Assessment of Tooth Vitality Using Pulse Oximeter

NCT03578549

Principal Investigator: Clauw, Daniel

Post-Doc Fellow: Dabiri, Darya

Draft or Version Number: 7.0

July 3, 2019

STATEMENT OF COMPLIANCE

The study will be conducted in accordance with the Code of Federal Regulations on the Protection of Human Subjects (45 CFR Part 46). All personnel involved in the conduct of this study have completed human subjects' protection training.

SIGNATURE PAGE

The signature below constitutes the approval of this protocol and the attachments, and provides the necessary assurances that this study will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements.

Principal Investigator or Clinical Site Investigator:

Signed: _____ Date: _____

Name: Daniel Clauw

Title: Professor, Department of Anesthesiology, University of Michigan

TABLE OF CONTENTS

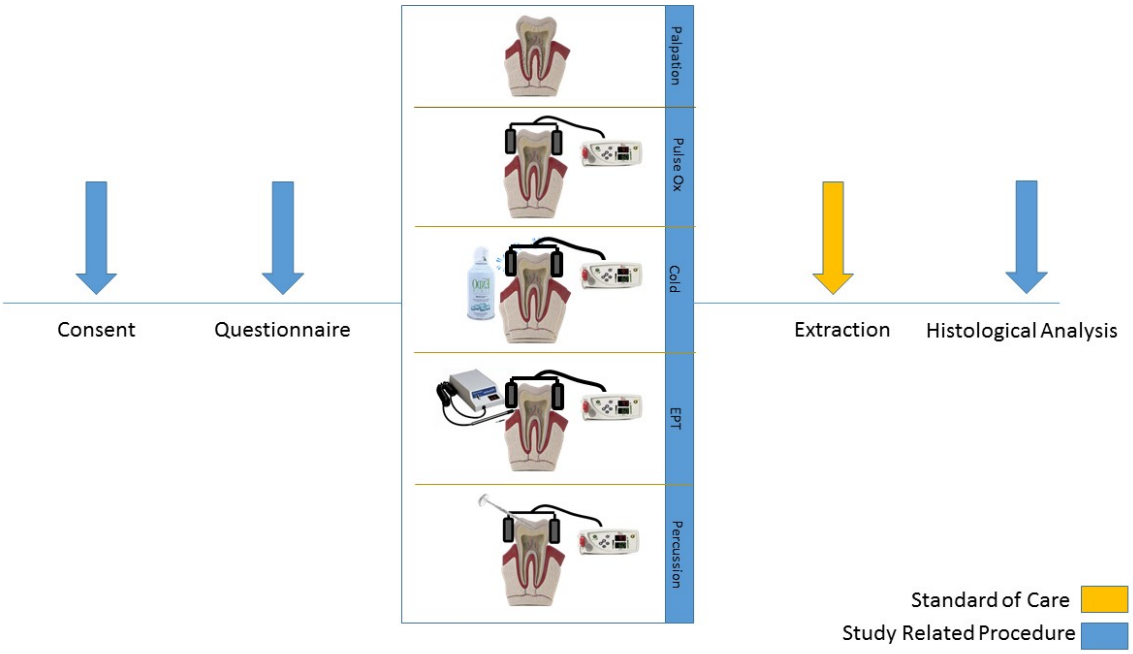
LIST OF ABBREVIATIONS

EPT	Electrical Pulp Test
PBF	Pulpal Blood Flow
LDF	Laser Doppler Flowmeter

PROTOCOL SUMMARY

Title:	Assessment of Tooth Vitality Using Pulse Oximeter
Précis:	The vitality of the pulp is determined according to the health of the vascular supply not the sensory fibers. Objective diagnostic devices that examine pulpal blood flow, such as pulse oximeter have shown promising results for the assessment of pulp vitality. This could be valuable not only in pulp necrosis but also in diagnosis of inflammatory status of the pulp. If blood flow in teeth is related to symptoms and/or pathology, this would likely have much broader applicability in dentistry, as dentists frequently struggle to answer questions such as "is the pain coming from the tooth or another structure?", and "is the tooth alive or dead". These are very important questions in a wide variety of individuals presenting with acute and chronic orofacial pain. In the present study we will measure pulpal blood flow using pulse oximeter in patients who are already undergoing extractions. Conventional tooth assessments (percussion, palpation, cold, and electrical pulp testing) and pulpal blood flow measurements would be used for teeth that are planned for extraction and also up to 2-5 additional teeth in each participant. We will then look to see whether these clinically measured physiologic indicators of tooth vitality are similar to the histological presentation of the extracted teeth
Objectives:	The objective of this study is to evaluate if pulse oximeter measurements are a reliable marker for patient's reported symptoms and/or histological evaluations.
Population:	35 participants
Site:	University of Michigan, Ann Arbor MI
Study Duration:	24 months
Subject Participation Duration:	Visit 1: approximately 45 minutes
Estimated Time to Complete Enrollment:	6 months

Schematic of Study Design:



Procedure

Study-Related Procedure

1) The researcher will gather information initially by asking if any of the teeth are hurting or sensitive to hot/cold
2) Participant will complete self-report questionaries' regarding their history of dental pain
3) Existing panoramic radiograph will be reviewed by a member of research team prior to choosing which teeth (less than 10) will be to be tested.
4) Blood pressure, and heart rate values recorded at this appointment will be obtained from the medical record by a member of research team prior to the extraction.
5) The researcher initially will palpate the gum around selected teeth and record any discomfort
6) A plastic clip, pulse oximeter will be placed over the teeth, one at a time, to measure blood flow by measuring the presence of pulse in these teeth for approximately for 15-30 seconds per tooth.
7) The clip will remain on the tooth for additional 2-3 minutes to continually measuring pulse while the following tests are taken place:
    a. Cold Testing: This test is used to assess if the tooth is "dead" or "alive" by measuring sensation of cold from the tooth. A small cotton ball will be sprayed with cold spray (called Endo-Ice) and then placed on the surface of that tooth for approximately 15 seconds or till participant reports any sensation of cold. There would be 30 seconds wait time prior to the next step.
    b. Electrical Pulp Testing: This test is used to assess if the tooth is "dead" or "alive" by measuring sensation of electrical pulses from the tooth. A small probe attached to an electrical pulp testing (EPT) device will be placed on the surface of that tooth for approximately 15 seconds or till participant reports any tingling sensation. There would be 30 seconds wait time prior to the next step.
    c. Percussion Testing: This test is used to assess if the tooth is "dead" or "alive". The end of the mouth mirror is used to gently tap on the tooth surface and record any discomfort. There would be 30 seconds wait time prior to removal of the clip.
8) Once the tests above are completed, the plastic clip will be removed from your tooth. The pulse oximeter sensors will be removed from the plastic clip and replace with the LDF sensor. The plastic clip will be placed back over the teeth previously tested to measure the blood flow with the LDF . Blood flow will be measured by the presence of a pulse in each tooth for approximately for 15-30 seconds. Once the measurement is obtained, the plastic clip will be removed from the tooth.
9) After obtaining participant's permission, researchers will obtain a de-identified copy of the participants dental radiographs
10) After planned extraction is completed by the oral surgery provider, the tooth/teeth from the standard of care procedure will be provided to the researcher for further analysis to determine of the tooth is "alive or dead". This process will occur at the University of Michigan, School Of Dentistry

Standard care procedures:

After completion of the study related procedures, patient's care is transferred to their treating clinician for dental extraction

Post-Extraction, Histological Analysis:

Upon completion of extraction, the teeth will be placed in formalin-containing bottle. After formalin fixation, the teeth will be prepared for histological examination using 10% EDTA for demineralization. Following that, the teeth are trimmed, embedded in paraplast and cut longitudinally to obtain 6um thick section. The sections will be stained with H&E and examined with a light microscope. The histological diagnosis is based on the classification used by Seltzer et al. as follows:

KEY ROLES AND CONTACT INFORMATION

Principal Investigator:

Daniel Clauw, Research Investigator,
Chronic Pain and Fatigue Research Center
Domino's Farms, Lobby M, PO Box 385
24 Frank Lloyd Wright Dr.
Ann Arbor, MI 48106, USA
(p) 734-998-0176
(f) 734-998-6900
dclauw@med.umich.edu

Institutions:

University of Michigan
Ann Arbor, MI 48109 USA

Key Personnel:

- **Daniel J. Clauw, M.D,** is a Professor of Medicine (Rheumatology), Anesthesiology, and Psychiatry at the University of Michigan (UM) and Director of the world renowned CPFRC. Dr. Clauw is an acknowledged expert in pain conditions and has greatly expanded the understanding of the neurobiological mechanisms of pain and fatigue in a number of chronic pain conditions. He has also been a prolific mentor, being the primary or co-mentor for 25 NIH K awardees, nearly all of which are now successful independent researchers. To this application he brings expertise in orafacial pain and clinical interventions including study design and analysis. He has expertise in the techniques described in this application including measurement of clinical pain, surrogates of centralized pain, and psychophysical pain testing. He has served as the head of research networks and center grants investigating chronic pain, and is uniquely suited to facilitate contact with other experts and collaborators throughout the training and research portions this study. Dr. Clauw will meet regularly with the candidate weekly in person and is available for additional informal meetings and ad hoc discussions.
- **Steven E. Harte, Ph.D.** is an Assistant Research Scientist in the Departments of Anesthesiology and Medicine, and is a core faculty member of the pain research at the University of Michigan. Dr. Harte's current research uses psychophysical measures and

advanced neuroimaging techniques to investigate the pathophysiology of chronic pain. He has been integral in developing and validating novel pain assessment methods and devices for research and clinical applications of psychophysical testing, including in large multi-site, longitudinal investigations of chronic pain. To this application Dr. Harte will provide the candidate mentorship on the use of unconventional sensory measurements of pain for both assessment of acute pain and chronic dental pain. He will meet regularly with the candidate once per month and on an ad hoc basis.

- **Darya Dabiri, DMD, MS.** Dr. Dabiri obtained her dental degree from the University of British Columbia in 2008. She then pursued dual training in pediatric dentistry and endodontics at the University of Toledo Medical Center and University of Michigan respectively. She has had numerous publication in both basic sciences and epidemiological studies looking at host/immune responses in viral myocarditis and early childhood caries in the developing countries. She pursued training in Master of Clinical Research at the University of Michigan and also a K-12 training grant through mentorship of the CPFRC faculty and the support of National Institute of Dental Research. Her research is devoted to better understand ways to take better care of children with endodontic issues in particular to establish a non-subjective tooth vitality testing device utilizing a variety of techniques. Dr. Dabiri will be involved with the collection, analysis and interpretation of the data that is being collected in the present study.
- **Grant Kruger, Ph.D.** is an Assistant Research Scientist at the Engineering Department at the University of Michigan. He is an expert in biomedical device design and manufacturing; control systems; mathematic modeling, analysis and prediction; software engineering; embedded systems development; metrology; process monitoring and fault diagnosis; digital signal processing; intelligent maintenance systems. He has a longstanding collaborative relationship with Drs. Clauw and Harte and together they have developed and patented several medical devices that perform quantitative sensory testing, similar to that proposed in this study. He will be integral in developing a pulse oximeter holder design.

INTRODUCTION: BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

1.1 Background Information

The ideal test to assess pulp status in diagnosis of odontogenic source of pain should provide a simple, objective, standardized, reproducible, non-painful, non-injurious, accurate, and inexpensive way of assessing the condition of the pulp tissue. (Gutmann 2011) With our current subjective measurements for pain assessments, diagnosis and treatment of dental pain in young children and children (any age) with cognitive impairment (CI) is very challenging. Studies have shown that children with cognitive impairments appear to be more likely to experience significant pain on a regular basis than unimpaired children. (Oberlander, O'Donnell et al. 1999, Massaro, Ronfani et al. 2014) In dentistry, self-report of pain is one of the essential tool for pain assessment and identification of origin of the pain. However, non-verbal children either due to young age of CI are unable to self-report reliably and that by itself challenge the clinician to accurately identify the source of pain.

Previous studies have examined non-subjective diagnostic devices such as the pulse oximeter, laser Doppler flowmetry and crown surface temperature change, to assess pulp vitality. Despite their promising results, these techniques has not become incorporated into dental diagnosis standard of care. (Fanibunda 1986, Fanibunda 1986, Schnettler and Wallace 1991, Fratkin, Kenny et al. 1999, Radhakrishnan, Munshi et al. 2002, Gopikrishna, Tinagupta et al. 2007, Jafarzadeh 2009, Anusha, Madhusudhana et al. 2017)

Pulse oximeter has been one of the earliest methods and most studied. The principle of pulse oximeter design is the fact that the arterial component of blood is pulsatile in nature (time varying). The purpose of using pulse oximeter is to measure the percentage of oxygenated hemoglobin (HbO2) to the total hemoglobin (Hb)- total oxygenated and deoxygenated hemoglobin. Therefore, when light is emitted through LED transmission on the human body (eg finger), the amount of light that passes through after attenuation from various tissues, it reaches to veins and arteries and can indirectly indicate pulsatile component which overcome the contact component.

Previous studies have shown the potential application of conventional pulse oximeter for assessment of the presence of dental pulp vascularity using a modified probe for teeth. (Alghaithy and Qualtrough 2017) It also appears that there is a correlation between oxygen saturation of pulpal circulation with systemic blood using pulse oximeter.(Schnettler and Wallace 1991)

Objective diagnostic devices such as pulse oximeter could be valuable not only in pulp necrosis but also in diagnosis of inflammatory status of the pulp. If blood flow in teeth is related to symptoms and/or pathology, this would likely have much broader applicability in dentistry, as dentists frequently struggle to answer questions such as “is the pain coming from the tooth or another structure?”, and “is the tooth alive or dead”. These are very important questions in a wide variety of individuals presenting with acute and chronic orofacial pain. In the present study we will measure pulpal blood flow using pulse oximeter in patients who are already undergoing extractions. We will then look to see whether these clinically measured physiologic indicators of tooth vitality are similar to the histological presentation of the extracted teeth.

1.2 Rationale

The purpose of this study is to assess tooth health by using a pulse oximeter which measures the blood flow within the tooth. We hypothesize that if we can measure both sensation and blood flow in a tooth, we will be able to better determine if the tooth is "alive or dead". In the future, this method may help determine which teeth are diseased and require dental treatments.
The objective of the study is to measure sensation as well as blood flow in a tooth, for potential use in dentistry.
Our specific aims are:

Aim - 1: To measure pulpal blood flow using pulse oximeter and laser Doppler Flowmeter (LDF)

Aim - 2: To measure tooth vitality using Cold, Electrical Pulp tester (EPT), percussion and palpation testing

Aim - 3: To assess histological presentation of the pulp the extracted teeth

Aim - 4: To evaluate the relationships between the Aims 1-3 to determine if pulpal blood flow is a reliable indicator of tooth vitality.

1.3 Potential Risks and Benefits

1.3.1 Potential Risks

Hand-held pulse oximeter:
The pulse oximeter is a non-invasive diagnostic device intended to measure systematic blood oxygenation and pulse rate. In this study, we will use a portable pulse oximeter (CMS60D) to measure blood flow in the tooth pulp. Clinical decisions will not be based on the results obtained from this test. The signal emitted from the sensor is light, and there is little to no risk associated from the pulse oximeter to human tissue. The pulse oximeter is battery operated and the sensors are isolated in plastic sheaths.

Infection control measures will be implemented for all items entering the oral cavity. The pulse oximeter sensors will be cleaned with disinfecting wipes (Colorox Healthcare Hydrogen Peroxide Cleaner Disinfectant, Colorox Company, Latham, NY) and then individually wrapped with a latex free, translucent, elastic barrier (CurelasticTM, Steri-Shield Products, Las Vegas, NV). The insulated wiring harness that connects the sensors to the body of the pulse oximeter will also be cleaned with disinfecting wipes and then covered with a barrier film (Universal Cover Barrier, Patterson Dental, Saint Paul, MN). A custom made sensor holder will be used to hold the pulse oximeter sensors onto the teeth (see below for additional details). The sensor holder will be cleaned with disinfecting wipes prior to use.

A new set of sensor holder will be used for each individual patient. We will plan to re-use the sensors total of 1-2 times. At each time, then sensors and its connecting wires will be wiped using

alcohol wipe and then covered using protection barrier, "blue tape". After each use, the blue tape would be removed and the sensors and its connectors be wiped again with alcohol

Laser Doppler Flowmeter (LDF):

The laser Doppler Flowmeter is a non-invasive diagnostic device intended to provide measurement of perfusion. This device is similar to pulse oximetry it identifies the number and velocity of blood cells circulating through vasculature. In this study, we will use a portable Laser Doppler Flowmeter, manufactured by Perimed Inc. to measure blood flow in the tooth. Clinical decisions will not be based on the results obtained from this test. According to PeriFlux System 5000 Manual, The PeriFlux PF 5000 Main Unit contains a diode laser emitting continuous divergent radiation. This laser light is transmitted to the PF 5000 LDF Unit(s). The PeriFlux System 5000 is classified in USA as a class 1 laser which covers the lowest risk laser. The biomedical engineering unit qualified this LDF unit in October of 2013 and tagged it with the hospital tag 464212.

The PeriFlux System 5000 laser Doppler measures micro-circulatory blood flow. Laser light is applied to the tissue through fiber optics. The laser light hits moving blood cells in the microvasculature, and this scatters the light and causes the laser light to change frequency due to Doppler shift. The frequency change will depend on the amount of blood cells in the illuminated area and the speed of those blood cells. Part of the laser light is returned to a photo detector in the instrument. The value from the photo detector is electronically processed and the signal is converted into a perfusion rate.

Infection control measures will be implemented for all items entering the oral cavity. The LDF sensors will be cleaned with disinfecting wipes (Colorox Healthcare Hydrogen Peroxide Cleaner Disinfectant, Colorox Company, Latham, NY) and then individually wrapped with a latex free, translucent, elastic barrier (CurelasticTM, Steri-Shield Products, Las Vegas, NV). The insulated wiring harness that connects the sensors to the body of the LDF will also be cleaned with disinfecting wipes and then covered with a barrier film (Universal Cover Barrier, Patterson Dental, Saint Paul, MN). A custom made sensor holder will be used to hold the LDF sensors onto the teeth and/or supporting gingival. The sensor holder will be cleaned with disinfecting wipes prior to use.

A new set of sensor holder will be used for each individual patient. We will plan to re-use the sensors total of 1-2 times. At each time, then sensors and its connecting wires will be wiped using alcohol wipe and then covered using protection barrier, "blue tape". After each use, the blue tape would be removed and the sensors and its connectors be wiped again with alcohol

<u>Pulse oximeter sensor holder:</u>
The sensor holder is designed to hold the sensors securely around the teeth. The sensor holders are designed in various sizes to accommodate variation in dental anatomies. The first iteration of the sensor holder (i.e., “Design-1”) is 3D printed using the Form2Lab Printer (see photo on page 42).

The material used is Dental LT Clear Resin (FormsLab, Somerville, MA) as it can produce accurate, biocompatible parts ideal for this application. Dental LT Clear is tested at NAMSA (Chasse sur Rhône, France) and is certified biocompatible (EN-ISO 10993-1:2009/AC:2010).

Design-1 includes external wings for placement of cotton rolls to increase comfort for the patient. The interface between the sensor and the teeth/gingiva is covered with a thick layer (5-8mm) of clear Memosil 2 (Kulzer, Hanau, Germany). We have planned on including this interface for comfort and also to increase the surface area since the conventional pulse oximeter is against skin and muscle and we plan to place it over the tooth. It has round edges and can be additionally supported on the tooth using OpalDam paste (Ultradent Products, South Jordan, UT) to minimize any risk of accidental abrasion on the adjacent soft tissue. The sensor holder will be disinfected in an ethanol solution between each subject.

There is a small chance that a participant may feel slight pressure on their tooth when the clamp is placed or that it might pinch the gingival tissue a little. If this occurs, the clamp will be repositioned or removed and another size clamp will be used. Some participants might experience pressure from the sensor holder around the tooth following the test. If a subject finds any pressures unbearable or intolerable, he or she may ask the operator to stop the test and the pressure will be immediately removed from the tooth.

Dental pain questionnaire:
There is possibility of discomfort associated with being asked personal questions about history of dental and your overall wellbeing. You may refuse to answer any questions on the questionnaire of survey that may be uncomfortable.

Cold testing:
One of the potential risk of cold testing is temporary mild pain or discomfort at the site of testing. If a subject finds the test unbearable or intolerable, he or she may ask the operator to stop the test and the cotton ball will be immediately removed from the tooth.

Electrical Pulp testing (EPT):
One of the potential risk of EPT testing is temporary mild pain or discomfort at the site of testing. If a subject finds the test unbearable or intolerable, he or she may ask the operator to stop the test and the probe will be immediately removed from the tooth.

Percussion:

One of the potential risk of percussion testing is temporary mild pain or discomfort at the site of testing. If a subject finds the test unbearable or intolerable, he or she may ask the operator to stop the test.

1.3.2 Potential Benefits

Participants are not expected to receive any personal benefits from being in this study.

OBJECTIVES

1.4 Study Objectives

The purpose of this study is to assess tooth health by using a pulse oximeter which measures the blood flow within the tooth. We hypothesize that if we can measure both sensation and blood flow in a tooth, we will be able to better determine if the tooth is "alive or dead". In the future, this method may help determine which teeth are diseased and require dental treatments.

Our specific aims are:

Aim - 1: To measure pulpal blood flow using pulse oximeter and Laser Doppler Flowmeter

Aim - 2: To measure tooth vitality using Cold, Electrical Pulp tester (EPT) , percussion and palpation testing

Aim - 3: To assess histological presentation of the pulp the extracted teeth

Aim - 4: To evaluate the relationships between the Aims 1-3 to determine if pulpal blood flow is a reliable indicator of tooth vitality.

1.5 Study Outcome Measures

Primary Outcomes Measures: Correlation Between Subjective and Objective Measurements of Pulpal Status

- *Subjective Measurements Pulpal Status*: Conventional pulpal diagnosis using cold test [Endo Ice, Coltene/Whaledent, Cuyahoga Falls, OH, USA] and Electrical Pulp Test -EPT S[Kerr Vitality Scanner, SybronEndo] testing and recorded, along with vertical and lateral percussion and palpation responses. Conventional pulpal diagnosis assessment would be used for teeth that are planned for extraction and additional teeth (10 total) in each participant

- *Objective Measurements Pulpal Status:* Measurements obtained from pulse oximeter gives a series of reading of oxygen saturation level, and pulse. Pulse oximeter measurements would be used for teeth that are planned for extraction and also up to 2-5 additional teeth in each participant

Exploratory Outcomes:

- *Systemic Vital Sign Measurements:* Blood pressure and pulse recording will be obtained from the recorded values from the participant's chart.

- *Histological Assessment of the Extracted Teeth:* Upon completion of extraction, the teeth will be placed in formalin-containing bottle. After formalin fixation, the teeth will be prepared for histological examination using 10% EDTA for demineralization. Following that, the teeth are trimmed, embedded in paraplast and cut longitudinally to obtain 6um thick section. The sections were stained with H&E and examined with a light microscope.

- *Laser Doppler Flowmeter (LDF):* Measurements obtained from LDF pulse oximeter gives a series of reading of blood velocity. LDF measurements would be used for teeth that are planned for extraction and also up to 2-5 additional teeth in each participant

STUDY DESIGN

Standard Care Procedures:

Participants may receive the following treatment procedures whether he/she join this study or not:

- Review of medical and dental history
- Oral examinations
- Dental Procedures (e.g., extractions) planned by his/her oral surgery provider

Study-Related Procedure

- The researcher will gather information initially by asking if any of the teeth are hurting or sensitive to hot/cold
- Participants will complete self-report questionaries' regarding their history of dental pain
- Existing panoramic radiograph will be reviewed by a member of research team prior to choosing which teeth (less than 10) will be to be tested.
- Blood pressure, and heart rate values would be recorded on this appointment from your medical record by a member of research team prior to the extraction.
- The researcher initially will palpate the gum around selected teeth and record any discomfort
- A plastic clip, pulse oximeter will be placed over the teeth, one at a time, to measure blood flow by measuring the presence of pulse in these teeth for approximately for 15-30 seconds.
- The clip will remain on the tooth for additional 2-3 minutes to continually measuring pulse while the following tests are taken place:
    - **Cold Testing**: This test is used to assess if the tooth is "dead" or "alive" by measuring sensation of cold from the tooth. A small cotton ball will be sprayed with cold spray (called Endo-Ice) and then placed on the surface of that tooth for approximately 15 seconds or till participant reports any sensation of cold. There would be 30 seconds wait time prior to the next step.
    - **Electrical Pulp Testing:** This test is used to assess if the tooth is "dead" or "alive" by measuring sensation of electrical pulses from the tooth. A small probe attached to an electrical pulp testing (EPT) device will be placed on the surface of that tooth for approximately 15 seconds or till participant reports any tingling sensation. There would be 30 seconds wait time prior to the next step.
    - **Percussion Testing:** This test is used to assess if the tooth is "dead" or "alive". The end of the mouth mirror is used to gently tap on the tooth surface and record any discomfort. There would be 30 seconds wait time prior to removal of the clip.
    -
- Once the tests above are completed, the plastic clip will be removed from your tooth. The pulse oximeter sensors will be removed from the plastic clip and replace with the LDF sensor. The plastic clip will be placed back over the teeth previously tested to measure the blood flow with the LDF . Blood flow will be measured by the presence of a pulse in each tooth for approximately for 15-30 seconds. Once the measurement is obtained, the plastic clip will be removed from the tooth.

- After obtaining participant's permission, researchers will obtain a de-identified copy of the participants dental radiographs
- After planned extraction is completed by the oral surgery provider, the tooth/teeth from the standard of care procedure will be provided to the researcher for further analysis to determine of the tooth is "alive or dead". This process will occur at the University of Michigan School of Dentistry Oral Surgery Clinics.
- Upon completion of extraction, the teeth will be placed in formalin-containing bottle. After formalin fixation, the teeth will be prepared for histological examination using 10% EDTA for demineralization. Following that, the teeth are trimmed, embedded in paraplast and cut longitudinally to obtain 6um thick section. The sections were stained with H&E and examined with a light microscope. The histological diagnosis are based on the classification used by Seltzer et al. as follow:

    1. Normal Pulp: Intact, uninflamed pulp
    2. Transitional Stage: Intact pulp with scattered inflammatory cells
    3. Pulpitis: inflammatory process affecting the coronal and radicular
    4. Pulp Necrosis: All pulp tissue is necrotic

STUDY ENROLLMENT AND WITHDRAWAL

1.6 Subject Inclusion Criteria

In order to be eligible to participate in this study, you must meet the following criteria:

1. Must be a patient of the University of Michigan School of Dentistry Oral Surgery Clinics
2. Must be scheduled for at least one tooth extraction at Oral Surgery Clinic
3. Must be 18 to 65 years old
4. Must be able to understand and willing to cooperate with all study procedures
5. Must be able to sign an IRB-approved written consent
6. Must have at least two teeth with an intact crown, without any full metallic crown coverage, and/or previous root canal treatments
7. Must have no history of spontaneous pain, and/or lingering pain to cold/hot in teeth that are not going to be extracted on that day.
8. If one of the teeth mentioned in inclusion #6 is being extracted, be willing to give your extracted tooth/teeth for further analysis to determine of the tooth is “alive or dead”

1.7 Subject Exclusion Criteria

An individual who meets any of the following criteria will be excluded from participation in this study:

- If participant self-reports liver, kidney, heart, blood, metabolic or systemic disease which may make execution of the protocol or interpretation of the results difficult
- If participant has severe physical impairments (e.g., complete blindness or deafness) and/or cognitive impairments (e.g., dementia) that precludes participation in the procedures outlined in this proposal
- If participant is or could be pregnant
- If participant is currently receiving radiation
- If participant not able to hold mouth open for extended period of time

1.8 Strategies for Recruitment and Retention

Recruitment Process:

Subject candidates will be identified by screening clinic schedules for extraction treatments and reviewing the subject candidate’s dental records as prescreening activities. Subject Withdrawal

1.8.1 Reasons for Withdrawal

Subject participation is strictly voluntary and there is no treatment associated with the study; therefore, a subject may withdraw from further participation in the study without penalty or harm. Any reason(s) the subject may give for terminating his or her participation will be kept

confidential. No further information will be required of the subject and the subject will be compensated for their participation undergone prior to termination.

Study personnel will be authorized to release a subject from further study participation if:

- The researcher believes that it is not in the subject's best interest to stay in the study.
- The subject becomes ineligible to participate.
- The subject's condition changes such that he or she needs treatment that is not allowed while taking part in the study.
- The subject does not follow instructions from the researchers.
- The study is suspended or canceled.

1.8.2 Handling of Subject Withdrawals

No further information will be required of the subject. Clinical data collected prior to the point of withdrawal will be used in the study. Subjects who do not complete the study will be replaced.

1.9 Premature Termination or Suspension of Study

This study may be suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party. If the study is prematurely terminated or suspended, the principal investigator will promptly inform the IRB and will provide the reason(s) for suspension or termination.

Circumstances that may warrant termination include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to subjects
- Insufficient adherence to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination of futility

STUDY SCHEDULE

1.10 Screening

Subject candidates will be identified by screening clinic schedules for dental extraction treatments and reviewing the subject candidate's dental records. Patients that appear to qualify during the pre-screening process will be contacted directly in person on the day of their regular extraction consult appointment.

1.11 Enrollment/Baseline (Day 0, visit 1)

Subject candidates will be approached at the time when they present to the dental clinic for the consultation prior to their dental extraction. Consent will be obtained when patient showed interest in participation in the study.

1.12 Study Visit (Procedure Appointment)

Study visit will take approximately 30-45 minutes. This visit includes completing study related procedures including completing the questionnaire by the participant, and performing vitality testings' including cold, EPT, percussion, palpation and pulse oximeter recordings.

1.13 Withdrawal Visit

There are many reasons why the researchers may need to end an individual's participation in the study. Some examples are:

- ✓ The researcher believes that it is not in your best interest to stay in the study.
- ✓ You become ineligible to participate.
- ✓ You do not follow instructions from the researchers.
- ✓ The study is suspended or canceled.

STUDY PROCEDURES/EVALUATIONS:

1.14 Study Procedures/Evaluations

Study procedures will be evaluated continuously throughout this study to evaluate adherence to the protocol.

1.15 Laboratory Procedures/Evaluations

Upon completion of extraction, the teeth will be placed in formalin-containing bottle. After formalin fixation, the teeth will be prepared for histological examination using 10% EDTA for demineralization. Following that, the teeth are trimmed, embedded in paraplast and cut longitudinally to obtain 6um thick section. The sections were stained with H&E and examined with a light microscope. The histological diagnosis are based on the classification used by Seltzer et al. as follow:

1. Normal Pulp: Intact, uninflamed pulp
2. Transitional Stage: Intact pulp with scattered inflammatory cells
3. Pulpitis: inflammatory process affecting the coronal and radicular
4. Pulp Necrosis: All pulp tissue is necrotic

1.16 Study Specific Biospecimens

1.16.1 Specimen Collection Procedures

After the study related procedures are completed, dental care is transferred to the Oral Surgeon

1.16.2 Specimen Preparation, Handling, and Storage

Extracted teeth will be collected in “biohazard specimen transfer bag” and labels with their specific ID number. They will then be transferred from oral surgery on the same day to the pathology laboratory at the school of dentistry for histology.

1.16.3 Specimen Shipment

There is no shipment required for this study. All the specimen histological evaluation will be done at the dental school.

1.17 Questionnaire Administration

History of dental and orofacial pain will be collected in person usingvalidated questionnaires;

1. DePaQ (Validated Dental Pain Questionnaire)
2. Fibromyalgia Survey Questionnaire (FSQ)

ASSESSMENT OF SAFETY

1.18 Specification of Safety Parameters

Safety monitoring for this study will focus on unanticipated problems involving risks to participants, including unanticipated problems that meet the definition of an adverse event.

1.18.1 Unanticipated Problems

The Office for Human Research Protections (OHRP) considers unanticipated problems involving risks to subjects or others to include, in general, any incident, experience, or outcome that meets **all** of the following criteria:

7. unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied;
8. related or possibly related to participation in the research (possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
9. suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

1.18.2 Serious Adverse Events

An adverse event (AE) is defined as: "Any untoward or unfavorable medical occurrence in a human subject, including any abnormal sign, symptom, or disease, temporarily associated with the subject's participation in the research, whether or not considered related to the subject's participation in the research." AE reporting is explicit to any AEs with a causal relation to the study procedure (pulp testing, use of pulse oximeter). Any complications due to the standard of care extraction procedure will not be reported as these AEs are not as a result of a casual relation to the study procedure. Only AEs that occur during the investigational device testing would be reported. An adverse finding can include a significant change in baseline symptoms, abnormal assessments or any combination of these.

Examples of AEs are as follows:

- Changes in the general condition of the patient
- Subjective symptoms offered by or elicited from the patient
- Objective signs observed by the Investigator or other study personnel
- All concurrent diseases that occur after the start of the study, including any change in severity or frequency of preexisting dental pain.

Any participant that has an adverse event will be followed weekly until either the event has resolved or the event is considered stable by the participant and the research team. Adverse events that persist for an extended duration (such as over the duration of the study) will also be followed weekly until either the event is resolved or considered stable by the PI and the research participant.

1.19 Reporting Procedures

All adverse events, regardless of attribution, will be recorded by the Principal Investigator (PI) and/or a study team member in the patient's research file using the AE CRF. In addition, AEs will be tracked in aggregate using the AE tracking log. This log will also document corrective actions required and attribute severity, relatedness and expectedness.

The PI will review and acknowledge (by signing the CRF) all recorded adverse events. The U-M IRB will receive at least an annual summary of all AEs in aggregate. AEs will be reported within 7-days of the occurrence notification. Unanticipated problems or privacy violations/breach of confidentiality will be treated as serious and also reported within 7-days of occurrence to the appropriate oversight body.

STUDY OVERSIGHT

The PI retains primary responsibility for oversight. In addition, the Institutional Review Board of the University of Michigan will review and approve the protocol, study procedures and continuing reviews.

Study staff will permit authorized representatives and regulatory agencies to examine (and when required by applicable law, to copy) research records for the purposes of quality assurance reviews, audits, and evaluation of the study safety, progress and data validity.

CLINICAL SITE MONITORING

Because the study meets IRB approval, it is continually reviewed by IRB. This study is cross sectional and does not involve administration of any clinical treatments. The study involves no more than minimal risk to participants. For these reasons, it is our belief that additional monitoring of the site is not necessary.

STATISTICAL CONSIDERATIONS

1.20 Study Hypotheses

The calculations are based on a comparison of the test results and "true" disease status. The sensitivity, specificity, and positive and negative predictive values of heat and cold application and electrical pulp tests will be calculated. Teeth are grouped into two categories, healthy, true disease (irreversible pulpitis and necrosis). A perfect diagnostic test would always be positive in the presence of disease and negative in the absence of disease. Therefore, sensitivity, specificity, and positive and negative predictive value have been developed to characterize test accuracy and to compute the benefits of test usage. In addition to that, clinical measurements of the extracted teeth will be compared with identification of the true disease by histological evaluation for the pulp status.

1.21 Sample Size Considerations

35 completer in total

1.22 Final Analysis Plan

To compare accuracy of pulp vitality between traditional tooth vitality testing (Cold and EPT) to pulse oximeter reading to determine if the pulse oximeter is a reliable indicator of tooth vitality. We will analyze the relationships between these clinical readings using Pearson correlations and/or Chi Square tests. We will evaluate repeatability of the measurements within a tooth for the pulse oximeter using Bland-Altman plots, intraclass correlation coefficients (ICCs), and the percentage of repeat measurements within +/- 0.5 mm along with a 95% confidence interval for the percentage. Pulse oximeter reading will be compared against the gold standard (Cold and EPT) assessment using paired t-tests, 95% confidence intervals for the difference from the gold standard with a 95% confidence interval. The clinical pulpal status will be compared to the histological status for the difference using ANOVA and compared for the percentage of measurements with +/- gold standard visual assessment of the pulp status using McNemar's tests.

SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA/DOCUMENTS

Study staff will maintain appropriate dental and research records for this study. Study staff will permit authorized representative and regulatory agencies to examine (and when required by applicable law, to copy) research records for the purposes of quality assurance reviews, audits and evaluation of the study safety, progress and data validity.

QUALITY CONTROL AND QUALITY ASSURANCE

Quality control will be continually monitored by periodic (semi-annual) reviews of the protocol and procedures used in the study. All questionnaire data is reviewed on the day of the participant visit for completeness. De-identified data is monitored after collection for various points of quality assurance.

ETHICS/PROTECTION OF HUMAN SUBJECTS:

1.23 Ethical Standard

The investigator will ensure that this study is conducted in full conformity with the principles set forth in The Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research, as drafted by the US National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research (April 18, 1979) and codified in 45 CFR Part 46 and by the Institutional Review Board requirements of the University of Michigan.

1.24 Institutional Review Board

The protocol, informed consent form(s), recruitment materials and all participant materials have been submitted to the IRB for review and approval. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented in the study. Please see accompanying IRB application and letters of approval, and Informed Consent document.

1.25 Informed Consent Process

Informed consent is a process that is initiated prior to the individual agreeing to participate in the study and continues throughout study participation. Extensive discussion of risks and possible benefits of study participation will be provided to participants and their families, if applicable. A consent form describing in detail the study procedures and risks will be given to the participant. Consent forms will be IRB-approved, and the participant is required to read and review the document or have the document read to him or her. The principle investigator will explain the research study to the participant and answer any questions that may arise. The participant will sign the informed consent document prior to any study-related assessments or procedures. Participants will be given the opportunity to discuss the study with their surrogates or think about it prior to agreeing to participate. They may withdraw consent at any time throughout the course of the study. A copy of the signed informed consent document will be given to participants for their records. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their clinical care will not be adversely affected if they decline to participate in this study.

The consent process will be documented in the clinical or research record.

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

Children below the age of 18 and cognitively impaired adults are excluded from the current study.

1.27 Participant Confidentiality

Participant confidentiality is strictly held in trust by the investigators, and study staff. This confidentiality is extended to cover testing of biological samples in addition to any study information relating to participants. The study protocol, documentation, data, and all other information generated will be held in strict confidence.

1.28 Future Use of Stored Specimens and Other Identifiable Data

The de-identified extracted teeth will be transferred for histological evaluation on the same day to the pathology laboratory at the school of dentistry.

DATA HANDLING AND RECORD KEEPING

Several measures have been taken to reduce the risk of breach of confidentiality. These include training of study team members, electronic and physical security measures for data capture and storage, and collecting a minimum of identifiable information for each individual. Breach of confidentiality will be considered a "definitely related" Serious Adverse Event. As such, it will be reported to the University of Michigan IRB within 7 days of occurrence, and a remediation plan will be put in place immediately.

1.29 Data Management Responsibilities

Data collection and accurate documentation are the responsibility of the principal investigator and study staff under the supervision of the principal investigator. All source documents and laboratory reports must be reviewed by the principal investigator and/or the study team and/or data entry staff, who will ensure that they are accurate and complete. Unanticipated problems must be reviewed by the investigator or designee.

1.30 Data Capture Methods

Data will be kept confidential, in part, by assigning each participant an ID number. This number will be used to keep track of each individual's data. Participant names will never be collected or stored with research data. Paper copies of the data from questionnaires and radiographic copy of each participant will be stored in a locked file cabinet in a locked office. Paper copies of the consent document and the payment processing forms (i.e. those containing subject names) will be stored in a separate locked filing cabinet away from the data. The PI (or a designee) will retain the keys to access these files.

Participants will complete paper-based questionnaires; no electronic survey completion will occur. Data will be verified and checked for completeness and initialed by the principle investigator prior to the completion of the visit. All data will be entered into a research database, REDCap and/or excel.

Study data will be collected and may be managed using an electronic data capture tools hosted at University of Michigan. REDCap (Research Electronic Data Capture) is a secure, web-based application designed to support data capture for research studies, providing: 1) an intuitive interface for validated data entry; 2) audit trails for tracking data manipulation and export procedures; 3) automated export procedures for seamless data downloads to common statistical packages; and 4) procedures for importing data from external sources.

1.31 Types of Data

Outcome measure data of cold testing, EPT, percussion, palpation testing and pulse oximeter reading will be collected in the study. Extracted teeth that meet the inclusion criteria will be also collected for histological evaluation.

1.32 Schedule and Content of Reports

De-identification of data occurs with the generation of study documents, including the assignment of a unique research ID to each participant's data.

1.33 Study Records Retention

Study records will be maintained for ten years from the date of the study is completed.

1.34 Protocol Deviations

A protocol deviation is any noncompliance with the clinical study protocol, Good Clinical Practice, or Manual of Procedures requirements. The noncompliance may be on the part of the subject, the investigator, or study staff. As a result of deviations, corrective actions are to be developed by the study staff and implemented promptly.

These practices are consistent with investigator obligations in::

- Compliance with Protocol, Sections 4.5.1, 4.5.2, 4.5.3, and 4.5.4.
- Quality Assurance and Quality Control, Section 5.1.1
- Noncompliance, Sections 5.20.1 and 5.20.2.

All deviations from the protocol must be addressed in study subject source documents and promptly reported the University of Michigan IRB, according to their requirements.

PUBLICATION

This study will be published in a peer-reviewed scientific journal after study completion.

LITERATURE REFERENCES

Alghaithy, R. A. and A. J. Qualtrough (2017). "Pulp sensibility and vitality tests for diagnosing pulpal health in permanent teeth: a critical review." Int Endod J **50**(2): 135-142.
Anusha, B., K. Madhusudhana, S. K. Chinni and Y. Paramesh (2017). "Assessment of Pulp Oxygen Saturation Levels by Pulse Oximetry for Pulpal Diseases -A Diagnostic Study." J Clin Diagn Res **11**(9): Zc36-zc39.
Fanibunda, K. B. (1986). "Diagnosis of tooth vitality by crown surface temperature measurement: a clinical evaluation." J Dent **14**(4): 160-164.
Fanibunda, K. B. (1986). "The feasibility of temperature measurement as a diagnostic procedure in human teeth." J Dent **14**(3): 126-129.
Fratkin, R. D., D. J. Kenny and D. H. Johnston (1999). "Evaluation of a laser Doppler flowmeter to assess blood flow in human primary incisor teeth." Pediatr Dent **21**(1): 53-56.
Gopikrishna, V., K. Tinagupta and D. Kandaswamy (2007). "Comparison of electrical, thermal, and pulse oximetry methods for assessing pulp vitality in recently traumatized teeth." J Endod **33**(5): 531-535.
Gutmann, J. L. (2011). "Diagnostic pulp testing in dentistry: how far have we come?" J Hist Dent **59**(3): 126-128.
Jafarzadeh, H. (2009). "Laser Doppler flowmetry in endodontics: a review." Int Endod J **42**(6): 476-490.
Massaro, M., L. Ronfani, G. Ferrara, L. Badina, R. Giorgi, F. D'Osualdo, A. Taddio and E. Barbi (2014). "A comparison of three scales for measuring pain in children with cognitive impairment." Acta Paediatr **103**(11): e495-500.
Oberlander, T. F., M. E. O'Donnell and C. J. Montgomery (1999). "Pain in children with significant neurological impairment." J Dev Behav Pediatr **20**(4): 235-243.
Radhakrishnan, S., A. K. Munshi and A. M. Hegde (2002). "Pulse oximetry: a diagnostic instrument in pulpal vitality testing." J Clin Pediatr Dent **26**(2): 141-145.
Schnettler, J. M. and J. A. Wallace (1991). "Pulse oximetry as a diagnostic tool of pulpal vitality." J Endod **17**(10): 488-490.

QUESTIONNAIRES

1. Where in the mouth and/or face region do you feel the pain you currently have? (You may tick more than 1 answer)	1	tooth/teeth (1)	☐
	2	gums (1)	☐
	3	tongue (0)	☐
	4	palate (0)	☐
	5	floor of mouth (0)	☐
	6	inside of cheek (0)	☐
	7	jaw (0)	☐
	8	jaw joint (0)	☐
	9	others (please specify: (0))	☐

2. How long have you had your current pain?	1	less than 1 week (1)	☐
	2	1 week or longer, but less than 4 weeks (2)	☐
	3	4 weeks or longer, but less than 6 months (3)	☐
	4	6 months or longer, but less than 1 year (4)	☐
	5	1 year or longer (5)	☐

3. How would you describe the intensity of your current pain AT ITS WORST?	1	Mild (1)	☐
	2	Discomforting (2)	☐
	3	Distressing (3)	☐
	4	Horrible (4)	☐
	5	Excruciating (5)	☐

4. Thinking about your current pain, how would you describe its pattern of occurrence?	1	Episodic: It comes and goes (1)	☐
	2	Continuous: It's constant (2)	☐

	Not at all (1)	A small extent (2)	Moderate extent (3)	A large extent (4)	Complete extent (5)
5. Please indicate the extent to which your pain radiates to the surrounding area:	☐	☐	☐	☐	☐

	Complete extent (5)	A large extent (4)	Moderate extent (3)	A small extent (2)	Not at all (1)
6. Please indicate the extent to which it is worse when you chew or eat on the side of your mouth with the pain:	☐	☐	☐	☐	☐

	Makes it a lot more painful (5)	Makes it a little more painful (4)	No effect (3)	Makes it a little better (2)	Makes it a lot better (1)
7. Please indicate the effect of eating or drinking something COLD:	☐	☐	☐	☐	☐
Please indicate the extent to which	**Not at all (1)**	**A small extent (2)**	**Moderate extent (3)**	**A large extent (4)**	**Complete extent (5)**
8. your gums have been swollen now or have been swollen recently:	☐	☐	☐	☐	☐
9. the tooth where you have the pain from feels loose:	☐	☐	☐	☐	☐
10. it is difficult to swallow now or has been difficult to swallow recently:	☐	☐	☐	☐	☐

Please indicate the extent to which	Not at all (1)	A small extent (2)	Moderate extent (3)	A large extent (4)	Complete extent (5)
11. the tooth where you have the pain from feels like it is sticking out a little:	☐	☐	☐	☐	☐

	Full extent (5)	A large extent (4)	Moderate extent (3)	A small extent (2)	Not at all (1)
12. Please indicate the extent to which you have had difficulties with sleeping:	☐	☐	☐	☐	☐

13. Which of the following word(s), if any, would you use to describe your current pain?	Yes (1)	No (0)
Exhausting	☐	☐
Electric shocks	☐	☐
Pulling	☐	☐
Numb	☐	☐

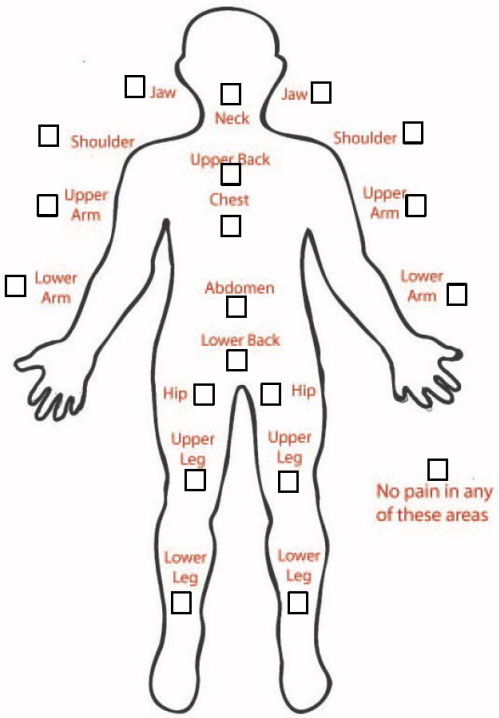
14. What other word(s), if any, would you use to describe your current pain? Please write in the space below:

Subject ID #: _____
Visit#: _____
Visit Date: _____

Fibromyalgia Survey Questionnaire (FSQ)

1. Please indicate below if you have had pain or tenderness over the past 7 days in each of the areas listed below.

Please make an X if you have had pain or tenderness. Be sure to mark both right and left sides separately.



2. Using the following scale, indicate for each item the level of severity over the past week by checking the appropriate box.

	No Problem	Slight or mild	Moderate	Severe
a. Fatigue	☐0	☐1	☐2	☐3
b. Trouble thinking or remembering	☐0	☐1	☐2	☐3
c. Waking up tired (unrefreshed)	☐0	☐1	☐2	☐3

3. During the past 6 months have you had any of the following symptoms?

a. Pain or cramps in lower abdomen	☐1 Yes	☐0 No
b. Depression	☐1 Yes	☐0 No
c. Headache	☐1 Yes	☐0 No

4. Overall, were the symptoms listed in questions 1-3 above, generally present for at least 3 months?

☐1 Yes ☐0 No

5. Do you have a disorder that would otherwise explain the pain?

☐1 Yes ☐0 No

FORMS

Assessment of Tooth Vitality Using Pulse Oximeter	MEASUREMENT READING	ID Number	FORM 2
	Date:		Examiner:

SPECIMENS	
How many teeth are selected?	
How many of them are treatment planned for extraction?	

MEASURMENTS		
Tooth #:	**LOCATION**	**READINGS**
Endo Ice (Cold)		
EPT		
Pulse Ox		
Laser Doppler Flowmeter		
Percussion		
Palpation		

MEASURMENTS		
Tooth #	**LOCATION**	**READING**
Endo Ice (Cold)		
EPT		
Pulse Ox		

Laser Doppler Flowmeter		
Percussion		
Palpation		

MEASURMENTS		
Tooth #	**LOCATION**	**READING**
Endo Ice (Cold)		
EPT		
Pulse Ox		
Laser Doppler Flowmeter		
Percussion		
Palpation		

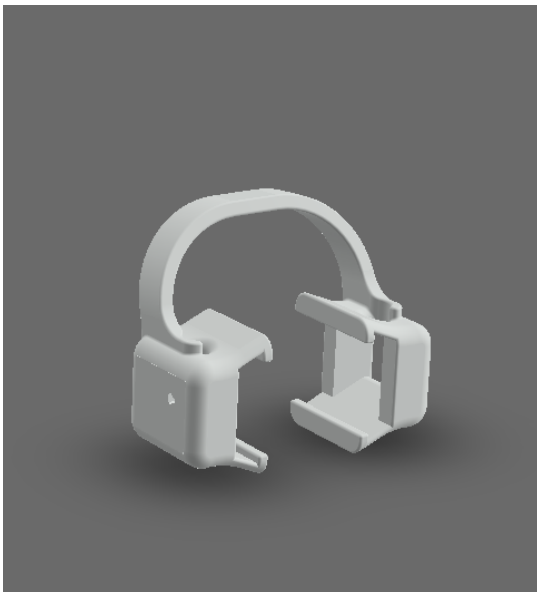
MEASURMENTS		
Tooth #	**LOCATION**	**READING**
Endo Ice (Cold)		
EPT		
Pulse Ox		
Laser Doppler Flowmeter		
Percussion		
Palpation		

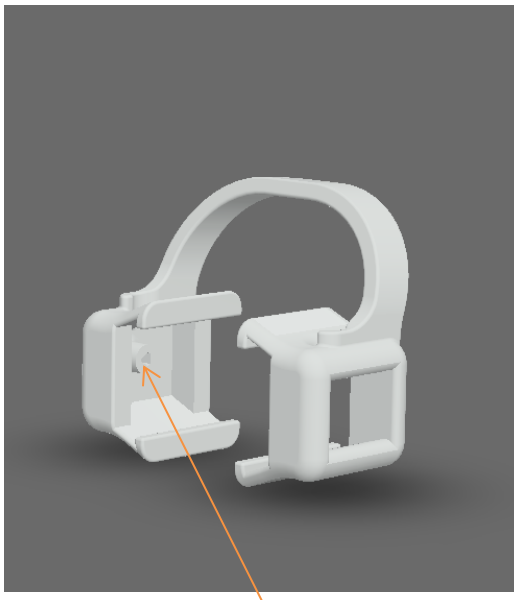
Assessment of Tooth Vitality Using Pulse Oximeter	Documentation of Specimens Collection	ID Number	FORM 3
	Date:		Name of Person Recording the Specimen:

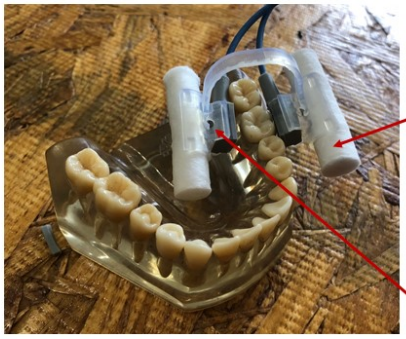
SPECIMENS		
Extracted tooth/teeth was/were collected	YES	NO
How many teeth were extracted?		
IF NOT collected, why?		
Date of tooth collection		
Are the extracted tooth/teeth placed in formalin-containing bottle?	YES	NO
Are the extracted tooth/teeth transferred to the School of Dentistry laboratory for further analysis?	YES	NO

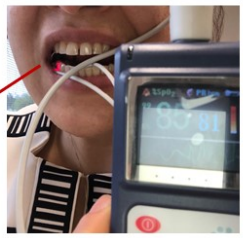
ADVERSE EVENTS		
Have there been any adverse events? **(if "Yes" complete AE form 2 and provide details in "Comments" section)**	YES	NO

COMMENTS




LDF probe holder


Cotton Roll
Perforation/Hole


Intraoral placement