MULTIDISCIPLINARY APPROACH TO PELVIC PAIN (MAPP)

TRANS-MAPP
QUANTITATIVE SENSORY TESTING (QST)
MANUAL OF OPERATING PROCEDURES

Sponsored by: The National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), National Institutes of Health (NIH), Department of Health and Human Services (DHHS)

MOP — VERSION 1.2

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Collaborating Sites: University of Michigan, University of California Los Angeles, Washington University at St. Louis, University of Washington

Prepared by: Megan Halvorson, Steven Harte, Henry Lai, Simon Haroutounian, Bruce Naliboff

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CONTACT INFORMATION

For general questions or concerns, call or email:

Steven Harte, Ph.D.
QST Workgroup Chair
734-998-7053 (office)
734-998-6939 (Karen Brown, Administrative Assistant)
seharte@med.umich.edu

If Dr. Harte is unavailable and the request is urgent, contact one of the following QST workgroup members or the MAPP DCC.

1. Simon Haroutounian, Ph.D.: simon.haroutounian@wustl.edu
2. Henry Lai, M.D.: laih@wudosis.wustl.edu
3. Dan Clauw, M.D.: dclauw@med.umich.edu
4. Megan Halvorson: meganeb@med.umich.edu

For MAST System technical issues and repairs, email:
masthelpdesk@umich.edu

QST Core Battery

The overall objective of the MAPP Phase II QST protocol is to provide a broader and more comprehensive set of QST measures into the Phase II MAPP studies. The following paradigms will be used to evaluate pain processing at different levels of the neuraxis:

a) *Generalized hypersensitivity* will be assessed using the MAST device on the thumb,

b) *Spinal segmental hypersensitivity* will be assessed using a pressure algometer and pinprick stimulator at the suprapubic area, and

c) *Conditioned pain modulation* will be used to determine the efficacy of supraspinal pain inhibitory mechanisms.

The core QST battery will be conducted at all discovery sites at the week 4 and the 6, 18, and 36 month clinic visits, and at all pre/post ATLAS visits, on all SPS participants. The core QST battery will also be conducted on all Control participants at the Eligibility/Screening Visit and Month 6 Visit.

University of Michigan is providing all necessary equipment required to perform the core QST battery. Sites will be responsible for purchasing disposable supplies, including protective gloves, foot covers, and cleaning supplies. The core battery requires approximately 75-90 minutes, including participant familiarization and training.

Pre-Visit Checklist

Supply List

- ☐ MAST System*
- ☐ Algometer *
- ☐ Metronome*
- ☐ Earphones (for metronome)
- ☐ PinPrick Stimulator (256 mN)*
- ☐ LX Immersion Circulator *
- ☐ Foot Bath*
- ☐ Water Pitcher
- ☐ 2x plastic foot covers
- ☐ Protective gloves for research coordinator (latex or similar)
- ☐ 3-4x towels (or paper towel)
- ☐ Padding to place under participant's forearm and knees while laying on exam table (hand towels acceptable)
- ☐ Pillow to place beneath participant's head during testing on exam table
- ☐ QST CRF
- ☐ Timer/Stopwatch
- ☐ Cleaning supplies
- ☐ Thermometer

Supplied by University of Michigan; all other items to be acquired by each site

Considerations for Testing Environment

- Proximity to a sink/drain for filling and emptying foot bath
- Access to exam table/cot on which participant will lay during Algometry and Temporal Summation tasks
- Chair for participant to sit in during other tasks
- Access to power outlet

Other QST Considerations

- "State" and pain-related self-report measures should be administered prior to QST. Trait and non-pain related measures can, if necessary, be administered during QST breaks. Contact the QST Workgroup for guidance and approval to administer self-report measures during QST.
- QST procedures should occur prior to pelvic exam, if possible. (If not possible, this should be recorded on the CRF. Ideally, the RC should plan the pelvic exam in the afternoon after QST and neuroimaging.)
- At least **2 hours** should pass between completion of QST and beginning of the Neuroimaging resting state scan.
- All devices should be charged and disinfected (as applicable) prior to use.
- Research Coordinators (RC) should wear gloves during QST procedures.
- Advise participants to wear comfortable, loose-fitting clothing for the QST procedures. Advise them that we will be testing their bare feet, an area between their pubic bone and belly button, forearm, and shoulder area.

- Participants are encouraged to void prior to initiation of QST procedures, if possible (this is to avoid compressing onto the full bladder, also coordinate with Biospecimen collection if possible). Participants may however also void during QST procedures as necessary.
- If significant breaks or other procedures occur between QST procedures, this should be consistent across all sites. Record deviations as necessary.
- **Familiarization/practice procedures for each QST method should be conducted at ALL SPS visits, and at any ATLAS visits that occur more than 6 months since a previous QST session**.

QST Pre-Procedure Diagnostic Questions

MAPP QST CRF

- All data except the MAST Ascending Test will be recorded on the "QST CRF" created by the UPENN DCC.
- At every visit, administer the "QST Pre-procedure Diagnostic Questions" located on the QST CRF to ensure that the participant is an appropriate candidate for testing. If the participant is not a candidate for one of the four procedures (MAST, algometer, Pinprick, CPM), they should still complete all other QST procedures for which they are appropriate.

Artificial Fingernail Status and History for MAST procedures

1. If participant responds "Yes," that artificial fingernails are worn, record whether or not they are willing to continue wearing artificial fingernails for the full duration of the MAPP II SPS Study.
    a. If NO, proceed with all QST tasks, but participant will be unable to participate in the MAST and the Conditioned Pain Modulation (CPM) tasks at future visits if they are not wearing artificial fingernails. Algometry and Temporal Summation may be conducted regardless of artificial fingernail status.
    b. If YES, proceed with all QST tasks. Confirm that participant is wearing artificial fingernails at future visits. If not, participant will be unable to participate in the MAST and the Conditioned Pain Modulation (CPM) tasks at future visits unless artificial fingernails are worn. Algometry and Temporal Summation may be conducted regardless of artificial fingernail status.
2. If a participant responds "YES," that they have started wearing artificial fingernails since previous clinic visit QST procedures, skip the MAST and CPM tasks. The participant should, however, complete the Algometry and Temporal Summation tasks.
3. If a participant responds "YES," that they have discontinued wearing artificial nails since previous clinic visit QST procedures, skip the MAST and the CPM tasks. The participant should, however, complete the Algometry and Temporal Summation Tasks.
4. Other considerations – recent removal of artificial fingernails at the time of the Baseline Visit.
    a. Those who infrequently wear artificial fingernails (e.g., 1 week or less for a special occasion, 1-2x per year) and have not worn them for at least 1 month prior to a study visit may proceed with testing. Participant should be made aware that they should not wear artificial fingernails during future visits involving QST. Questions regarding specific cases can be directed to the QST workgroup.
    b. Those who regularly wear artificial fingernails, but have recently removed them should be deferred from enrollment for a period of **6 months** to allow adequate time for the nailbed to fully recover. If they are enrolled, MAST and CPM tasks should be skipped. These participants should still participate in the Algometry and Temporal Summation tasks. Contact MAST Help Desk for guidance on a case-by-case basis.

Peripheral Neuropathy for MAST & Conditioned Pain Modulation Procedures

1. Participant has diagnosed peripheral neuropathy in the hands (upper extremity) which would interfere with MAST results, skip MAST and CPM procedures. Algometry and Temporal Summation should still be conducted.
2. Participant has diagnosed peripheral neuropathy in feet (lower extremity) which would interfere with CPM results, skip CPM procedures. MAST Test, Algometry, and Temporal Summation should still be conducted.

3. Participant reports sensory abnormalities in either the hands or the feet but does not have diagnosed upper or lower extremity neuropathy, respectively. Record this information on the CRF but conduct all QST procedures as normal.

Open Wounds on Feet for Conditioned Pain Modulation Procedures

1. If participant has significant open wound(s) on non-dominant foot (significance of wound determined by RC or site PI), but not on the dominant foot, the participant's dominant foot should instead be used for all CPM testing, including for the Painful Conditioning Stimulus Calibration (i.e., Hot Water Calibration). If wounds are healed at the future visit, the test may be conducted per protocol (i.e., conduct testing on non-dominant foot.)
2. If participant has significant open wound(s) on both feet, CPM testing should be skipped at this visit. All other QST procedures may be completed.
3. If participant has significant open wound(s) on dominant foot, but not on the non-dominant foot, conduct calibration/familiarization protocols on the non-dominant foot (and testing may occur on non-dominant foot per protocol.) If wounds are healed at the future visit, the test may be conducted per protocol (i.e., conduct testing on non-dominant foot.)

Thumb Injuries and Other Abnormalities

1. If participant has a missing, severely malformed, or injured thumb on which testing is to be performed, MAST testing should be conducted on the opposite thumb, provided it is not missing nor injured. For example, both familiarization and testing would be conducted on the dominant thumb if the non-dominant thumb was abnormal.
    a. **A rest interval of 5-10 minutes should be provided between the MAST familiarization protocol and the MAST test if testing is conducted on the same thumb to permit sufficient tissue recovery**.
    b. Record the thumb on which familiarization and testing occurred on the CRF.
2. If both thumbs are missing and/or injured, skip the MAST and the CPM tasks. The participant should, however, complete the Algometry and Temporal Summation Tasks.

Generalized Mechanical Sensitivity (MAST Test)

MAST Setup

1. Turn on all MAST System components and connect the computers (see MAST Operation Manual).

2. Connect the MAST handpiece to the "Right Thumb Device" side of the Device Configuration window (see MAST Operation Manual).

3. Confirm that the MAST System is working by applying a test pressure (0.25 kg is sufficient) from the Device Configuration window. You can use either your thumb or the supplied plastic test phantom for this test. The "STATE" information bar will indicate "OK" if the pressure was administered correctly.

4. Enter your participant demographic information into the MAST server: Participant ID, Site ID, Visit Number, and RC ID are required.

5. Confirm that the MAPP DCC username and password are entered in "SFTP Configuration" on the MAST server to allow data uploads.

MAST Familiarization Protocol (non-dominant thumb)

1. Participants will undergo a familiarization procedure/practice test prior to the actual test. The purpose of familiarization is: 1) to teach participants how to perform the task correctly, 2) reduce test anxiety, and 3) to acclimate participants to the sensations (pressures, sounds, etc.) experienced during the task.

2. Pressures are to be applied to the non-dominant thumb during the familiarization procedure, and to the dominant thumb for the actual Ascending Test.

3. RC will read**.** ***In a few moments, I will conduct a test to measure your sensitivity to pressure. We will start with a practice test before conducting the actual test so that you can become comfortable with this procedure.***

   During testing, pressures will be applied to your thumbnail for 5 seconds. AFTER each pressure is released, you will be asked to rate how painful it felt using a rating scale with numbers 0 through 100. This scale is shown here on this computer screen. A zero (0) means 'no pain' and a one hundred (100) means 'pain as bad as you could imagine.' To indicate your rating, you will use a stylus to press the location on the scale that best describes the intensity of the pain in your thumb. If you prefer, you may these arrows at the bottom of the screen to adjust your rating up or down. Once you have selected your rating, press the Green confirm button in the lower right corner of the screen. Keep in mind that there is no right or wrong response, and you can use any number from 0 to 100. Some pressures may be painful while others may not be. Remember, if it just feels like pressure but not pain, rate (0) zero. If any pressure is intolerable, let me know and I can release it immediately. You can also release the pressure yourself by pressing the 'STOP' button on your screen. You can also let me know if you feel your thumb is not in the correct position and needs to be adjusted. During testing, sit in a comfortable position and try to relax. This test usually takes 5-7 minutes to complete. Do you have any questions?"

4. Next, demonstrate how to properly hold the MAST handpiece, and assist the participant in doing so. The participant will hold the handpiece like a joystick while placing his or her thumb into the opening in the device, with their thumbnail positioned beneath the rubber tip. The plunger tip should contact the center of the thumbnail bed.

5. Instruct participant to keep his/her hand relaxed and to rest his/her thumb on the bottom surface of the thumb hole. They should never push the thumb upwards against the rubber tip during testing as this can interfere with pressure measurement.

6. Ensure that the participant is seated comfortably with the hand, forearm and elbow resting on a table. You may need to place a towel or pad underneath his/her arm, particularly around the elbow, to improve comfort. Instruct participant to not stand the handpiece upright after the test is completed. It is top heavy and will fall over. After testing, he/she should gently place handpiece down on its side. You don't want to perform the test very close to the edge of the table or in the "mid-air," since you don't want the participant to drop the device accidentally to the floor during testing.

7. One or two light "sample" pressures will be applied to the participant's thumbnail in advance of the familiarization procedure in order to ensure proper thumb positioning. To apply sample pressures, navigate:
    a. Server Computer → Menu → Device Configuration
    b. Set force to **0.25 kg**; set duration to **2 seconds** (if not already selected)
    c. Click the Green check mark to apply the pressure.
    d. The rubber tipped plunger in the MAST handpiece will lower to apply the pressure to the participant's thumbnail. The rubber tip should fall directly in the center of the thumbnail and not on the cuticle or the tip of the nail.

8. Once the participant's thumbnail is in the proper position, the familiarization procedure will be conducted. It is OK to speak to the participant during the familiarization test to ensure he/she understands the procedure and scale, and to answer questions as needed. However, avoid non-task related conversation.

9. To perform the Familiarization test, navigate to:
    a. Server computer → Testing Algorithms → Discrete Stimuli
    b. Select "**MAPP ASC**" from the menu of saved tests and click the Green checkmark to load the test
    c. This test of consists of a series of ascending pressures beginning at 0.50 kg/cm^2 and increasing in 0.50 kg/cm^2 increments. Each pressure is delivered for 5 seconds. After the pressure is released, a prompt will appear on the Client computer to rate the pain evoked by the preceding pressure using a 0-100 rating scale. Ratings will be entered directly into the Client computer.
    d. Start the test by selecting the PLAY button on the main screen on the server.
    e. RC will manually stop the test by pressing the STOP or PAUSE buttons at the first occurrence of one of three following possible stop criteria:
        i. Participant provides a **rating ≥ 50** on the 0-100 NRS, or
        ii. Participant asks you to stop the test and indicates that they are unable or unwilling to continue, or
        iii. After 10 kg/cm^2 of pressure has been applied.

10. If a participant requests a pressure to be released prematurely, ask them whether they want to continue testing after a short break or end the test at that time.

11. **After test completion, review that the participant understands of the procedure and provide additional guidance as necessary**. This is particularly important if he/she rated very low pressures (e.g., 0.5 kg/cm^2) as painful or rated the highest pressure that they could tolerate < 70 or 80. This *could* indicate poor understanding of the rating scale or user error when entering responses. In this event, it may be useful to explain that ratings less than 30 are usually interpreted as mild pain, ratings greater than 30 but less than 70 as moderate pain, and ratings over 70 as intense or severe pain. It is useful to monitor test progress in real time and address these issues immediately when they occur.

12. Familiarization data are not saved.

MAST Ascending Test (dominant thumb)

1. Following the familiarization procedure, you will conduct the MAST Ascending test on the participant's dominant thumb.

2. RC will read: ***"Now that we've completed the practice test, we are now going to conduct the actual test on your dominant thumb. Do you have any questions before we get started?"***

3. Apply 1-2 sample pressures from the Device Configuration window to ensure proper thumb placement in the device.

4. To perform the Ascending Series: hit the RESET button and then the PLAY button on the MAST Server. Note: You do not need to reselect the "MAPP ASC" testing algorithm if you conduct the Ascending test directly after the Familiarization procedure.

5. This test of consists of a series of ascending pressures beginning at 0.50 kg/cm^2 and increasing in 0.50 kg/cm^2 increments. Each pressure is delivered for 5 seconds. After the pressure is released, a prompt will appear on the Client computer to rate the pain evoked by the preceding pressure using a 0-100 rating scale. Ratings will be entered directly into the Client computer.

6. Although you don't have an active role in performing this test, you should closely monitor test progress from the Server computer so as to ensure accurate data collection and equipment function. Any unusual circumstances that cannot be corrected should be recorded in the "Notes" section of the MAST software or on the CRF. **It is also recommended that participant ratings after each stimulus are recorded on paper in real time.** This provides a backup of the data in the event of catastrophic equipment failure (e.g., hard drive crash) during testing and it also helps to maintain focus on the test procedure.

7. RC will manually stop the test by pressing the STOP or PAUSE buttons in the MAST software at the first occurrence of one of three following possible stop criteria:
    a. Participant provides a **rating ≥ 80** on the 0-100 NRS, or
    b. Participant asks you to stop the test and indicates that they are unable or unwilling to continue, or
    c. After 10 kg/cm^2 of pressure has been applied.

8. Save data locally (i.e., on MAST computer) by clicking the END TEST button in the MAST software, navigating to the folder in which the file should be saved, and then clicking SAVE. RC will access this data file later to calculate the Pain40 pressure CPM (described below).

9. Upload data file to the MAPP DCC server by following the prompts initiated after saving the data locally.

Making Corrections to MAST Data

If a participant enters a rating and then indicates that it is incorrect, there are two options for correcting the rating:

1. During the actual test, briefly pause the test sequence and scroll backwards on the main screen of the MAST server to where the error occurred. Participant ratings are populated in the bottom "Response" row. Change the value in the box that is incorrect and then hit the Play button to resume testing.

OR

2. Do not upload the data immediately after testing. Go to the saved .csv file, open it, correct the error, and then re-save (you'll get lots of prompts when trying to save a csv file in windows – just keep saving and answering in the affirmative until it ends). Then proceed to upload the file.

With either option, note what happened and the remediation in the participant's visit notes.

If incorrect data was already uploaded, send a change request to the DCC with the relevant information and the rating will be updated in the database.

Segmental Mechanical Sensitivity (Algometer Test)

Overview. A handheld, analog pressure algometer (FPK Algometer, Wagner Instruments, Greenwich, CT) with a 1 cm^2 flat rubber probe will be used to deliver quantifiable pressure stimuli to the **suprapubic area** (midway between the pubic symphysis and the umbilicus in the midline of the body – the most common referral site of pelvic pain. Algometry will also be performed at non-symptomatic control sites (forearm and trapezius)

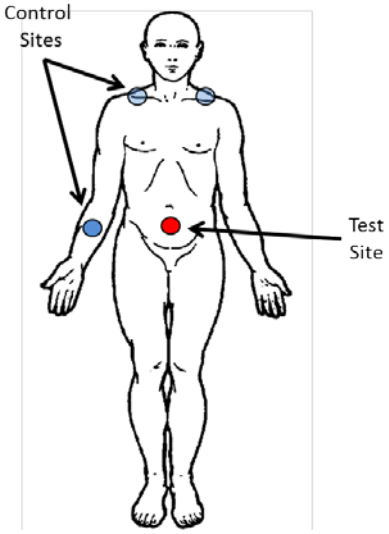


Figure 1. Algometry and temporal summation test and control sites.

General Setup and Instructions

1. Patients will first empty their bladder prior to testing to reduce the chance of algometer stimulation provoking unanticipated urine voiding, or compressing onto a full bladder.

2. <u>Identifying the forearm test site:</u> The forearm control site is located midway between the wrist and the elbow, with the participant's palm facing up, and the hand in a relaxed position (Fig 1).

3. <u>Identifying the suprapubic test site:</u> To locate the suprapubic testing site, the RC (or participant) is to first locate the umbilicus (i.e., belly button). Next, the participant will be asked to locate the pubic symphysis (i.e., "pubic bone") on their body and point to it. Testing at the suprapubic region is to be conducted at the midpoint of these two sites in the midline of the body, approximately over the bladder (Fig 1).

4. <u>Identifying the trapezius test site:</u> Located at the midpoint of the upper left and right trapezius, at the area that is most tender (i.e., the fibromyalgia tender point).

5. <u>Metronome</u>: The RC will maintain a consistent rate of pressure delivery with the algometer by using a metronome (Korg MA-1).
    a. Turn on metronome and install batteries
    b. Press yellow power button.
    c. Program metronome to tick/beep at a frequency of 1Hz (60 beats per minute) using the arrow buttons to set the tempo to 60 beats per minute (bpm). Note: Programming is only necessary the first time the metronome is used.
    d. Press the Start/Stop button to activate.
    e. Adjust volume using dial on right side of device.
    f. The device will now beat once per second. It should look like Fig 2.
    g. Press yellow power button to turn off device when testing is complete.
    h. RC will attach earphone to metronome during testing so that participants don't hear the ticks. RC is responsible for supplying his/her own earphones.

Figure 2. Metronome with 60 bpm setting.

6. Algometer: RC should be familiar with the operation and handling of the algometer. The picture below demonstrates the correct way to hold the algometer. Pressure should always be applied to soft tissue (muscle) and not bone. For a video demonstrating the proper method to apply pressure using an algometer, see "SI" at the following link: http://www.sciencedirect.com/science/article/pii/S0304395906001527

Figure 3. Holding the algometer.

Algometer Familiarization Protocol (non-dominant forearm)

1. Participants will undergo a familiarization procedure on the non-dominant forearm prior to data collection. Familiarization occurs while participant is seated in a chair facing the RC, so that he/she can clearly see the device prior to testing and ask questions.

2. RC will read: ***"The next set of tests will be conducted while you lay down. Before we do that, I would like to show you the devices we'll be using. First, I'll be using this pressure algometer to test of your sensitivity to pressure on your forearm and on an area located a few inches below your belly button. I'll ask you to rate the pain intensity of each pressure that I apply using the same 0-100 pain rating scale we used during the previous test, with 0 representing a sensation of pressure but no pain, and 100 representing 'pain as bad as you can imagine.' I will now apply a couple sample pressures on your forearm so that you can experience how it feels.***

3. RC will first apply a sample 2 kg pressure, then a sample 4 kg pressure, each for 5 seconds once the target pressure is achieved, to the non-dominant forearm site, and have the participant practice rating each pressure on a scale of 0-100. These data are not saved.

4. During the application of each pressure, **press and hold the "release-button"** located on the side of the algometer to maintain the amount of pressure applied during the 5 second stimulation period (otherwise the dial is locked).

5. The participant will be seated at this time and they should rest their arm on a table or other flat surface.

6. Be sure to press algometer tip into the center of the forearm muscle to avoid slippage of the rubber tip. It is also critical to avoid applying pressure to bone. Only soft tissue (muscle) should be stimulated with the algometer.

7. Note: It is suggested that Temporal Summation Familiarization (see page 19) occurs immediately following Algometry Familiarization (prior to the patient lying down on the exam table).

Forearm Algometry (dominant forearm)

1. Participant will lay supine on exam table. RC will provide pillow for participant's head, padding/pillow under the participant's knees for lumbar support/comfort (if requested), and padding (such as a hand towel) beneath the participant's dominant forearm (to cushion the elbow).

2. RC reads: ***"First, I'll start with the pressure test. After each pressure, I'll ask you to rate the pain intensity of the pressure from 0-100. Remember, if it just feels like pressure but not pain, rate (0) zero. If the test becomes too uncomfortable, please let me know, and we can stop at any time. A total of six pressures will be applied to your forearm. Also, I will be wearing headphones during the test so that I can listen to a beeping pattern that will help me apply the pressures with correct timing."***

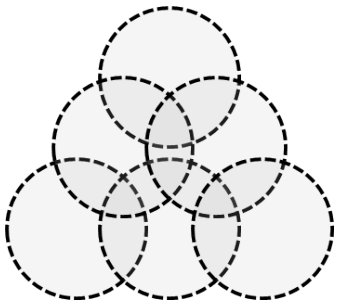
3. Participant will place dominant forearm on exam table with their palm up and their hand relaxed. Test site is the midpoint of the ulnar forearm.

4. RC administers six, **5 second** duration pressure stimuli (i.e., hold for 5 seconds after reaching the target pressure) to forearm with the release-button pressed, in the following **pseudo-random order,** with **15-20 seconds between** each administration of pressure:
   i. 2 kg
   ii. 2 kg
   iii. 4 kg
   iv. 2 kg
   v. 4 kg
   vi. 4 kg

5. Note: This order of pressures will be used for every participant, at every visit, at all MAPP sites. DO NOT CHANGE THE ORDER.

6. Apply pressure at a rate of approximately **0.5 kg/cm^2/second** using the 1 Hz metronome ticks as a guide. Thus, the algometer needle should pass a 0.5 kg mark at each tick until the target pressure is reached. **Headphones or earphones are to be worn by the RC during the test to prevent the participant from hearing the beeps.**

7. Each pressure should be applied in such a way that there is approximately 40-50% overlap with the previous pressure, such that a pyramid pattern similar to that shown to the right is created on the participant's skin at the test site.



8. Subjects will be asked to rate the pain intensity of each pressure using a 0-100 NRS. Record each rating on the QST CRF.

9. Note: The indentations and red marks caused by the algometer on the skin are normal and do not reflect lasting tissue damage. They should resolve within a couple hours.

Suprapubic Algometry

1. Participant will remain supine on exam table.

2. Participant will assist in locating the suprapubic site by identifying their pubic bone and belly button; testing will be conducted at the midpoint in the midline of the body. The participant need only expose enough skin at the suprapubic site for testing to be conducted.

3. RC reads: ***"Next, I will test the area below your belly button. To help me locate this area, I would like you to find your pubic bone. Using your hand, over your clothing, start at your waist and move down toward your groin pushing downward until you feel a bone – that is your pubic bone. Next, I'll have you point to your belly button. The area that I'll be testing is halfway between those two points, and I'll need you to expose just a small area of skin at that point so that I can conduct the test. Remember, if the test becomes too uncomfortable, please let me know, and we can stop at any time. A total of six pressures will be applied to this area."***

4. RC to administer six, **5 second** duration pressure stimuli (i.e., hold for 5 seconds after reaching the target pressure) to the suprapubic site, in the following **pseudo-random order**, with **15-20 seconds between** each pressure:
    i. 2 kg
    ii. 2 kg
    iii. 4 kg
    iv. 2 kg
    v. 4 kg
    vi. 4 kg

5. Note: This order of pressures will be used for every participant, at every visit, at all MAPP sites. DO NOT CHANGE THE ORDER.

6. Apply pressure at a rate of approximately **0.5 kg/cm^2/s** using the metronome as a guide.

7. Subjects will be asked to rate the pain intensity of each pressure using a 0-100 NRS. Record each rating on the QST CRF.

Trapezius Algometry (Pressure Pain Threshold)

1. Trapezius algometry is conducted **AFTER** the PinPrick test (see page 18).

2. Participant will be asked to get up from exam table and return to a seated position. It is critical that the RC has access to the back of the participant (i.e., chair should not be against a wall) and that the RC stands taller than the participant's shoulders (i.e., participant should not be seated on a high stool or exam bed).

3. RC reads: ***For this next test, I'll be applying a gradually increasing pressure on your shoulders. Rather than having you rate the pain, I would like you to indicate the moment the sensation changes from a feeling of pressure to a feeling of pain. You can say something like "pain" or "now" or "stop" when this occurs. Once you start to feel any pain, even a small amount, please tell me and that's when I'll stop. First, I'll apply a practice pressure to your right shoulder and you can tell me when you start to feel pain.***

4. RC will apply a test/familiarization pressure on the right trapezius first and have the participant practice identifying their pain threshold. The test pressure should be applied approximately 2 inches lateral to the midpoint of the upper trapezius, so as to avoid sensitization of the test site. The test should be conducted on bare skin (not over clothing) over muscle, not bone. These data are not recorded.

5. Note: Unlike the application of 5 second pressures at the suprapubic region and forearm, DO NOT press the release-button located on the side of the algometer during the trapezius threshold measurement as the highest pressure that elicited pain needs to be recorded.

6. A sequence of gradually increasing pressures will then be applied at the trapezius test site, which is located at the midpoint of the upper left and right trapezius. To identify the correct test site, gently palpate the trapezius muscle with your fingers until the participants identifies the area that is most tender. Apply the pressure directly at that site.

7. Pressure will be increased at rate of approximately **0.5 kg/cm^2/s** (using the metronome with earphones), until participant identifies his/her pain threshold.

8. This procedure will be repeated two times on each side in the following order:
    a. Left trapezius
    b. Right trapezius
    c. Left trapezius
    d. Right trapezius

9. Record pain threshold for each pressure application in "kg" on the QST CRF.

Temporal Summation (PinPrick Test)

Overview. Temporal summation refers to an increased perception of pain in response to sequential stimuli of equal physical strength. It is a QST model of neural plasticity and central hyper-excitability that is thought to reflect the progressive increase in neuronal firing of dorsal horn neurons in response to repetitive nociceptive C-fiber stimulation (i.e., windup). In this test, we will use a fixed intensity 256 mN pinprick stimulus (PinPrick Stimulator, MRC Systems GmbH) to evaluate temporal summation.

PinPrick Test Setup

1. Participant will be asked to lie supine (i.e., on their back) on the exam table (with same padding under knees and head as suggested above for algometer test).

2. Metronome will continue to tick at a frequency of 1 Hz (i.e., 60 bpm / 1 beat per second).

3. Testing sites: dominant forearm and suprapubic area (same as algometer test).

4. PinPrick Stimulator:
    a. This stimulator applies 256 mN of force via a calibrated blunt needle. The needle will not puncture the skin. **It is extremely important not to bend the tip of the stimulator**. Bending the stimulator tip will disrupt the sliding action and calibration, and potentially alter the applied force. Stimulators must be sent to Germany for repairs.
    b. **For the IRB, consent documents, and instructions, the PinPrick stimulator will be referred to as a "pointed skin probe" to reduce anxiety regarding this test.**
    c. The pinprick stimulator requires disinfection between participants. Disinfection procedures can be found on Page 28.
    d. It is critical that all RCs learn the proper method to hold and apply the pinprick stimulator. It is held similar to a pen and is applied perpendicular to the skin (see Fig. 4). For a video demonstrating the proper method to use the stimulator, see "SI" at the following link: http://www.sciencedirect.com/science/article/pii/S0304395906001527.

Figure 4. Pinprick stimulator**.**

PinPrick Familiarization Protocol (non-dominant forearm)

1. The PinPrick Familiarization Protocol occurs immediately following the Algometry Familiarization Protocol, while participant is in a seated position.

2. RC will read: ***"For the second procedure I will be using this pointed skin probe to stimulate your forearm and the area below your belly button. As you can see, this device has a pointed end; however, it will not pierce or stick the skin.***
Similar to the previous procedure, I'll also be asking you to rate the intensity of this sensation. First, I'll apply a single stimulus and ask you to rate the pain intensity from 0-100, with 0 indicating 'no pain' or any kind of pricking or stinging sensation´, and 100 indicating 'pain as bad as you can imagine.' Next, I'll apply a series of ten stimuli in a row, and then ask you to rate the overall pain intensity of the entire series of ten stimuli using the same scale. I would now like to give you a practice test on your forearm. Do you have any questions before we proceed?

3. Familiarization will occur on non-dominant forearm. The forearm control site is located on the ulnar forearm midway between the wrist and elbow, 1 cm below the Algometry test site.

4. Using the PinPrick Stimulator, apply a single stimulus perpendicularly to the skin for approximately **0.5 seconds.** Participant will be asked to rate the pain intensity of the single stimulus using 0-100 NRS. Following a 5-second pause, a train of 10 identical pinprick stimuli (256 mN) will be applied with a **frequency of 1Hz within an area of 1 cm^2**. Participant will be asked to provide an overall rating of the pain intensity of the series of 10 pinpricks using 0-100 NRS.

5. It is not necessary to record the pain ratings collected during the Familiarization Protocol.

6. Review procedure and confirm participant understands. Ask if participant needs to use restroom. Assist participant getting onto the exam bed.

Forearm PinPrick Test (dominant forearm)

1. Test will occur on dominant forearm AFTER the Algometry test while patient is still supine. The forearm control site is located on the ulnar forearm, 1 cm below the midpoint of the wrist and elbow (and 1 cm below the Algometry test site).

2. RC will read: ***"Next we'll conduct the test using the pointed skin probe. Remember, I'll apply a single stimulus and have you rate the pain intensity of that single stimulus from 0-100. After that, I'll apply a series of ten stimuli and have you rate the overall pain intensity of the entire series of ten. I'll repeat this test a total of three times at both your forearm and at that the area below your belly button. After the testing is done, I'll also be asking you to rate any lasting pain you have at the site for a few seconds. If test becomes too painful, let me know, and we can stop at any time."***

3. Using the PinPrick Stimulator, apply a single stimulus perpendicularly to the skin for approximately **0.5 seconds**. Participant will be asked to rate the pain intensity of the single stimulus using 0-100 NRS. Following a 5 second pause, a train of 10 identical pinprick stimuli (256 mN) will be applied with a **frequency of 1Hz within an area of 1 cm^2** (using the metronome with earphones). Participant will be asked to provide an overall rating of the pain intensity of the series of 10 pinpricks using 0-100 NRS.

4. Repeat step #3 two more times at the forearm site within the same 1 cm^2 area, for a total of 3 cycles (or 33 stimulations) at the dominant forearm site. Each cycle should be separated by at least 10 seconds. Record ratings on QST CRF.

5. Pain After-sensations: At 15- and 30-seconds following the last train of 10 stimuli, participants will also be asked to rate any residual pain sensation in the testing area using the 0-100 NRS. This will require a stopwatch or timer, or the metronome can be used to track time. Record ratings on QST CRF.

Suprapubic PinPrick Test

1. Although the formal familiarization procedure described above has already been conducted, RC will deliver one or two single pinprick stimuli at the suprapubic region to reduce anxiety and acclimate the participant to the sensation produced by the pinprick in this area

2. Conduct the PinPrick test 1 cm above the Algometry test site at the suprapubic region. The marks left by algometer should be sufficient to locate the correct region without participant help.

3. Using the PinPrick Stimulator, apply a single stimulus perpendicularly to the skin for approximately **0.5 seconds**. Participant will be asked to rate the pain intensity of the single stimulus using 0-100 NRS. Following a 5 second pause, a train of 10 identical pinprick stimuli (256 mN) will be applied with a **frequency of 1Hz within an area of 1 cm^2** (using the metronome with earphones) Participant will be asked to provide an overall rating of the pain intensity of the series of 10 pinpricks using 0-100 NRS.

4. Repeat step #3 two more times within the same 1 cm^2 area, for a total of 3 cycles (or 33 stimulations) at the suprapubic site. Record ratings on QST CRF.

5. Pain After-sensations: At 15- and 30-seconds following the last train of 10 stimuli, participants will also be asked to rate any residual pain sensation in the testing area using the 0-100 NRS. Record ratings on QST CRF.

6. Have participant return to chair for Trapezius algometry (see page 17).

Conditioned Pain Modulation (CPM)

Overview. CPM testing requires a "conditioning" stimulus to evoke endogenous analgesia and a "test" stimulus to determine the efficiency of the endogenous analgesia. Here, thumbnail pressure pain produced by the MAST will serve as the test stimulus, and a hot foot bath will serve as the conditioning stimulus. An innocuous neutral temperature foot bath will serve as a control or "sham" conditioning stimulus.

Pre-CPM

1. Immediately following the Trapezius Algometry procedure, RC will ask participant to remove shoes and socks, and place plastic foot covers on both feet. Later, the RC will assist in securing foot covers with ties to prevent slippage.

2. While participant removes shoes and socks, calculate Pain40 from the MAST ascending data using the "*Pain Calculator*" excel file located on the MAST server. To do this:
    a. Open MAPP data folder on Desktop and double-click the .csv file containing the ascending data from that participant.
    b. Copy the ratings located in column "R Rating" (second from last)
    c. Paste ratings into *Pain Calculator* excel file and record Pain40 value on QST CRF.

Foot Bath / Immersion Circulator Setup

1. It is suggested that the RC setup the Foot Bath and Immersion Circulator prior to or at the beginning of the study visit (before the QST session).

2. It is suggested to position foot bath in the area where testing will occur prior to filling it with water, as the foot bath is heavy when filled. Placing a towel beneath the foot bath prior to filling makes for easier movement of the foot bath.

3. Attach the immersion circulator to the side of the foot bath, perpendicular to participant's foot to avoid direct water flow onto the foot (Fig 5).
    a. The circulator should rest approximately 0.5 inches above the foot platform. It should not rest on the foot platform.
    b. Use the screw on the back of the immersion circulator to secure it in place. DO NOT tighten the screw too tightly as this could crack the foot bath.

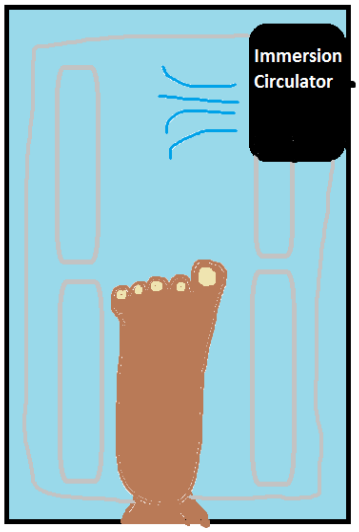


Figure 5. Foot bath configuration.

4. Fill water bath with lukewarm water high enough to fully cover the minimum fill line on the immersion circulator (approximately 5 inches above the base of the foot bath). Using a pitcher to transfer water from the sink to the foot bath is one suggested method of transfer. Placing a line indicating the correct water depth on the exterior of the foot bath with tape or a marker is also recommended.

5. Plug in the immersion circulator following institutional guidelines.

6. Turn the immersion circulator ON by flipping the switch on the back of the device.

7. Press the “Menu” button and use the arrows set the temperature to **32.0° Celsius (C)**. Press the “Play” button to start the circulator. Add more water to the foot bath if the circulator makes a loud gurgling noise.

8. **Cover the display so the participant cannot see the temperature** at any point during the testing. A piece of thick paper or cardboard taped to the side of the immersion circulator works well.

9. Obtain 3-4 towels to keep nearby, so participant may dry feet after testing, and cover/insulate feet between tests.

MAST Pain40 Calibration (dominant thumb)

1. The CPM test stimulus will be a 30 second pressure calibrated for each individual to evoke a pain rating of 40/100. We first estimate this value from the MAST ascending data. Here, we check to determine the accuracy of that estimation and adjust it as necessary.

2. RC will read: ***“For the final series of tests, I will again be applying pressure to your dominant thumb. In the first test, I will apply a series of 5 second pressures and ask you to rate the pain intensity of each one from 0-100. This time you won’t need to enter your ratings into a computer, but instead you will say your pain rating out loud and I will record it. Any questions?”***

3. On the MAST server, first navigate to Menu → Device Configuration and first apply a 0.25 kg/2-s sample pressure to ensure proper thumb placement (repeated as needed).

4. Under “Device Configuration” apply the initially calculated Pain40 pressure to the dominant thumbnail for 5-s and ask participant to rate it verbally using 0-100 NRS.
    a. If rating is 35-45, proceed with “CPM 1.”
    b. If rating is $\leq$ 34, increase Pain40 pressure by 0.25-1.5 kg, and repeat test as needed until rating of 35-45 achieved.
    c. If rating is $\geq$ 46, decrease Pain40 pressure by 0.25-1.5 kg, and repeat test as needed until rating of 35-45 achieved.
    d. A final rating between 30 and 50 is acceptable.
    e. Record Initial Pain 40 pressure and rating, Final adjusted Pain40 pressure and rating, and number of adjustments required on QST CRF.
    f. Note: If multiple adjustments are necessary, wait 3-5 minutes before proceeding to CPM 1.

CPM 1: Test Stimulus Alone (MAST - dominant thumb)

1. RC will read: ***"Next, you'll receive continuous pressure on your thumbnail for 30 seconds. Every 10 seconds, I will ask you to rate your thumb pain using the same 0-100 scale. I can stop the test at any time if the pressure becomes intolerable. Do you have any questions?"***

2. Apply 0.25 kg/2-s sample pressures as needed to ensure proper thumb placement in MAST handpiece, if necessary.

3. On the MAST server, navigate to Testing Algorithms → Discrete Stimuli and select the **"MAPP CPM 1"** test. This test will apply a continuous 30 second pressure to the selected thumb. Note: Do not navigate to the "Conditioned Pain Modulation" option under Testing Algorithms as this is a different test.
    a. Select dominant (left or right) thumb (this should be the same as the ascending test).
    b. Enter the **adjusted Pain40** pressure value in the "Start Intensity" and "End Intensity" box.
    c. Press the Green check mark to load the test.
    d. **Uncheck the "Enable Rating" box on the MAST main screen**.
    e. Press PLAY to start the test.

4. A "1" will be located on the MAST display in the "Rate Time" row at each 10 second interval which requires a pain rating. Request a pain rating at each time by saying: ***"Prepare to rate the pain on the thumb***" about 1-2 seconds prior to the rating followed by ***"Rate your thumb pain now"*** at the exact time.
    a. Participant will verbally report his/her thumb pain intensity rating 3 times (every 10 seconds during thumbnail pressure) and these will be recorded in the QST CRF. These data will not be saved in the MAST computer.
    b. **Stop the test manually** after obtaining the 3 pain ratings by hitting the STOP or PAUSE buttons.
    c. **Note:** Continuous thumbnail pressure pain tends to summate of over time. It is not uncommon for pain ratings to increase during the 30 seconds. Do not stop testing unless the participant requests the test to stop because it is unbearable or they are rating the pain as 100. If you do stop prematurely, request a pain rating at the stopping point after the pressure is released. At least two pain ratings should be obtained for the analysis. If two ratings are collected, move on to CPM 2 and 3. Follow the same procedure for MAST testing in CPM 2 and CPM 3
    d. If the pressure used for this test is unbearable (or rated greater than 80) and the participant is unwilling or unable to endure it again (i.e., for CPM 2 and 3), it will be necessary to recalibrate the Pain40 pressure level and repeat CPM 1. The CPM 1 procedure should only be repeated one time. Following re-calibration and retesting, confirm that participant will be able to tolerate the new Pain40 pressure for several additional tests. If the pain is still unbearable or if the participant is unwilling to continue, skip CPM for this visit only.

5. Wait **ten (10)** minutes before conducting CPM 2.
    a. During this break, the next instructions should be reviewed with the participant and the participant can feel the neutral water foot bath with his/her hand if desired.
    b. At this time, the RC should also secure the plastic foot covers using the included ties.

CPM 2: Test Stimulus (Dominant Thumb) + Neutral Conditioning Stimulus (non-dominant foot)

1. Participant should again hold the MAST handset with their dominant hand on the testing table, and prepare to place their non-dominant foot in the 32.0°C foot bath for 60 seconds, with the bottom of their foot resting on the slotted foot rest. Apply sample pressures to thumbnail to ensure proper thumb placement. Participant should be wearing protective plastic foot covers.

2. RC will read: ***"For the next test, you will receive thumbnail pressure while having your foot submersed in water. Once we get started, I'll ask you to place your non-dominant foot into a foot bath filled with lukewarm water for 60 seconds. During the first 30 seconds, I'll ask you to rate your foot pain from 0-100. After 30 seconds, while your foot is still in the water, pressure will be applied to your dominant thumb. During that time, I will ask you to rate only the intensity of your thumb pain. I'll remind you of this during the test as well. If you find the test too painful and would like to stop early, remember you can remove your foot from the water at any time, or say 'stop' if you would like me to remove the pressure from your thumb. Do you have any questions?"***

3. On the MAST server, navigate to Testing Algorithms → Discrete Stimuli and select the "MAPP CPM 2/3" test. This test will apply a continuous 30 second pressure to the selected thumb.
    a. Select dominant (left or right) thumb.
    b. Enter the **adjusted Pain40** pressure value in the "Start Intensity" and "End Intensity" box
    c. Press the Green check mark to load the test.
    d. **Uncheck the "Enable Rating" box on the MAST main screen.**

4. Instruct participant to immerse non-dominant foot in the 32° C foot bath and, at the same time, press PLAY on the MAST server. Pressure delivery will initiate after a 30 second delay.

5. Using a timer, ask the participant to verbally rate the intensity of pain in his/her foot produced by the water at 10- and 25-seconds. Record ratings on the QST CRF.

6. Pressure will be applied to the dominant thumbnail for 30 seconds concurrent to the last 30 seconds of the neutral water foot bath.
    a. A "1" will be located on the MAST display in the "Rate Time" row at each 10 second interval which requires a pain rating (i.e., 40-, 50-, and 60-seconds). Request a pain rating at each time by saying: ***"Prepare to rate the pain on the thumb***" about 1-2 seconds prior to the rating followed by ***"Rate your thumb pain now"*** at the exact time.
    b. Participant will verbally report his/her thumb pain rating 3 times total (every 10 seconds during thumbnail pressure) and these will be recorded in the QST CRF. These data will not be saved in the MAST computer.
    c. Stop the test manually after obtaining the 3 pain ratings by hitting the STOP or PAUSE buttons.

7. Instruct participant to remove foot from water. Obtain final pain intensity rating of foot bath immediately following foot withdrawal (~60 seconds) and record on QST CRF.

8. Foot should be dried and wrapped in a towel to stay warm between tests. Plastic foot covers should be left in place on both feet.

9. **IMMEDIATELY:** Increase water temperature of foot bath to 44.5°C, while keeping display hidden from participant. Press "Stop" and then "Menu" on the immersion circulator, use arrow buttons to increase temperature, and then press the "Play" button to initiate heating.

Hot Water Calibration (dominant foot)

1. The time needed for the water bath temperate to increase from neutral to painful (i.e., from 32°C to 44.5°C) is approximately 10-12 minutes. During that break, instructions for the next test should be reviewed with participant. Once water bath has reached 44.5°C, calibration may begin.

2. RC will read: ***"Now, I'll be having you rate the painfulness of a hot water foot bath. The water temperature will be similar to a hot shower or hot tub, but it is not hot enough to cause a burn. You're welcome to use your hand to feel the water before we get started. In a few moments, I'll ask you to place your dominant foot in the hot water bath for 30 seconds. Every 10 seconds, I'll ask you to rate the pain in your foot using the same 0-100 scale. You can remove your foot from the water at any time if the pain becomes intolerable."***

3. Participant will be asked to place their dominant foot into the hot water bath for 30 seconds and rate the pain intensity of the foot bath at 10-, 20-, and 30-seconds.

4. Record the Initial hot water temperature (**44.5 °C**) and the Initial hot water pain rating (rating given at 30 seconds) on the QST CRF. Ratings provided at 10- and 20-seconds need not be recorded during the calibration.

5. After 30 seconds, participant may remove foot from water.
    a. If final rating (at 30 seconds) is between **30-70/100** → proceed with CPM 3.
    b. If final rating (at 30 seconds) is:
        i. **≤ 15/100**: increase water temperature 2°C and repeat calibration test immediately after target water temperature has been reached (i.e., increase from 44.5°C to 46.5°C). **DO NOT INCREASE TEMPERATURE BEYOND 46.5°C.**
        ii. **16-29/100**: increase water temperature 1°C and repeat calibration test immediately after target water temperature has been reached (i.e., increase from 44.5°C to 45.5°C).
        iii. If a participant does not find the 46.5°C maximum allowed water temperature painful at a **minimum level of 30/100**, reposition the immersion circulator to provide direct water current over the foot. The temperature remains the same, yet the turbulence will increase the perceived pain intensity. Continue CPM testing even if participant does not find this painful. Indicate that immersion circulator was repositioned on CRF.
    c. If final rating (at 30 seconds) is:
        i. **71-80/100**: decrease water temperature 1°C and repeat calibration test immediately once water temperature has been reached (i.e., decrease from 44.5°C to 43.5°C).

ii. **81-100/100**: decrease water temperature 2-3°C and repeat calibration test immediately once water temperature has been reached (i.e., decrease from 44.5°C to 43.5°C [or 42.5°C if judgment calls for it]).
iii. Tips for decreasing water temperature:
    1. Add small amount of ice and stir, *or*
    2. Using pitcher, remove a few cups of water from the water bath and replace with a few cups of colder tap water.

6. Continue calibration until you've determined a water temperature that is likely to elicit a rating of 30-70 on the 0-100 NRS and will be tolerated for 60 seconds. Note: the optimal pain rating of the hot water foot bath is 50-60.

7. Record final Hot Water Temperature and Hot Water Pain Rating on the QST CRF.

8. Immediately proceed with CPM 3 after appropriate conditioning stimulus intensity (i.e., hot water temperature) has been determined.

CPM 3: Test Stimulus (Dominant Thumb) + Painful Conditioning Stimulus (non-dominant foot)

1. Participant should again hold the MAST handset with their dominant hand, and prepare to place their non-dominant foot in the hot water bath for 60 seconds, with the bottom of their foot resting on the slotted foot rest. Apply sample pressures to thumbnail to ensure proper thumb placement. Participant should be wearing protective plastic foot covers.

2. RC will read: ***For our last test, you will again receive thumbnail pressure while having your foot submersed in water. This time, the water bath will still be hot, but again it is not hot enough to cause a burn. Once we get started, I'll ask you to place your non-dominant foot into the foot bath for 60 seconds. During the first 30 seconds, I'll ask you rate the pain in your foot from 0-100. After 30 seconds, while your foot is still in the water, pressure will be applied to your dominant thumb. During that time, I will ask you to rate only the intensity of your thumb pain. As before, we can stop the test at any time if it becomes unbearable. Do you have any questions?***

3. On the MAST server, press the **RESET** button to reload the "MAPP CPM 2/3" test. This test will apply a continuous 30 second pressure to the dominant thumb at the previously select Pain40 pressure level.

4. Instruct participant to immerse non-dominant foot into the hot foot bath at the temperature determined during the calibration phase, and at the same time, press PLAY on the MAST server. Pressure delivery will initiate after a 30 second delay.

5. Using a timer, ask the participant to verbally rate the intensity of pain in his/her foot produced by the water at 10- and 25-seconds. Record ratings on the QST CRF.

6. Pressure will be applied to the dominant thumbnail for 30 seconds concurrent to the last 30 seconds of the hot foot bath.
    a. A "1" will be located on the MAST display in the "Rate Time" row at each 10 second interval which requires a pain rating (i.e., 40-, 50-, and 60-seconds). Request a pain rating at each time by saying: ***"Prepare to rate the pain on the thumb***" about 1-2

seconds prior to the rating followed by ***"Rate your thumb pain now"*** at the exact time.

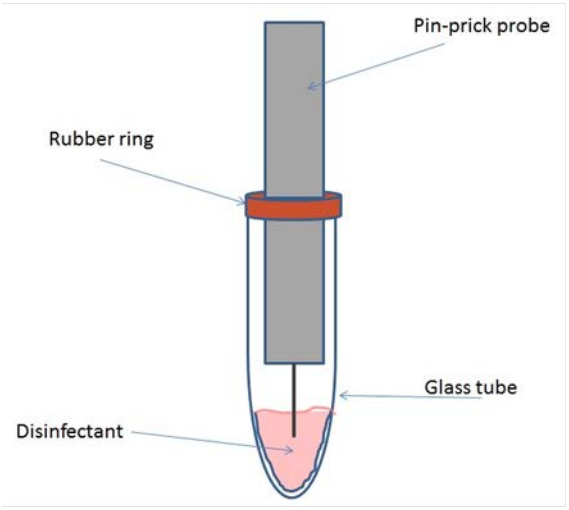
b. Participant will verbally report his/her thumb pain rating a total of 3 times (every 10 seconds during thumbnail pressure) and these will be recorded in the QST CRF. This data will not be saved in the MAST computer.
c. **Stop the test manually after obtaining the 3 pain ratings by hitting the STOP or PAUSE buttons**.

7. Instruct participant to remove foot from water. Obtain final water pain intensity rating immediately following foot withdrawal (~60 seconds) and record on QST CRF.

8. Remove and dispose of plastic foot covers and provide participant a towel to dry his/her feet.

Post-Visit Checklist and Supplies

- ☐ Disinfect PinPrick Stimulator for 10 minutes by submerging the tip (only) in approved disinfectant solution using the supplied tube and storage rack. Add 3.5 mL of **diluted** ECOLAB neutral disinfectant into the glass tube. Place the rubber ring on the stimulator (down until the word *THE*) before submerging in disinfectant (rubber ring can be left in place permanently). Put on and remove the rubber ring ONLY from the **blunt** end of the probe. Rinsing is not necessary.



- ☐ Clean algometer tip with alcohol swabs or sanitizing wipes.

- ☐ Clean MAST handpiece and stylus <u>as needed</u> with a very slightly damp cloth using water. Apply water to the cloth only. Do not apply water directly to the MAST. Only wipe handle and thumb hole. Avoid getting moisture near the power switch, recharging port, or ventilation slots. If contaminated (e.g., sick patient sneezed on unit), clean with sanitizing wipes (Lysol or Clorox Wipes, or similar). DO NOT USE products containing alcohol, bleach or ammonia.

- ☐ Empty water bath.
  - o First turn OFF the immersion circulator, carefully disconnect it from the water bath, and gently shake out any water in the device onto towels. Allow it to thoroughly air dry before packing away.
  - o It is suggested that a pitcher be used to remove most of the water before picking up the water bath, to make it easier to lift. Pour out the water, and allow to thoroughly air dry. Wash as needed with mild dish soap and warm water. Do not use solutions containing bleach or ammonia.

- ☐ Upload MAST Test data file to DCC, if not already done.

Disposable Supplies (each site required to purchase)

1. PinPrick Disinfectant
    - ECOLAB Neutral Disinfectant Cleaner, #14541, 1.3 L (~ $40-100).
    - Available at the following website or through your institutional supplier at a lower cost
    - https://catalog.nationalew.com/catalog/CatalogSearch.aspx?Value=14541&Type=Anything
    - Directions for diluting ECOLAB neutral disinfectant: The disinfectant comes in a concentrated solution than needs to be diluted before use. The standard dilution is: 1:128 – i.e. add ½ ounce (approx. 15 ml) of the solution in 0.5 gallon (approx. 1900 mL) tap water. The diluted solution can be kept in a sealed plastic container (e.g., specimen container, such as for 24-h urine collection) for 6 months.
    - Follow OSHA and institutional guidelines regarding handling, storage, and disposal of ECOLAB Neutral Disinfectant Cleaner (refer to MSDS for details).

2. CPM Foot Covers
    - CLEAR PLASTIC BOOT SHOE COVERS 16" TALL WITH TIES; Product Code: 13-BT805, (500/CASE) $43.95
    - http://www.saraglove.com/Clear-Plastic-Polyethylene-16-Boot-Covers-p/13-bt805.htm?sku=13+BT805+XL+C2&vfsku=13.BT805.XL.C2&Click=6866&vfsku=13.BT805.XL.C2&gpla=pla&gclid=CjwKEAjw3YipBRDL2bHhjLmFkQsSJADtzktjzZ2LcoPUgva-JBtXkJ_T7Ew-p0Ekrs1iNMf1dr_UABoCATPw_wcB\

Equipment Scheduled Maintenance

1. **MAST Handpiece**
    - **MAST handpiece should be returned to University of Michigan for re-calibration after approximately 50% of patients have completed their 6 month visit**. Contact the MAST Help Desk to schedule this service. Under normal circumstances, devices will be returned within 5 days of receipt.
    - Sites may request more frequent calibration checks as needed.
    - **Rubber tips should be monitored regularly and replaced (by the RC) if they appear cracked or damaged.** Several extra tips are supplied with each MAST system. Additional tips can be ordered free of charge from University of Michigan. Tips screw easily into the plunger end and can be removed and replaced by hand.
    - Rubber tips should be routinely checked prior to testing to ensure they are securely tightened. Loose tips will result in inaccurate force delivery and data collection. Contact the MAST Help Desk for additional guidance if necessary.

2. **Algometer**
    - No scheduled maintenance should be required for the duration of the MAPP study. However, please contact the QST Workgroup if you notice unusual operation or the rubber tip becomes displaced or damaged.

3. **PinPrick Stimulator**
    - The Pinprick Stimulator is extremely delicate and should be handled with care to prevent the needle from getting bent or damaged.
    - The QST Workgroup will continually monitor needle wear at several sites and will provide additional guidance regarding maintenance as necessary.
    - Contact the QST Workgroup immediately if the needle becomes damaged.

4. **Foot Bath/Immersion Circulator**
    - Water temperature should be checked every month using a thermometer. Fill the foot bath as normal and set temperature to 46.5°C. Record temperature in the middle of the foot bath (in the normal location of the foot during testing). Temperature should be within +/- 0.5 degrees of target.
    - **To extend the life of the foot bath, hot water should be removed as quickly as possible after the CPM procedure is completed.**
    - Foot bath should be regularly monitored for cracks in the plastic.

Appendix 1: Order of Procedures

Participant seated

1. QST Pre-Procedure Diagnostic Questions
2. MAST Familiarization and Practice Test (non-dominant thumb)
3. MAST Ascending Test (dominant thumb)
4. Algometer Familiarization (non-dominant forearm)
5. PinPrick Familiarization (non-dominant forearm)

Participant supine

6. Forearm Algometer Test (dominant forearm)
7. Suprapubic Algometer Test
8. Forearm PinPrick Test (dominant forearm)
9. Suprapubic PinPrick Test

Participant returns to seated position

10. Trapezius Algometer Test (pain threshold, left and right trapezius)
11. Participant removes shoes and socks, and puts on foot covers
12. Pain40 calibration (MAST dominant thumb)
13. CPM 1: Test Stimulus Only (MAST dominant thumb)

10 min break

14. CPM 2: Test Stimulus (MAST dominant thumb) + Neutral Water (non-dominant foot)

10 min break

15. Hot Water Calibration (dominant foot)
16. CPM 3: Test Stimulus (MAST dominant thumb) + Hot Water (non-dominant foot)

Appendix 2: Participant Instructional Scripts

(In Chronological Order)

MAST Familiarization Protocol (non-dominant thumb)

RC will read. ***In a few moments, I will conduct a test to measure your sensitivity to pressure. We will start with a practice test before conducting the actual test so that you can become comfortable with this procedure.***

During testing, pressures will be applied to your thumbnail for 5 seconds. AFTER each pressure is released, you will be asked to rate how painful it felt using a rating scale with numbers 0 through 100. This scale is shown here on this computer screen. A zero (0) means 'no pain' and a one hundred (100) means 'pain as bad as you could imagine.' To indicate your rating, you will use a stylus to press the location on the scale that best describes the intensity of the pain in your thumb. If you prefer, you may these arrows at the bottom of the screen to adjust your rating up or down. Once you have selected your rating, press the Green confirm button in the lower right corner of the screen. Keep in mind that there is no right or wrong response, and you can use any number from 0 to 100. Some pressures may be painful while others may not be. Remember, if it just feels like pressure but not pain, rate (0) zero. If any pressure is intolerable, let me know and I can release it immediately. You can also release the pressure yourself by pressing the 'STOP' button on your screen. You can also let me know if you feel your thumb is not in the correct position and needs to be adjusted. During testing, sit in a comfortable position and try to relax. This test usually takes 5-7 minutes to complete. Do you have any questions?"

MAST Ascending Test (dominant thumb)

RC will read: ***"Now that we've completed the practice test, we are now going to conduct the actual test on your dominant thumb. Do you have any questions before we get started?"***

Algometer Familiarization Protocol (non-dominant forearm)

RC will read: ***"The next set of tests will be conducted while you lay down. Before we do that, I would like to show you the devices we'll be using. First, I'll be using this pressure algometer to test of your sensitivity to pressure on your forearm and on an area located a few inches below your belly button. I'll ask you to rate the pain intensity of each pressure that I apply using the same 0-100 pain rating scale we used during the previous test, with 0 representing a sensation of pressure but no pain, and 100 representing 'pain as bad as you can imagine.' I will now apply a couple sample pressures on your forearm so that you can experience how it feels."***

Algometer Test

Forearm Algometry

RC reads: ***"First, I'll start with the pressure test. After each pressure, I'll ask you to rate the pain intensity of the pressure from 0-100. Remember, if it just feels like pressure but not pain, rate (0) zero. If the test becomes too uncomfortable, please let me know, and we can stop at any time. A total of six pressures will be applied to your forearm. Also, I will be wearing headphones during the test so that I can listen to a beeping pattern that will help me apply the pressures with correct timing."***

Suprapubic Algometry

RC reads: ***"Next, I will test the area below your belly button. To help me locate this area, I would like you to find your pubic bone. Using your hand, over your clothing, start at your waist and move down toward your groin pushing downward until you feel a bone – that is your pubic bone. Next, I'll have you point to your belly button. The area that I'll be testing is halfway between those two points, and I'll need you to expose just a small area of skin at that point so that I can conduct the test. Remember, if the test becomes too uncomfortable, please let me know, and we can stop at any time. A total of six pressures will be applied to this area."***

Trapezius Algometry (Pressure Pain Threshold)

RC reads: "***For this next test, I'll be applying a gradually increasing pressure on your shoulders. Rather than having you rate the pain, I would like you to indicate the moment the sensation changes from a feeling of pressure to a feeling of pain. You can say something like "pain" or "now" or "stop" when this occurs. Once you start to feel any pain, even a small amount, please tell me and that's when I'll stop. First, I'll apply a practice pressure to your right shoulder and you can tell me when you start to feel pain."***

PinPrick Familiarization Protocol (non-dominant forearm)

RC will read: ***"For the second procedure I will be using this <u>pointed skin probe</u> to stimulate your forearm and the area below your belly button. As you can see, this device has a pointed end; however, it will not pierce or stick the skin.***

Similar to the previous procedure, I'll also be asking you to rate the intensity of this sensation. First, I'll apply a single stimulus and ask you to rate the pain intensity from 0-100, with 0 indicating 'no pain' or any kind of pricking or stinging sensation´, and 100 indicating 'pain as bad as you can imagine.' Next, I'll apply a series of ten stimuli in a row, and then ask you to rate the overall pain intensity of the entire series of ten stimuli using the same scale. I would now like to give you a practice test on your forearm. Do you have any questions before we proceed?

Forearm PinPrick Test; Suprapubic PinPrick Test

RC will read: ***"Next we'll conduct the test using the pointed skin probe. Remember, I'll apply a single stimulus and have you rate the pain intensity of that single stimulus from 0-100. After that, I'll apply a series of ten stimuli and have you rate the overall pain intensity of the entire series of ten. I'll repeat this test a total of three times at both your forearm and at that the area below your belly button. After the testing is done, I'll also be asking you to rate any lasting pain you have at the site for a few seconds. If test becomes too painful, let me know, and we can stop at any time."***

Conditioned Pain Modulation (CPM)

MAST Pain40 Calibration

RC will read: ***"For the final series of tests, I will again be applying pressure to your dominant thumb. In the first test, I will apply a series of 5 second pressures and ask you to rate the pain intensity of each one from 0-100. This time you won't need to enter your ratings into a computer, but instead you will say your pain rating out loud and I will record it. Any questions?"***

CPM 1: Test Stimulus Alone (MAST dominant thumb)

RC will read: ***"Next, you'll receive continuous pressure on your thumbnail for 30 seconds. Every 10 seconds, I will ask you to rate your thumb pain using the same 0-100 scale. I can stop the test at any time if the pressure becomes intolerable. Do you have any questions?"***

CPM 2: Test Stimulus (Dominant Thumb) + Neutral Water Conditioning Stimulus (non-dominant foot)

RC will read: ***"For the next test, you will receive thumbnail pressure while having your foot submersed in water. Once we get started, I'll ask you to place your non-dominant foot into a foot bath filled with lukewarm water for 60 seconds. During the first 30 seconds, I'll ask you to rate your foot pain from 0-100. After 30 seconds, while your foot is still in the water, pressure will be applied to your dominant thumb. During that time, I will ask you to rate only the intensity of your thumb pain. I'll remind you of this during the test as well. If you find the test too painful and would like to stop early, remember you can remove your foot from the water at any time, or say 'stop' if you would like me to remove the pressure from your thumb. Do you have any questions?"***

Hot Water Calibration (dominant foot)

RC will read: ***"Now, I'll be having you rate the painfulness of a hot water foot bath. The water temperature will be similar to a hot shower or hot tub, but it is not hot enough to cause a burn. You're welcome to use your hand to feel the water before we get started. In a few moments, I'll ask you to place your dominant foot in the hot water bath for 30 seconds. Every 10 seconds, I'll ask you to rate the pain in your foot using the same 0-100 scale. You can remove your foot from the water at any time if the pain becomes intolerable."***

CPM 3: Test Stimulus (Dominant Thumb) + Painful Hot Water Conditioning Stimulus (non-dominant foot)

RC will read: ***For our last test, you will again receive thumbnail pressure while having your foot submersed in water. This time, the water bath will still be hot, but again it is not hot enough to cause a burn. Once we get started, I'll ask you to place your non-dominant foot into the foot bath for 60 seconds. During the first 30 seconds, I'll ask you rate the pain in your foot from 0-100. After 30 seconds, while your foot is still in the water, pressure will be applied to your dominant thumb. During that time, I will ask you to rate only the intensity of your thumb pain. As before, we can stop the test at any time if it becomes unbearable. Do you have any questions?***