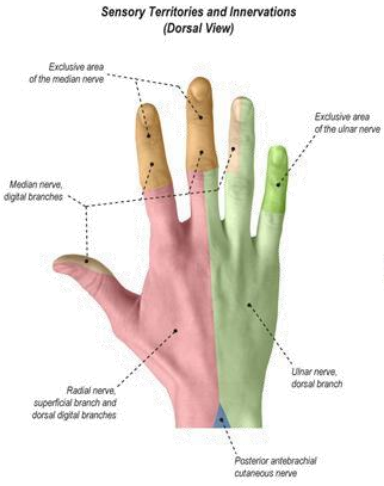
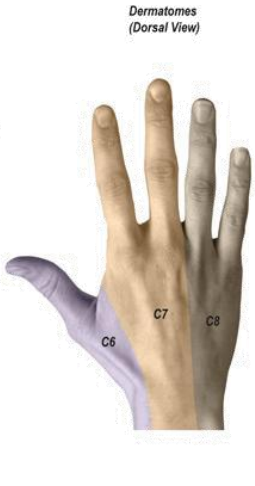
Appendix 1. Procedure and verbal instructions for performing quantitative sensory testing.

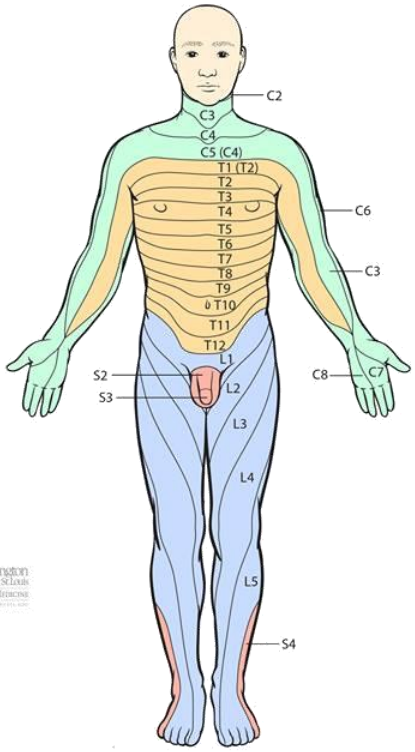
STANDARDIZED PROTOCOL

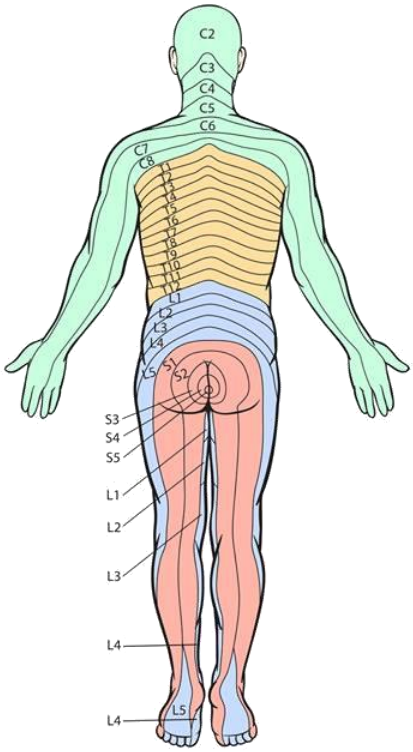
General instructions

- ✓ Complete the entire protocol in the suggested order.
- ✓ The experimenter must not wear perfume.
- ✓ Ask participants to remove their jewellery from around the areas to be tested.
- ✓ Prepare the thermode and the Medoc Main Station software (turn the device on before you start the program).
- ✓ Identify the areas to be tested with a washable marker.
- ✓ In these areas, draw a 1 cm^2 circle in an area without hair (this area will be used for the mechanical-DT).
- ✓ Photograph the areas tested to make sure the same areas are retested at each visit.
- ✓ Test the non-painful area(s) first followed by the painful area(s).
- ✓ Participants must keep their eyes closed for the duration of the tests.
- ✓ Be alert to signs of pain in your participant (if you suspect that a spike of pain may have influenced the participant's concentration and confirm this with his/her you should repeat the trial).
- ✓ If too much pain is generated during a test, consider reducing the number of trials or allow the patient to take a short break. Sometimes, participants need to get up and move because their limbs go numb.
- ✓ If you decide to reduce the number of trials due to pain intensity, write "TMP" (too much pain) at the appropriate place.
- ✓ If the threshold is not reachable, write "NA" (not applicable) at the appropriate place.
- ✓ Be aware that sensory perception may be different from one dermatome/cutaneous innervation area to another (i.e., the C8 dermatome area on the hand may present allodynia, but not the C7 area). If you detect significant differences between your trials, it may mean that the sensory profiles of these two areas are different. Try to test areas that have the same sensory profile.









Thermal Stimuli

TSA-II NeuroSensory Analyzer
(Medoc, Ramat Yishai, Israel)

Thermode
(Medoc, Ramat Yishai, Israel)

1. COLD DETECTION THRESHOLD

- Practice the test on another part of the body first until the participant understands the task.
- Use the TSA-II thermode (3 x 3 cm).
- Do not attach the thermode; hold it firmly in place but without more pressure than the weight of the thermode.
- Choose the Medoc program: Cold detection threshold.
- Baseline temperature of 32°C.
- Descending ramp of 0.5°C/s.
- Minimum temperature of 0°C.
- Ascending ramp of 1°C/s to come back to the baseline temperature of 32°C.
- Repeat the test 3 times on each area by moving the thermode slightly between each trial and using an ISI variable of 5 to 8 s (in order to avoid sensitization of the area due to a spatio-temporal summation).
- The threshold is calculated by averaging the temperatures of the 3 trials.
- Do not touch the participant with anything other than the thermode (wires, etc.) during the test.
- Help the participant to determine the beginning of the test with a countdown: “The test will start in 3, 2, 1, 0”.
- Before each trial, ask: “How is the temperature of the thermode? Neutral, cold or warm?” (The patient should reply “neutral”. If not, ask him/her to rub the tested area with his/her hand to normalize it).

INSTRUCTIONS

1. I will place a thermode on your skin.
2. This device can control temperature variations very precisely.
3. I will ask you to close your eyes and concentrate on the temperature.
4. During the test, the temperature of the thermode will cool down.
5. As soon as you feel the slightest cooling sensation, please press the mouse button.
6. The goal here is not to test your endurance but to evaluate your ability to detect the sensation of cold.
7. I will repeat the test 3 times on each area.
8. Are you ready? Please close your eyes and concentrate on the temperature.

2. HEAT DETECTION THRESHOLD

- Practice the test on another part of the body first until the participant understands the task.
- Use the TSA-II thermode (3 x 3 cm).
- Do not attach the thermode; hold it firmly in place but without more pressure than the weight of the thermode.
- Choose the Medoc program: Heat detection threshold.
- Baseline temperature of 32°C with an ascending ramp of 0.5°C/s.
- Maximum temperature of 50°C.
- Descending ramp of 1°C/s to come back to the baseline temperature of 32°C.
- Repeat the test 3 times on each area by moving the thermode slightly between each trial and using an ISI variable of 5 to 8 s (in order to avoid sensitization of the area due to a spatio-temporal summation).
- The threshold is calculated by averaging the temperatures of the 3 trials.
- Do not touch the participant with anything other than the thermode (wires, etc.) during the test.
- Help the participant to determine the beginning of the test with a countdown: "The test will start in 3, 2, 1, 0".
- Before each trial, ask: "How is the temperature of the thermode? Neutral, cold or warm?" (The patient should reply "neutral". If not, ask him to rub the tested area with his hands to normalize it).

INSTRUCTIONS

1. I will ask you to close your eyes and concentrate on the temperature.
2. During the test, the temperature of the thermode will warm up.
3. As soon as you feel the slightest warming sensation, please press the mouse button.
4. The goal here is not to test your endurance but to evaluate your ability to detect the sensation of heat.
5. I will repeat the test 3 times on each area.
6. Are you ready? Please close your eyes and concentrate on the temperature.

3. COLD PAIN THRESHOLD

- Practice the test on another part of the body first until the participant understands the task.
- Use the TSA-II thermode (3 x 3 cm).
- Do not attach the thermode; hold it firmly in place but without more pressure than the weight of the thermode.
- Choose the Medoc program: Cold pain threshold.
- Baseline temperature of 32°C with a descending ramp of 1°C/s.
- Minimum temperature of 0°C.
- Ascending ramp of 8°C/s to come back to the baseline temperature of 32°C.
- Repeat the test 3 times on each area by moving the thermode slightly between each trial and using an ISI variable of 5 to 8 s (in order to avoid sensitization of the area due to a spatio-temporal summation).
- The threshold is calculated by averaging the temperatures of the 3 trials.
- Do not touch the participant with anything other than the thermode (wires, etc.) during the test.
- Help the participant to determine the beginning of the test with a countdown: "The test will start in 3, 2, 1, 0".
- Before each trial, ask: "How is the temperature of the thermode? Neutral, cold or warm?" (The patient should reply "neutral". If not, ask him to rub the tested area with his hands to normalize it).

INSTRUCTIONS

1. Every 30 seconds, I will ask you to close your eyes and concentrate on the temperature.
2. At this point, the temperature will begin to cool down.
3. It will become increasingly cold. Please press the mouse button as soon as you feel a painful sensation on top of the cold sensation: in other words, the sensation will change from cold to an uncomfortable sensation (pinching or burning).

4. As soon as you feel the first sensation of pinching or burning, even if this sensation is perfectly bearable, you must press the mouse button.
5. When you press the mouse button, the thermode will return to the baseline temperature.
6. I will repeat the test 3 times on each area.
7. The goal here is not to test your endurance but to evaluate your ability to detect a painful cold sensation.
8. Are you ready? Please close your eyes and concentrate on the temperature.

4. HEAT PAIN THRESHOLD

- Practice the test on another part of the body first until the participant understands the task.
- Use the TSA-II thermode (3 x 3 cm).
- Do not attach the thermode; hold it firmly in place but without more pressure than the weight of the thermode.
- Choose the Medoc program: Heat pain threshold.
- Baseline temperature of 32°C.
- Ascending ramp of 1°C/s.
- Maximum temperature of 50°C.
- Descending ramp of 8°C/s to come back to the baseline temperature of 32°C.
- Repeat the test 3 times on each area by moving the thermode slightly between each trial and using an ISI variable of 5 to 8 s (in order to avoid sensitization of the area due to a spatio-temporal summation).
- The threshold is calculated by averaging the temperatures of the 3 trials.
- Do not touch the participant with anything other than the thermode (wires, etc.) during the test.
- Help the participant to determine the beginning of the test with a countdown: “The test will start in 3, 2, 1, 0”.
- Before each trial, ask: “How is the temperature of the thermode? Neutral, cold or warm?” (The patient should reply “neutral”. If not ask him to rub the tested area with his hands to normalize it).

INSTRUCTIONS

1. Every 30 seconds, I will ask you to close your eyes and concentrate on the temperature.
2. At this point, the temperature will begin to warm up.
3. It will become increasingly hot. Please press the mouse button as soon as you feel a painful sensation on top of the heat sensation: in other words, the sensation will change from warm to an uncomfortable sensation (pinching or burning).
4. As soon as you feel the first sensation of pinching or burning, even if this sensation is perfectly bearable, you must press the mouse button.
5. When you press the mouse button, the thermode will return to the baseline temperature.
6. I will repeat the test 3 times on each area.
7. The goal here is not to test your endurance but to evaluate your ability to detect a painful heat sensation.
8. Are you ready? Please close your eyes and concentrate on the temperature.

Mechanical Stimuli

5. MECHANICAL DETECTION THRESHOLD

- Practice the test on another part of the body first until the participant understands the task.
- Use von Frey monofilaments (Bioseb).
- This is a set of 20 nylon filaments that range in force from 0.008 g to 300 g according to a logarithmic scale.
- The monofilament is pressed against the skin with sufficient force to cause it to bend and form a U-shape.
- The larger the filament, the greater the force applied.
- Present the stimulus only within the 1 cm^2 circle on a hairless area of the skin, waiting 5 to 8 seconds between each stimulus (make sure you do not touch hair with the monofilament because you will activate the sensory hair follicle receptor and confuse the participant with another stimulus).
- The contact time is 1 s.
- The filaments exert their nominal force when they begin to bend: Do not go beyond this force or you risk damaging the filaments.
- Inform the subject each time you are going to touch his/her skin with a filament by saying the word "now" and present the stimulus within one second. After the stimulation, ask the participant if he/she felt something. Test for false positives (randomly every 3 to 5 trials): do the movement without touching the skin to make sure that the subject is not simply responding to the feeling of movement. During the test itself, note the number of false positives on the answer sheet.
- Start with the 4.08 filament (1.0 g).
    - If the subject is able to detect the 4.08 (1.0 g) filament, repeat the stimulation using a thinner filament [3.84 (0.6 g)]. Continue with a lower strength filament until the participant does not feel the sensation of touch and note the force (g) of the last filament detected. Next, present the last filament detected. If the subject does not feel a sensation of touch with this filament, increase the force until a sensation is detected. Note the force of this filament. Repeat these manipulations until you have crossed the threshold 6 times (3 times down and 3 times up). The threshold is the average of the 6 forces (g) recorded.
    - If the subject does not detect the 4.08 (1.0 g) filament, repeat the stimulation using a thicker filament [4.17 (1.4 g)]. Continue with a higher strength filament until the participant detects a sensation of touch and note the force (g) of the first filament detected. Next, repeat the stimulation using a thinner filament. Continue with a lower strength filament until the participant does not feel the sensation of touch and note the force (g) of the last filament detected. Repeat these manipulations until you have crossed the threshold 6 times (3 times down and 3 times up). The threshold is the average of the 6 forces (g) recorded.

Size	1,65	2,36	2,44	2,83	3,22	3,61	3,84	4,08	4,17	4,31	4,56	4,74	4,93	5,07	5,18	5,46	5,88	6,1	6,45	6,65
Force (g)	0,008	0,02	0,04	0,07	0,16	0,4	0,6	1	1,4	2	4	6	8	10	15	26	60	100	180	300

INSTRUCTIONS

1. During the test, I will ask you to keep your eyes closed.
2. During the test, I will press thin filaments against your skin.
3. Your task is to focus only on the sensation caused by the filaments.
4. Before each test, I will say the word "now" and in the next second, I will touch your skin with one of these filaments.
5. Sometimes I will touch your skin and sometimes I will not.
6. You must tell me if you felt the filament touching your skin or not.
7. During the test, it will become more and more difficult to feel the filaments.
8. Then, the order will be reversed, and it will become easier to feel the filaments.
9. If you think I touched your skin after I said "now", please say "yes", but if you did not feel the sensation, please say "no."
10. Do you understand the instructions?
11. Please close your eyes and concentrate on the sensation of touch.

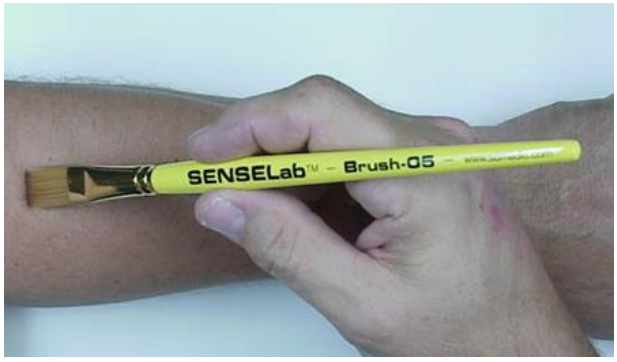
Von Frey Monofilaments
(Bioseb, Pinellas Park, Florida, United States)

6. MECHANICAL DYNAMIC ALLODYNIA

- Practice the test on another part of the body first until the participant understands the task.
- Use a SENSELab Brush-05 (Somedic).
- Bring the brush in contact with the skin and move it (distance of approximately 2 cm) until the brush filaments bend slightly.
- After each trial, ask the patient to score the pain intensity using the visual analogue scale (VAS)
- Test each area 5 times.
- Each trial must be performed at different locations in the area tested.
- Wait 5 to 8 seconds between each trial (to avoid sensitization of the area tested due to spatio-temporal summation of the stimulus).
- Calculate and note the average of the VAS score of the 5 trials.

INSTRUCTIONS:

1. During the test, I will ask you to keep your eyes closed.
2. I will move the hairs of this brush on your skin after I say "now".
3. After each time I apply the brush on your skin, you will have to evaluate the sensation on this visual scale.
4. I will repeat the test 5 times on each area.
5. Do you understand the instructions?
6. Please close your eyes and focus on the sensation of the brush.



SENSELab Brush-05
(Somedic AB, Hörby, Sweden)

7. VIBRATION DETECTION THRESHOLD

- Practice the test on another part of the body first until the participant understands the task.
- Use the VSA-3000 biothesiometer.
- Choose the Medoc program: Heat pain threshold.
- Select a fixed frequency of 100 Hz.
- Select an ascending ramp of 1 µ/s.
- Repeat the test 3 times on each area by moving the biothesiometer slightly between each trial and using an ISI variable of 5 to 8 s (in order to avoid sensitization of the area due to a spatio-temporal summation).
- The threshold is calculated by averaging the vibration (µm) of the 3 trials.
- Do not touch the participant with anything other than the biothesiometer (wires, etc.) during the test.
- Help the participant to determine the beginning of the test with a countdown: "The test will start in 3, 2, 1, 0".

INSTRUCTIONS

1. I will place a biothesiometer on your skin.
2. This device can control changes in vibration intensity very precisely.
3. I will ask you to close your eyes and focus on the vibration.
4. During the test, the sensation of vibration will increase.
5. As soon as you feel the first sensation of vibration, please press the mouse button.
6. I will repeat the test 3 times on each area.
7. Do you understand the instructions? Please close your eyes and focus on the sensation of vibration.

TSA-II NeuroSensory Analyzer
(Medoc, Ramat Yishai, Israel)

Hand-held VSA
(Medoc, Ramat Yishai, Israel)

8. PRESSURE PAIN THRESHOLD

- Practice the test on another part of the body first until the participant understands the task.
- Use a Wagner Force Ten FDX Digital Algometer.
- Use a metronome to help you apply an ascending ramp of 0.5 kg/cm² s.
- Stop at 11 kg.
- Repeat the test 3 times on each area by moving the algometer slightly between each trial (in order to avoid sensitization of the area due to a spatio-temporal summation).
- Wait 1 minute between each trial.
- The threshold is calculated by averaging the force (kg) of the 3 trials.
- Help the participant to determine the beginning of the test with a countdown: "The test will start in 3, 2, 1, 0".

- If you are testing an area that is not supported by a surface, use your free hand as a support (see the picture below).

INSTRUCTIONS

1. *This device is an algometer and I will use it to evaluate your sensitivity to deep pain.*
2. *I will place the algometer on your skin and gradually increase the pressure.*
3. *Please say "stop" as soon as the pressure starts to be painful.*
4. *This is not a pain tolerance test.*
5. *So, you must say "stop" as soon as the pressure starts to be painful.*
6. *I will repeat the test 3 times on each area.*
7. *Do you understand the instructions? Please close your eyes and focus on the sensation of pressure.*

Wagner Force Ten™ FDX Digital Algometer
(Wagner, Greenwich, Connecticut, United States)

9. MECHANICAL WIND-UP RATIO OF TEMPORAL SUMMATION OF PAIN

- Practice the test on another part of the body first until the participant understands the task.
- Use the Owen Mumford Neuropen with Neurotips that produce a calibrated force of 40 g.
- For the single pinprick stimulus, press the Neuropen on the skin until the indicator is in the white square (which means that the force applied is 40 g).
- After each trial, ask the participant to score the pain intensity using the visual analogue scale (VAS).
- Note the score.
- Then apply a series of 10 repetitive stimuli and ask the participant to score the pain intensity using the VAS (use a metronome to help you to apply 1 stimulus per second within an area of 1 cm^2).
- Note the score.
- Repeat the test (1 stimulus followed by the series of 10 stimuli) 5 times on each area.
- Each trial (1 stimulus followed by the series of 10 stimuli) must be performed at different locations in the area tested.
- Wait 30 seconds between each trial.

- To calculate the ratio, subtract the score obtained after the series of 10 stimuli from the score obtained after the single stimulus.
- The mean ratio is calculated by averaging the ratio of the 5 trials.

INSTRUCTIONS FOR THE SINGLE PINPRICK STIMULUS

1. I will apply this instrument (Neuropen) to your skin once after I say "now" for 1 second and you will have to evaluate the intensity of the sensation felt on this visual scale.

2. The test can be stopped at any time if you find that it is too painful.

3. Do you understand the instructions? Please close your eyes and focus on the sensation.

INSTRUCTIONS FOR THE SERIES OF 10 REPETITIVE PINPRICK STIMULI

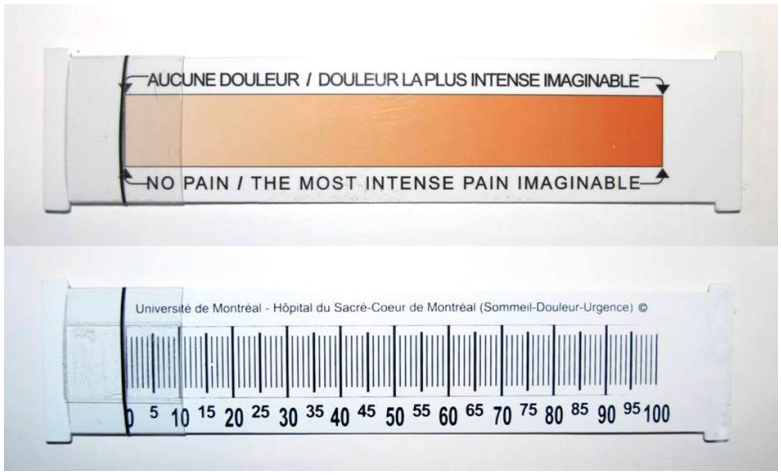
1. Now I am going to apply a series of 10 stimuli to your skin after I say "now".

2. After the series, I will ask you to evaluate the intensity of the sensation felt on this visual scale.

3. The test can be stopped at any time if you find that it is too painful.

4. Do you understand the instructions? Please close your eyes and focus on the sensation.

Owen Mumford Neuropen and Neurotips
(Owen Mumford, Saint-Marcel, France)



Visual Analogue Scale
(Raoul Daoust, Montreal, Quebec, Canada)