and Preoperative Assessments

Baseline assessments will be performed after written informed consent has been obtained and prior to the index surgical procedure. Data collected at baseline are obtained as part of standard preoperative evaluation and include, as available:

Demographic information

Relevant medical history and comorbidities

Physical examination findings

Preoperative imaging studies, including MRI and cervical spine radiographs, as clinically indicated

Patient-reported outcome measures, including:

Neck Disability Index (NDI)

Visual Analog Scale (VAS) for neck and arm pain

Short Form-12 (SF-12) Health Survey

Osteoporosis risk assessment or prior bone mineral density testing, if available as part of routine care

No additional testing is required solely for research purposes.

3.8.2 Intraoperative Assessments

Intraoperative assessments consist exclusively of biospecimen collection performed during the index ACDF procedure, as described in Section 3.7. Specimens are obtained under sterile conditions and do not alter surgical technique, operative duration, or perioperative management.

Intraoperative data abstracted for study purposes may include:

Operative level(s)

Implant type and configuration

Duration of surgery

Estimated blood loss

These data are obtained from standard operative documentation.

3.8.3 Postoperative and Follow-Up Assessments

Postoperative follow-up assessments will occur as part of routine clinical care at the following approximate time points:

- 6 weeks
- 6 months
- 12 months
- 24 months

At each follow-up visit, available clinical and radiographic data will be abstracted for study purposes, including:

- Patient-reported outcome measures:
- Neck Disability Index (NDI)
- Visual Analog Scale (VAS) for neck and arm pain
- Short Form-12 (SF-12) Health Survey
- Documentation of postoperative complications or adverse events
- Assessment of treatment satisfaction, when available
- Radiographic imaging obtained as part of standard of care follow-up, as clinically indicated, including neutral lateral radiographs and, where obtained per standard clinical practice, flexion-extension radiographs
- Standard of care CT scans of the cervical spine obtained at approximately 6 months and 12 months postoperatively for assessment of fusion progression and evaluation of potential radiolucency around screws
- Radiographic assessments will be interpreted per routine clinical practice and recorded for exploratory correlation with microbiologic findings.

3.8.4 Missed or Unscheduled Visits

If a participant misses a scheduled follow-up visit, reasonable efforts will be made to capture available clinical data within the allowable visit window. Data obtained from unscheduled visits or alternative clinical encounters may be included if relevant and available.

Participants who discontinue follow-up will be documented in accordance with Section 3.6. No additional procedures will be required for participants who withdraw or are lost to follow-up.

3.8.5 Summary Table of Procedures

A summary table outlining the timing of data collection and assessments is provided in the Schedule of Procedures table included in this protocol. This table serves as a reference and does not represent mandatory study visits beyond standard clinical care:

Schedule of Procedures Table

Evaluation	Baseline (within 90 days of surgery)	Perioperativ e prior to discharge	6 weeks ± 14 days	6 months ± 30 days	12 months ± 60 days	24 months ± 60 days
History & Physical	X					
Informed Consent	X					
SCORE Questionnaire	X					
MRI	X					
X-Rays: FLEX/EXT	X					
X-Rays: Neutral LAT	X		X	X	X	X
CT Scan				X		X
Assessment of Cultures		X				
VAS Neck /Arm Pain	X		X	X	X	X
VAS Satisfaction			X	X	X	X
NDI	X		X	X	X	X
SF-12	X		X	X	X	X

3.9 Patient Reported Outcome Measures

The outcomes of this study are designed to characterize the presence, composition, and clinical relevance of bacterial communities associated with cervical spine instrumentation in patients undergoing primary anterior cervical discectomy and fusion (ACDF). Outcomes will be assessed using a combination of standard microbiologic culture results, metagenomic sequencing data, and routinely collected clinical and radiographic information.

3.9.1 Primary Outcome Measure

The primary outcome of this study is:

- Detection of bacterial presence (positive vs negative) at each anatomic site as determined by:
 - Conventional culture methods, and
 - Metagenomic next-generation sequencing (mNGS)

3.9.2 Secondary Outcome Measures

Secondary outcomes include:

- Microbial diversity and composition at each sampling site, including alpha and beta diversity metrics.
- Concordance and discordance between culture-based microbiology and mNGS results.
- Identification of low-abundance or unculturable organisms detected by mNGS but not by standard culture.
- Associations between microbiologic findings and:
 - Patient-reported outcomes (NDI, VAS-Neck, SF-12)
 - Radiographic fusion status
 - Radiographic evidence of screw lucency or loosening

3.10 Follow-up Schedule

Subjects will undergo standard postoperative follow-up at:

- Baseline (preoperative)
- 6 weeks
- 6 months
- 12 months
- 24 months

At each follow-up interval, clinical outcomes and imaging studies will be collected as outlined in the protocol. No additional visits beyond standard clinical care are required for study participation.

3.10.1 Clinical Data Collection

Clinical data collected longitudinally will include, as available within routine care:

- Patient-reported outcome measures (see appendix):
 - Neck Disability Index (NDI)
 - Visual Analog Scale for neck pain (VAS-Neck)
 - Short Form Health Survey (SF-12)
- Documentation of postoperative complications or adverse events
- Relevant clinical notes pertaining to postoperative recovery

3.10.2 Radiographic Assessment

Radiographic outcomes will be assessed using imaging obtained as part of standard postoperative follow-up and may include plain radiographs and CT imaging as clinically indicated.

Radiographic data abstracted for study purposes include:

- Fusion status
- Evidence of screw lucency or loosening
- Other implant-related findings relevant to clinical outcomes

As part of standard of care follow-up, subjects may undergo cervical spine CT scans at approximately 6 months and 12 months postoperatively to assess fusion progression and potential radiolucency around screws. Standard postoperative radiographs, including neutral lateral views and other radiographic views obtained per routine clinical practice, will also be collected when available.

Radiographic assessments will be interpreted per routine clinical practice and recorded for exploratory correlation with microbiologic findings.

3.10.3 Study Burden

Participation in this study does not alter the standard clinical follow-up schedule and does not increase the number of clinical visits, imaging studies, or procedures required of participants.

3.11 Statistical Analysis Plan and Sample Size Justification

3.11.1 Overview

This is a prospective, observational study designed to characterize the bacterial microbiome associated with primary ACDF and to compare conventional culture based microbiology with metagenomic next generation sequencing (mNGS). Analyses will be primarily descriptive and comparative and will be interpreted in the context of an exploratory, hypothesis generating study design.

Descriptive statistics will be summarized for all measurements. Counts and percentages will be calculated for categorical variables, including bacterial detection status, organism categories, fusion status, screw lucency or loosening status, sex, and race or ethnicity. Continuous variables, including patient reported outcomes and radiographic measurements, will be summarized using mean and standard deviation or median and interquartile range, as appropriate based on distribution.

All statistical tests will be two sided. The target significance level will be 0.05 unless otherwise specified.

3.11.2 Operationalization of Outcomes

Primary and secondary outcomes will be operationalized as follows.

Primary microbiologic outcome:

- Bacterial detection status at each anatomic sampling site, defined as positive versus negative by:
 - conventional culture based methods (Pathway A), and
 - metagenomic next generation sequencing (mNGS; Pathway B)

Secondary microbiologic outcomes:

- Organism identification, defined as the organism category or taxonomic assignment produced by each method for each sample
- Strain-level comparisons between different swab cultures
- Agreement between the two methods in organism identification across samples

Secondary clinical and radiographic outcomes:

- Patient reported outcomes collected during routine clinical follow up, including NDI, VAS Neck, and SF 12
- Radiographic outcomes assessed during routine follow up, including fusion status and radiographic evidence of screw lucency or loosening

3.11.3 Analyses by Aim

Aim 1: To assess the prevalence of subclinical pathogens in primary cervical fusion patients by comparing standard microbiology methods with next-generation sequencing and metagenomics.

Because each sample is evaluated by both detection methods, the primary comparison is paired. McNemar's test will be used to evaluate the difference in paired bacterial detection status (positive versus negative) between conventional culture and mNGS. Results will be summarized using 2 by 2 contingency tables, paired proportions, and the direction of

discordance.

Aim 2: To characterize the types of bacteria identified by each method. This aim concentrates on evaluating the diversity and species of bacteria detected through standard microbiology versus metagenomics next-generation sequencing. Agreement between conventional culture and mNGS in organism identification will be evaluated using weighted kappa statistics. Kappa values will be reported with confidence intervals, and agreement will also be described descriptively by organism categories and sampling sites.

Aim 3: Evaluate the clinical outcomes of patients after the primary surgery with cervical instrumentation and at follow-up visits.

Subjects will be categorized into groups based on bacterial detection status using a pre specified definition (for example, any positive detection by either method at one or more operative site samples, versus no detection across operative site samples). Continuous outcomes such as NDI, SF 12, VAS Neck, and selected radiographic continuous measures will be compared between groups using an independent samples t test when assumptions of normality are met, or the Wilcoxon rank sum test when distributions are non normal.

Aim 4: To assess screw or hardware integration radiographically at follow-up.

Categorical radiographic outcomes, including fusion status and presence of screw lucency or loosening, will be compared between bacterial detection groups using chi square tests when cell counts are sufficient, or Fisher's exact test when expected cell counts are small.

3.11.4 Multivariable and Confounding Assessment

Potential confounders will be evaluated in exploratory multivariable analyses as appropriate based on sample size and event rates. Candidate covariates may include age, sex, BMI, smoking status, diabetes status, number of operative levels, and other clinically relevant baseline variables available from routine clinical documentation.

For continuous outcomes, linear regression models may be used. For binary outcomes such as fusion status or screw lucency or loosening, logistic regression models may be used. Model selection will be parsimonious and guided by clinical plausibility and the number of outcome events to avoid overfitting.

3.11.5 Missing Data

Missing data will not be imputed. Analyses will be conducted using available data, and the extent and pattern of missingness will be summarized descriptively.

3.11.6 Sample Size and Power Justification

The target enrollment for this study is 76 patients. This sample size is intended to support the primary microbiologic comparison and allow for anticipated attrition.

Aim 1 sample size justification:

A sample size of 68 patients achieves 90 percent power to detect a difference (P_1 minus P_0) of 0.20 using a two sided binomial test with target significance level of 0.05. The actual significance level achieved is 0.0349 under the assumption that the population proportion under the null hypothesis is 0.40. To account for an estimated attrition rate of 10 percent, the total enrollment target is increased to 76 patients.

Aim 2 sample size justification:

Agreement between conventional culture and mNGS for organism identification will be assessed using the kappa statistic. Under assumptions of nine organism categories with frequencies of 0.05, 0.10, 0.05, 0.30, 0.30, 0.05, 0.05, 0.05, and 0.05, a total of 155 samples achieves 80 percent power to detect a true kappa value of 0.90 in a test of H_0 : kappa equals 0.80 versus H_1 : kappa not equal to 0.80 at a two sided significance level of 0.05.

Because multiple sampling sites will be collected per enrolled subject, the planned sampling scheme will provide the required number of samples for the agreement analysis. Conservatively assuming five samples per patient and treating samples as independent for the purpose of this calculation, 35 patients would yield approximately 175 samples. Accounting for 10 percent attrition, at least 35 patients will be required to meet the sample based target for Aim 2. The overall enrollment target of 76 patients therefore satisfies both Aim 1 and Aim 2 sample size requirements.

4. RISK ANALYSIS

4.1 General Risk Assessment

This study is a prospective, observational investigation involving patients undergoing primary anterior cervical discectomy and fusion (ACDF) as part of standard clinical care. No experimental surgical procedures, devices, medications, or alterations to clinical management are introduced as part of this study.

All surgical, perioperative, and postoperative care is performed according to standard institutional practice and is independent of study participation.

4.1.1 Risks Associated With Standard Surgical Care

Participants in this study are subject to the same risks inherent to ACDF surgery as any patient undergoing this procedure, regardless of research participation. These risks include, but are not limited to:

- Superficial or deep surgical site infection
- Pneumonia or atelectasis
- Thromboembolic events, including deep vein thrombosis or pulmonary embolism
- Hemorrhage or hematoma formation
- Injury to blood vessels, nerves, or surrounding soft tissues
- Dysphagia, dysphonia, or hoarseness
- Neurologic injury, including weakness, numbness, or paralysis
- Cerebrospinal fluid leak
- Implant-related complications, including loosening or nonunion
- Persistent or recurrent neck or arm pain
- Adverse reactions to anesthesia
- Cardiopulmonary events, including myocardial infarction or stroke
- Death

These risks are not increased by participation in this study.

4.1.2 Risks Associated with Research Procedures

Research-related activities in this study consist of:

- Collection of intraoperative swab specimens from surgical field surfaces that are already exposed during routine surgery
 - Collection of clinical, radiographic, and patient-reported outcome data obtained during standard clinical care
- No additional incisions, tissue biopsies, implants, medications, imaging studies, or clinical visits are required for research purposes.

The incremental risks associated with study participation are therefore minimal and include:

- A theoretical risk of contamination or prolonged operative time related to specimen collection. This risk is minimized by standardized sterile technique and integration of specimen collection into routine surgical workflow without altering surgical steps.
- A minimal risk of breach of confidentiality related to collection and storage of research data and biospecimens.

No direct physical risk is associated with metagenomic sequencing, as sequencing is performed only on collected specimens and does not involve any intervention in the participant.

4.2 Methods to Minimize Risk

Multiple safeguards are in place to minimize risks to participants:

- No deviation from standard surgical technique or perioperative management
- No additional invasive procedures beyond standard of care including standardized radiographs.
- Strict sterile technique during specimen collection
- Standardized specimen collection protocols to minimize operative disruption
- Use of study identifiers only on biospecimens and data
- Secure storage of data and biospecimens with access limited to IRB-approved study personnel
- Separation of identifiers from research data via secure linkage files
- No use of research sequencing results for clinical decision-making unless independently confirmed through clinically validated testing

All investigators and research staff are trained in human subjects research, Good Clinical Practice, and institutional data security requirements.

4.3 Potential Benefits

4.3.1 Benefits to Individual Participants

Participants are not expected to receive direct clinical benefit from participation in this study beyond the care they would otherwise receive as part of standard clinical management.

Research-related metagenomic sequencing results are investigational and will not be used to guide clinical care. There is opportunity to have further clarity of a patient's clinical situation and understanding their bacterial presence may improve their clinical outcome from the primary surgery.

4.3.2 Benefits to Society and Scientific Knowledge

This study has the potential to provide important scientific and clinical insights, including:

- Improved understanding of occult bacterial presence associated with cervical spine instrumentation
- Identification of microbial signatures that may contribute to aseptic loosening, persistent pain, or implant-related complications
- Improved comparison between traditional culture-based microbiology and metagenomic sequencing in spine surgery
- Foundational data to inform future diagnostic strategies, risk stratification, and preventive approaches in spine surgery

These insights may ultimately benefit future patients undergoing cervical spine surgery.

4.4 Risk–Benefit Assessment and Justification

The risks associated with participation in this study are minimal and are largely limited to theoretical risks related to specimen handling and data confidentiality. No additional clinical risk is introduced beyond standard surgical care.

Given the minimal incremental risk and the potential to significantly advance understanding of postoperative spine complications, the overall risk–benefit ratio is favorable.

This study is ethically justified and appropriate for conduct in accordance with institutional policies, federal regulations, and principles of human subjects research.

5. PRIVACY AND CONFIDENTIALITY

5.1 Confidentiality and HIPAA Considerations

All study activities will be conducted in compliance with the Health Insurance Portability and Accountability Act (HIPAA), applicable federal and state regulations, and institutional policies governing the protection of human subject privacy and confidentiality.

Protected health information (PHI) will be accessed and used only as necessary to identify eligible participants, obtain informed consent, collect study-related data, and conduct follow-up assessments. Access to PHI will be limited to IRB-approved study personnel who have completed required human subjects and HIPAA training.

Participants will be assigned a unique study identification number upon enrollment. This study ID will be used for all study-related data collection and biospecimen labeling. Direct identifiers will not appear on research datasets, laboratory specimens, or analytic files.

The linkage between study identification numbers and participant identifiers will be maintained in a secure enrollment log. This linkage file will be stored separately from study data and biospecimens and will be accessible only to authorized study personnel.

5.2 Data Capture and Source Documentation

Study data will be obtained from routine clinical documentation, operative reports, imaging studies, laboratory results, and patient-reported outcome instruments collected as part of standard clinical care.

Data will be abstracted into study-specific case report forms or electronic data capture systems approved by the institution. Source documentation will be maintained in accordance with Good Clinical Practice (GCP) and institutional requirements to allow for data verification and monitoring, if applicable.

Only de-identified or coded data will be used for analysis. Any data shared with collaborating laboratories or external investigators will be limited to the minimum necessary and will not include direct identifiers.

5.3 Data Storage, Access, and Security

Electronic study data will be stored on secure, password-protected institutional servers that comply with Hospital for Special Surgery information security standards. Access will be role-based and restricted to IRB-approved study personnel.

Physical study documents, including consent forms and regulatory files, will be stored in locked cabinets within secure research offices. Electronic files will be protected by institutional encryption, firewall protections, and regular backup procedures.

Study data will be retained for the duration required by institutional policy and applicable regulations. At the conclusion of the retention period, data will be securely archived or destroyed in accordance with institutional procedures.

5.4 Biospecimen Storage and De-Identification

All biospecimens collected for research purposes will be labeled using study identification numbers only. No direct identifiers will be included on specimen containers.

Biospecimens designated for metagenomic analysis will be stored in secure, access-controlled laboratory freezers at Hospital for Special Surgery or approved collaborating facilities. Transfer of specimens to external laboratories will occur

under approved material transfer agreements and using coded identifiers only.

Any future research use of stored biospecimens or associated data will be limited to IRB-approved purposes consistent with participant consent. No biospecimens will be used for purposes beyond the scope of approved research without additional IRB approval, as required.

6. ADVERSE EVENTS AND SAFETY REPORTING

6.1 Definitions

For the purposes of this study, adverse events will be defined and classified in accordance with institutional policy and federal regulations.

An Adverse Event (AE) is any untoward medical occurrence in a participant temporally associated with participation in the study, whether or not it is considered related to the study.

A Serious Adverse Event (SAE) is any adverse event that:

- Results in death
- Is life-threatening
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability or incapacity
- Results in a congenital anomaly or birth defect
- Is otherwise considered a significant medical event based on clinical judgment

An Unanticipated Problem Involving Risks to Subjects or Others (UPIRSO) is any incident, experience, or outcome that:

- Is unexpected in nature, severity, or frequency
- Is related or possibly related to participation in the research
- Suggests that the research places subjects or others at a greater risk of harm than previously recognized

6.2 Collection and Documentation of Adverse Events

This study is observational and does not alter standard clinical care, surgical technique, perioperative management, or postoperative follow-up. No investigational drugs or devices are administered as part of this protocol.

Adverse events will be identified through routine clinical follow-up, review of the medical record, operative reports, postoperative notes, and patient-reported information obtained during standard clinical encounters.

All adverse events and serious adverse events that occur during the study period will be documented in the study records, including:

- Description of the event
- Date of onset and resolution
- Severity
- Relationship to the surgical procedure
- Outcome

Adverse events that are expected risks of anterior cervical discectomy and fusion or routine postoperative care will be recorded but are not considered study-related unless there is clear evidence to suggest otherwise.

6.3 Attribution and Relationship to Study Participation

Because this study does not introduce any intervention beyond standard of care, adverse events are not expected to be caused by participation in the research.

The only study-specific activities include intraoperative swab collection and data abstraction. Swab collection is performed under sterile conditions during routine surgical exposure and does not alter operative technique, operative time, or patient management.

Accordingly:

- Adverse events related to surgery or postoperative recovery will be attributed to standard clinical care
- Adverse events related to research activities are not anticipated
- Any adverse event determined to be possibly related to study participation will be documented and evaluated

6.4 Reporting Requirements and Timelines

All serious adverse events and unanticipated problems involving risks to subjects or others will be reported to the Institutional Review Board in accordance with Hospital for Special Surgery reporting requirements and timelines.

The Principal Investigator is responsible for determining whether an adverse event meets criteria for expedited reporting.

Events that require reporting include:

- Serious adverse events that are unexpected and possibly related to study participation
- Unanticipated problems involving risks to subjects or others

Routine postoperative complications that are expected and related to standard surgical care do not require expedited IRB reporting unless they meet criteria for an unanticipated problem.

6.5 Safety Oversight

Given the observational nature of this study and the absence of investigational interventions, a formal privacy monitoring board is not required.

Participant safety will be overseen by the treating clinical team as part of standard postoperative care. Any concerns regarding participant safety or unexpected risks identified during the course of the study will be promptly addressed and reported as required.

7. STUDY ADMINISTRATION

7.1 Roles and Responsibilities

The study will be conducted under the oversight of the Principal Investigator (PI), who is responsible for the overall conduct of the study, ensuring compliance with the approved protocol, institutional policies, and applicable regulatory requirements.

Co-investigators and study staff will assist with study activities as delegated by the PI. Responsibilities may include subject screening and enrollment, informed consent, data abstraction, biospecimen handling, coordination with collaborating laboratories, and maintenance of regulatory documentation.

All study personnel will perform only those tasks for which they are appropriately trained and authorized. A delegation of authority log will be maintained and kept current throughout the study.

7.2 Training and Delegation of Authority

All study personnel will complete required institutional training prior to participation in study activities, including:

- Human subjects protection training
- HIPAA and privacy training
- Any additional training required by Hospital for Special Surgery or collaborating institutions

Study-specific training will be provided as needed to ensure consistent implementation of study procedures, including informed consent, specimen handling, labeling, and data entry.

Delegation of study responsibilities will be documented, and changes in study personnel or responsibilities will be updated promptly in the study records and reported to the IRB as required.

7.3 Protocol Deviations and Violations

A protocol deviation is defined as any departure from the approved protocol that does not increase risk to participants or compromise their rights, safety, or welfare.

A protocol violation is defined as a deviation that may affect participant safety, rights, or data integrity.

All protocol deviations and violations will be documented in the study records. Deviations or violations that meet institutional criteria for reporting will be submitted to the IRB in accordance with Hospital for Special Surgery policies and timelines.

Corrective and preventive actions will be implemented as appropriate to minimize recurrence.

7.4 Record Retention

Study records, including regulatory documents, consent forms, source documents, and research data, will be retained in accordance with institutional policy and applicable federal and state regulations.

Records will be maintained for the required retention period following study completion or termination. After this period, records will be securely archived or destroyed in accordance with institutional procedures.

7.5 Study Close-Out

The study will be considered complete after all enrolled participants have completed required follow-up, all study data have been collected and finalized, and all regulatory obligations have been met.

A final study report or closure notification will be submitted to the Institutional Review Board in accordance with Hospital for Special Surgery requirements. No further study-related activities will occur following IRB-approved study closure.