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Document Name	RAD Version
CIP and Appendices METAL CONCENTRATION	10.0

Title	CIP MDT16060SD1701 LP2 Metal Concentration ver6.0 2019 07 08

APPROVALS

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Clinical Investigation Plan

Clinical Investigation Plan/Study Title	An Investigation of The Metal Concentration In Subjects Implanted With The Prestige LP™ Cervical Disc At Two Contiguous Levels In The Cervical Spine
Clinical Investigation Plan Identifier	MDT16060SD1701
Study Product Name	Prestige LP™ Two Level Cervical Disc
Sponsor/Local Sponsor	Medtronic Spine 1800 Pyramid Place Memphis, TN 38122
Medcial Monitor	[redacted] Senior Clinical Program Manager [redacted]
Document Version	Version 6.0, 08 JUL 2018

Confidentiality Statement

1. Investigator Statement

Study product Name	Prestige LP™ Two Level Cervical Disc
Sponsor	Medtronic Spine
Clinical Investigation Plan Identifier	MDT16060SD1701
Version Number/Date	Version 6.0, 08 JUL 2019

I have read the protocol, including all appendices, and I agree that it contains all necessary details for me and my staff to conduct this study as described. I will conduct this study as outlined herein and will make a reasonable effort to complete the study within the time designated.

I agree to comply with International Conference on Harmonization Guidelines on Good Clinical Practice, United States Food and Drug Administration Code of Federal Regulations (parts 11, 50, 54, and 56). I agree to ensure that the confidential information contained in this document will not be used for any purpose other than the evaluation and conduct of the clinical investigation without the prior written consent of Medtronic.

I will provide all study personnel under my supervision copies of the protocol and access to all information provided by Medtronic. I will discuss this material with them to ensure that they are fully informed about the products and the study.

Investigator's Signature:	
Investigator's Name:	
Institution:	
Date:	

Table of Contents

2. Glossary

Term	Definition
AE	adverse event
Al	Aluminum
BMD	bone mineral density
CBC	complete blood count
CFR	Code of Federal Regulations
CIP	Clinical Investigational Plan
CP Ti	Unalloyed Commercially Pure Titanium
CT	Computed Tomography
eCRF	Case Report Form
DEXA	dual energy x-ray absorptiometry
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HCO	Health Care Organization
HCP	Health Care Professional
HIPAA	Health Insurance Portability Accountability Act
ICF	informed consent form
ICH	International Conference on Harmonization
IDE	Investigational Device Exemption
IRB/EC	Institutional Review Board/Ethics Committee
Mg	Milligram
mL	Milliliter
MRI	Magnetic Resonance Imaging
NSAID	nonsteroidal anti-inflammatory drug
PCS	Physical Component Score
PTEG	Polyethylene-terephthalate-glycol
PI	Principal Investigator
SAE	Serious Adverse Event
SF-36	Quality of Life Questionnaire
SSED	Summary of Safety and Effectiveness Data
Ti	Titanium
V	Vanadium

3. Synopsis

Title	An Investigation of The Metal Concentration In Subjects Implanted With The Prestige LP™ Cervical Disc At Two Contiguous Levels In The Cervical Spine
Clinical Study Type	Post-Approval Study
Product Name	Prestige LP™ Cervical Disc
Local Sponsor	Medtronic Spine 1800 Pyramid Place Memphis, TN 38122
Indication under investigation	The Prestige LP™ Cervical Disc is indicated for skeletally mature patients for reconstruction of the disc from C3-C7 following discectomy at one level or two contiguous levels for intractable radiculopathy (arm pain and/or a neurological deficit) with or without neck pain, or myelopathy due to abnormality localized to the levels of the disc space and at least one of the following conditions confirmed by imaging (CT, MRI, X-rays): herniated nucleus pulposus, spondylosis (defined by the presence of osteophytes), and/or visible loss of disc height as compared to contiguous levels. The Prestige LP™ Cervical Disc is implanted using an anterior approach. Patients should have failed at least 6 weeks of non-operative treatment or have had the presence of progressive symptoms or signs of nerve root/spinal cord compression in the face of continued non-operative management prior to implantation of the Prestige LP™ Cervical Disc. This indication is approved for use in the United States.
Investigation Purpose	To assess metal concentrations present in the blood serum of subjects before and after disc implantation.
Product Status	The Prestige LP™ Two Level Disc is approved for use in the U.S.

Primary Objective	The primary objective of this clinical study will be to assess metal concentrations present in the blood serum of subjects who receive surgical treatment with the Prestige LP™ Cervical Disc.	
Outcomes	**ENDPOINTS**	**MEASUREMENTS**
Primary Measurements	Concentrations of Ti, V and Al in the blood serum	Serum samples assessment for titanium, vanadium and aluminum concentrations
Secondary Measurements	Overall Success	
	Neck Disability Index (NDI) Success Change of NDI score from baseline	NDI Questionnaire
	Change of SF-36 PCS score from baseline	SF-36 Health Survey Physical Component Summary
	Change of Neck Pain Score from baseline	Neck Pain Numerical Rating Scale (NRS)
	Change of Arm Pain score from baseline	Arm Pain Numerical Rating Scale (NRS)
	Adverse Event(s)	AE Reports
	Secondary Surgeries	Second Surgery Reports
	Neurological success	Neurological Assessment
Other Measurement(s)	Work Status (Employment Status/Time to return to work)	Work Status
	Pain medication use	Pain medication
	Estimated blood loss [including whether a transfusion was performed], Length of surgery, Length of hospital stay	Surgery data
Study Design	30 patients will be enrolled to receive two-level treatment for degenerative disc disease with the Prestige LP™ Cervical Disc at two contiguous levels. Blood samples for metal concentration analysis will be drawn at the preoperative visit, 6 week, and 12 month postoperative time points.	

Treatment Groups	Single treatment group with patients received the Prestige LP™ Cervical Disc at two contiguous levels.
Sample Size	30 subjects will be enrolled at up to 5 sites in the United States. Subjects will be followed for 12 months postoperatively.
Inclusion Criteria	**Key Inclusion Criteria:** A subject must meet all of the following inclusion criteria to participate in this study: I.1. Has cervical degenerative disc disease at two (2) contiguous cervical levels (from C3 to C7) requiring surgical treatment and involving intractable radiculopathy, myelopathy or both; I.2. Has a herniated disc and/or osteophyte formation at each level to be treated that is producing symptomatic nerve root and/or spinal cord compression. The condition is documented by patient history (e.g., neck and/or arm pain, functional deficit and/or neurological deficit), and the requirement for surgical treatment is evidenced by radiographic studies (e.g., CT, MRI, x-rays, etc.); I.3. Has been unresponsive to non-operative treatment for at least six weeks or has the presence of progressive symptoms or signs of nerve root/spinal cord compression in the face of continued non-operative therapy; I.4. Has no previous surgical intervention at the involved levels or any other planned/staged surgical procedure at the involved levels; I.5. Has preoperative neck pain score ≥ 8 based on the preoperative Neck and Arm Pain Questionnaire; I.6. Must be at least 18 years of age and be skeletally mature at the time of surgery; I.7. If a female of childbearing potential, subject is non-pregnant, non-nursing, and agrees not to become pregnant during the study period; I.8. Is willing to comply with the study plan and sign the Subject Informed Consent Form.
Exclusion Criteria	**Key Exclusion Criteria:** A subject will be excluded from participating in this study for any of the following reasons:

	E.1 Has a cervical spine condition other that symptomatic cervical degenerative disease requiring surgical treatment at the involved levels; E.2 Has documented or diagnosed cervical instability relative to contiguous segments at either level, defined by dynamic (flexion/extension) radiographs showing: a. Sagittal plane translation > 3.5 mm, or b. Sagittal plan angulation >20° E.3 Has more than two cervical levels requiring surgical treatment; E.4 Has severe pathology of the facet joints of the involved vertebral bodies; E.5 Has had previously surgical intervention at either one or both of the involved levels. E.6 Has been previously diagnosed with osteopenia or osteomalacia; E.7 Has any of the following that may be associated with a diagnosis of osteoporosis (If "Yes" to any of the below risk factors, a Dual Energy X-Ray Absorptiometry (DEXA) Scan will be required to determine eligibility.): a. Postmenopausal non-Black female over 60 years of age who weighs less than 140 pounds. b. Postmenopausal female who has sustained a non-traumatic hip, spine or wrist fracture. c. Male over the age of 70. d. Male over the age of 60 who has sustained a non-traumatic hip or spine fracture. If the level of Bone Mineral Density (BMD) is a T-score of -3.5 or lower (i.e., -3.6, -3.7, etc.) or a T-score of -2.5 or lower (-2.6, -2.7, etc.) with vertebral crush fracture, then the patient is excluded from the study; E.8 Has presence of spinal metastases;

	E.9 Has overt or active bacterial infection, either local or systemic; E.10 Has chronic or acute renal failure or prior history of renal disease; E.11 Has a documented allergy or intolerance to, titanium, or a titanium alloy; E.12 Is mentally incompetent. (If questionable, obtain psychiatric consult); E.13 Is a prisoner; E.14 Is pregnant; E.15 Has received drugs that may interfere with bone metabolism within two weeks prior to the planned date of spinal surgery (e.g., steroids or methotrexate), excluding routine perioperative anti-inflammatory drugs; E.16 Has history of an endocrine or metabolic disorder known to affect osteogenesis (e.g., Paget's Disease, renal osteodystrophy, Ehlers-Danlos Syndrome, or osteogenesis imperfecta); E.17 Has a condition that requires postoperative medications that interfere with the stability of the implant, such as steroids. (This does not include low-dose aspirin for prophylactic anticoagulation and routine perioperative anti-inflammatory drugs.); E.18 Has received treatment with an investigational therapy within 28 days prior to implantation surgery or such treatment is planned during the 16 weeks following implantation; E.19 Is currently taking or has had chronic usage of certain prescription medications (e.g., Cloxacillin, an antibiotic used for prophylaxis against surgical infections, and/or Clotrimazole;

Study Procedures and Assessments	Blood collection for metal concentration testing will be performed pre-operatively and post-operatively at 6 weeks and 12 months.
Safety Assessments	Adverse events (AE) will be evaluated during and after surgery to determine the safety associated with the subject's treatment and study procedures. This data will be reported to the FDA on an annual basis until the end of the study.
Statistical Methods	Descriptive statistics will be used to summarize data from this study. Changes in metal concentration and other scores from preoperative may be assessed by using paired t-test for normally distributed data or Wilcoxon signed rank test for not normally distributed data.

4. Introduction

4.1 Background

Arthroplasty is an alternative to spinal fusion that may provide preservation of motion at the treated levels. While fusion has been the treatment of choice for degenerative disc disease, disc replacement is a viable option for patients who do not respond to non-surgical treatment of their damaged discs.

The Prestige LP™ Cervical Disc is an artificial cervical disc comprising of two low-profile metal plates that interface through a ball and trough mechanism, permitting segmental spinal motion. The Prestige LP™ Cervical was approved by the Food and Drug Administration (FDA) to be used at a single and two contiguous levels.

The articulation of two metallic components has tendency to generate metal concentrations in very small quantities, as observed in the Prestige LP™ Cervical Disc single level metal concentration study. However, no metal concentration data is available for when the disc is implanted at two contiguous levels as the concentrations of metal were not assessed during the Prestige LP™ Cervical Disc Two Level Investigational Device Exemption (IDE) Study.

4.2 Purpose

This is a protocol for a human research study and should be conducted according to the protocol, US and international standards of Good Clinical Practice (GCP) and International Conference on Harmonization (ICH) Guidelines, applicable government regulations, and institutional review board (IRB)/ethics committee (EC) policies and procedures.

This clinical study will include human patients diagnosed with degenerative disc disease at two (2) contiguous levels in the cervical spine from C3 to C7.

This study will investigate the titanium, vanadium, and aluminum concentrations present in blood serum samples obtained from patients enrolled in the study that undergo surgical treatment with the Prestige LP™ Cervical Disc at two contiguous levels in the spine. The study will be conducted at up to five clinical sites, limited to a patient population within the United States (U.S.) only. All investigative sites in this clinical study will follow the same clinical study protocol.

4.2.1 Summary of Non-Clinical Data

Information pertaining to the "Summary of Non-Clinical Data" can be located in the Summary of Safety and Effectiveness Data (SSED) of the Prestige LP™ Cervical Disc at FDA's website.

4.2.2 Summary of Clinical Data

Information pertaining to the "Summary of Clinical Data" can be located in the SSED of the Prestige LP™ Cervical Disc at FDA's website.

5 Study Objectives and Measurements

5.1 Objectives

This clinical study will assess the metal concentrations present in the blood serum of patients who receive surgical treatment with the Prestige LP™ Cervical Disc at two contiguous cervical levels from C3-C7. The information obtained from this clinical investigation will be used to support the post market surveillance for a PMA supplement for the Prestige LP™ Cervical Disc implanted at two contiguous levels. This clinical study is expected to begin within six months after FDA approval of the Prestige LP™ Cervical Disc study.

The timeline presented below provides information relevant to major study milestones and the Sponsor expectations as to when they will begin:

Study Timeline Prestige LP™ Two Level Cervical Disc Metal Concentration Study	Sponsor Expectations	Dates based on Feb. 13, 2018 Protocol Approval date by FDA	Latest Estimated Sponsor Expectations
Expected date to study initiate	Pending FDA Approval of Prestige LP™ Two Level CervicalDisc Metal Concentration study protocol update (P090029/S009): 1 month following FDA Approval	July 2018	Completed
Expected IRB approvals per month	1 per month	1 per month July-Sept 2018 based on 5sites	Completed
Expected date of initiation of subject enrollment	1 month following site IRB approval	May 4, 2018	June 6, 2018
Expected number of subjects enrolled per month	5	5	1
Expected date to subject enrollment completion	6 months following IRB approval of last site initiated	March 2019	June 2021

Study Timeline Prestige LP™ Two Level Cervical Disc Metal Concentration Study	Sponsor Expectations	Dates based on Feb. 13, 2018 Protocol Approval date by FDA	Latest Estimated Sponsor Expectations
Expected date to complete follow up of all study participants	26 months following last subject enrollment	May 2021	August 2022

5.1.1 Primary Objective(s)

The primary objective of this clinical study is to assess the metal concentrations in the blood serum of patients implanted with the Prestige LP™ Cervical Disc at two contiguous cervical levels for the presence of metal concentrations. The metal to be assessed will include:

- Titanium (Ti)
- Vanadium (V)
- Aluminum (Al)

The metal concentrations will be assessed at the following time points: preoperative, 6 weeks and 12 months post index surgery.

5.1.2 Secondary Objective(s)

Secondary objectives of this study are:

- To evaluate overall success rate with overall success defined as:
  - NDI score improvement of at least 15 points from baseline
  - Maintenance or improvement in neurological status
  - No serious AE classified as implant associated/related or implant/surgical procedure associated/related
  - No secondary surgical procedure classified as "failure".
- To assess the postoperative Neck Disability Index (NDI) score improvement from baseline
- To evaluate NDI success rate where NDI success is defined as at least 15-point improvement from the baseline
- To evaluate neurological success rate where neurological success is defined as maintenance or improvement of neurologic status from baseline
- To assess the safety of the Prestige LP™ Cervical Disc as reported through AEs and secondary surgeries
- To assess the postoperative Neck and Arm Pain score change from baseline
- To assess the postoperative SF-36 PCS score change from baseline

5.2 Study Measurements

- In this study, titanium, vanadium and aluminum metal concentrations will be assessed at preoperative, 6 weeks, and 12 months. The NDI, neurological success, adverse events (AEs), and secondary surgeries will be evaluated to consider overall success at 12 months. The neck and arm pain scores and SF-36 score will also be assessed. In addition to these measurements, the change in metal concentration at different time points with respect to baseline (preoperative) will be evaluated.

5.2.1 Primary Measurements

- Concentrations of Ti, V and Al in the blood serum at preoperative, 6 week and 12 month timepoints of subjects who receive surgical treatment with the Prestige LP™ Cervical Disc at two contiguous levels from C3-C7.

5.2.2 Secondary Measurements

Secondary measurements include:

- Overall success. Overall, subjects enrolled in the study who receive the Prestige LP™ Cervical Disc at two contiguous levels will be considered an overall success if all the following are met:
  - NDI Score improvement of at least 15 points from baseline
  - Maintenance or improvement in neurological status
  - No serious AE classified as implant associated/related or implant/surgical procedure associated/related
  - No secondary procedure classified as a "failure"
- NDI success
- Change of NDI score from baseline
- Change of Neck pain score from baseline
- Change of Arm pain score from baseline
- Change of SF-36 score from baseline
- Neurological success
- Adverse Events
- Secondary surgery at index level

5.2.3 Other Measurements

- Work status (i.e., employment status/return to work)
- Pain medication use
- Surgery data (e.g., estimated blood loss [including whether a transfusion was performed], length of surgery, length of hospital stay)

- Collect information on permanent metal implants, exposure to metal ions in working environment and injections, supplements and/or vitamins containing titanium, aluminum and vanadium.

6 Study Design

This will be a prospective study that will be conducted at up to five sites in the U.S. The subjects will be required to provide informed consent to participate in the study and meet all the inclusion and exclusion criteria as stated in Section 8.4 and 8.5 of this clinical study protocol.

6.1 Treatment Groups

All subjects enrolled in this study will receive the same treatment with the Prestige LP™ Cervical Disc at two contiguous levels. Data will be collected from subjects with conditions, medical history or environments that would contribute to additional metal ion exposure. This includes subjects working in a profession where exposure to metal ions may be increased, subjects taking injections or supplements containing metal ions or subjects who have permanent metal implants. No subjects will be randomized for this study.

6.2 Study Duration and Sites

The duration of this study will be approximately Four years based on an estimated enrollment period of thirty months and continued follow-up for 12 months for each subject enrolled. The study will continue until every enrolled subject has reached 12 months following surgery. At that time, the study will be considered complete, the final results will be determined, and a final report will be prepared.

6.3 Rationale

No formal testing to assess metal concentrations in the blood serum was performed during the IDE study, for patients implanted with the Prestige LP™ Cervical Disc at two contiguous levels.

As a condition of approval, the FDA requested the sponsor to investigate the metal concentrations in patients implanted with the Prestige LP™ Cervical Disc at two contiguous levels in the cervical spine in a post approval study.

7 Product Description

7.1 General

The Prestige LP™ Cervical Disc device is a dynamic device made of titanium alloy/titanium carbide composite (Ti-6AL-4V/10 wt.% TiC), also referred to as titanium ceramic composite, that is inserted into the intervertebral disc space of the cervical spine. The device is comprised of two low-profile metal plates that interface through a ball and trough mechanism, permitting segmental spinal motion.

The Prestige LP™ Cervical Disc is commercially available in various sizes to accommodate the intervertebral disc space and to engage the contiguous vertebral bodies.

7.2 Description of Product

The Prestige LP™ Cervical Disc, shown in Figure 1 below, is a two-piece articulating device that is inserted into the intervertebral disc space as a single unit at two contiguous cervical levels using an anterior approach. The device is manufactured from a titanium ceramic composite (titanium alloy (Ti-6Al-4V) with 10% Titanium Carbide) and consists of two metal plates which function via a ball and trough mechanism. The superior component of the implant contains the ball portion of the mechanism, and the inferior component contains the trough portion. These two features engage to create an interface designed to allow for motion after implantation. Each component is affixed to the adjacent vertebral body by two rail geometries incorporating anti-migration teeth, which are press fit into two pre-drilled holes in the vertebral bone. The portion of the flat surface between the rails and contacting the vertebral endplate contains commercial pure titanium (CP Ti) plasma thermal sprayed coating designed to permit bony on-growth for additional device incorporation. The remaining portion of the flat surface is titanium ceramic roughened to enhance fixation.

The device is available in various sizes to accommodate varied subject anatomy and the intervertebral disc space and to engage the contiguous vertebral bodies. The available components of the Prestige LP™ Cervical Disc are shown in Table 1 below. The Prestige LP™ Cervical Disc is provided sterile via a gamma ray method.

Figure 1: Two Schematic Views of the Prestige LP™ Cervical Disc

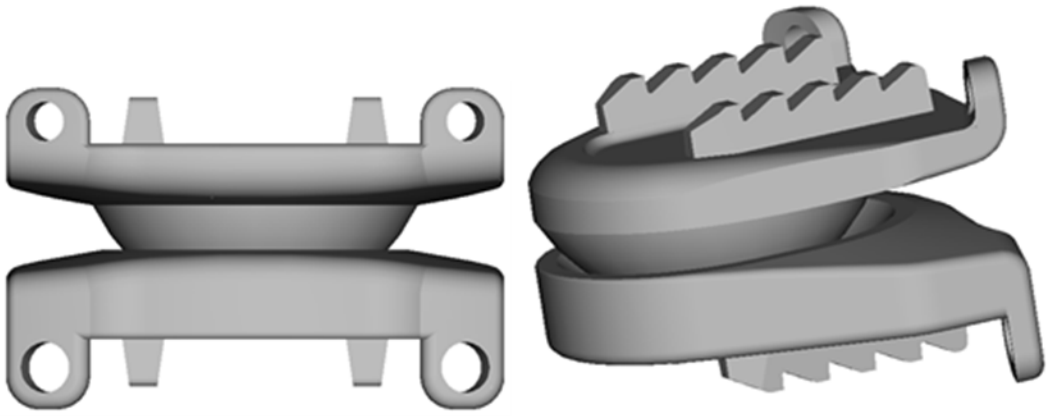

Table 1: Prestige LP™ Cervical Disc Device Sizes

Catalog Number	Size
6972250	5mm x 12mm
6972450	5mm x 14mm
6972650	5mm x 16mm
6972260	6mm x 12mm
6972460	6mm x 14mm
6972660	6mm x 16mm
6972860	6mm x 18mm
6972470	7mm x 14mm
6972670	7mm x 16mm
6972870	7mm x 18mm
6972480	8mm x 14mm
6972680	8mm x 16mm
6972880	8mm x 18mm

7.3 Study Product Packaging and Labeling

The Prestige LP™ Cervical Disc is provided in a sterile package ready for use. The Prestige LP™ Cervical Disc device is placed in a Polyethylene-terephthalate-glycol (PETG) retainer and lid to hold the inferior and superior components in place. The retainer assembly and device are placed packaged in a dual PETG tray with Tyvek configuration. The assembly and subject labels are placed inside a carton and sealed. The product label is applied to the outside of the carton.

8 Selection of Subjects

8.1 Study Population

A total number of 30 subjects will be enrolled in this study.

8.2 Subject Enrollment

A subject is considered enrolled in this clinical study at the time at which he/she signs the subject Informed Consent. All subjects must sign the Informed Consent prior to undergoing any evaluations or procedures that are purely research-related (defined as those that would not be done if the subject was not participating in this study) or collection of study data.

8.3 Inclusion Criteria

A subject must meet all the following inclusion criteria to participate in this study:

I.1 Has cervical degenerative disc disease at two (2) contiguous cervical levels (from C3 to C7) requiring surgical treatment and involving intractable radiculopathy, myelopathy or both;

I.2 Has a herniated disc and/or osteophyte formation at each level to be treated that is producing symptomatic nerve root and/or spinal cord compression. The condition is documented by patient history (e.g., neck and/or arm pain, functional deficit and/or neurological deficit), and the requirement for surgical treatment is evidenced by radiographic studies (e.g., CT, MRI, x-rays, etc.);

I.3 Has been unresponsive to non-operative treatment for at least six weeks or has the presence of progressive symptoms or signs of nerve root/spinal cord compression in the face of continued non-operative therapy;

I.4 Has no previous surgical intervention at the involved levels or any other planned/staged surgical procedure at the involved levels

I.5 Has preoperative neck pain score ≥ 8 based on the preoperative Neck and Arm Pain Questionnaire;

I.6 Must be at least 18 years of age and be skeletally mature at the time of surgery;

I.7 If a female of childbearing potential, subject is non-pregnant, non-nursing, and agrees not to become pregnant during the study period;

I.8 Is willing to comply with the study plan and sign the Subject Informed Consent Form.

8.4 Exclusion Criteria

A subject will be excluded from participating in this study for any of the following reasons:

E.1 Has a cervical spine condition other than symptomatic cervical degenerative disease requiring surgical treatment at the involved levels;

E.2 Has documented or diagnosed cervical instability relative to contiguous segments at either level, defined by dynamic (flexion/extension) radiographs showing:

a. Sagittal plane translation > 3.5 mm, or
b. Sagittal plane angulation > 20°

E.3 Has more than two cervical levels requiring surgical treatment;

E.4 Has severe pathology of the facet joints of the involved vertebral bodies;
E.5 Has had previous surgical intervention at either one or both of the involved levels;
E.6 Has been previously diagnosed with osteopenia or osteomalacia;
E.7 Has any of the following that may be associated with a diagnosis of osteoporosis (If "Yes" to any of the below risk factors, a DEXA Scan will be required to determine eligibility.):
   a. Postmenopausal non-Black female over 60 years of age who weighs less than 140 pounds.
   b. Postmenopausal female who has sustained a non-traumatic hip, spine or wrist fracture.
   c. Male over the age of 70.
   d. Male over the age of 60 who has sustained a non-traumatic hip or spine fracture. If the level of BMD is a T score of -3.5 or lower (i.e., -3.6, -3.7, etc.) or a T score of -2.5 or lower (i.e., -2.6, -2.7, etc.) with vertebral crush fracture, then the subject is excluded from the study.
E.8 Has the presence of spinal metastases;
E.9 Has overt or active bacterial infection, either local or systemic;
E.10 Has chronic or acute renal failure or prior history of renal disease;
E.11 Has a documented allergy or intolerance to titanium, or a titanium alloy;
E.12 Is mentally incompetent (if questionable, obtain psychiatric consult);
E.13 Is a prisoner;
E.14 Is pregnant;
E.15 Has received drugs that may interfere with bone metabolism within two weeks prior to the planned date of spinal surgery (e.g., steroids or methotrexate), excluding routine perioperative anti-inflammatory drugs;
E.16 Has history of an endocrine or metabolic disorder known to affect osteogenesis (e.g., Paget's Disease, renal osteodystrophy, Ehlers-Danlos Syndrome, or osteogenesis imperfecta);
E.17 Has a condition that requires postoperative medications that interfere with the stability of the Implant, such as steroids. (This does not include low-dose aspirin for prophylactic anticoagulation and routine perioperative anti-inflammatory drugs.);
E.18 Has received treatment with an investigational therapy within 28 days prior to implantation surgery or such treatment is planned during the 16 weeks following implantation.
E.19 Is currently taking or has had chronic usage of certain prescription medications (e.g., Cloxacillin, and antibiotic used for prophylaxis against surgical infections, and/or Clotrimazole).

8.5 Withdrawal Criteria

A subject may be withdrawn, if any of the following occur during the study:

- Subject refuses treatment
- Subject requests withdrawal from the study
- Subject withdraws consent
- AE makes the continuation of the subject impossible or inadvisable.
- Subject lost to follow-up.
- Subject discovered after enrollment not to have met the protocol entrance criteria.
- Subject refuses to comply with required study procedures.
- Use of any additional experimental drug or device or participation in another clinical study.
- The study Sponsor terminates the Investigator site or the study.

A subject may end his/her participation in a study at any time. If a subject withdraws, the Investigator is to make a reasonable effort to determine the reason for the subject's withdrawal from the study and to complete termination procedures. The clinical site will make a minimum of three (3) attempts to contact subjects who miss a scheduled study visit. Telephone calls, registered letters, and offers of transportation to the study site are considered reasonable effort. Documentation of attempts to reach the subject is to be document in the subject's medical and study record.

Subjects who withdraw from the study will not be replaced. Subjects who request withdrawal from the study will be asked their reason(s). Reason(s) for subject withdrawal will be recorded on the electronic case repot form (eCRF) for all subjects that sign the informed consent.

If voluntary withdrawal occurs, the follow-up of these subjects will be according to the standard of care at the site. Data collection according to the study protocol will discontinue for subjects who withdraw and/or are withdrawn.

8.6 Strategies for Recruitment and Retention

Original Investigational Sites from the Prestige LP™ Two-Level IDE Study (P04-12) will be contacted to inform the Investigators and Sub-Investigators of this study and assess his/her willingness to participate. If up to five qualified sites cannot be selected from the original sites in the previous two-level study, other potential sites will be contacted and evaluated for participation.

9 Study Procedures and Visits

A narrative description of the various evaluations performed at each visit follows, organized by study phase. Study visits have been designed to occur on a schedule consistent with the standard of care for this type of treatment.

All subjects must give their informed consent in accordance with the informed consent regulations in Title 21 of the Code of Federal Regulations (CFR) 50.

9.1 Schedule of Events

Table 2: Schedule of Study Assessments

Procedure	Pre-/Peri-operative						
	Pre-operative	Surgery/ Hospital Discharge	6 wks. ±2 wks.	3mos. ± 2 wks.	6 mos. ± 1 mo.	12 mos. ± 2mo.	Study Exit
Preoperative Information							
Confirm patient eligibility	X						
Obtain informed consent	X						
Obtain HIPAA Authorization	X						
Case Report Forms							
Informed Consent	X						
Inclusion/Exclusion Criteria	X						
Demographics	X						
Medical and Surgical History	X						
Neurological Status	X		X	X	X	X	
Blood Collection for Metal Concentration Testing	X		X			X	
Neck and Arm Pain Questionnaire	X		X	X	X	X	
Surgery Data		X					
Device Identification		X					
Hospital Discharge		X					
Subject Visits			X	X	X	X	
Medications	X						
SF-36 PCS Health Survey	X		X	X	X	X	
Neck Disability Index	X		X	X	X	X	
Study Exit							X
Secondary Surgery Intervention (if applicable)			X	X	X	X	

Adverse Event (if applicable)		X	X	X	X	X	
Protocol Deviation (if applicable)	X	X	X	X	X	X	
Device Deficiency (if applicable)		X	X	X	X	X	
DEXA Scan*	X						

*A DEXA Scan was only required if the patient had a risk factor that may be associated with a diagnosis of osteoporosis.

9.2 Screening Procedures

After preliminary screening, if the subject is deemed eligible for further consideration in this study, the following procedures will be performed:

- The subject will be presented full information about the clinical study and asked if he/she would like to participate.
- If the subject agrees, he/she will be asked to sign the Informed Consent Form (ICF) and Health Insurance Portability Accountability Act (HIPAA) authorization form. No study procedures will be carried out until the subject has signed the ICF/HIPAA form(s).

Once the ICF/HIPAA form(s) are signed, the following procedures will be carried out:

- A copy of the signed ICF/HIPAA form(s) will be provided to the subject and/or to his/her legal representative.
- The subject will be evaluated for any remaining eligibility questions per the inclusion/exclusion criteria.
- The subject will be assigned a unique, sequential clinical study number.

NOTE: Screening and enrollment can both occur at one visit, or they can be split into multiple visits.

If after enrollment, the subject is discovered not to have met the study entrance criteria, as defined in the protocol, the subject will be exited from the study.

9.3 Study Procedures

Preoperatively:

- The patient will be asked to provide basic demographic and medical history data (including cancer history).
- The patient will be asked to provide information about his/her nicotine use, employment/work status, pain medication use (including chronic nonsteroidal anti-inflammatory drug (NSAID) use).

- The patient will undergo the following assessments: Neck Disability Index (NDI), Neurological Status and Neck & Arm Pain.
- The patient will undergo an abbreviated physical examination.
- The patient will have a blood serum sample drawn for laboratory analysis to assess the Ti, Al and V metal concentration prior to the devices being implanted.

Surgery/Discharge Visit:

- Surgery data (i.e., estimated blood loss [including whether a transfusion was performed], length of surgery, and length of hospital stay) will be collected
- Any reported AEs will be documented.

9.4 Follow up Procedures

Follow up visits (6 week, 3 month, 6 month, 12 month):

- The patient will be asked about his/her nicotine use, employment/work status, pain medication use (including chronic NSAID use).
- The patient will undergo/complete the following assessments: NDI, Neurological status, and Neck and Arm Pain. The patient will be asked if they have experienced any AEs, second surgery procedures, or other protocol-specified events of interest (e.g., cancer, pregnancies).
- The patient will have a blood serum sample drawn for laboratory analysis to assess the Ti, Al and V metal concentrations at preoperative, 6 week and 12 month follow up visits.

9.5 Unscheduled Visits

For this study, only visits as designated per the "Schedule of Events" will be required. However, if any AEs or second surgeries are identified during the study, these will be reported.

9.6 Early Termination/Discontinuation Visit

Subjects may withdraw voluntarily from participation in the study at any time. Subjects may also withdraw voluntarily from receiving the study product Prestige LP™ Cervical Disc for any reason.

Prior to completion of the surgical treatment, an investigator may decide to forego the study treatment based on his or her medical judgment. Examples of why the surgery may not be completed as intended include but are not limited to: adverse events (AEs) or complications that occur during surgery, technical difficulties during the surgery, and anatomical findings that prevent implantation of the device(s). The reason for the termination of the study treatment by the investigator should be recorded on the Subject Disposition Electronic Case Report Form. In addition, if the termination of the study is a result of an adverse event, the event will be recorded on an Adverse Event Electronic Case Report Form and included in a summary of discontinuations, but

the subject will not be followed for efficacy and will not be counted in the total number of study subjects.

Follow up visits for the duration of the study period are required. If a subject is discontinued from the surgery after surgery, follow up visits will not be continued.

For a subject to be identified as discontinued, the following two conditions must be met: 1) the subject or his/her representative must notify the investigational site in writing, by telephone, or in person, and 2) no data is obtained on the subject after notification of discontinuation. The reason for the discontinuation will then be recorded on the Subject Disposition Electronic Case Report Form. Missed visits after discontinuation in the study will not constitute a protocol deviation.

Subjects who fail to return for follow up visits or who do not respond to efforts in scheduling evaluations will not be considered discontinued unless the study site has been officially notified of discontinuation.

9.7 Subject Consent

This study will be conducted in full compliance with the informed consent regulations in 21 CFR 50. All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. This consent form will be submitted with the protocol for review and approval by an IRB(s). The Investigator is responsible for obtaining the formal consent of a subject (competent to do so and not under duress), using the IRB-approved Informed Consent Form (ICF); and this consent will be obtained before the subject undergoes any study procedure(s). This ICF must be signed by the subject or legally acceptable representative, and the Investigator-designated study personnel obtaining the consent. A copy of the signed ICF will be given to the subject and the original retained by the investigator with the site's copy of the eCRFs.

The informed consent form must be reviewed and approved by the Sponsor and the governing IRB prior to initiation of the study at a clinical site.

9.8 Planned Study Closure

Study closure is defined as closure of a clinical study that occurs when Medtronic and/or regulatory requirements have been satisfied per the Clinical Investigational Plan and/or by a decision by Medtronic or regulatory authority, whichever occurs first. Study Closure is a process initiated by distribution of an initial study closure letter. The study closure process is complete upon distribution of the Final Report or after final payments, whichever occurs last.

9.9 Unblinding Procedures

This is a single arm study; therefore, no subjects will be randomized and unblinding of subjects will not be needed.

9.10 Monitoring Subject Compliance

Subject follow-up is crucial to the outcome of the clinical study. Subjects should be informed of the importance of returning for follow-up visits within the prescribed windows.

9.11 Measures to Minimize Bias

This is a single arm study and all subjects will receive the same surgical treatment. Subjects enrolled in this study must meet all inclusion criteria and no exclusion criteria as stated in this protocol in Section 8.3 and 8.4 respectively. No study site will be allowed to enroll more than one third of the total enrollment.

This study will be run in compliance with 21 CFR 54 with financial disclosures being obtained from all participating Investigators, by the Sponsor at the beginning of the study and at the completion of the study. If any changes occur during the course of the study, it is the Investigator's responsibility to notify the Sponsor and provide a new financial disclosure to document the change.

9.12 Study Supplies

The study Sponsor will provide each study site with any required binders for keeping of study related documents as applicable.

9.13 Medication Compliance

9.13.1 Permitted Treatment(s)

- Unless not medically feasible, the use of NSAIDs is recommended for two weeks postoperatively.
- The use of non-steroidal anti-inflammatory drugs such as; Toradol (ketorolac) and PO Naprosyn (naproxen) is acceptable.
- Use of a soft cervical collar is recommended as needed.

9.13.2 Post-Operative Prohibited Treatment(s)

- Electrical bone growth stimulation should not be used for treatment of the cervical spine during the 12-month follow-up period.
- Current use of or previous chronic use of prescription drugs [e.g., Cloxacillin, an antibiotic used for prophylaxis against surgical infections, and/or Clotrimazole].
-

9.14 Assessment of Efficacy

Efficacy will be assessed using the secondary measurements of this study.

9.14.1 Efficacy Measures

The efficacy measures will be determined by the measurements listed in the sections below.

9.14.2 Pain/Disability Status

The self-administered Neck Disability Index (NDI) Questionnaire (Vernon and Mior, 1991)[1] will be used. Success for each individual subject will be defined as pain/disability improvement postoperatively according to the following definition:

Preoperative Score-Postoperative Score >= 15

Success in this category is a criterion for overall success.

9.14.3 Pain Status

Neck Pain Score

Numerical rating scales adapted in part from Measuring Health (McDowell and Newell, 1996)[2] will be used to evaluate neck pain. This method of analyzing pain is further discussed in "Clinical applications of visual analogue scales; a critical review."[3] In this clinical trial, neck pain is described as a composite of pain intensity and duration and is derived by adding the numerical rating scores from the pain intensity and duration scales.

Arm Pain Score

Numerical rating scales as described above will also be used to measure arm pain.

9.15 Assessment of Safety

The criteria outlined in this section will be used to evaluate each subject during surgery and postoperatively to determine the safety associated with the subject's treatment. The safety of the Prestige LP™ Cervical Disc treatment and metal levels will be assessed and reported to the FDA.

9.15.1 Safety Endpoint(s)

9.15.2 Neurological Status

Neurological status will be evaluated preoperatively and postoperatively. Neurological status is based on motor function, sensory function, and reflexes. Each of the categories is comprised of a

[1] Vernon, H and Mior S (1991). The Neck Disability Index: A Study of reliability and validity. J. Manipulative Physio Ther 1991; 14:409-415.
[2] McDowell, I. and Newell, C. (1996). Measuring Health; a Guide to Rating Scales and Questionnaires (2nd Edition). New Your: Oxford University Press.
[3] McCormack HM, Horne D. and Sheather S. Clinical applications of visual analogue scales: a critical review. Phychol Med 1988; 18:1007-1019.

number of elements. The measurements will be recorded on the Neurological Status Electronic Case Report Form.

Postoperative evaluations of each element on the Neurological Status Electronic Case Report Form will be compared to the preoperative evaluations. Success will be defined as maintenance or improvement in each element for the time period evaluated.

9.15.3 Adverse Events

All reported AE information, as defined in Section 11, for a patient after surgery and throughout their participation in the study will be included in the study database and will be analyzed. This analyzed data will be reported to the FDA on an annual basis until the end of the study. AEs related to Medtronic products will be entered into the quality system, at which time further determinations on reporting requirements will be made.

The follow-up report should give full details of the experience, including an assessment of the relationship to the test article(s), and will be sent to the Sponsor promptly. SAEs will then be reported to regulatory authorities by the Sponsor.

9.15.4 Device Deficiency

A device deficiency is inadequacy of a medical device with respect to its identity, quality, durability, reliability, safety, or performance. Note: Device deficiencies include malfunctions, use errors, and inadequate labeling. If a device deficiency causes an adverse event to the subject, then an Adverse Event Electronic Case Report Form is completed. If a device deficiency does not cause an adverse event, then complete the Device Deficiency Electronic Case Report Form.

9.16 Clinical Laboratory Tests

All subjects enrolled in this clinical study, who undergo surgical treatment with the Prestige LP™ Cervical Discs will be required to have their blood drawn at specified time intervals, to be analyzed for metal concentrations. Each blood serum sample will be analyzed for the concentration of titanium, vanadium and aluminum. The metal concentrations at each postoperative evaluation will be summarized using descriptive statistics and will be compared against the preoperative baseline measurements. Guidelines specific to the blood collection procedure and metal concentration testing procedure are provided as an attachment to this protocol in Appendix I.

9.17 Pregnancy Reporting

The Investigator must report all pregnancies to the Sponsor immediately after becoming aware of the event. Subjects who become pregnant during the study will be followed until the completion of pregnancy and lactation (if applicable), and outcomes will be collected on both mother and child. The pregnancy will not be considered an adverse event, unless a complication resulting from the pregnancy is reported.

9.18 Cancer Reporting

In the event that a subject experiences an AE involving a cancer diagnosis during the study, the Investigator should make every attempt to provide the pathology report (if available); histopathology; other co-morbid medical diagnoses; applicable laboratory reports (e.g., complete blood count (CBC); and the age of onset. The Investigator must report all AEs involving a cancer diagnosis to the Sponsor or Sponsor's representative immediately after becoming aware of the event.

As part of study enrollment, each subject will be asked to provide information on his/her cancer history.

10 Risks and Benefits

10.1 Potential Risks

The potential risks in this study may include but are not limited to:

- **Surgical procedure (any):** wound, local and/or systemic reactions and/or infection and/or adverse reactions to anesthesia
- **Surgical procedure (spine):** dysphagia, dysphonia, hoarseness and other tracheal, esophageal or pharyngeal conditions
- **Artificial Disc Device:** loosening, bending or breakage of the components and/or other difficulties with the position of the implant
- **X-rays:** known risks to participating in the study include subject exposure to radiation from cervical spine x-rays
- **Blood Draw:** The risks involved in taking blood include, but are not limited to: swelling, bruising, or infection around the vein where blood is collected. During and after this procedure, the subject may feel dizzy or may faint.

10.2 Potential Benefits

The potential benefits from this study allow the Sponsor to assess potential safety concerns related to the presence of metal concentrations in the blood of subjects who undergo surgical treatment with the Prestige LP™ Cervical Disc.

10.3 Risk-Benefit Rationale

Any risks associated with participation in this clinical study will be minimized and managed in accordance with and full compliance to 21 CFR 50, 56.

11 Adverse Events and Device Deficiencies

11.1 Definitions/Classifications

An AE is any untoward medical occurrence that does not necessarily have to have a casual relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (e.g., abnormal laboratory finding, symptom, or disease temporally associated with the use of a product, whether or not considered related to the product(s) under study. This definition includes events related to the procedures involved and/or worsening of pre-existing conditions. Pre-existing conditions for which the condition has not worsened, will not be reportable as an adverse event.

AEs are intended to be volunteered by patients or observed by the investigator and are to be recorded as appropriate within the electronic data capture (EDC) database. Each event will be classified according to the criteria defined in this protocol. If possible, a specific diagnosis should be recorded rather than a listing of individual signs and symptoms. AE reporting processes will be carried out in compliance with the protocol, the governing IRB, and the requirements of FDA and other applicable regulatory authorities.

11.1.1Severity of Adverse Events

The severity of adverse events should be assessed according to the World Health Organization (WHO) Recommendations for Grading of Acute and Subacute Toxic Effects. The following definitions should be used for events shat are not defined in the WHO Toxicity Scale.

Mild (Grade 1): The adverse event is noticeable to the subject but does not interfere with routing activity. The adverse event does not require removal of the implant(s).

Mild (Grade 2): The adverse event interferes with routine activity but responds to symptomatic therapy or rest. The adverse event does not require remove of the implant(s).

Severe (Grade 3): The adverse event significantly limits the subject's ability to perform routine activities despite symptomatic therapy and may require hospitalization. In addition, the adverse event may require removal of the implant(s).

Life-threatening (Grade 4): The adverse event requires removal of the implant(s), or the subject is at immediate risk of death, even if not related to the implant(s).

Medical and scientific judgement should be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed in the definition above.

An Adverse Event that is of Grade 3 or Grade 4 will be considered a serious adverse event (SAE). All serious adverse events (SAEs) should be reported immediately to the Sponsor. The immediate

reports should be followed promptly by detailed, written reports. The immediate and follow-up reports should identify patients by unique code numbers assigned to the study subjects rather than by the subjects' names, personal identification numbers, and/or addresses. The investigator should also comply with the applicable regulatory requirement(s) related to the reporting of unexpected serious adverse drug reactions to the regulatory authority(ies) and the IRB.

The follow-up report should give full details of the experience, including an assessment of the relationship to the test article(s), and will be sent to the Sponsor promptly. SAEs will then be reported to regulatory authorities by the Sponsor.

11.1.2 Assessment of Related Adverse Events

The relationship between an AE and the implant/procedure will be assessed by the Investigator, and by the Sponsor on the basis of the following definitions:

Implant Associated/Related: AE for which there is a reasonable possibility that the event may have been caused by one or more components of the implant.

Surgical Procedure Associated/Related: AE for which there is a reasonable possibility that the event may have been caused primarily by the surgical procedure.

Implant/Surgical Procedure Associated/Related: AE for which there is a reasonable possibility that the event may have been caused by both the implant(s) and the surgical procedure.

Undetermined: AE for which sufficient information is not available at the time of the event to determine causality.

Not Related (NR): AE for which sufficient information exists to indicate that the etiology is unrelated to the implant(s) or surgical procedure.

All AEs are required to be entered in a timely fashion, into the EDC on the Adverse Event Electronic Case Report From and each will be classified according to the aforementioned criteria. The Investigator should pay particular attention to the subject's postoperative neurological evaluations. It is important that any new, significant neurological deficits be reported as an AE. In addition, in the event that a subject experience an AE involving a malignancy, the investigator should make every attempt to provide a pathology report describing the location or the tumor; the histopathology of the tumor; other comorbid medical diagnosis; and the age of onset, gender, and ethnicity of the subject. Any information regarding the subject's family history of cancer should also be provided, if available. Any subject with a malignancy will be followed until the subject completes the study. Periodic updates should be provided by the clinical site to the Sponsor.

11.1.3 Secondary Surgical Interventions

Some AEs or treatment failures may lead to a secondary surgical intervention at an operated index level. Information on the types of secondary surgical interventions is provided in the section below. Secondary Surgical Interventions will be classified by Medtronic. All patients undergoing a secondary surgical intervention at any operated index level will be followed.

- **Revision:** A procedure that adjusts or in any way modifies either one or both of the original implant configurations (e.g., adjusting position of the original configuration, removal with replacement with the same type of study implant). A revision will be classified as a treatment "failure".**Removal:** A procedure that removes one or more components of the original implant configuration without replacement with the same type of trial implant. Removals will be classified as either elective or non-elective. An elective removal is a procedure that removes the Prestige LP™ Cervical Disc(s) at the discretion of the investigator and/or the patient. An elective removal will not be classified as a treatment "failure." An elective removal is not considered an SAE. A non-elective removal is any removal other than an elective removal and is considered an SAE and will be classified as a treatment "failure."

NOTE: In the event that an implant requires a removal (explant), specific information and instructions are to be followed for this process. The instructions and information regarding the process is provided in the "Procedure for Retrieval, Handling and Packaging" included as an attachment to this protocol in **Appendix II**. All explanted devices will be analyzed along with histological evaluation of any retrieved peri-prosthetic tissue under the Post-approval Device Failure study (Explant Analysis) for the two-level indication.

- **Supplemental Fixation:** A procedure at the involved level(s) in which additional spinal devices not approved as part of the protocol are placed. A supplemental fixation will be classified as a treatment "failure."
- **Reoperation:** Any surgical procedure at the involved level(s) that does not remove, modify, or add any components and is not considered a removal, revision, or supplemental fixation. A reoperation, elective removal or other surgical procedure will not be classified as a treatment "failure."
- **Other:** Any surgical procedure not classified as a revision, removal, supplemental fixation or reoperation.

11.2 Reporting of Adverse Events

AE reporting processes will be carried out in compliance with the protocol, the governing IRB, and the requirements of FDA and other applicable authorities.

All adverse events will be considered reportable for this study.

Adverse events must be recorded in the subject's medical record and on the Adverse Event and/or Device Deficiency eCRF and promptly reported to Medtronic. IRB/EC reporting must be completed in accordance with the policies of the governing IRB/EC.

It is the responsibility of the Investigator to identify the occurrence of adverse events and device deficiencies and to ensure the required information is accurately documented on the eCRF.
Reports of adverse events and device deficiencies will include the following information, at a minimum:

- Date of event
- Diagnosis or description of the event
- Assessment of the seriousness and relationship to the device and/or surgical procedure
- Treatment provided
- Outcome and date of resolution

The clinical course of each adverse event must be followed until resolution, subject discontinuation from the study or last study follow up visit, whichever comes first. "Not resolved" adverse events and device deficiencies must be assessed at each protocol required visit, and new or updated information must be documented on the Adverse Event and/or Device Deficiency eCRF and promptly reported to Medtronic and, if applicable, to the IRB. At time of study exit the status of all unresolved Adverse Events should be evaluated and should reflect subject' status at time of study exit.

If necessary, the Investigator may report to the sponsor initially by telephone or e-mail and follow-up with completed eCRFs and, upon request of Medtronic, de-identified source documentation regarding the event (e.g., physician/nurse notes or summaries) should be provided to Medtronic.

Medtronic safety personnel will promptly review all reported adverse events and device deficiencies and if necessary request clarification and/or additional information from the Investigator. In addition, aggregate safety data will be reviewed and analyzed to identify potential safety issues, signals, and trends at minimum, annually after the first subject is enrolled.

12 Data Review Committees

Due to the nature of this study, no data review committees will be convened for this study.

13 Statistical Design and Methods

Since there is not statistical hypothesis, the analyses will be descriptive in nature. In general, the categorial variables will be summarized using frequency tables and continuous variables will be summarized using mean, standard deviation, median, minimum, and maximum.

All data analyses will be based on observed data; missing data will not be imputed.

Changes in metal concentration and other scores from preoperative will be assessed by using paired t-test for normally distributed data or Wilcoxon signed rank test for not normally distributed data.

13.1 Analyses of the Measurements

13.1.1 Primary Measurement Analysis

- The concentrations of titanium, aluminum and vanadium at different time points will be summarized using mean, standard deviation, median, minimum, and maximum.
- Changes in metal concentrations at different time points with respect to preoperative concentrations will be summarized using mean, standard deviation, median, minimum, and maximum. In addition, paired t-test for normally distributed data or Wilcoxon signed rank test for not normally distributed data will be carried out to test whether the change from the baseline is significant.
- The titanium concentration data collected for this study will also be presented in the table along with the titanium concentration data collected in the Prestige LP™ one-level metal concentration study.

13.1.2 Secondary Measurement Analysis

For secondary measurement, including overall success, NDI success and neurological success, success rate will be summarized in frequency tables. The success rate at other visits will also be summarized. In addition, change of NDI score, neck pain score and SF-36 PCS score at post-op visits from baseline will be summarized using mean, standard deviation, median, minimum and maximum. Paired t-test or Wilcoxon signed rank test will be carried out to test whether the change from the baseline is significant.

The relationship between the metal concentration and clinical variables will be assessed at the end of the study. Simple correlation analysis will be performed for the relationship between metal concentrations and continuous outcome scores, including NDI, neck pain, arm pain and SF-36 PCS scores by evaluation time point. The relationship between metal concentrations and binary outcomes, including overall success and neurological success will be assessed by comparing metal concentrations in the success and failure groups of subjects' statuses by evaluation time point.

The cumulative rate of AEs and secondary surgeries will be derived using survival analysis.

13.1.3 Other Measurement Analysis

Data analysis from other measurements collected from the study will include:

- Work status (i.e., employment status/return to work)
- Pain medication use
- Surgery data (e.g., estimated blood loss [including whether a transfusion was performed], length of surgery, length of hospital stay)

In addition, at the end of the study, clinical outcomes for this study will be presented along with similar information up to two (2) years from the Prestige LP™ two-level IDE study in the summary tables.

13.2 Handling of Missing Data

All data analysis will be based on observed data; missing data will not be imputed.

13.3 Interim Analysis(es)

The available metal concentration data, along with clinical data, will be summarized and reported to FDA via the study progress reports and the final study report. No formal interim analysis is planned.

14 Ethics

14.1 Statement(s) of Compliance

This study is to be conducted according to the protocol, international standards of Good Clinical Practice (International Conference on Harmonization (ICH) guidelines), applicable government regulations (FDA Code of Federal Regulations, Title 21 for device studies), and institutional research policies and procedures.

This study will be conducted under a protocol reviewed and approved by an IRB; the study is to be conducted by scientifically and medically qualified persons; the benefits of the study are in proportion to the risks; the rights and welfare of the subjects will be respected; the physicians conducting the study will ensure that the hazards do not outweigh the potential benefits; and the results to be reported will be accurate.

Subjects will be compensated for their participation in the study; the compensation, if allowed will not exceed an amount which would influence their participation.

15 Study Administration

15.1 Source Data/Documents

All case report form (CRF) information should be traceable to original patient records (source documents). Source documents will be reviewed during the course of monitoring to verify the accuracy of CRFs. This review will be conducted according to the Sponsor's monitoring guidelines (or those of its representative). The Investigator(s)/Institution(s) will permit study-related monitoring, audits, IRB(s) review, and regulatory inspection(s) by providing direct access to source data/documentation. Source documents to evaluate safety data may be requested by the Sponsor.

15.2 Monitoring

Monitoring for this clinical study will be in compliance with ICH Guidelines.

The clinical monitoring for this trial will be conducted by Medtronic or a qualified designee (contract research organization (CRO)).

Sites will be required to provide redacted copies of source documents to Medtronic as noted in Section 15.1 of this protocol. Source documents must be available for regulatory review by Medtronic and/or the FDA. Each site will maintain records of communications with Medtronic clinical staff on study progress, discrepancy resolutions, or any other issues needing resolution.

The monitor will review the available study data according to the monitoring plan. Monitoring will include review and resolution of missing or inconsistent results and source document checks (i.e., comparison of submitted study results to original reports) to assure the accuracy of the reported data. This data will be reviewed via remote and on-site monitoring visits.

Prior to the enrollment of the first subject at each clinical site, a pre-study visit with the investigator and the study staff will be conducted. The purpose of the visits is to assess the site's ability to conduct the clinical trial as per FDA/ICH/GCP and Sponsor guidelines.

15.3 Study Sites

To ensure that the Investigator and his/her staff understand and accept their defined responsibilities, the monitor will maintain regular correspondence with the site and may be present during the course of the study to verify the acceptability of the facilities, compliance with the study plan and relevant FDA regulations and the maintenance of complete records. Monitoring will include review and resolution of missing or inconsistent results and source document checks (i.e. comparison of submitted study results to original reports) to assure the accuracy of the reported data.

15.4 Institutional Review Board (IRB)

This study will be conducted in full compliance with the IRB regulations in 21 CFR 56. This protocol will not be initiated unless it has been reviewed and approved by, and remains open to continuing review by, an IRB meeting the requirements of 21 CFR 56. The IRB shall review and have authority to approve, require modification in (to secure approval), or disapprove the protocol. The IRB shall notify the Investigator and the Institution in writing of its decision. The IRB shall require that the information given to subjects as part of the informed consent is in accordance with 21 CFR 50.25. The IRB shall conduct continuing reviews of the protocol at intervals appropriate to the degree of risk, but not less than once per year. Copies of all reports to and correspondence between the Investigator and the IRB/EC must be provided to the Sponsor. Further, at the completion or early termination of the study, a final report should be provided to the IRB by the Investigator within 90 days.

15.5 Data Management

Subject data will be recorded in a limited access secure electronic data capture (EDC) system. The clinical database will reside on a production server. All changes made to the clinical data will be captured in an electronic audit trail and will be available for review by the Sponsor and/or its representative. The associated software and database have been designed to meet regulatory compliance requirements for deployment as part of the validated system compliant with laws and regulations applicable to the conduct of clinical studies that use electronic records and signatures. Database backups are performed regularly.

The Investigator will provide his/her electronic or written signature on the appropriate CRFs in compliance with 21 CFR 11. Changes to data previously submitted to the Sponsor require a new electronic or written signature by the Investigator acknowledging and approving the changes.

Visual and/or electronic data review will be performed to identify possible data discrepancies. Manual and/or automatic queries will be created in the EDC system and will be issued to the site for appropriate response. Site staff will be responsible for resolving all queries in the database. Use of electronic records will be compliance with 21 CFR 11.

15.6 Data Management Responsibilities

Data collection is the responsibility of the clinical study staff at the site under the supervision of the site Principle Investigator. During the study, the Investigator must maintain complete and accurate documentation of the study.

15.7 Data Capture Methods

This study will use an electronic Case Report Form (eCRF) system. Guidelines and training will be provided to each investigative site on the use of the eCRF system.

The Investigator will provide his/her electronic signature on the appropriate electronic case report forms (eCRFs) in compliance with 21 CFR 11. Changes to data previously submitted to the Sponsor require a new electronic signature by the Investigator acknowledging and approving the changes.

Visual and/or electronic data review will be performed to identify possible data discrepancies. Manual and/or automatic queries will be created in the EDC system and will be issued to the site for appropriate response. Site staff will be responsible for resolving all queries in the database.

15.8 Timing/Reports

The investigator/institution should maintain the trial documents as specified in Essential Documents for the Conduct of a Clinical Trial (ICH-GCP E6) and as required by the applicable regulatory requirement(s). The investigator/institution should submit written summaries of the status of the trail to the IRB annually, or more frequently, if requested by the IRB.

15.9 Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

In the event that a subject revokes authorization to collect or use PHI, the Investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (i.e., that the subject is alive) at the end of their scheduled study period.

15.10 Liability

The study is fully financed by Medtronic, Inc. and is insured. Upon request, a certificate of insurance can be provided.

15.11 CIP Amendments

When a change to the CIP is required, the Sponsor standard operating procedures (SOPs) will be followed to make the appropriate changes and receive approval. A description of changes made between the previous and current versions will be summarized within the protocol in Version History.

Any amendment(s) to the Protocol must be made and approved by Medtronic and submitted to the IRB by each clinical site as appropriate; protocol amendment approval must be obtained prior to implementation of the amendment. Administrative amendments to the Clinical Investigation Plan will be submitted to the IRB/EC and appropriate regulatory authorities for notification, if applicable.

15.12 Record Retention

The Sponsor and/or Sponsor's representative will maintain all records related to this study. Records must be retained at each clinical site and by the Sponsor and/or Sponsor's representative for a period of two (2) years after the date the study is completed or terminated or until records are no longer needed to support a regulatory submission, whichever is longer. All study records may be subject to regulatory inspection. Medtronic should be contacted for any questions regarding record retention.

15.13 Protocol Deviations

A study protocol deviation is an instance when the Investigator or site personnel did not conduct the study according to the protocol, clinical investigational plan (CIP), or Investigator agreement. Any protocol deviations initiated without Sponsor approval that may affect the scientific soundness of the study, or affect the rights, safety, or welfare of study subjects, must be reported to the Sponsor and to the Investigator's IRB as soon as possible, but no later than five (5) working days of the protocol deviation.

15.14 Publication and Use of Information

All information not previously published concerning the Prestige LP™ Cervical Disc and Medtronic, Inc. is considered confidential and is the sole property of Medtronic, Inc. Site personnel agree to use this information only in connection with this study and will not use it for other purposes without written permission from Medtronic, Inc.

15.14.1 Publication Policy

It is agreed that, before publication of any study-related data, the sponsor will be given the opportunity to review and comment upon any manuscript that contains data derived from this study. Additionally, the following guidelines will apply:

- An Investigator publication steering committee will be formed to assist with development of publication plans and resulting publications.
- In general, no publication will precede the publication of the primary measurements.
- In general, publications will be developed from complete/finalized datasets.
- Investigators are obligated to provide Medtronic with an opportunity to review any publication developed from data derived from this study.
- Authorship for any publications developed from Medtronic Spinal sponsored research, should be in accordance with the International Committee of Medical Journal Editors authorship guidelines.

- Medtronic will not financially compensate health care professionals (HCPs) or health care organizations (HCOs) for writing or editing activities on scientific publications related to research sponsored by Medtronic.
- A legal agreement must be in place to address the use of Medtronic research data and cover rights and obligations related to the publication effort.

15.15 Suspension or Early Termination

Termination of the study is discontinuance, by sponsor or by withdrawal of IRB or FDA approval before completion. This is possible for the whole study or for a single center. Study suspension is a temporary postponement of study activities related to enrollment. This is possible for the whole study or a single center.

15.15.1 Criteria for Study-Wide Termination of Suspension

Possible reasons for considering study suspension or termination of the study for all centers include but are not limited to:

- AEs and device deficiencies associated with the system or product which might endanger the safety or welfare of subjects.
- Observed/suspected performance different from the product's design intent.
- Decision by Medtronic or regulatory body (medically/ethically justifiable) where the study is operating under regulatory body authority

15.15.2 Criteria for Investigator/Center Termination or Suspension

Possible reasons for clinical Investigator or center termination or suspension include but are not limited to:

- Failure to obtain initial IRB approval or annual renewal of the study
- Consistent non-compliance to the CIP (e.g., failure to adhere to inclusion/exclusion criteria, failure to follow subjects per scheduled follow-ups, etc.)
- Lack of enrollment
- Noncompliance to regulations and the terms of the Clinical Trial Agreement (e.g. failure to submit data in a timely manner, failure to follow-up on data queries and monitoring findings in a timely manner, etc.
- IRB suspension of the center
- Fraud or fraudulent misconduct (as defined by local law and regulations)
- Investigator request (e.g., no longer able to support the study)

15.15.3 Medtronic-Initiated Termination or Suspension

In the case of study termination or suspension for reasons other than a temporary IRB approval lapse, the investigator will promptly inform the IRB.

In the case of study termination, the investigator must inform the subjects and may inform the personal physician of the subjects to ensure appropriate care and follow-up is provided.

In the case of a study suspension, subject enrollment must stop until the suspension is lifted by Medtronic. Subjects already enrolled should continue to be followed out of consideration of their safety, rights, and welfare.

15.15.4 Investigator-Initiated Termination or Suspension

The Investigator will promptly inform:

- Medtronic and provide a detailed written explanation of the termination or suspension
- The institution (where required per regulatory requirements)
- The Institutional Review Board
- The subjects and may inform the personal physicians of the subject to ensure appropriate care and follow-up is provided.

In the case of study suspension:

- Subject enrollment must stop until the suspension is lifted
- Subjects already enrolled should continue to be followed out of consideration of their safety, rights, and welfare

15.15.5 Institutional Review Board-Initiated Termination or Suspension

The Investigator will promptly inform:

- Medtronic and provide a detailed written explanation of the termination or suspension within five (5) business days
- The institution (where required per regulatory requirements)
- The subjects and may inform the personal physicians of the subjects, with the rationale for the study termination or suspension

In the case of a study suspension:

- Subject enrollment must stop until the IRB suspension is lifted
- Subjects already enrolled should continue to be followed in accordance with IRB policy or its determination that an overriding safety concern or ethical issue is involved.

15.16 Post-Study Follow-Up

The Sponsor of this study does not anticipate any "Post-Study Follow-Up." However, because this study is regulated by the FDA, there may be a need for subjects to return for additional follow-up beyond the 12-month visit period. Any subject follow-up that is subsequently required after the completion of the 12-month visit will require subjects to be re-consented.

16 References

1. Vernon, H and Mior S. (1991). The Neck Disability Index: A study of reliability and validity. J. Manipulative Physio Ther 1991: 14:409-415.
2. McDowell, I. and Newell, C. (1996). Measuring Health: A Guide to Rating Scales and Questionnaires (2nd Edition). New York: Oxford University Press.
3. McCormack HM, Horne D. and Sheather S. Clinical applications of visual analogue scales: a critical review. Phychol Med 1988; 18:1007-1019.

17 Appendices

Appendix I:

Metal Concentration Analysis Protocol

Appendix II:

Procedures For Retrieval, Handling and Packaging

Appendix III:

Sponsor Information

Appendix IV:

Prestige LP™ Cervical Disc Information For Use

18 Version History

Version	Summary of Changes	Author(s)/Title
4.0	• Transferred document to new CIP template 056-F275. Administrative changes made; section numbering has changed to satisfy template requirements. • Investigation Purpose in Synopsis is new to satisfy template requirements.	[redacted]

	• Product Status in Synopsis is new to satisfy template requirements. • Study Procedures and Assessments in Synopsis is new to satisfy template requirements. • Safety Assessments is new in the synopsis to satisfy template requirements.	
5.0	• Added clarifying verbiage in Indication under Investigation section of Synopsis • Removed 3 month, 6 month and 24 month blood serum sample collection timepoints. • Added to Glossary: CIP – Clinical Investigation Plan HCO – Health Care Organization HCP – Health Care Professional SOP – Standard Operating Procedure • Removed from Glossary: MedDRA – Medical Dictionary for Regulatory Activities • Removed "or contiguous levels" form Inclusion Criteria 1.4 • Removed Inclusion Criteria 1.5 • Removed Exclusion Criteria E.4 • Removed "or contiguous levels" from Exclusion Criteria E.6 • Removed Exclusion Criteria E.11 • Removed Exclusion Criteria E.12 • Removed Exclusion Criteria E.18 • Removed Exclusion Criteria E.19 • Removed Exclusion Criteria E.24 • Removed Exclusion Criteria E.26 • Removed Exclusion Criteria E.27	[redacted]

	• Removed 3 month and 24 month blood sampling timepoints. • Timeline table updated to reflect Dates based on February 14, 2018 Protocol Approval date by FDA and Latest timeline estimates. • Section 12 Data Review Committees was added to satisfy new template requirements. • Section 15.11 CIP Amendments was added to satisfy new template requirements. • Appendix I: Updated Medtronic Contact Information. • Appendix III: Added protocol version number and date to clarify approvers for Version 4.0, 28-FEB-2018. • Appendix III: Added a new table of approvers for Version 5.0, 19-APR-2019.	
6.0	• Update Section 5.2.3 Other Measurements to include data collection on permanent metal implants, exposure to metal ions in work environment and injections and supplements and/or vitamins containing titanium, aluminum and vanadium. • Update section 6.1 Treatment Groups to clarify metal ion data collection.	[redacted]

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This document records information about the Sponsor, as required by ICH GCP E6 (RI). If there are updates to the information contained in this document, it will be provided to sites and IRB's separate from this version of the CIP.

Clinical Investigation Plan Approval: Sponsor personnel responsible for the CIP approval. Internal approvals of this CIP will be kept in the Sponsor Trial Master File. Below are all Appendices

APPENDICES

Appendix I:

- **Metal Concentration Analysis Protocol**

Appendix II:

- **Procedures For Retrieval, Handling And Packaging**

Appendix III

- **Sponsor Information**

Appendix IV

- **Prestige LP™ Cervical Disc Information For Use**

APPENDIX I

METAL CONCENTRATION ANALYSIS PROTOCOL

TRACE METAL ANALYSIS LABORATORY - DEPARTMENT OF ORTHOPEDIC SURGERY

RUSH UNIVERSITY MEDICAL CENTER

INTRODUCTION

Analysis for titanium and vanadium concentrations in serum will be performed by the Trace Metal Analysis Laboratory (TMAL), Department of Orthopedic Surgery at Rush University Medical Center, following a serum testing protocol developed by the lab. Aluminum in serum will be determined by the Metals Laboratory of Mayo Medical Laboratories Rochester MN. The protocol is divided into two parts:

A. Blood Collection Procedure

B. Metal Concentration Testing Procedure.

METAL CONCENTRATION

A. COLLECTION GUIDELINES PROVIDED BY RUSH UNIVERSITY MEDICAL CENTER-DEPARTMENT OF ORTHOPEDIC SURGERY

INTRODUCTION

Blood to be analyzed for trace metal concentrations is susceptible to contamination from the environment, collection techniques, collection vessels, etc. Specimens must be collected and processed as described below to prevent and/or greatly minimize contamination.

Samples should be collected using the blood draw kit provided by the Trace Metal Analysis laboratory of Rush University Medical Center or Medtronic. One of these kits will always be available at the investigational site as a backup. For the discussion on the following techniques and procedures, please contact one of the staff at the Trace Metal Analysis Laboratory (TMAL) at Rush University Medical Center.

The equipment provided for the collection and storage of the serum is element specific. Different collection tubes as well as storage tubes are provided. The trace metal analysis laboratory at Rush uses S-monovette (Sarstedt) blood collection tubes which look like a syringe and the metals laboratory of Mayo Medical laboratories uses a blue top blood collection tube from a specified manufacturer (Covidien Royal Blue Monoject tube). In addition, for the aluminum testing the blood must be the first collected in the venipuncture.

This document includes:

1. Instructions for blood collection and separation for the subjects enrolled in the Prestige LP™ (Two Level) Metal Concentration Study.

i. Items needed for blood separation at your site. This is a list of equipment that must be available in your facility (or somewhere nearby) in order for the protocols to be completed.

ii. Items supplied by Rush University Medical Center for blood collection and separation.

iii. Standard operating practice for the collection of blood to be analyzed for trace metals.

iv. Standard operating procedure for blood separation.

v. Mailing blood to be analyzed for Trace Metals. Shipping directions

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Contact information at RUSH University Medical Center:

[redacted]

Contact information at Medtronic:

[redacted]

I. Items Needed for Blood Separation at Your Site

1. Personnel to collect and separate the blood.
    a. Training will be provided

2. A Class II, Type A, B or C biological safety cabinet (BSC) (class 100 laminar flow cabinet with a HEPPA filter also referred to as ISO class 5). They are also referred to as biological safety cabinets. There are many versions of this and they all look slightly different; however, they all do the same thing. They protect the 'product' from contamination and also protect the user from the sample. To make sure it provides the "class 100 atmosphere," it is usually best to call the manufacturer to make sure, or look at the manual.

3. A clinical centrifuge, which is able to accommodate the blood collection tubes we use (similar to 16X100 culture tube), or one that accommodates 50ml conical centrifuge tubes (the blood collection tubes fit into these 50ml centrifuge tubes and the tubes provide a containment area for the blood collection tubes and prevent contamination).

4. At least a -20°C freezer that can be used for temporary storage of the biological samples before they are sent to Rush University Medical Center. Samples stored for longer than 3 months should be stored -60°C to -80°C.

5. Access to dry ice and a styrofoam container for shipping part of the samples back to Department of Orthopedic Surgery at Rush University Medical Center.

6. A supply of 16-18mOhm de-ionized water (from Millipore system, Barnstead, or similar).

7. A waste vessel

II. <u>Items to be supplied by the trace metals analysis laboratory at RUSH University Medical Center - if performing blood separation at your site</u>

Draw Kit(s): The kits contain the following:

- Nitrile gloves-powder free size small
- (may be substituted with any nitrile powder free glove)
- Tourniquet
- Alcohol wipe
- 2X2 gauze
- Adhesive bandage
- B-D Vacutainer Safety- lockblood collection set with attached holder 21G, ref: 368652
- Blood collection tubes:
- One: Covidien Royal Blue Monoject tube ref: 8881 307006
    - Silicone Coated tubes, Silicone coated Royal Blue stopper, no additive,
    - 7 ml draw (provided to Rush by Mayo Medical Laboratories -
    - Metals laboratory) tube size: 13mm X 100MM, tube length without stopped 97 mm
- Three: Sarstedt Neutral S-Monovette® 7.5 ml draw (referred as syringe) ref:01.1728.001
    - Tube size: Outer diameter 15 mm, cap diameter 19 mm, Tube length without screw cap 92 mm

Storage tube Kit(s):

- Storage tubes/vials:

- One: Metal Free Storage vial (supplied by the Metals Laboratory of the Mayo Medical Laboratories)
- Three: 5 ml storage tubes from the Trace Metal Analysis Laboratory at Rush
- Labels (n=4)

Other supplies as needed:

- Parafilm
- Lab marker
- Clean gloves
- 1-125 ml acid washed jar
- Tube Rack
- lab coat
- Extra supplies for draw kit, storage tube kit, etc.
- Storage box for the samples
- 1 pipette for blood separation: 200-1000 µl capacity (such as Labsystems Finnpipette from Fisher 21-377- 1000 or any equivalent such as Pipetman, Eppendorf, etc.). Use only clear (colorless) acid-washed tips.
- Acid washed clear (colorless) tips in a tray
- Labels

Note: a vessel to be used as a waste container will be needed during the separation process.

Pipettors should not have exposed metal parts.

III. <u>Standard Operating Practice for the Collection of Blood to be Analyzed for Trace Metals</u>

MATERIALS: Draw Kit - in a biohazard bag

- Nitrile gloves-powder free size small

(may be substituted with any nitrile powder free glove)

- Tourniquet

- Alcohol wipe
- 2X2 gauze
- Adhesive bandage
- B-D Vacutainer Safety-lock blood collection set with attached holder 21G, ref: 368652
- Blood collection tubes:
- One: Covidien Royal Blue Monoject tube ref: 8881 307006, Silicone Coated tubes, Silicone coated Royal Blue stopper, no additive,7 ml draw (provided to Rush by Mayo Medical Laboratories - Metals laboratory) tube size: 13mm X 100MM, tube length without stopped 97 mm
- Three: Sarstedt Neutral S-Monovette® 7.5 ml draw (referred as syringe) ref:01.1728.001, Tube size: Outer diameter 15 mm, cap diameter 19 mm, Tube length without screw cap 92 mm

Please familiarize yourself with the method and materials described below prior to drawing any blood samples. Some of the equipment is not the typical equipment encountered by phlebotomists. Also, the syringes are not evacuated tubes ("vacutainers®"); they are made into evacuated tubes by pulling on the piston until it snaps into place.

The blood collection tubes used in this protocol contain no additives and do not require any inversion.

Observe standard phlebotomy safety protocols.

Training will be provided to designated phlebotomist.

i. Use only powder-free gloves.

ii. Label the syringes 1, 2, 3 in the order that they will be drawn (if not already pre-numbered).

iii. Label the syringes with the subject's identification code, date and time

iv. Label the Covidien Royal Blue Monoject tube with the subject's identification code, date and time.

v. Syringes- Pull the piston straight back until it snaps into its locking position. (the white plastic inter-sleeve should securely locked at the bottom of the tube)

vi. Snap off the piston. The syringe is now evacuated (like a "vacutainer® tube").

vii. Prep the subject and perform the venipuncture,

Note: You will get a flashback of blood into the tubing when you are in the vein.

viii. Attach Covidien Royal Blue Monoject tube to the adapter/tube holder, blood will flow freely into the tube. Fill the tube. Detach the tube from the tube holder.

- This tube must be drawn first as per Mayo instructions.

ix. Attach syringe #1 (see "Additional important information" bellow) to the adapter/tube holder, blood will flow freely into the syringe.

x. After filling the syringe disconnect it and attach the next syringe in order.

xi. After drawing the last syringe, disconnect the syringe from the adapter **before** removing the needle from the vein. This sequence prevents any air from entering the syringe.

xii. Take care of the subject.

xiii. Keep the drawn samples upright. The tubes/syringes **do not** require inversion.

Additional important information:

It is very important that the samples are collected in the given order.

Syringe #1 is the flushing syringe. The blood that flows into this syringe is used to flush the needle and the adapter. If at any time during the drawing process the vein is lost after the blue-top tube is drawn and another needle stick is needed to complete the protocol, blood has to be drawn into a flushing syringe before the remaining syringes are filled. The new needle and adapter must be flushed with blood before continuing the process.

In instances where a smaller gauge needle is needed, use the 21 gauge Safety-Lok™ Blood Collection set (BD).

[redacted]

IV. Standard Operating Practice for the Separation of Blood

Training will be provided to designated personnel responsible for blood separation.

General Guidelines

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All of the following procedures, except centrifugation, are to be done under a Class II laminar flow biological safety cabinet (Type A, B or C, laminar flow biological safety cabinet with a HEPPA filter) wearing Clean gloves provided by Rush laboratory.

While working under the laminar flow hood, clean gloves must be worn at all times. This is to avoid dust from your hands/arms falling into any vessels and contaminating their contents. Keep in mind that the air flow of most the cabinets is downward.

Prior to any pipetting, read the instructions on how to properly use the pipette. Always pipette with slow even strokes.

All vessels used, or those that will come in contact with a vessel that will be used in the preparation of samples, are acid-washed. These vessels will be supplied to you acid-washed. However, it is your responsibility to assure that they stay clean.

Position the equipment/materials in such manner that crossing above an open container is avoided.

Upon receiving the drawn blood, note the time that the specimen was drawn. Then, follow to the following procedure:

I. Allow the blood to clot naturally for at least 30 minutes but no more than 4 hours (see important information in Note 1 and Note 4 below).

Blood should be centrifuged and separated promptly upon clotting.

II. Centrifuge the specimens for 25 minutes to enable separation of the serum from the cellular fractions.

1. Centrifugation maybe a two-step process, due to the different blood collection tubes involved.

III. Transport the specimens, in an upright position, to the class-100 biological safety cabinet where separation will be performed.

IV. Attach an identification label (Brady® label) on the storage tubes/vials.

Refer to Note 2: Label the storage tubes

II. Separation procedures

a. Blue top tube- Mayo method

- Remove the stopper from the royal blue top collection tube and also unscrew the cap of the metal free storage vial.

- Carefully pour the serum into the storage vial avoiding any cellular components of blood.
- Screw the cap onto the metal free storage vial.

b. Syringes- Rush method

- This method requires pipetting Please refer to the following steps.

c. Fill one 125 ml polypropylene jar with de-ionized H_2O.

d. Fill one acid-washed 15 ml graduated tube with de-ionized water.

e. Prepare the discard vessel nearby.

f. Place the blood collection tubes and the storage tubes in the plastic test tube rack provided.

g. Separate one syringe at a time. Starting with syringe #1

h. Take syringe #1 and unscrew the screw cap and place the cap on a piece of Parafilm™; it may be needed for re-centrifugation.

i. Unscrew the cap of the corresponding storage tube and place it on a piece of Parafilm™;

j. Dial the pipette to 1000 µl. Affix a tip on the pipette.

k. Clean the tip. Pick up deionized water (DiH2O) from the jar by immersing the tip as deep as possible into the jar, but not going past the ridges, and discard it into the waste vessel. The waste vessel has not been acid-washed, so great care must be taken to avoid contact with the waste vessel. Do not touch it with the tip or allow the discarded liquid to splash back on the tip. Repeat 1 more time if needed.

- If the tip becomes contaminated at any time, it must be discarded and a new one must be used. If water droplets adhere on the internal or external surface of the tip and one more pick up and dispensing **does not** eliminate the droplets, discard the tip and use a new one.

l. Dial the pipette to 500 µl. Pick up DiH2O from the 15 ml acid-washed tube one last time and discard it. This is a final rinsing step and makes sure you have dialed down the pipette. The tip is now cleaned and is ready to use.

m. Using the prepared tip, pick up serum from the blood collection tube and transfer it to the properly labeled acid washed storage tube. Transfer all of the serum to the storage tube.

- Pipetting of serum should always be done very slowly. If the tip becomes contaminated with packed cells, its contents should be dispensed back into the blood collection tube and the tip should be discarded. The blood is now mixed to some degree and must be re-centrifuged to transfer the rest of the serum. Centrifuge for 10 minutes then proceed to transfer the rest of serum to the storage tube using a new cleaned tip.

n. Discard the used tip and screw the cap on the storage tube.

o. Repeat steps g to n to separate the serum of the next blood collection tube.

- It is essential that a new and cleaned pipette tip is used with each of the blood collection tubes. You must use clean a tip and use it to separate blood collected in tube #1, then discard it. Clean a new tip and use it to separate blood from tube #2 etc.. Also, as mentioned above if re-centrifugation is required a new cleaned tip should be used. The above described procedure prevents cross-contamination.

p. Discard the blood collection tubes and the cell fractions.

q. Check to make sure the caps are screwed on **TIGHTLY** on the storage tubes and that the storage tubes are labeled properly.

r. Seal the seam between the screw cap and the storage tube using Parafilm™.

s. Freeze the sample in an **upright** position until they are shipped to Rush. Do not freeze the tubes on their side or upside down.

Additional information and useful hints:

Note 1: Blood should be allowed to clot naturally in the tubes collected.

The blue top tube contains a clot activator (it is coated with silicone) to accelerate the clotting process. Centrifuge and separate the serum promptly upon clotting

The syringes contain no additives or activators and thus clotting may take longer than in the blue-top tube. Wait until the blood has clotted in the syringes before centrifuging them.

Note 2: Labeling the storage tubes

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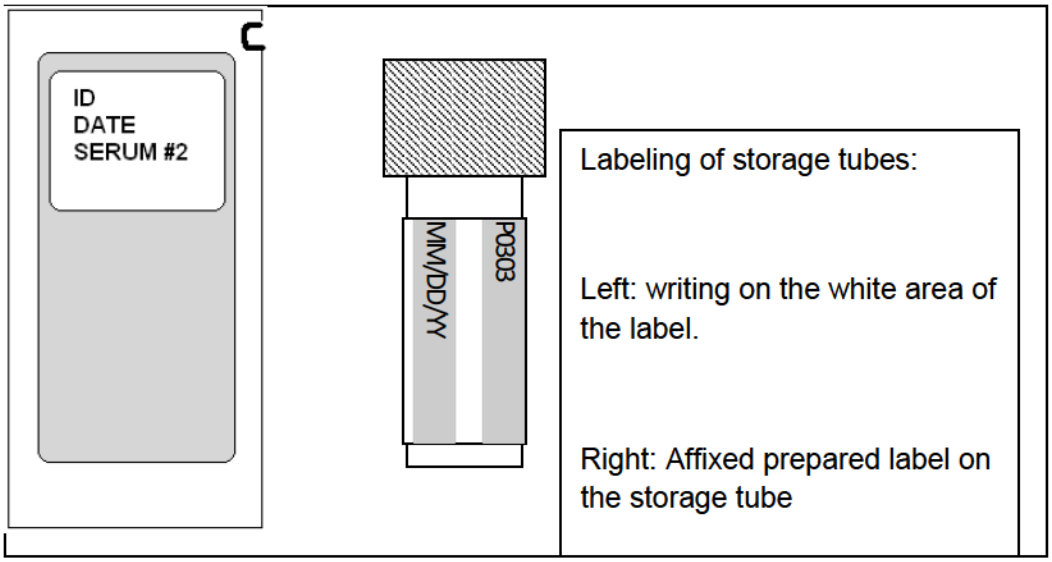
There are two types of storage tubes one is 13 mm x 82 mm PP tube with a blue label "Mayo Metal Free" wording on it. There are three PP storage tubes 15.3 mm X 57 mm with no labels. Both types have been acid washed and need to be properly labeled

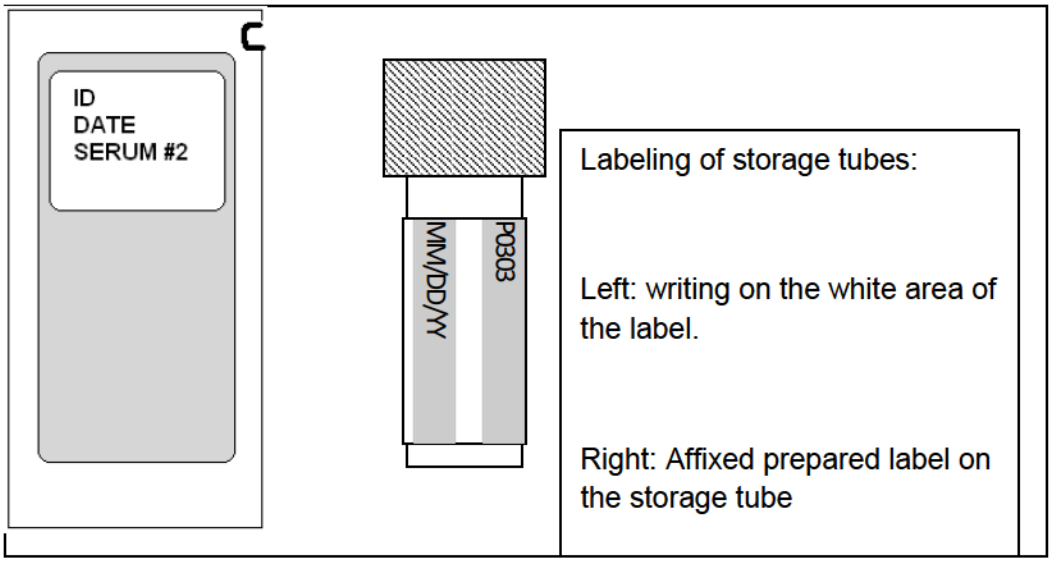
Labels will be provided in the kits. The labels should include the following

- The study assigned number (e.g. PXXX.)
- Date of collection
- Contents
    - For Mayo tube –serum for Al
    - For Rush serum #1 or S1, either is fine. This is serum from syringe #,1, S2 for syringe # 2 and S3 for syringe #3

Do not write directly on the storage tube. Write on the white area of the label before affixing the label to the storage tube.

- Affixing the labeled label onto the storage tube:
- Peel off the backing by the white area. Place the white portion of the label onto the circumference of the tube first and then wrap the clear plastic portion over the white portion. This action seals the written area of the label. It also allows an unrestricted visibility area of the tube for sampling.
- Make sure of the following:
- The entire label fits on the tube (i.e. it is not touching the ridge at the bottom of the tube or does not hang off the end of the tube), and that the label is placed on the tube smoothly (i.e. no bubbles).


ID
DATE
SERUM #2
MM/DD/YY
P0303
Labeling of storage tubes:
Left: writing on the white area of the label.
Right: Affixed prepared label on the storage tube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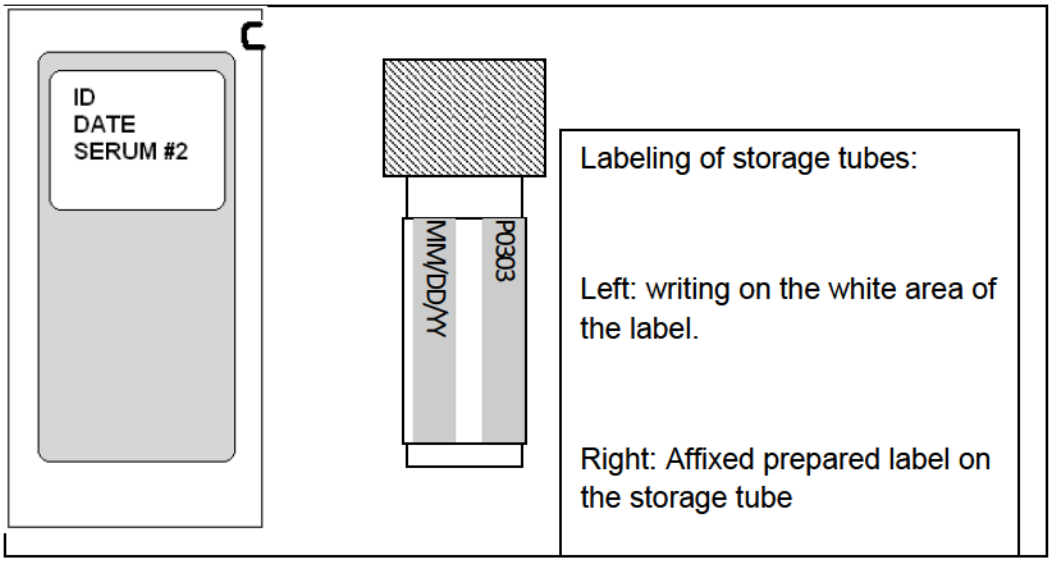
P0303
Labeling of storage tubes:
Left: writing on the white area of the label.
Right: Affixed prepared label on the storage tube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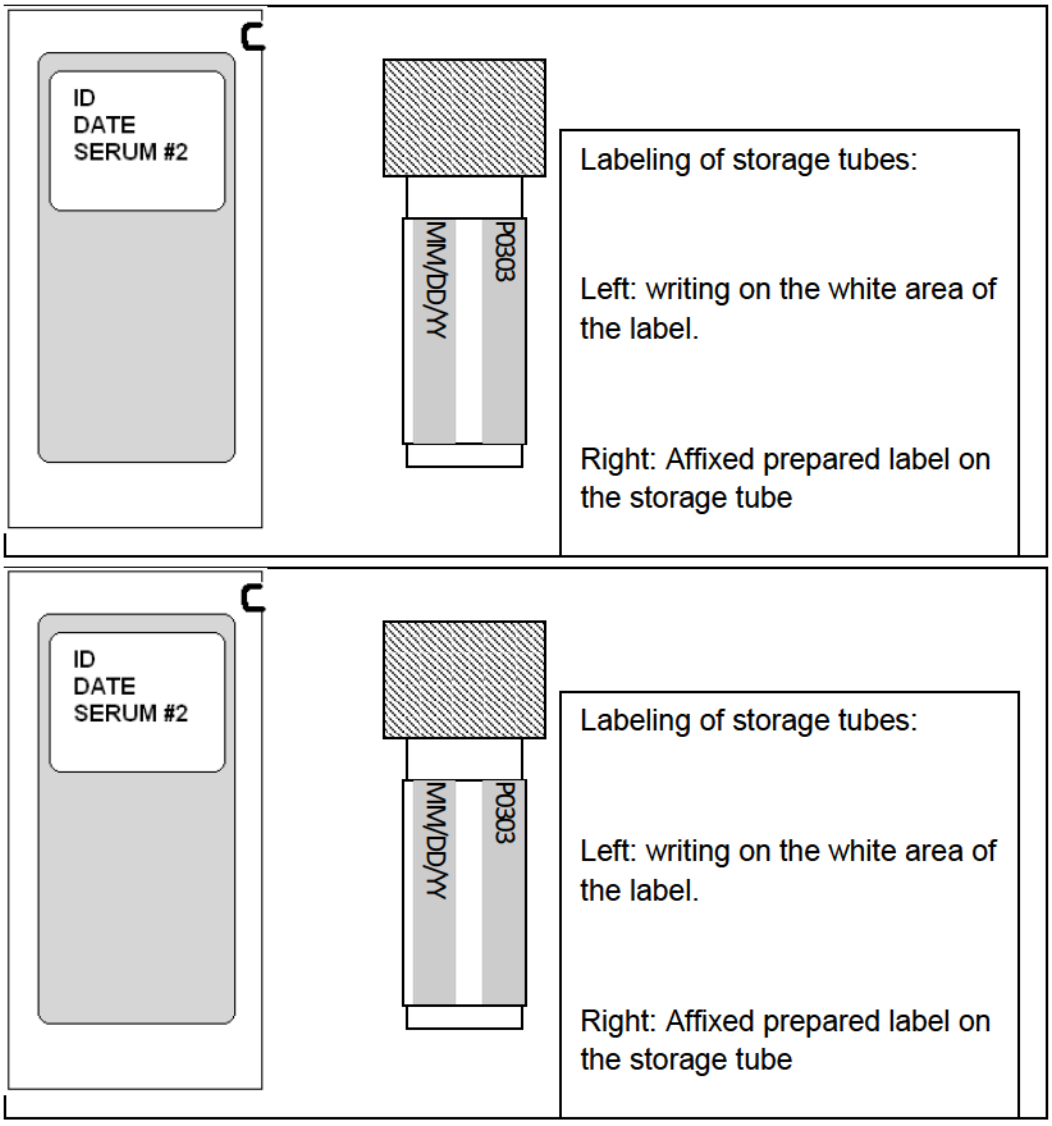
P0303
Labeling of storage tubes:
Left: writing on the white area of the label.
Right: Affixed prepared label on the storage tube



Note 4: **useful hints for use during separation**

- Recently it has been taking a bit longer for blood to clot due to the increase use and dosage of anticoagulants such as aspirin, Warfarin etc. When clotting has proceeded successfully, the blood is a solid mass. When tilting the tube carefully the mass does not move. It is ready to spin. While clotting, the blood may take several forms. Two layers may be visible in the blood collection tube: the top serum layer and the red cell layer. If the red cells portion is solid and does not flow when the tube is tilted (slowly and carefully!), the tubes are ready to spin. In others, there is a solid red mass mixed with a liquid yellowish mass on the top, in others the serum becomes a solid mass but the cell is not yet solid. In all these cases, it is best to wait until both fractions, cells and serum are solid before spinning. HOWEVER, cases exist where one or

both of the fractions may not solidify. The important thing to remember is that centrifugation should be done within 4 hours after collection. Small amount of cell lyses can occur due to the blood draw process; this is unavoidable.

- While pipetting serum you will notice that bubbles form in the tip and may get worse with use. This is normal and expected occurrence. It is also the reason why the pipette is dialed to 500µl for the separation. These bubbles will travel beyond this ½ way mark but with slow even pipetting technique will not go beyond the cleaned portion of the tip.

- Sometimes a fibrin clot, a solid mass of serum, may be present in the serum. In this case, a cleaned tip may be used to puncture the fibrin clot and squeeze the serum out of the clot, by pressing the tip against the clot and the wall. Be firm, but very careful, because the tip may fall off the pipette. The blood must be re-spun following this procedure because the serum and packed cells have re-mixed. Spin for 10 minutes and proceed with the separation. A new pipette tip must be used to continue separation.

- Due to the extensive use of plastic material, static charges sometimes develop in the vessels and surrounding atmosphere. This can result in the inability to properly clean the tips; water droplets sticking in the internal and external surfaces of the tip are the usual consequence. The following are a few ways to prevent the buildup of static electricity.

    - When preparing your working area, use a dampened wipe (Kimwipes™, Alphawipes®, etc) to wipe down the surface you will be using. Wipe in one direction only while constantly finding a clean surface on the wiping medium.

    - After taking the vessels/supplies needed from the protective packaging and placing them in the hood, change your gloves.

    - Before you position the vessels in their final arrangements and starting the separating process.

    - If a jar and its contents become statically charged, empty the jar and refill it with new DiH2O. If at any time DiH2O travels up into the cone of the pipette, immediately discard the tip and clean the cone of the pipette using a Q-tip™. This occurs when uneven strokes are used during pipetting.

V. Mailing samples to be analyzed for trace metals

i. The serum should be in their designated tubes. The caps of the storage tubes should be well tightened and seam between the cap and body of the tube covered with Parafilm™.

ii. Samples will be shipped to different locations.

a. Adhere to the department of transportation rules and regulations when shipping these clinical samples.

iii. Shipping to Mayo:

a. The serum sample collected for the aluminum testing should be sent to Mayo in the box provided, by FEDEX priority next day shipping. With appropriate labeling as a clinical sample, UN 3733 Biological samples category B (noninfectious)

b. Shipping address:

Mayo Medical Laboratories
Metals laboratory
[redacted]

iv. Shipping Samples to department of Orthopedic Surgery at Rush University Medical Center

a. A box will be provided to you that you can use to store the storage tubes in after separation. This box should be kept in at least a -20°C freezer until the samples are ready to be shipped to Rush University Medical Center. Samples should be kept upright

b. The instructions below are to be used as a guide, please follow Department of Transportation rules and regulations for shipping biological samples and the use of dry ice.

c. Once the samples are ready to be shipped:

I. The box will need to be placed in a plastic bag, and then placed in a Styrofoam container containing dry ice. The amount of dry ice will depend on the size of container, but it should be big enough to fit approximately 5 lbs of dry ice (or more if needed). The Styrofoam box should be placed inside another box (just a plain cardboard box).

II. Alternatively, the storage tubes can be placed in bubble wrap bags/ pockets, and the pockets placed in a plastic bag.

d. In both methods described above enough packing material and/or wrapping method should be employed to prevent the individual samples from flying around in the styrofoam box or bump against each other and cause breakage. Reminder: extreme temperatures cause brittle fracture.

e. The outside of the mailing box will need to be labeled with a dry ice sticker (provided in binder) and appropriate UN 3373 sticker Biological Substance category B (noninfectious).

f. Ship it by Federal Express next day. You **do** have to say that it contains non-infectious human samples. You must follow the DOT rules and regulations for shipping biological samples and the use of dry ice. Please do not mail on Friday or Saturday since no laboratory personnel will be present to receive the package on Saturday or Sunday. Reminder: the building is not open on weekends or on holidays. During holiday times call the laboratory to assure personnel will be in the laboratory to receive the shipment. Please **notify** Medtronic or RUSH when samples have been shipped.

Shipping Address:

[redacted]

[redacted]

B. METAL CONCENTRATION TESTING PROCEDURE

METAL CONCENTRATION TESTING PROCEDURE

Introduction

Metal concentrations (titanium and vanadium) in serum will be tested by the Trace Metal Analysis Laboratory at RUSH University Medical Center, following a serum testing protocol developed by the lab.

Instrumentation

- Titanium and vanadium is measured with a single instrument, a Thermo Finnigan Element 2 - Sector Field High Resolution Inductively Coupled Plasma Mass Spectrometry (SF-HR-ICP-MS or HR-ICP-MS).
- The instrument is equipped with platinum cones, PFA nebulizer and spray chamber, and a sapphire injector.
- The instrument is run with high purity argon gas (>99.999% pure).
- The isotope ^{47}Ti (AMU=46.95176) will be monitored in this protocol.

- An internal standard ^{45}Sc (AMU=44.95591) will be used to monitor potential drifts in the ICP-MS response during testing. All solutions tested contain the internal standard at 1 ng/mL.
- All measurements will be made in medium resolution mode (m/Δm ~4000) to separate and resolve the analyte ^{47}Ti from polyatomic spectral interferences.
- The instrument will be optimized by the operator prior to every run, using a 1 ng/mL multi-element standard purchased from High Purity Standards in serum matrix, matching the matrix being tested.

Materials

- All vials, tubes, bottles, flasks and pipette tips will be acid-washed with trace metal grade nitric acid prior to use.
- Solution preparation measurements will be performed with calibrated micropipettes.
- All solutions will be prepared with deionized water (16 to18MΩ) from a MilliQ system.
- Ultrapure grade nitric acid (e.g. Ultrex II, JT Baker) and ultrapure grade hydrofluoric acid (e.g.TAMAPURE AA-10) will be used in the preparation of solutions.
- All sample handling and preparation of solutions will be performed in class 100 clean rooms in a biological safety cabinet.
- Stock standards of inorganic titanium, vanadium and scandium will be purchased from High Purity Standards to prepare the calibrators.

Solutions and Calibrators

- A set of calibrators will be made every 12-18 months. These calibrators will be made from inorganic titanium and vanadium standards purchased from High Purity Standards. A list of available calibrators is provided in the table below.

Table 1: Titanium calibrators (ng/ml)

Ti concentration in vial	0.03	0.25	0.5	1	2	4	8	16	32	160
V concentration in vial	0.005	0.009	0.019	0.037	0.075	0.15	0.3	0.6	1.2	6

- Stability and accuracy of the calibrators will be checked against a previous set, as well as every 3 months by generating a curve using all the calibrators in serum matrix and analyzing a 1 ng/ml multi-element standard from a different lot purchased from High Purity Standards.

- The calibrators will be used to test both subject samples and QCs.

- An internal standard solution will also be prepared from inorganic scandium purchased from High Purity Standards.

Sample Measurement

- Serum samples will be diluted 10-fold in 0.2% HNO_3TrHF and tested using the Methods of Additions. This will involve each sample being tested neat (no spike) and with three calibration spikes of increasing concentrations. All samples will also be spiked with an internal standard, 1ng/mL scandium. The analyte measurement will be divided by the internal standard measurement and then blank corrected.

- The concentration of the analyte in the sample will be computed from the slope and intercept of the standard addition calibration curve. The acceptable linearity of the standard additions curve will be r^2 value greater than0.998. One standard additions curve is generated per serum sample.

Quality Control

- Seronorm™ Trace Elements Serum (TES) L-1 and L-2, as well as an in-house serum QCs, will be analyzed as quality controls during every run to determine the lab's performance and accuracy.

- Seronorm™ vials will be reconstituted following the manufacturer's protocol using 16 to18MΩ water, transferred to acid-washed storage tubes, and then stored in ultralow freezers. Please note that the Seronorm™ TES does not provide certified values for titanium only information values, are available. A vial-to-vial variation in titanium concentrations is also acknowledged by the manufacturer (Seronorm™).

- The analyte concentrations of titanium the in-house serum QC's have been determined to be 0.922 $\pm$ 0.08 for Reference QC, 1.83 $\pm$ 0.08 ng/ml for the Low QC and 304.6 $\pm$ 12.27 ng/ml for the high QC.

- The analyte concentrations of vanadium the in-house serum QC's have been determined to be 0.289 $\pm$ 0.016 for Reference QC, 0.085 $\pm$ 0.004 ng/ml for the Low QC and 9.617 $\pm$ 0.426 ng/ml for the high QC.

- The Seronorm™ TES L-1 and L-2 will be tested at the beginning of the analytical run. In addition, either one of the Seronorm™ QCs or an in-house QC will be reanalyzed every 10-12 samples. The acceptance criteria is as recommended by Seronorm™ or within allowable total error of 20% for the in-house QC

- QC samples will be analyzed by the method of additions using the same calibrators used to test subject samples as described above.

Established Performance Goals

The Allowable Total Error for this method is set at 20%. Performance goals for both accuracy and precision are set at 10%. Method validation studies were performed to determine the following performance parameters of this method, and are detailed in section 9 of this document.

- Limit of Quantitation

The limit of quantitation was determined following CLSI guideline EP17-A2 and is set at 0.3 ng/mL for Ti.

- Accuracy

  Recovery - As determined by a recovery study in serum matrix, the systematic error for titanium is -2.49% and for vanadium is -1.78%, within an allowable systematic error of 10%.

- Inter-lab Corroboration – Accuracy

  The TMAL performed an inter-lab comparison of values with Mayo Medical Laboratories (MML), Rochester, MNin 2013. Serum samples from 15 subjects were tested by both the laboratories. The samples were collected from 2011 through 2013; the integrity of the samples was maintained by following the TMAL collection and storage protocol. Analyte concentrations of the samples ranged from 1 to 40 ng/mL for Ti and from 0.01 to 17 ng/ml for V.

  MML provided results following their standard protocol using DRC-ICP-MS for Titanium, as well as ICP-OES for Titanium.

  The TMAL tested the samples across 11 different runs from 2011 through 2013 using method of additions. The calibrators were made from inorganic titanium and vanadium standard of purchased from High Purity Standards.

  Comparing TMAL and MML's resulted in the following linearity. For Titanium using DRC-ICP-MS, r^2=0.9976, slope=1.0455, and using ICP-OES, r^2=0.994, slope=0.980. For Vanadium using DRC-ICP-MS, r^2 =1, slope = 0.9559.

- Reference Material Accuracy

The TMAL analyzes the Seronorm™ Trace Elements Serum (TES) L-1 and L-2 as quality controls every run to determine performance and the lab's accuracy.

Seronorm™ vials are reconstituted following the manufacturer's protocol using 18MΩ water, transferred to acid-washed storage tubes and stored in -80C freezers.

The Seronorm™ TES does not provide certified values for Titanium but only information values, at 11.2 and 12.9 ng/mL for Ti and at 0.96 and 1.01 ng/ml for V. A vial-to-vial variation in titanium concentrations is also acknowledged by Seronorm™.

Seronorm™ samples are analyzed by method of additions. The calibrators are made from inorganic titanium and vanadium standard purchased from High Purity Standards. This same calibrator set is used to test the serum samples.

Data compiled since January 2013 includes 40 separate runs, on a single instrument. The determined mean is within 10% of the Seronorm information value, but due to the vial-to-vial variation, data presented below (Figures 1-4) is color coded by vial. The vials are reconstituted to 3mL, and the TMAL can only test each vial up to three times.

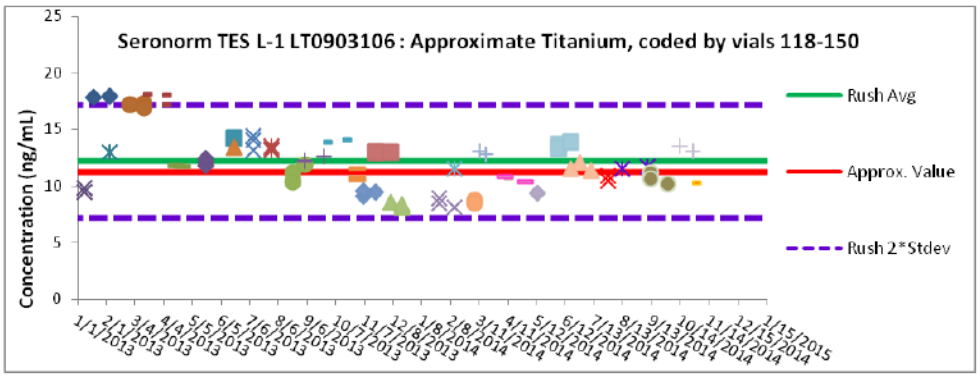


Figure 1: Trace Elements Serum Level 1 titanium concentration results since 2013. The values are color coded by vials. Vials purchased from Seronorm are reconstituted to 3mL and allow up to three tests by the TMAL. Seronorm acknowledges a vial-to-vial variation in titanium and only provides an approximate concentration of 11.2 ng/mL Ti.

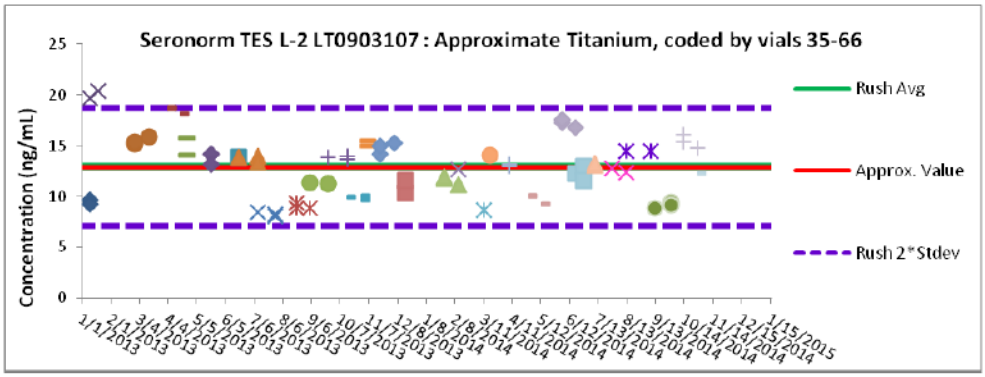


Figure 2: Trace Elements Serum Level 1 titanium concentration results since 2013. The values are color coded by vials. Vials purchased from Seronorm are reconstituted to 3mL and allow up to three tests by the TMAL. Seronorm acknowledges a vial-to-vial variation in titanium and only provides an approximate concentration of 11.2 ng/mL Ti

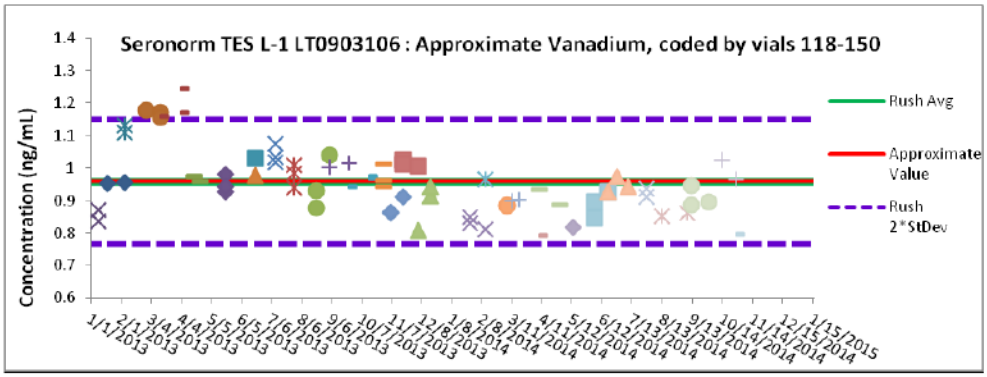


Figure 3: Trace Elements Serum Level 1 vanadium concentration results since 2013. The values are color coded by vials. Vials purchased from Seronorm are reconstituted to 3mL and allow up to three tests by the TMAL. Seronorm acknowledges a vial-to-vial variation in vanadium and only provides an approximate concentration of 0.96 ng/mL Ti

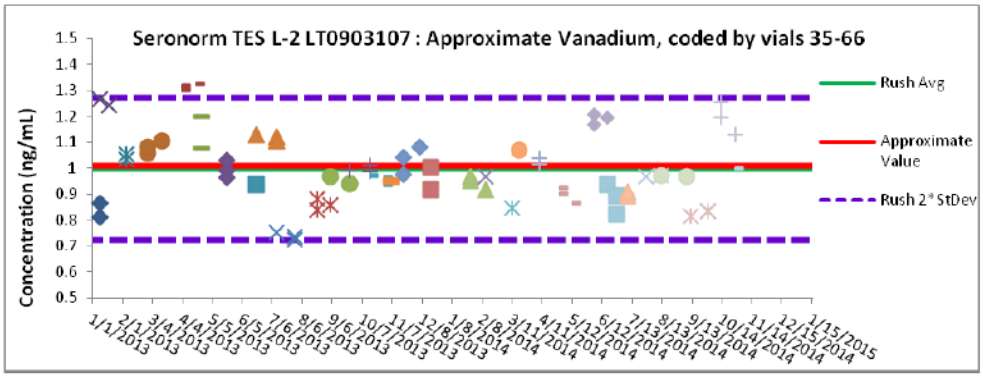


Figure 4: Trace Elements Serum Level 2 vanadium concentration results since 2013. The values are color coded by vials. Vials purchased from Seronorm are reconstituted to 3mL and allow up to three tests by the TMAL. Seronorm acknowledges a vial-to-vial variation in vanadium and only provides an approximate concentration of 1.01 ng/mL Ti.

- Precision

  As determined by a within-run precision study in serum matrix, the random error for titanium is 3.34% and for vanadium is 2.48%, within an allowable random error of 10%.

- Reproducibility

  The TMAL analyzes an in-house serum quality control every run and compiles the data to determine within-lab precision/reproducibility/stability. A baseline metal concentration level serum pool of 280mL was collected from a single person and spiked with inorganic titanium purchased from High Purity Standards. The approximate concentration of titanium was 4.5 ng/ml and 0.2 ng/ml of vanadium. The QC sample was poured into 56 acid-washed vials and has since been is stored in identical environment (-70 C) as the samples.

  The QC sample has been tested, over a seven-year period by multiple technicians using calibrants from different lots. All the testing was performed on a single instrument.

  For 130 tests from 2008 to 2014, the statistics (mean± SD) are Titanium (4.52 ± 0.19 ng/ml, CV = 4.23% and vanadium 0.19±0.01 ng/ml, CV=4.57%.

- Proficiency Testing

  The TMAL participates in the Quebec Multielement External Comparison Quality Assessment Scheme (QMEQAS). Unfortunately, at the present time titanium in serum is not an element included in the scheme in the New York Department of Health

Biomonitoring PT program for Trace Elements (formerly NYS DOH PT program for Trace Elements) and the CAP (College of American Pathologists) proficiency PT program. As with the QMEQAS the CAP PT program does not include titanium in the samples. A plot of the lab's performance in vanadium in serum is included below (Figure 5). Note: In 2011 and in 2012, only one out of the three events had serum included in the scheme.

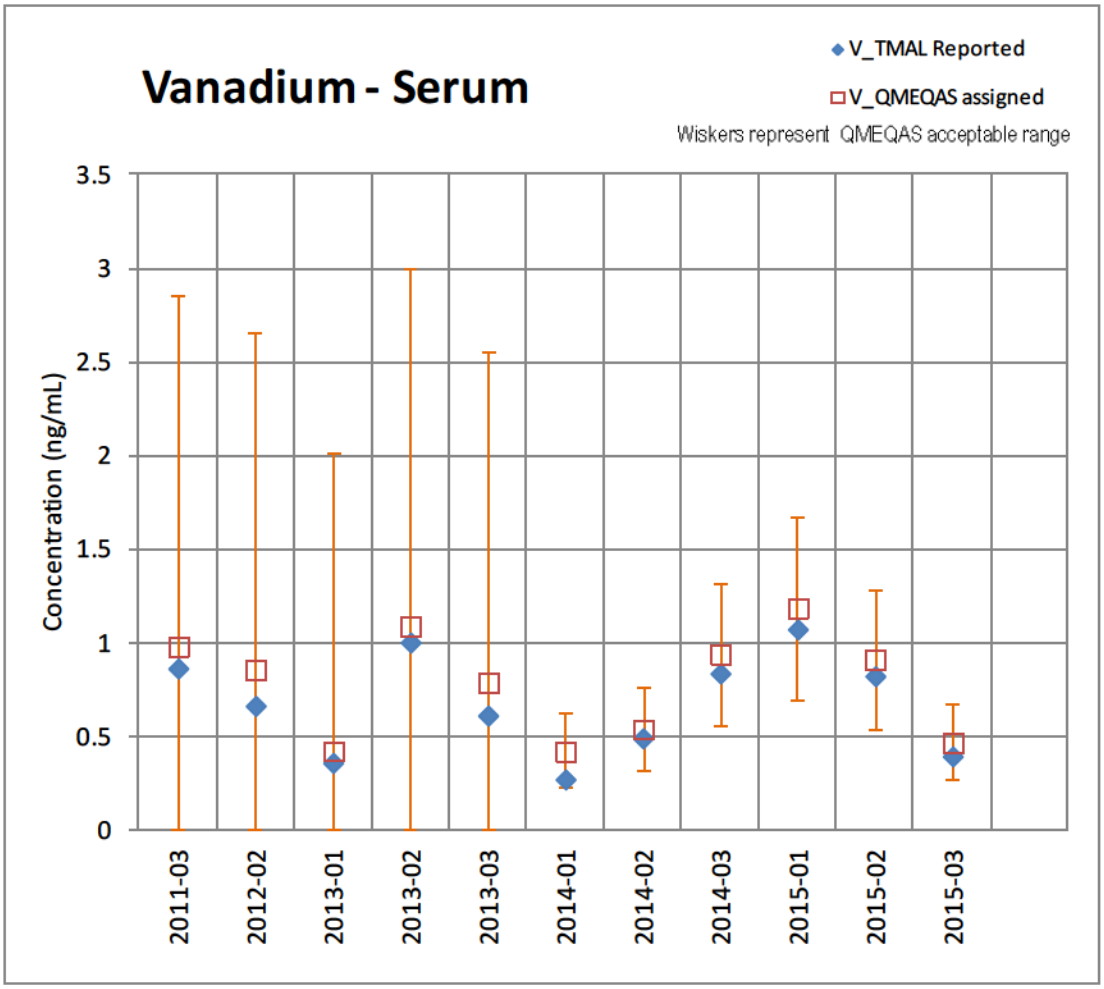


Figure 5: QMEQAS results for Vanadium in Serum. Only one event in 2011 and in 2012 had serum included in the scheme.

The laboratory also participated in the New York State proficiency program. A plot of the laboratories performance is shown below in figure 6.

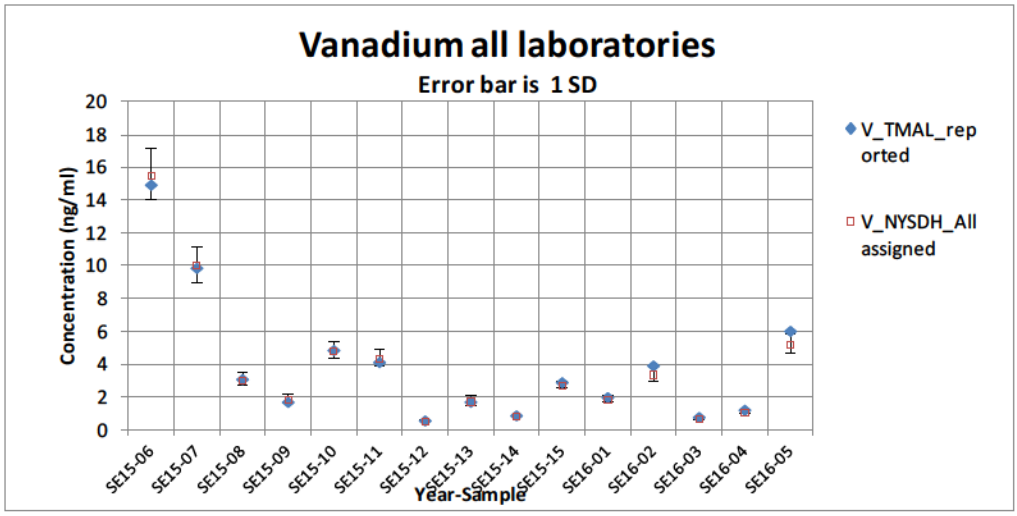


Figure 6: New York State Department of Health Trace Elements proficiency testing program.

- Sample Stability
  Serum samples are stored in ultra-low freezer (-70C). Table 2 below lists statistics for yearly intervals of our in-house quality control with concentration of Ti = 4.5 ng/ml and V=0.2 ng/ml, tested by our standard protocol using the method of additions, using the same set of calibrators as those used to test subject samples. The in-house QC is stored in identical environment (-70 C) as the samples.

Table 2: Statistics For Yearly Intervals

years	Number of tests	Ti Mean + SD, CV	V Mean + SD, CV
2008	7	4.38 ± 0.11, CV =2.47%	0.19± 0.003, CV = 1.84 %
2009	26	4.49 ± 0.30, CV =6.60%	0.19± 0.01, CV = 6.18 %
2010	14	4.51 ± 0.24, CV = 5.42%	0.20± 0.01, CV = 3.79 %
2011	31	4.54 ± 0.11, CV = 2.45%	0.20± 0.01, CV = 4.11 %
2012	11	4.45 ± 0.16, CV = 3.65%	0.20± 0.01, CV = 3.70 %
2013	25	4.58 ± 0.13, CV = 2.93%	0.20± 0.01, CV = 3.50%

2014	21	4.56 ± 0.15, CV = 3.33%	0.20 ± 0.01, CV = 3.72 %
2015	47	4.51 ± 0.12, CV = 2.64%	0.20 ± 0.01, CV = 3.62 %
2008-2015	182	4.52 ± 0.17, CV = 3.83%	0.20± 0.01, CV = 4.28 %

- Reference Range

The TMAL, in an internal study, has analyzed serum from 311 subjects with no implants, using the same collection, separation and testing protocols.

Reference range determined for titanium in serum is <0.7 ng/ml

Reference range determined for vanadium in serum is <0.2 ng/ml

Reference range provided for Aluminum provided by MML is 0-6 ng/ml[1]

Prospective studies, such as the Prestige LP metal concentration study, include a pre-operative value which for each subject will serve as the baseline for the post-operative values. The pre-operative values can also serve as a "reference" or baseline values for subjects in similar circumstances, where pre-operative values do not exist. Post-operative values of subjects with well-functioning implants and metal concentration levels reported in the literature can also be used to compare values from subjects which will undergo explantation of implants.

Method Validation Studies

- Linearity by Dilution

A baseline metal concentration level serum pool was collected from a single person and spiked with inorganic titanium and vanadium from a custom multi-element standard purchased from High Purity Standards. The analyte concentration of the spiked pool was 2000 ng/mL Ti and 100 ng/ml V.

Dilutions at 2x, 5x, 10x, 50x, and 100x were made with 18MΩ water. Three replicates were tested of each dilution and the original spiked pool, analyzed by method of additions. The calibrators are made from inorganic titanium and vanadium standard purchased from High Purity Standards.

The linearity by dilution study was conducted on a single test day, on a single instrument.

[1] http://www.mayomedicallaboratories.com/test-catalog/Clinical+and+Interpretive/8373

Results are linear within an allowable systematic error of 10%.

- Linearity by Addition

  A baseline metal concentration level serum pool was collected from a single person, separated into 5 pools and spiked with inorganic titanium and vanadium from a custom multi-element standard purchased from High Purity Standards. Analyte concentrations of the spiked pools range from 400 to 2000 ng/mL Ti and from 20 to 100 ng/ml V.

  Three replicates were tested of each spiked pool, as well as the original unspiked serum, and quantified by external calibration. The calibration curve was in serum matrix spiked with inorganic titanium and vanadium from a different lot, also purchased from High Purity Standards.

  The linearity by addition study was conducted on a single test day, on a single instrument.

  Results are linear within an allowable systematic error of 10%. The TMAL reportable range upper limit for Titanium is 2000ng/mL and for Vanadium is 100 ng/mL.

- Within-Run Precision

  A baseline metal concentration level serum pool was collected from a single person, and spiked with inorganic titanium and vanadium purchased from High Purity Standards. The analyte concentration of the spiked pool was 14.93 ng/mL Ti and 0.56 ng/ml V.

  Eleven samples of the pool were analyzed by method of additions. The calibrators are made from inorganic titanium and vanadium standard purchased from High Purity Standards.

  The mean, standard deviation, and coefficient of variation were calculated from the results. The CV for titanium and vanadium is 3.34% and 2.48%, within an allowable random error of 10%.

- Within-Lab Precision

  The TMAL analyzes an in-house serum quality control every run and compiles the data to determine within-lab precision. A baseline metal concentration level serum pool of 280mL was collected from a single person and spiked with inorganic titanium purchased from High Purity Standards. The approximate concentration of titanium was 4.5 ng/ml and of vanadium was 0.2 ng/ml. The QC was poured into 56 acid-washed vials and has since been is stored in identical environment (-70 C) as the samples.

QC samples are analyzed by method of additions. The calibrators are made from inorganic titanium and vanadium standard purchased from High Purity Standards.

Data compiled since January 2013 includes 32 separate runs, on a single instrument. The mean, standard deviation, and coefficient of variation were calculated from the results. The CV for titanium and vanadium is 3.43% and 3.66%, within an allowable random error of 10%.

- Recovery

  Three baseline metal concentration level serum pools were collected from a single person and each pool was spiked with 10 different concentrations of inorganic titanium and vanadium purchased from High Purity Standards. The spike concentrations were 0.0625 to 80 ng/mL Ti, and from 0.00234 to 3 ng/ml V.

  Three replicates were tested of the unspiked serum and at each spike concentration, and quantified by external calibration. The calibration curve was in serum matrix spiked with inorganic titanium and vanadium from a different lot, also purchased from High Purity Standards.

  The study was conducted on three different test days and on a single instrument.

  The mean recovery was calculated for each spike concentration and the proportional systematic error for titanium and vanadium was -2.49% and -1.78% respectively, within in an allowable systematic error of 10%.

- Limit of Quantitation

  The TMAL determined the limit of quantitation (LOQ) by following the CLSI guideline EP17-A2 “Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline – Second Edition.”

  The study was conducted over three different analytical runs on a single instrument with four independent serum samples, two reagent lots purchased from High Purity Standards (HPS), and a total of 36 replicates per reagent lot. The TMAL used recovery of standards for the estimation of bias, using a third reagent lot purchased from HPS.

  The LOQ is set at 0.3ng/mL for Ti, and 0.02 ng/ml for V. The total error was computed following Westgard model for each reagent lot, and passed within 20% allowable total error.

APPENDIX II

PROCEDURES FOR RETREIVE, HANDLING AND PACKAGING

This document provides information pertaining to the removal of a device. The investigator is responsible to follow the procedures outlined in the protocol for retrieving the devices and periprosthetic tissue sample(s).

Notify the sponsor as soon as possible prior to a scheduled or anticipated explantation of the devices.

Clinical history of the subject and implant information must be collected in the Prestige LP™ (Two-level) explant form. This form will be included in the retrieval kit provided to the investigational site by Medtronic upon receiving the notification of a scheduled or anticipated explant.

Prior to explantation of the devices, obtain the following radiographs: Anterior/Posterior, Lateral, and Flexion/Extension views. All radiographs should be copied and saved in DICOM format (if possible) and forwarded to Medtronic in a timely manner

PHOTOGRAPHS AND LABELING OF THE EXPLANT

Several photographs of the devices *in situ* prior to removal should be taken.

Every effort should be made to mark both the superior and inferior components (ideally with a fine osteotome at 12 o'clock mid-line position if possible) prior to removal in order to preserve the *in-situ* orientation. Immediately after, please take photographs of the mark(s) and document the orientation for both the components (superior/inferior, anterior/posterior, right/left to anatomic orientation of device) at both the operative levels from where the implants were retrieved. This is particularly important if any components are misaligned in any way due to migration or surgical mal-positioning and identified at the time of revision surgery. If the 12 o'clock position is not accessible and another marking position is used, a photographic record as well as a written note of the position should be made.

Several photographs should be taken of the disc space once the device is explanted.

Immediately following the explantation of the device, each component should be rinsed free of excess blood and placed on a clean surgical towel for photographs to be taken. A surgery label should include the subject ID/number, date and operative level the implant was retrieved from using the labels provided. Several photographs should be taken of the explanted device components including the superior (female component) and inferior (male component). One photo should be taken at a magnification that allows all of the removed components to be viewed together, and subsequent photos should be taken of individual components at a magnification that allows their features to be clearly

seen, including the laser markings and any obvious wear or damage to the parts. The retrieved components should be photographed on both sides. This information should be adequately labeled and should accompany the explanted components.

A detailed record of any damage caused to the components during extraction should be created in the presence of the operating surgeon (when possible). Any photographs of this damage can also be helpful.

COLLECTION OF TISSUE NEAR THE IMPLANT

Collect several small peri-prosthetic tissue samples taken from different anatomic locations, i.e. anterior, posterior, right lateral, left lateral, inferior endplate, superior endplate, overlying soft tissues etc. from both operative levels where the implants were retrieved. Photograph each tissue sample along with a label (provided) that identifies the subject ID/number, date and operative level the implant, and anatomic location the tissue was retrieved from.

Place each tissue sample into an individual cassette. Label each cassette with the location from which the tissue was retrieved (i.e. anterior, posterior etc.) as well as the implanted level.

Place each individual cassette into a small jar filled with 10% neutral formalin and secure the cap tightly; then place each small jar inside a large jar and secure the cap. Place the label used in the photographs on the side of each large specimen jar.

LABELING, FIXATION, PACKAGING AND SHIPPING OF RETRIEVED IMPLANT, TISSUE SAMPLES

Each explanted component should be gently wiped with surgical swabs to remove excess blood and fluids before being placed into separate jars filled with 10% neutral buffered formalin at least twice the volume of the implant. This includes both the superior component and the inferior components retrieved from both the operative levels. Affix the label used in the photos on the side of each large jar containing explanted components.

Place any individual labeled tissue cassettes with bone/implant interfacing tissue in separate jars of 10% neutral buffered formalin. Place each small jar inside a large jar and secure the cap. Place the label used in the photographs on the side of each large specimen jar

NOTE: Care should be taken during any subsequent handling of the specimens to avoid damage to the parts such as rubbing the articulating surfaces together, dropping, or knocking the parts or allowing tissue attached to the implant to dry out.

When handling, packaging and shipping the retrieved components, place each individual component of all explanted devices into separate small jars filled with formalin and secure the caps tightly and then place each small jar into a larger jar.

Notify the Medtronic Clinical Department study specific Clinical Trial Associate (CTA) for the Prestige LP™ Two Level study, with the name of the person returning the devices, date of implantation, date of explantation, date of return of the explant, reason for explantation, catalog/lot number of the explanted device, and operative levels the implants were retrieved from. The investigational site is responsible for shipping the explanted devices and periprosthetic tissue within five (5) days.

[redacted]

Note: Special shipping labels may be required by FedEx, depending on the quantity and type of liquid used to store the implants. These labels will be provided in the retrieval kit. For more information, please check with Medtronic designee or your local FedEx shipping location.

ANALYSIS

Peri-prosthetic tissues associated with the Prestige LP™ Cervical Disc will undergo histological evaluation. The histological evaluation will be conducted according to an explant protocol developed by Jeffrey M. Toth, PhD., Professor of Orthopaedic Surgery and Director of Biomaterials Research at the Medical College of Wisconsin. Dr. Toth will thoroughly photograph the devices and explanted tissues, perform high resolution radiography on explanted tissue samples, and produce histology slides of the periprosthetic tissues with a histological evaluation of the host response to the device and particulate debris (if present) according to ASTM F981-04.

Following photographic documentation of periprosthetic tissues and all device components, all device components will be dried, left intact, individually packaged, and sent to Exponent at the following address for non-histological implant analysis:

[redacted]

APPENDIX III

Sponsor Information

Protocol approvers: Biostatistician, Clinical Leadership, Clinical Quality Compliance, Clinical Study Manager, Medical Writer, Monitor, Program Lead, Regulatory, Safety, Therapy SME

Medtronic Spine

1800 Pyramid Place SS-46 | Memphis, TN 38132

Name	Title
[redacted]	Sr. Clinical Research Director
[redacted]	Clinical Program Manager, Medical Advisor
[redacted]	As delegated for the Clinical Program Manager
[redacted]	Prin. Clinical Research Specialist, Clinical Study Manager
[redacted]	Prin. Clinical Quality Specialist
[redacted]	Sr. Statistics Manager
[redacted]	Prin. Clinical Research Monitor
[redacted]	Regulatory Affairs Director
[redacted]	Sr. Clinical Research Manager, Safety
[redacted]	Sr. Clinical Project Manager, Medical Advisor

Medical Advisor:

[redacted]

APPENDIX IV

PRESTIGE LP CERVICAL DISC INFORMATION FOR USE