Persons using assistive technology may not be able to fully access information in this file. For assistance, e-mail AgingResearchBiobank@imsweb.com. Include the website and filename in your message.

Quantitative Sensory Testing

TABLE OF CONTENTS

1. Background and rationale

In MOST, we will assess measures related to sensory testing. Individuals experience differing levels of pain for a given injury or degree of inflammation. Changes in pain pathways in response to tissue injury or inflammation can account for such differences. To develop rational approaches to the prevention or management of pain among persons with knee osteoarthritis (OA) requires an understanding of the mechanisms that underlie abnormal pain sensitivity. In the prior MOST Study, we found that pain sensitization was associated with pain severity and with certain MRI features of OA. With this MOST renewal, we will now have the opportunity to assess additional quantitative sensory testing measures to provide insights into transitions in pain severity, and from acute to chronic pain.

We will first screen participants for peripheral neuropathy. We will assess temporal summation (a measure of central sensitization) using a punctate probe set. We will then assess pressure pain threshold using a pressure algometer as the primary measure of pain sensitivity. We will assess pressure pain threshold at the knee (patella) as a measure of peripheral sensitization, and at the wrist (a site remote from the knee which itself is not affected by OA typically), as a measure of an individual's underlying predisposition to pain and/or central sensitization. We will also evaluate descending pain pathways through assessment of conditioned pain modulation.

2. Equipment and supplies

- von Frey monofilament, 10g (to screen for peripheral neuropathy)
- Punctate probe set (from Mike Young, UNC): 7 probes in each set
    - Ideally, each site should have 2 full probe sets and one additional set of preloaded plastic tips, in addition to extra wires for replacements
- Pressure Algometer (FDIX25 digital algometer)
- 2 Small rectangular bean bags (e.g., AliMed Inc., Reorder # 95-506) or a rolled up washcloth (covered by paper towel or table paper)
- Blood pressure cuff
- Stress ball (with MOST logo)
- Stop watch (+/- metronome or metronome app on smart phone set to 60 beats per minute, with no time signature or 1/4 time)
- Magic marker (black) or other appropriate marking tool
- Disposable shorts or examination gown (if participant forgets to bring shorts)
- Examination gloves
- Examination table

2.1 Service and maintenance

2.1.1 von Frey filaments

Log the date that the new filaments are first used and mark expiration date (6 months from when received) on filaments. Discard filaments when they are 6 months old and replace with new ones. Log the date that the filaments are replaced. Prior to each use visually monitor for bent or broken filaments.

2.1.2 Punctate probe set

Visually monitor for bent or broken filaments prior to each use. Replace as needed following detailed instructions in Appendix 1.

Equipment contact information:
University of North Carolina at Chapel Hill
School of Dentistry, CPRI Electronics Shop

2.1.3 Pressure algometer

Contact information:
Wagner Instruments
P.O. Box 1217, Greenwich, CT, 06836-1217
Tel: 1-800-345-4188; 1-203-698-9681
sales@wagenerinstruments.com

Contact person: Bill Wagner

2.2 Software

None.

2.3 Calibration

2.3.1 Calibration of algometer against certified weights

The algometer should be calibrated monthly against a balance beam scale using certified weights of 10 and 25 pounds.

At UAB clinical center:
The certified weights should be placed on the scale and the scale balanced to assure the scales' calibration for both weights. The scale should then be set at each of the two weights, starting with 5 pounds. One technician should press down onto the scale with the rubber pad of the algometer, keeping the device vertical and with peak hold on. The other technician should watch the balance beam and let the technician with the algometer know when balance is achieved. The reading on the algometer should fall within +/- .5 pounds for the 10 pound weight, and +/- 1

pound for the 25 pound weight. This procedure should be repeated for each of the other two weights and the results recorded on the MOST Algometer Calibration Log.

At U-Iowa clinical center:
Follow instructions under UAB, except U-Iowa examiners will use a calibrated balance beam scale, so the first step of calibrating the scale with the certified weights is not necessary. (The balance beam scale must be calibrated annually by a certified Weights and Measures Department and monthly following the instructions for calibrating for linearity under section 2.2 of Operations Manual, Chapter 3S, Weight.)

2.3.2 Examiner monthly algometer calibration

Examiners performing pain sensitivity testing must be tested on a monthly basis to insure that they are applying algometer pressure at a consistent rate of 5 kg at 10 seconds +/- 1 second and 7 kg at 14 seconds +/- 1 second. The MOST Algometer Monthly Examiner Calibration Log (located on the MOST study website under Documents & Forms > Equipment) must be completed each time examiner calibration is completed.

The instructions/log is also posted on the MOST study website under Documents & Forms > Equipment.

3. Safety issues and exclusions

3.1 Safety issues

Although rare, there is the potential for skin irritation and redness or bruising during testing. Bruising could potentially result from application of the pressure algometer during pressure pain threshold testing. Although very rare, skin puncture with temporal summation probe could potentially occur (in ongoing study of rheumatoid arthritis with older adults on prednisone, we have not had any skin breakage issues); this can be minimized by slighting varying the location of applying the probe on the skin during temporal summation testing.

3.2 Exclusions and determination of anatomic sites to use/test

3.2.1 Testing at the knee

If the participant has had unilateral amputation of the leg above the knee, test the native limb. If both legs have been amputated above the knee, test the wrist only. If the participant has had unilateral or bilateral knee replacements within the past 3 months, do not test the patella of the knee on the limb(s) with the replacement; only the tibial tuberosity of the replaced knee will be tested; the patella can be tested if the knee replacement was >3 months ago. Areas with open or healing skin wounds will also need to be excluded. Recent surgical scars (within the past 3 months) over the patella or tibial tuberosity would exclude those particular sites from testing. Healed skin wounds or healed surgical scars are *not* exclusions.

3.2.2 Testing at the wrist

Fracture evaluation
If the right wrist has been not been broken in the last 6 months, this wrist is eligible for full testing after further evaluation. If, however, it has been broken in the last 6 months, this wrist is not eligible for pressure pain threshold (PPT) testing and the left wrist should be evaluated. If the left wrist has not been broken in the last 6 months, then this wrist is eligible for full testing after further evaluation. If, however, the left wrist was also broken in the last 6 months, it is also not eligible for pressure pain threshold testing. Whichever wrist from the fracture evaluation is eligible for further evaluation should then be assessed for any other reasons as to why it cannot be tested. If both wrists were broken, then they also must next be evaluated for eligibility for the remaining pain sensitivity tests.

Evaluation of other exclusions for testing at the wrist
Next evaluate the wrists for any other reason they cannot be tested such as presence of cast, other irremovable item covering the skin, regular use of splint/brace, open or healing skin wounds. Healed skin wounds or healed surgical scars are *not* exclusions. If both wrists are eligible for further evaluation, then first evaluate the right wrist. If the right wrist is ineligible, then evaluate the left wrist. Whichever wrist is eligible will have the quantitative sensory testing performed, and if there were no exclusions due to prior wrist fracture, pressure pain threshold will also be performed. If both wrists are ineligible, then do not perform any of the tests (pressure pain threshold or other quantitative sensory testing measures) on the upper extremity.

Evaluation of exclusions for blood pressure measurement
Another factor in the determination of which wrist to use will be determined by which arm can have a blood pressure cuff inflated for conditioned pain modulation (CPM) assessment. Ideally, the left arm should be used for blood pressure cuff inflation to allow the right wrist to be used for this protocol. Please see detailed CPM exclusions in Section 4.1.6.

The arm-specific exclusions for blood pressure cuff inflation are:

- Lymphedema
- Takayasu's arteritis
- Arteriovenous fistula for hemodialysis

If the left arm meets any of these exclusions but the right arm does not have an exclusion, then the right arm must be used for blood pressure cuff inflation. ***This would mean that the LEFT wrist should be used for temporal summation, PPT and CPM. This is because the arm used for blood pressure cuff inflation MUST be contralateral to the wrist being tested.***

IMPORTANT NOTE: If the only arm that is eligible for blood pressure cuff inflation is ipsilateral to the only wrist that can be tested, then only perform temporal summation and PPT testing, as CPM cannot be performed.

4. Participant preparation

Testing should be performed once the participant has had an opportunity to rest quietly for 2 minutes.

Note that both knees will be tested, and that the distal radioulnar joint will serve as a reference site. Please refer to Section 3.2 for the exclusions to testing of these sites.

Before starting any procedure, and before performing anatomic landmarking, determine which knees are eligible for testing, and most importantly, which wrist is eligible for testing based on both the wrist exclusions and the blood pressure cuff exclusions. The wrist contralateral (i.e., opposite) to the arm to be used for blood pressure cuff inflation must be used for all of the procedures below. ***If both wrists and both arms are eligible, perform testing on right wrist (distal radioulnar joint) (with left arm to be used for blood pressure cuff inflation).***

Also determine whether subject is a MOST-SENS participant, and if so, which knee is the index knee (refer to Data from Prior Visits Report). The index knee should be specifically noted and consider marking the index knee with an additional 'dot' to identify it as the knee that will undergo the second PPT during the CPM procedure.

Anatomic sites for testing will need to be marked. The approximate center of the patella and distal radioulnar joint will all be identified with a black magic marker. To identify each landmark, please refer to Appendix 2. If not already marked, place an 'x' in the required areas to facilitate testing. For a MOST-SENS participant, consider additionally marking a 'dot' on the index knee to facilitate identification of the correct index knee for the second CPM during the CPM procedure.

4.1 Detailed measurement procedures

4.1.1 Peripheral neuropathy screen and quantitative sensory testing

Screening for peripheral neuropathy will take place using the 10g von Frey monofilament. Quantitative sensory testing will be assessed in a standardized manner using punctate probes to assess temporal summation, and an algometer to assess pressure pain threshold (PPT) and conditioned pain modulation (CPM). Temporal summation will only be assessed at the wrist (distal radioulnar joint). PPT will be assessed at the wrist (distal radioulnar joint) and knee (patella), while CPM and will only be assessed at the wrist (distal radioulnar joint) for all except for those who are MOST-SENS participants, who will also have CPM assessed at the index patella (i.e., the knee with knee replacement that occurred within the past 24 months).

4.1.2 Order of testing

1. Peripheral neuropathy screen with 10g von Frey monofilament
2. Temporal summation (2 trials)
3. PPT
4. CPM

These measures should generally not be painful, although there may be some transient discomfort for some. They may, however, evoke painful responses in the presence of, for example, peripheral or central sensitization.

4.1.3 General instructions

Equipment (see Section 2)

- One von Frey filament, strength 10 gram
- Pressure Algometer (FDIX25)
- Punctate Probe set (see additional equipment in section 2.1.2)
- Blood pressure cuff
- Stress ball
- Stop watch, metronome app (set to 60 beats per minute, 1/4 time or no time signature)

At the beginning of each day, place each of the instruments to be used on a small tray table, in order of use (e.g., 10 g monofilament, punctate probe set, pressure algometer, blood pressure cuff). All items that touch the skin (10 g von Frey monofilament, punctate probes, algometer tip) must be cleaned with alcohol after use on each participant. For the punctate probe set, only the wires can be routinely disinfected. This can be accomplished by placing the storage rack into a shallow pan of isopropyl alcohol (70% or higher) such that only the tips of the probes are immersed. Do not spray disinfectant onto the probes. DO NOT ALLOW LIQUID TO ENTER THE BODY OF THE PROBES. (Avoid corrosive chemicals like chlorine bleach. Corrosion and liquid residue inside the probes create friction and will result in inaccurate forces.) This cleaning procedure should take place between each participant assessment. Place the wire portion of the probes into the tray containing an appropriate level of alcohol. The volume needed should be pre-determined and measured in a graduated cylinder (i.e., determine level of fluid needed to just cover the wire tips, then measure that volume in a graduated cylinder, and mark the plastic tray with a horizontal line as a visual cue). The wire tips should sit in the tray with alcohol for 30-60 seconds. Then the tray should be removed from alcohol to allow the tips to air dry. Once air dry, return the probes to their upright position to avoid damaging the wire tips.

The pressure algometer should only be turned on when ready for use. At the end of the day, plug in the algometer to recharge it. If the algometer reads low battery, the device may be used while plugged in.

Participant positioning

The participant should wear shorts and a shirt that allows the wrist to be exposed as pressure from rolled up garments (pant leg or sleeve) will interfere with the testing. Have the participant lie in a supine position on an exam table with the head of the table elevated to about 25 to 30 degrees. The legs should be extended, with one to two pillows as support under the knees as needed. The support underneath the knee must be firm enough so as to not allow the knee to sink into the support when applying pressure with the algometer. The wrist that is being tested should be comfortably resting on a side table, with palm facing down. The side table height should be adjusted such that the forearm is resting comfortably on it. Be sure that the side table is at a secure height and won't move down with pressure. The arm being tested should be abducted

slightly (no more than 30°). The forearm from the wrist proximally should be supported by a small rectangular beanbag (e.g., AliMed Inc., Reorder # 95-506; note that a rice bag should not be used since rice can be displaced causing forearm to sink), and a small beanbag or a rolled up washcloth (covered by paper towel or table paper) should be used underneath the palm such that the wrist is flat on the support and the fingers are comfortably positioned. Ensure the forearm is positioned on the table such that the examiner has sufficient room to place his/her forearm on the table for support without touching the participant's skin. The participant will be asked to have their eyes closed during each testing procedure after first having the demonstration of what the instrument looks like, what you will be doing with it, and for some, what it feels like on the dorsum of their right wrist with their eyes open.

The nature and the purpose of the tests should first be explained to the participant (see Script in Section 4.1.4).

4.1.4 Peripheral neuropathy screening with 10 gram monofilament

The peripheral neuropathy screening test is completed first before the other quantitative sensory tests.

For this examination the participant is lying on the examination table with their knees bent and the soles of their feet flat on the exam table.

The monofilament should initially be prestressed (4 to 6 perpendicular applications to the dorsum of the examiner's first finger).

Script: "We are first going to assess the sensation in your feet. Before doing this test in your feet, I'm going to touch the skin on the back of your wrist with this plastic bristle so you know what to expect." **(**Show how filament bends, then touch the filament to the back of the participant's hand.)

Script: "Now I'll touch you briefly with the bristle just above the big toe **while you have your eyes closed**." Please say 'Now' every time you feel the bristle touch your skin."

Apply the filament to the dorsum of the great toe midway between the nail fold and the DIP joint (See Appendix 3). Do not hold the toe directly during the test.

Test the right toe first. Briefly (<1 second) apply the filament 10 times perpendicularly with an even pressure. When the filament bends, the force of 10 grams has been applied. Wait several seconds between each touch, ***varying the time slightly***.

A correct response requires a 'Now' while the filament is touching the toe or just as it is being removed. Count the number of trials completed on each toe and the number of times the participant did not respond to the stimulus on the toe. Record the data on the data collection form.

Repeat the test on the left toe.

After testing both toes, tell the participant they did fine. Do not discuss their results until the quantitative sensory testing is complete. Record the results for each toe on the Participant Results Report.

4.1.5 Temporal summation using punctate probe set

Description
The set consists of 7 probes with flat-end wire tips of 0.2 mm (0.008 inches) diameter. Each probe has a different weight inside to provide the following approximate forces when applied to the skin in a vertical orientation:

1. 8 mN (~ 0.8 grams)
2. 16 mN (~ 1.6 grams)
3. 32 mN (~ 3.3 grams)
4. 64 mN (~ 6.5 grams)
5. 128 mN (~ 13 grams)
6. 256 mN (~ 26 grams)
7. 512 mN (~ 52 grams)

Delivery of stimuli
The examiner should be positioned comfortably in a seated position with his/her forearm supported by the table such that s/he is not touching the participant. The probe must be held vertically during stimulation to achieve accurate force delivery and to ensure that the weight does not become stuck. Ensure that the probe is not held at an angle, like a pen; the wire must touch the skin in an absolute vertical orientation. It is also important to bring the probe to the subject's skin slowly. To accomplish this, bring the tip of the wire to just a few millimeters above the skin before commencing to touch the skin. If the tip contacts the skin at appreciable velocity additional inertial force will be added to the intended gravitational force (and can cause the probe to bend/break). Once contact is made the probe body should be **slowly** lowered until the internal weight is being entirely supported by the subject (i.e., the barrel should move down a little) and hold for a moment before lifting up from the skin. Only raise the probe's wire a few millimeters above the skin to prevent inappropriate excessive velocity when applying the next touch. Do not allow the white plastic tip or the metal barrel touch the skin. The examiner should also not allow his/her forearm or hand to touch the participant during this procedure. If the subject moves during stimulation the delicate wire tip may become bent.

When applying the probe-tip on the skin, make sure that only the tip touches the skin gently, without jabbing. The probe must be vertically oriented in line with gravity for the proper weight to be applied. The examiner must assure that her/his arm is stabilized, so there will be no lateral movement of the probe-tip as it touches the skin, and to ensure that his/her arm does not touch the participant.

Procedure starting with probe #5 (*prior to September 2017, procedure started with probe #1):
After showing the participant the probe, the participant's eyes should be closed for this exam. Starting with probe #5 (128mN), touch the probe to the skin of the distal radioulnar joint. Ask the participant to rate his/her pain on a scale of 0-10. If the pain rating is <4/10, then move on to

the next probe and repeat. Continue the same procedure with each probe in succession until a pain rating of ≥4/10 is obtained. The full temporal summation test will be performed using the probe at which the participant provides the pain rating of ≥4/10. If none of probes #5-#7 provide a pain rating of ≥4/10, then use the highest weight for the temporal summation test (probe #7). Using the selected probe, wait 10 seconds before applying the train of 10 stimuli, one per second (1Hz) (use a metronome). Ensure that the probe slides down the punctate filament only part way (i.e., the neck of the pen should not touch the skin), and do not lift the filament more than 0.5 cm off the skin in between touches to avoid too much force when applying the touches. For the 10-second trials, vary the position slightly along the 'x' marking the site (but do not vary the rate). At the end of the train of 10 stimuli, ask the participant to rate their pain; repeat the pain rating question 15- and 30-seconds after the end of the trial. This whole procedure will then be repeated again (i.e., two trials).

***IMPORTANT NOTE*: Rule for going back to lower numbered probes:**

- If pain rating is >=8/10 with probe #5, then go to probe #4 and follow procedure above.
- If pain rating is still >=8/10 with probe #4, then go to probe #3 and follow procedure above.
- If pain rating is still >=8/10 with probe #3, then go to probe #2 and follow procedure above.
- If pain rating is still >=8/10 with probe #2, then go to probe #1 and follow procedure above.

General Introduction (please read to the participant)
Script: "The next set of tests will help us get a better understanding of what may cause pain in people with knee osteoarthritis. I'm going to be touching your wrists and knees with various devices while your eyes are closed, and will be asking if you experience any pain. We understand that people have different ideas of what pain or discomfort means to them. We want you to think about what pain means to you, and respond to our questions accordingly. Pain is a personal experience. There are no right or wrong answers. We want to know if what you feel is painful to you. If you do experience pain, I will ask you to rate this pain using this scale (optional: show QST Response Card #1) with which you are already familiar. Zero means no pain and 10 means the worst pain you can imagine."

All tests are performed with the eyes closed. However, the participant will have their eyes open when you are showing them the instrument and showing them what you are going to do with it on their wrist.

Script: "Now I'm going to touch the skin on your wrist with this probe (show item). You can see that the tip slides back into the barrel (turn pen upside down to show tip retract into barrel) when it touches your skin. I'm first going to touch your wrist once and then ask you to rate *any pain* you may have had on the 0-10 scale. I will then repeat the test with other probes. Please close your eyes. Ready?"

Touch the wrist (distal radioulnar joint) once with probe #5 and then say:
Script: "Please rate ***any pain you may have had*** at your wrist from this test."

If the pain rating was <4/10, repeat the above step with the next probe, and continue repeating with subsequent probes until a pain rating of ≥4/10 is reported (**Note:** when starting with probe #5, please refer to "Important Note: Rule for going back to lower numbered probes" above on page 10). Using the probe that provided the pain rating of ≥4/10 (or probe #7 if none of them produced a pain rating of ≥4/10), wait at least 10 seconds (about the time it takes to say the script) and then apply the appropriate probe repetitively on the skin at a rate of once per second (1Hz) for 10 seconds. The participant's eyes should be closed for this exam.

Script: "Now I'm going to touch your skin several times over 10 seconds. When I'm finished, I will ask you to rate the maximal pain you may have experienced during this test. I will also ask you to rate any pain again a few moments after the end of the test."

Start the stopwatch (and metronome if needed) at the beginning of the trial, using it to guide the rate and to time the total trial of 10 applications of the probe once per second (be sure to count 10 applications – the # of touches is the key stimulus here). Vary the position of the probe placement on the skin slightly so as not to damage the skin by touching the skin in exactly the same spot for all 10 applications. Allow the stopwatch to continue once the 10 applications is over to monitor the 15- and 30-second recovery period after the trial is completed (for timing of the final questions).

At the conclusion of the trial, ask the participant to rate their maximal pain they had during the test:

Script: "Please rate *the maximal pain you may have experienced* at your wrist from this test."

Script: "I will ask you again in a few seconds."

After 15 seconds post-completion of the trial has passed, say:
Script: "Please rate *any pain you may be experiencing right now* at your wrist."

Script: "I will ask you again in a few seconds."

After 30 seconds post-completion of the trial has passed, say:
Script: "Please rate *any pain you may be experiencing right now* at your wrist."

Script: "Now I am going to repeat this test."

Perform a second trial of the temporal summation assessment using the same probe as you did for trial #1 at the distal radioulnar joint approximately 0.5cm proximal to the original test site.

4.1.6 Pressure pain threshold and conditioned pain modulation

The pressure pain threshold (PPT) protocol is expanded to include conditioned pain modulation, which assesses the efficiency or functioning of descending pain inhibitory systems.

Equipment (See Section 2)

- Pressure Algometer (FDIX25)
- Blood pressure cuff
- Stress ball (with MOST logo)
- Stopwatch

As before, the pressure pain threshold will be performed at the distal radioulnar joint and the center of the patella. The order of the anatomic sites to be tested will be the knee first (***right*** patella followed by the ***left*** patella), and then the ***right*** *distal radioulnar joint* (or **left** wrist if right wrist is excluded).

Before having started the QST protocol, the examiner should have already determined eligibility for including the conditioned pain modulation (CPM) protocol with the PPT measurement, which is primarily related to blood pressure cuff inflation, and whether or not the subject is a MOST-SENS participant, and if so, which is the index knee to be tested with the CPM protocol. The CPM protocol requires blood pressure cuff inflation, preferably in the *left* arm; if there are exclusions for using the left arm, then the *right* arm can be used***. Please note: if the right arm must be used for blood pressure cuff inflation, then the LEFT wrist should be used for PPT.***

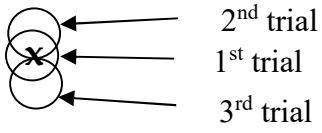
Exclusions for CPM

- By self-report:
  - MI within 30 days
  - Severe peripheral vascular disease
- Any exclusions that were relevant for blood pressure measurement (see Operations Manual, Chapter 3D, Blood Pressure)
- Exclusions for *left* arm for BP measurement (then use *right arm for blood pressure cuff inflation and left wrist for PPT and CPM*)
  - Lymphedema (e.g., post-mastectomy for breast cancer)
  - Takayasu's arteritis (regardless of disease activity) (because this disease results in pulselessness/poor blood flow due to arterial narrowing)
  - Arteriovenous fistula for hemodialysis

To prepare the algometer, ensure the arrows point to 'C,' to "Peak" (use Scroll/Peak button to set this), and to "kgf" (use Escape/Units button to set this). Hit 'Select/Zero' button in between readings (after having recorded the reading on the data collection form).

The instrument looks like this:

For each site to be tested, center the rubber tip over the 'x' that is marking the site to be tested for the 1st trial. For the 2nd trial, move the center of the rubber tip superiorly such that the bottom edge of the rubber tip is in the middle of the 1st circle. For the 3rd trial, move the center of the rubber tip inferiorly such that the top edge of the rubber tip is touching the bottom of the 2nd circle and is in the middle of the 1st circle:



For each site being tested, try to ensure that the examiners arms are supported (e.g., both elbows on the examining table) since 'free' arms are more prone to movement and it is more difficult to control the pressure algometer. This may require sitting on a chair, or standing across from the knee being tested. The device should be held with both hands on either side of the device, and thumbs on the top to provide adequate control and pressure.

For all measures, the participant should start by lying supine with their legs extended and right arm (or left arm if right arm can't be tested) resting comfortably on a flat surface of a table, with the elbow at 90 degrees.

Script: "For the last test I'm going to place this device on your wrist and your knees. During this test, pressure will gradually be applied. We are interested in learning the amount of pressure at which you *first* begin to experience slight pain. As soon as the pressure from the test first produces slight pain, say 'pain.' We are not interested in how long you can tolerate the pain, but rather when the pressure first becomes slightly painful."

Script: "Please tell me your understanding of what will occur during the test and what we'd like you to do."

(Wait for participant's response; reinforce the following script: "when pressure FIRST becomes slightly painful" as needed.)

Eyes remain open for this test.

Script: "Ok, I'm going to start at your right knee."

Apply the Pain Test FDIX25 Algometer at each test site: center of the patella of both knees, followed by the distal radioulnar joint of the eligible wrist (preferably right).

Place the tip of the algometer perpendicular to the skin, and apply steady and increasing pressure at a rate of 0.5 kg/sec starting at 0.

(Tell participant before starting each trial so that they are primed to start concentrating as soon as the pressure begins e.g., script: "I'm starting the first/second/third test now." After each test: script: "I'm just going to write that down.")
Record the pressure reading at the point at which the participant reports "pain" on the data collection form and remove the algometer from skin at that point. If 9 kg is reached on the algometer without the participant reporting "now," terminate the trial and record as 9.99 kg.

Repeat each measurement three times at each site.

Script: "I'll now repeat this at your left knee."

Repeat each measurement three times at each site.

Script: "I'll now repeat this at your [right/left] wrist."

Threshold levels at each anatomic site will be performed in the same order for each participant. Anatomical order* of exam is as follows:

1. Right side exam (participant supine):
   Patella

2. Left side exam (participant supine):
   Patella

3. Right side (participant sitting):
   Distal radioulnar joint (use left if right wrist not eligible, or if left arm not eligible for blood pressure cuff inflation)

*wrist is assessed last because the wrist will have CPM assessed

Conditioned Pain Modulation (CPM)
Immediately upon completing the PPT at the wrist, the CPM protocol will be initiated. Please note, prior to initiation of the Quantitative Sensory Testing protocol, it should have already been determined which arm is eligible for BP cuff inflation (preferably left arm), and whether or not the subject is a MOST-SENS subject. If it is a MOST-SENS subject, then the index knee needs to be identified (i.e., which knee had the knee replacement within the past 24 months) as this

index knee also needs to have PPT performed again; a 'dot' should have been placed on the index knee to identify it as the knee requiring the second PPT assessment with the CPM protocol.

Script: "We are now going to repeat the measurement at this wrist to see if your pressure threshold changes in response to inflating a blood pressure cuff on your arm and squeezing a soft ball with your hand. After I inflate the cuff, I will ask you to squeeze the ball 10 times. I will then ask you to rate any pain or discomfort you may have in your *forearm* on a scale of 0-10. I may ask you to repeat squeezing the soft ball until your level is ready for us to repeat the pressure threshold."

If the right wrist had temporal summation and PPT performed, then the left arm should be used for BP assessment. Elevate the index arm to chest level. Place the blood pressure cuff around the middle of the upper arm as for blood pressure measurement, with the lower edge of the cuff ~3cm proximal to the antecubital fossa. Inflate the cuff to 10 mmHg above the systolic BP (see Participant Results Report for blood pressure systolic measurement).

Start the timer set to 2 minutes. Ask the subject to squeeze the stress ball 10 times and then rate their pain on a 0-10 scale.

Script: "Please rate any pain or discomfort you may have in your forearm now on a 0-10 scale?"

- If pain is <4/10, ask subject to continue with another set of 5 hand exercises; repeat pain rating and hand exercies as needed until ii), iii), or iv) occurs.
- If pain ≥4/10, subject can discontinue hand exercises →go to PPT
- If pain is unbearable and subject requests cuff deflation →deflate cuff, go to PPT
- If cuff has been inflated for 2 minutes without pain ≥4/10 → PPT

Record number of hand squeezes required prior to proceeding to PPT. If the participant does more than 99 hand squeezes, record 99 on the form.

Record pain rating prior to proceeding to PPT.

Record PPT at the wrist (opposite to the arm with the blood pressure cuff inflated) [3 trials, as per the initial assessment]. Unless the pain is unbearable and the subject requests cuff deflation (see iii above), the cuff remains inflated on the participant's arm while the PPT is assessed.

For MOST-SENS subjects
The PPT will also be assessed at the index patella (knee that had a knee replacement within the prior 24 months). Again, unless the pain is unbearable and the subject requests cuff deflation (see iii above), the cuff remains inflated on the participant's arm while the PPT is assessed at the index patella.

Safety
- Cuff inflation to last no longer than 5 minutes
- Stopping rules for cuff inflation, whichever occurs first:

- Completion of PPT measurements;
- Upon subject request;
- Maximum of 5 minutes of cuff inflation (i.e., the maximal duration of the entire test)

5. Alert values, follow-up and reporting to participants

5.1 Peripheral neuropathy results and alert values

Peripheral neuropathy testing results:

- Normal: 8+/10 correct responses
- Reduced sensation: 1 to 7/10 correct responses
- Absent sensation: 0/10 correct responses

Participants with absent sensation in either toe should be told to contact their primary care provider regarding reduced sensitivity to touch in the big toe area of the body. Peripheral neuropathy results for each side (Normal; Reduced Sensation; or Absent sensation) will be documented on the Participant Results Report.

6. Quality assurance

6.1 Training and certification

"Master" examiners will train the clinic staff who will be administering the quantitative sensory tests. Clinic staff require no special qualifications or experience to perform this testing. Staff will be initially certified following the below certification requirements. The Pressure Pain Threshold exam will require a second 'master' certification by the 'master' examiner. Staff will be retrained and recertified midway through each examination cycle. Experience in musculoskeletal examinations is preferred but not required. Training should include:

- Read and study manual
- Attend MOST training session on techniques (or observe administration by experienced examiner)
- Practice on other staff or volunteers
- Discuss problems and questions with local expert or QC officer
- Suggestion: Use metronome for training temporal summation and pressure algometry

6.2 Certification requirements

- Complete training requirements
- Pass algometer calibration test
- (5 kg at 10 seconds +/- 1 second and 7 kg at 14 seconds +/- 1 second)
- Conduct exam on two volunteers:
    - According to protocol, as demonstrated by completed QC checklist

6.3 Quality assurance checklist

General preparation for tests

- ☐ Participant wearing shorts
- ☐ Participant's shoes and socks removed
- ☐ Participant positioned properly on examination table
- ☐ All anatomic landmarks found and marked properly

Exclusions

- ☐ All exclusions correctly assessed and documented

Peripheral neuropathy

☐	Participant's feet appropriately warm for testing
☐	Filament appropriately prestressed
☐	Correctly describes testing procedure
Right ☐ **Left** ☐	Participant's eyes closed during testing
Right ☐ **Left** ☐	Correctly applies filament during testing
Right ☐ **Left** ☐	Correctly records whether entire set was completed
Right ☐ **Left** ☐	Correctly records number of times participant did not feel the filament (stimulus)

Temporal summation

☐	Correct script used to introduce test trial on wrist
☐	Participant asked to rate pain at wrist
☐	If rating at wrist greater than 0, participant asked if painful
☐	Correct probe used with rating of ≥4/10 **OR** highest probe if none of the probes elicited pain rating ≥4/10
☐	Correct script used to introduce 10-second test
☐	Probe applied on the skin at a rate of once per second for 10 stimuli
☐	Participant asked to rate maximal pain at wrist at the end of 10 stimuli
☐	If maximal pain at wrist greater than 0, participant asked if painful
☐	Participant asked to rate current pain 15-seconds post-test at wrist
☐	If current pain greater than 0 at wrist, participant asked if painful

Pressure pain threshold

☐ Correct script used to introduce test

☐ Algometer applied to distal radioulnar joint until participant reports slight pain (x3)

Right ☐ **Left** ☐ Algometer applied to patella until participant reports slight pain (x3)

Conditioned pain modulation

☐ Correct script used to introduce test

☐ Blood pressure cuff applied correctly

☐ Correctly proceeded to PPT after required pain rating or time limit

☐ Algometer applied to wrist until participant reports slight pain (x3)

☐ Correct duration of cuff inflation

☐ Reviews form for completeness

☐ Correctly completes and submits form

7. Data collection form

Please see the Overview of the 144-month Follow-up Visit Operations Manual for an overview of the data collection forms, information on whether each form is in REDCap or TELEForm, and where the forms can be accessed on the study website.

Appendix 1 Wire and probe instructions for temporal summation test

1. Equipment needed <u>for new wire insertion or replacement</u>

- Magnifying glass
- Head lamp
- Wire cutters (see recommendations below)*
- Forceps (see recommendations below) **
- Grip pliers

* Wire cutters: Xcelite model 64CG but these may not be available anymore. Two current models that would probably work are the Xcelite MS54 or Xcelite MS54J. They are available at Amazon or at MSC Industrial Supply (mscdirect.com). The best wire cutters to use for trimming are the miniature type intended for electronics work. The large ones sold at hardware stores may mash the cut end of the wire so much that it will not fit into the hole.

** Forceps used for handling the wires should have broad flat tips, preferably without serrations. BU uses the Erem model EROP2ASA which is inexpensive and available at Amazon.

2. Preparation for first use

Probes are packed with the wires and wire-holders separated from the weights. This prevents damage to the fragile wires during shipping.

Prepare work area: Have a clean work surface on which a flat white piece of paper is taped to the table to ensure that there is no slippage. Remove any clutter from the work area.

Review **Figure 1** to familiarize oneself with the various parts and terminology used for the probe parts.

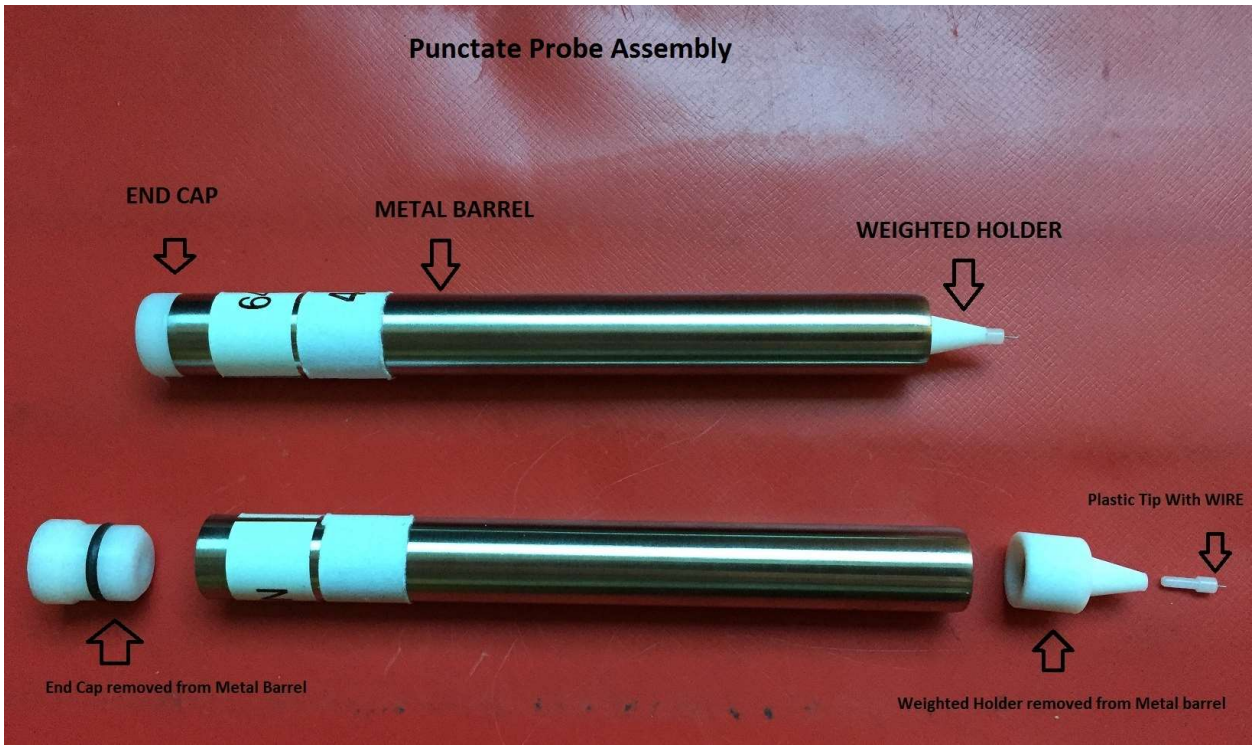


Figure 1 Punctate probe parts

Key terms (see labeled picture, **Figure 1**):

- End Cap
- Plastic tip
- Wire
- Weighted holder (each probe has a holder with a different weight)
- Metal barrel

Remove the end cap carefully with the grip pliers (or fingers if able). The cap is held in place by the friction of an O-ring. Apply the grip pliers to only the plastic cap; do not squeeze the metal barrel as it can affect the smooth movement of the weight inside. Remove cap with a twisting motion (do not try to simply pull). Carefully remove the weighted tip by sliding it out of the bottom of the barrel. Set it down on the flat work surface.

To insert a NEW plastic tip with wire, first use a spare plastic tip (that has no wire) to place into the plastic weighted holder. This is to ensure that the hole in the weighted holder has no defects. If the spare plastic tip fits easily, remove it and prepare to insert a plastic tip with wire (see below under ‘replacement of pre-loaded plastic tip with wire’). If the spare plastic tip cannot be inserted, contact Mike Young, University of North Carolina for a replacement weighted holder.

Carefully insert the wire/wire-holder assembly into the conical end of the weight. The wire-holder can be held with pliers or forceps if desired, but never use tools on delicate conical part of the weight. Reassemble the probe. Once assembled the probes should always be stored vertically in the modified test tube rack supplied with each set (Figure 2). Anytime a probe is held sideways or upside down it's possible that the weight may become stuck. Ensure that it moves freely before using on a participant.

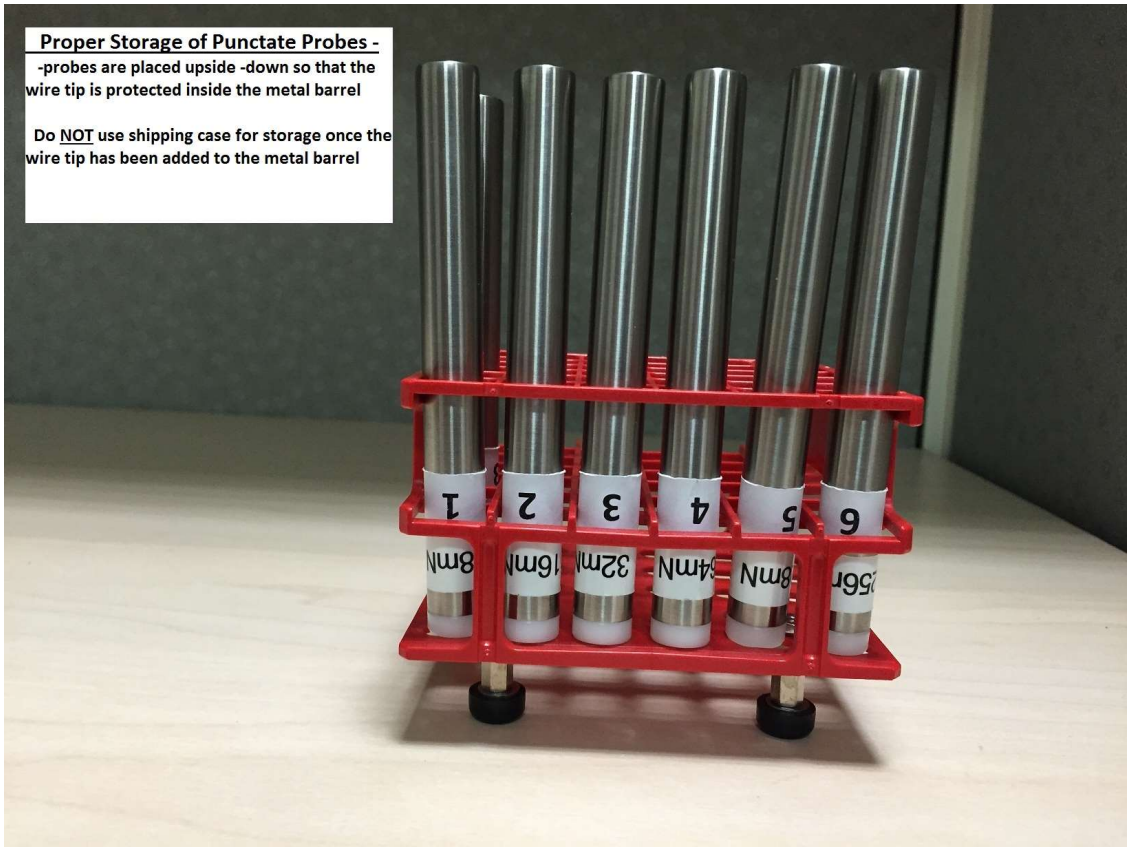


Figure 2 Probes stored vertically in rest tube rack provided

3. Replacement of bent wires

<u>For replacement of pre-loaded plastic tip with wire already in place</u>
Using the recommended forceps, with a gentle twisting motion, remove the plastic tip from the weighted holder. Set the plastic tip into the storage case for safe-keeping while replacing the wire.

Using the forceps, gently pick up a plastic tip with wire (preloaded) and place it into the hole of the weighted holder (which is sitting on the white paper). For probe #7, which has the largest weight, it may be more stable to do this step with the weighted holder sitting in the test tube rack. Once the bottom of the plastic tip has been inserted into the weighted holder, use the nails of your thumbs to symmetrically apply pressure to either side of the wire to firmly push the plastic tip the rest of the way into the weighted holder. Then place the weighted holder with wire back

into the metal barrel, and replace the plastic cap, ensuring it is pushed back into place. Be careful to not have the wire exposed outside of the barrel so as to not damage the wire. Store the probe upright in the test tube holder (i.e., so that the wire is not exposed). See **Figure 2.**

For replacement of the wires within a plastic tip

Once the plastic tip with damaged wire has been replaced as outlined above (under 'replacement of pre-loaded plastic tip with wire'), use the forceps to pull out the damaged wire and discard. Attempting to replace wires while the weight is still inside the tube is not recommended. A supply of spare 0.2 mm wires is provided with each set. Each wire has one "good" end that has been squared off/rounded and polished. The other end is ragged and sharp. The wires are stored in a block of foam with the good ends exposed. Consider taping a millimeter-marked tape measure to the bottom of the white paper on the work surface, or keeping a millimeter-marked ruler available.).

Use the forceps to hold a new wire, ensuring to keep track of the end of the wire that was facing up in the storage kit as this is the polished end that is the correct side to use for testing; the other end will need to be cut. Hold the forceps approximately 2-3mm from the polished end, and using the recommended wire-cutter, cut the wire approximately 2mm from the forceps (i.e., ~4-5mm from the good end; use the length of the old wire as a guide if needed). See **Figure 3**.

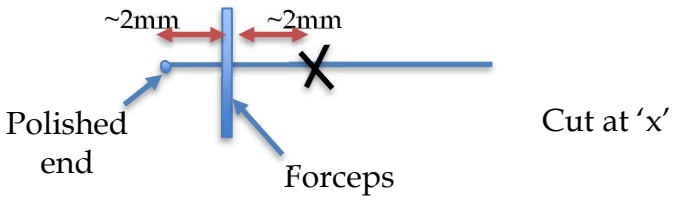


Figure 3 Cutting replacement wires that have a polished end

Carefully place the cut end into the plastic tip. Be careful not to bend the wire; do not apply force as it will cause the wire to kink. If the wire sticks out more than 2-3mm from the plastic tip, it will need to be cut further (otherwise it will be prone to bending when used). If the cut end of the wire does not fit into the plastic tip, it will need to be sanded down (see recommended polishing grit stone below in section regarding making new wires). Verify with a magnifying glass that the 'correct' end is facing up.

Each punctate probe set comes with extra plastic wire holders. These should be prepared in advance with wires inserted so that they may be readily used as replacements should a probe bend or break in the midst of a clinic visit.

4. Buying or making new wires

Additional wires with polished ends should be available for purchase from UNC for the immediate future, but long-term supplies cannot be assured. They are somewhat expensive because of the labor involved in hand polishing the tips.

Researchers who own several probe sets may find it economical to polish their own wires as needed. This is easy to do although some additional tools and supplies will be needed. Boston University (BU) can prepare new wires to ship to clinic sites. Please contact BU well in advance of using your last wires.

5. Tools and supplies

A small hand tool called a pin vise is needed to hold the wire while polishing the end. We recommend Starrett model 50609 (available at MSC as part #86406121 for around $25; see equipment list).

The wire we use is stainless steel type 316 LVM with a spring temper and a diameter of 0.008 inches. It can be purchased in long straight bundles from Amazon by searching for ASIN part number B001381C0U (for bundles of 30-inch length) or B001383E6K (for bundles of 60-inch length). They are shipped uncoiled in protective plastic tubes. Inexpensive polishing stones made of corundum (aluminum oxide) abrasive can be purchased from MSC Industrial Supply. We use three grades in succession from coarsest to finest:

- 600 grit (MSC part #05094057)
- 900 grit (MSC part #51032647)
- 1200 grit (MSC part #51032670)

The progress of polishing can be monitored easily with a hand lens of at least 10X magnification. The "Hastings Triplet" magnifiers manufactured by Edmund Optics (edmundoptics.com) work very well for this task. They cost approximately $75. 12.5X magnification (Edmund Optics part #59-818) or 20X magnification (Edmund Optics part #36-067).

6. The polishing procedure

The reason polishing is needed is because wire cutters typically leave a sharp, chisel-shaped wedge of metal on the end of a cut wire that would easily penetrate the subject's skin. The object is to grind off that damaged part of the wire, leaving an end that is flat and smooth.

Start by cutting a piece of wire about 6 inches long, being careful not to kink it. Load the wire into the jaws of the pin vise, making sure that it's centered. It will extend out the back of the hollow handle. The easiest way to do this is to start with the vise jaws closed, then open them a tiny bit at a time until you can just barely insert the wire into the space between the four jaws. Tighten the vise when just 1 or 2 millimeters of wire remain extended beyond the jaws. That gives enough support to allow grinding the end of the wire against an abrasive polishing stone without kinking it. First grind off the entire damaged end of the wire using the coarsest stone (600 grit). Hold the pin vise perpendicular to the surface of the stone while moving in small circles. Only modest pressure is needed. Pressing too hard may gouge the stone. Light gray lines left on the surface of the stone demonstrate that you're removing metal from the wire. To determine if you've removed enough metal view the wire end-on with a high-power hand lens. Very bright light will be needed. If adhering corundum particles obscure the metal surface it may

be helpful to push the wire through a sheet of facial tissue a few times to dislodge them. (Note that corundum dust also sticks to fingers. Don't touch the surface of the magnifier lens, eyeglasses, etc. The particles will scratch optical surfaces.) If the wire looks perfectly circular in cross section when viewed end-on then, you have ground away the entire damaged portion left by the wire cutter. The end will still be somewhat rough however because you've been using a coarse stone.

Switch to the 900 grit stone, which will leave the end of the wire a little bit smoother. Then use the 1200 grit. Be especially careful not to press too hard on these finer stones since they have a consistency almost like chalk. The 1200 grit stone should leave the end of the wire with a gray matt finish and no prominent gouges. If it looks flat, smooth, and perfectly round in cross section then the polishing is completed. Loosen the vise jaws barely enough to allow the wire to be advanced about a centimeter and then tighten again. Cut off the newly polished wire tip while leaving 1 or 2 mm extending beyond the jaws. In this way it's possible to make several polished tips before having to load another length of wire into the vise. With practice it should be possible to polish about 3 tips per hour. Store the newly made wire tips in a block of foam with the polished ends pointing outward.

Appendix 2 Anatomic landmarks

1. Background and rationale

For efficiency and consistency, the following anatomic landmarks will be identified and marked on participants at the relevant examination station:

Center of patella (knee cap)
- Quantitative sensory testing (PPT; also CPM if MOST-SENS participant)

Tibial tuberosity (TT)
- Full limb x-ray

Distal radioulnar joint
- Quantitative sensory testing (temporal summation, PPT)

Marking will be done with a black magic marker or other appropriate marking device before the participant begins examinations requiring the anatomical marking. For QST, the right/left patella, and right only radioulnar joint (unless left wrist must be used due to exclusions) will be marked with an "X" so examiners performing the examinations can easily identify the correct locations on the study participant. The right/left patella will be marked with a 1 to 1½ inch "X" and the l radioulnar joint will be marked with a ½ inch "X." For MOST-SENS participants, a 'dot' will be placed on the index knee.

For the full-limb x-ray, an *X-spot* radio-opaque adhesive marker with a 1 mm lead sphere will be placed on the marked right/left tibial tuberosity just prior to the full limb x-ray.

2. Equipment and supplies

- Magic marker (black) or other appropriate marking tool
- *X-spot* radio-opaque adhesive marker

3. Safety issues and exclusions

3.1 Safety issues

Magic Marker (or other appropriate marking tool)
- Take care when marking the anatomical landmark sites, especially if there is skin irritation, rash, or a wound in the area. These marks can be removed with an alcohol wipe.

X-spot radio-opaque adhesive marker
- Participants with sensitivity to adhesives may develop skin irritation from the adhesive-backed *X-spot* marker. Remove the *X-spot* marker from the participant's leg immediately after the x-ray exam is completed.

3.2 Exclusions

Participants with a lower extremity amputation may not have a tibial tuberosity or patella to mark and will therefore be excluded from marking that location.

4. Participant preparation

The participant should be wearing shorts to allow easy access to all of the anatomic sites for landmarking. Before you begin, have the participant supine. Marking of all sites should be done while the participant is supine. It is essential that the tibial tuberosity is marked while the participant is supine to insure proper placement of the *X-spot* radio-marker which will be applied bilaterally on top of the X mark which identifies the tibial tuberosity just prior to knee x-ray. The pain sensitivity and x-ray examinations are done with the participant standing or lying on an exam table with the leg extended.

5. Identification and marking of anatomical sites

5.1 Center of patella

Locate the midline and center of the patella (knee cap) by placing fingers and thumb all around the patella (see Figure 1). The examiner identifies the superior, inferior, medial, and lateral borders of the patella and marks the center of the patella with an "X." For MOST-SENS participants, additionally mark the index knee with a 'dot'.

5.2 Tibial tuberosity

The participant needs to be supine and their shorts on so the examiner can feel boney landmarks. As the examiner runs their hand from the top of the knee slowly down the front of the leg, they will feel the kneecap (patella) at the top and then a flat ropey like structure (the patellar tendon). Just below (inferior to) the tendon is a hard boney prominence, the tibial tuberosity (also called the tibial tubercle) (see Appendix 1). In some people this boney prominence is large, extending an inch or so from side to side or from its top to bottom. With the participant seated mark the "X" at the top of the tibial tuberosity, just where it joins with the patellar tendon. If the tuberosity extends from side to side, please mark the middle, where the tendon joins the bone. Do this first for one leg and then for the other leg. An *X-spot* radio-opaque marker will be placed on the marked location of the right and left tibial tuberosity just prior to the full limb x-ray.

5.3 Distal radioulnar joint

Mark the dorsal side of the distal radial-ulnar joint. The joint is the space in between the two boney prominences of the wrist. The distal ulnar styloid process is easy to identify, it is the large boney prominence on the dorsal small finger side of the wrist. The dorsal distal radial-ulnar joint is the space between the ulna and radius bones (see Figure 2).

Figure 1 Patella, Tibial Tuberosity

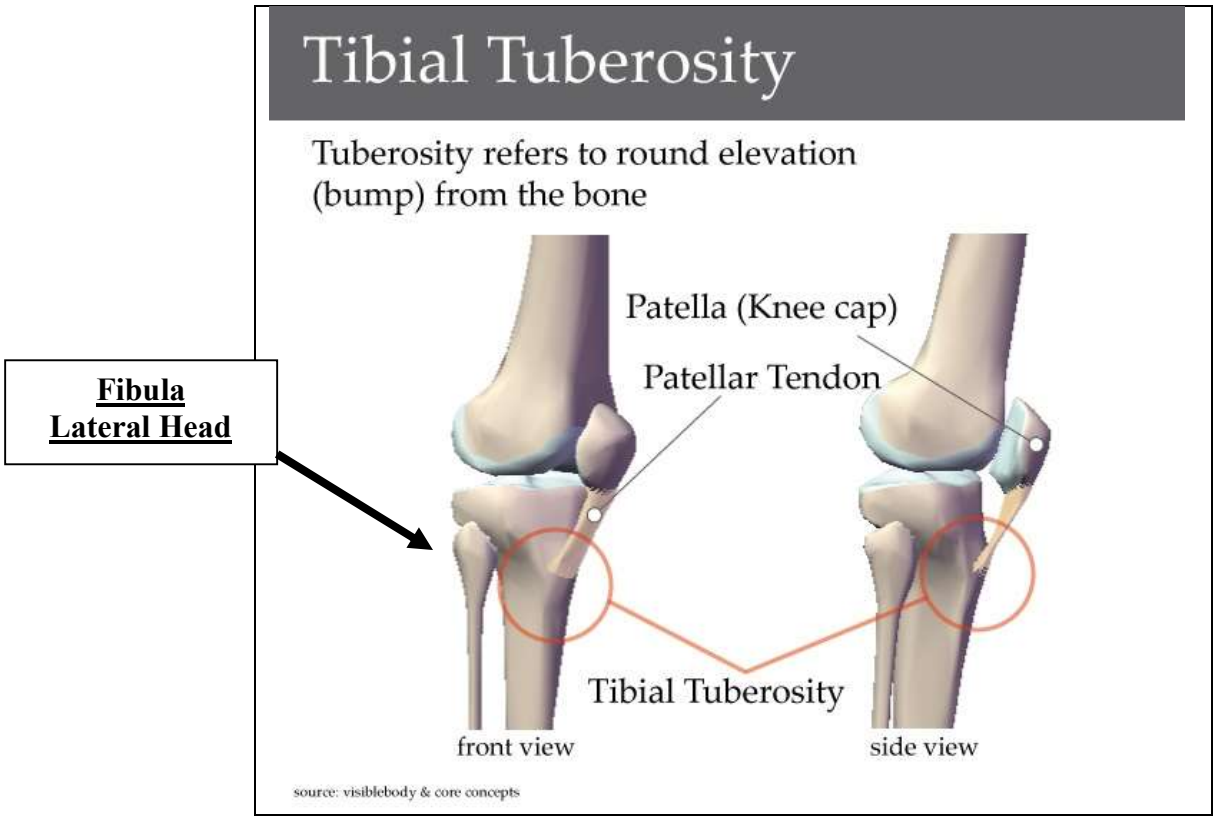
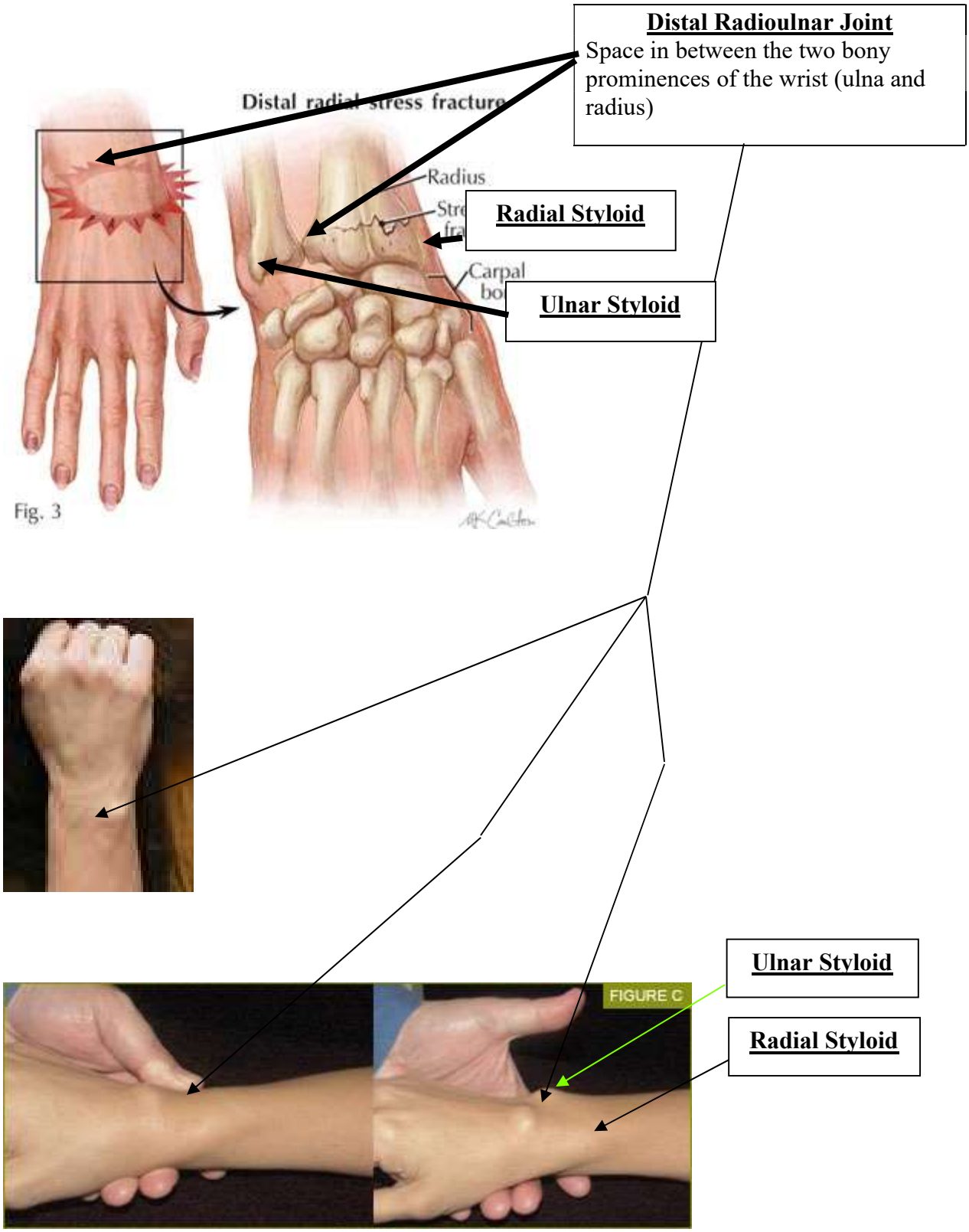
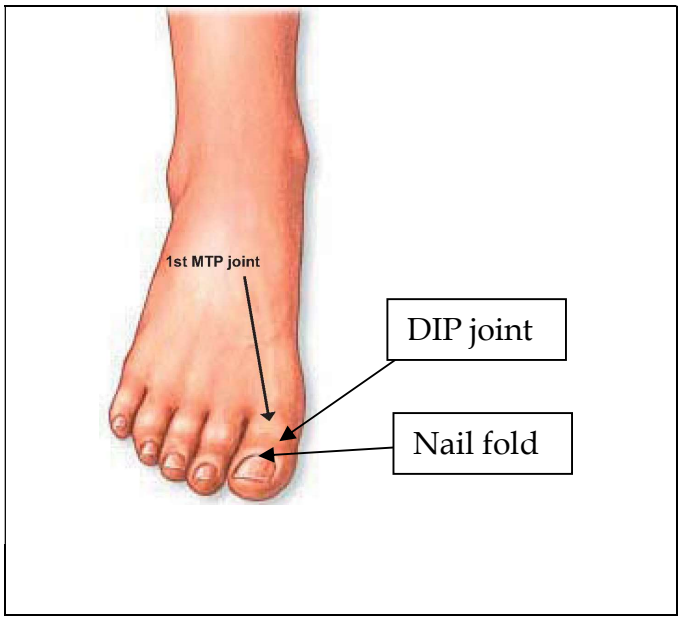


Figure 2 Radioulnar Joint



Appendix 3 Peripheral neuropathy test location: great toe



Peripheral neuropathy screening with the 10 gram monofilament will be done on the dorsum of each great toe midway between the nail fold and the DIP (distal interphalangeal) joint.