Motor Unit Number Index (MUNIX) Instructions & Qualification Process
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General procedure

MUNIX is a two-step measurement and will be performed from the APB, ADM, BB, TA and EDB muscles after supramaximal distal stimulation of the median, ulnar, musculocutaneous and peroneal nerves. Stimulation and recordings will be performed according to the following guidelines.

Particular attention should be paid to electrode position and temperature (not less than 29 degrees Celsius on the dorsum of hands and not less than 27 degrees Celsius on the dorsum of the feet). To ensure consistency in follow-up measurements the placement of electrodes and distance between the active and reference electrode will be standardized according to the provided photographic documentation. The recording electrode position will be adjusted several times to achieve maximal amplitude and risetime and a sharp negative take-off of the compound motor action potential (CMAP). This is one of the most critical steps and must be performed carefully.

Qualification

To ensure quality of measurements, each MUNIX investigator must have attended an in-person training session. This training session is mandatory for ENCALS participants and highly recommended for other researchers who want to implement this method! This recommendation results from former experience and according to the scientific literature. (Ref. 10-12) Prior to measuring study patients, each investigator has to study 4 normal subjects (e.g. staff members) twice at their center corresponding to the following protocol.

The whole set of 6 muscles (6 muscles assessed unilaterally) will be measured twice with at least 30 minutes between each session. Electrodes will be completely removed and any traces of electrode placement should be deleted/avoided. Optionally, both sides may be measured in one subject. For each set of 5 muscles, a separate session should be selected.

All measurements of each volunteer may be performed on the same day.

EMG-reports (resp. raw data if needed) will be sent to the central MUNIX coordination site (Neuromuscular Diseases Unit/ ALS clinic, Kantonsspital St. Gallen) by E-Mail (christoph.neuwirth@kssg.ch). One reviewer (C.N.) will evaluate all data for quality and review the MUNIX values and test-retest variability of each investigator. Investigators will be qualified and able to continue with the study if variability of measurements is less than 20%. This essential condition has already been used in a recent MUNE und MUNIX longitudinal ALS-studies.
Manual

Record CMAP

Please see below for more detailed instructions on maximizing CMAP. Electrode placement is described in detail for each individual muscle at the end of this manual of instructions. To record a CMAP connect the recording electrodes to channel 1 of the amplifier. Record the compound muscle action potential (CMAP) using supramaximal stimulation (Figure 1). Change the position of the ‘active’ or ‘E1’ electrode to obtain the CMAP with highest amplitude. It should not have an initial positive deflection and the expected shape. The markers ‘1’, ‘2’ and ‘3’ are placed at the onset, negative peak and baseline crossing of the CMAP. You can adjust their positions using the mouse, if necessary. The gain can also be adjusted if needed. The results table will show the associated measurements of the CMAP. If CMAP amplitude is less than 0.5 mV, results are probably unreliable and you can stop the MUNIX investigation in that muscle.

Figure 1

Maximizing CMAP

Place recording, reference, and ground electrodes appropriately for each muscle (see below for pictures of electrode placement for each muscle). In hand muscles and the EDB, reference electrode should be placed far distally. Procedures are identical to standard motor nerve conduction studies. After positioning the stimulator, gradually increase current intensity until CMAP reaches a plateau. Increase the current intensity by additional 20% to ensure supramaximal stimulation. After supramaximal stimulation has been achieved, replace the recording electrode several times until maximal CMAP amplitude has been recorded. This step is critical to the success of the study! Retest should be done blinded to previous CMAP results in the qualification process!

Be aware that positioning of the hand (pronation) or flexion/abduction of the fingers/thumb can have influence on the shape and amplitude of CMAP, therefore please use the same positions as those depicted for electrode placement. When maximal CMAP is obtained, please press F10 to export the CMAP to the word report. This has to be done for each measurement.
Recording SIP

After recording the CMAP, push the ‘Next’ or ‘Trace ▼’ key, as appropriate. The system will automatically change settings for the SIP recordings. Note the monitor trace in the bottom right corner. It should appear flat indicating no significant noise and interference (Figure 2).

- Push the ‘Switch’ or ‘Acquire ON’ button. You will see the EMG activity in the monitor trace (bottom right) and ‘Long trace’ (bottom left) windows. The signal amplitude should be greater than 200 µV and contain spikes representing EMG activity. When a suitable signal is seen, push the ‘Acquire’ or ‘Switch’ button to stop recording.
- Note the SIP measurements in the result table. The graph will display the data point of ‘Ideal case motor unit count (ICMUC)’ versus the SIP area.
- Push the ‘Next’ or ‘Trace ▼’ button to begin recording the next SIP epoch. Repeat the above 2 steps to get the necessary SIP epochs.

Figure 2

When the system has collected 3 SIP epochs that are accepted for analysis, the regression line is drawn, and the results of MUNIX calculations are updated (Figure 3).
The system is capable of recording up to 29 SIP epochs (Figure 4). Record the SIP using 5 force levels ranging from slight to maximum force of contraction. It is important to collect data in the low and mid-range of force. Perform 2 to 4 trials to collect at least 20 included SIP epochs or more.

**Figure 3**

**Figure 4**

**Generation of appropriate SIPs**

To accurately calculate MUNIX for each muscle it is important to capture an appropriate range of force during collection of SIPs (see above). The patient is asked to gradually increase force, receiving acoustic feedback. Motor unit potentials should gradually increase. The investigator provides resistance (see pictures below). The contraction must be isometric! Avoid any limb movement. Care should also be taken for the examiner not to touch the recording and reference electrodes. SIPs with a quality index below 1.0, ICMUC > 100 and SIP area < 20 mVms will not
be included for the MUNIX calculation. **SIPs with baseline shift, tremor or bimodal pattern with very low volume conducted activity** should not be recorded or may exceptionally be deleted/excluded manually.

**Hints**

- SIP recordings that do not fulfil the quality criteria will be excluded automatically by the software and should not be manually included.
- Avoid measurements with tremor, baseline-shift, or obvious change of innervation/force level within the single SIP recording epoch (500ms).
- Do not record SIPs too fast, resulting in SIP epochs of $<500$ms.
- Try to get a wide range of measurements at distinguishable different force levels. This can be estimated with a wide range of the provided “quality index”, starting at lower values above 1.0
- SIP recordings at lower/intermediate force levels are more important for proper MUNIX calculation than maximal force levels.
- However, in the biceps, tibial anterior, and FDI muscles, initial higher force levels might be required to obtain appropriate SIP recordings. Minimal SIP amplitude should be $> 0.2$ mV.
**Review results**
Click on the ‘Results’ (Figure 5) key to review the tabulated data (Figure 5). The graph is shown under the table. The data points excluded from analysis are shown using open circles in the plot. Note the indication of exclusion in the result table. By default SIP signals that produce ICMUC > 100 and/or Quality (=SIP area/ CMAP area) < 1 are automatically excluded. User can manually include or exclude the various recordings. Exit the screen by clicking on the ‘Results’ key in the bottom left of the screen.

![Figure 5](image)

One can also review the recordings using the scroll bar in the long trace window. In Figure 6, the ‘SIP 15’ epoch has excessive power-line frequency interference. Exclude this by removing the checkmark in the ‘Include’ column of the result table. Need of excluding SIPs should be the exception and only applicable to clearly polluted or faulty SIP recordings!

![Figure 6](image)
Click on ‘Exit Test’ to return to test menu of the system (Figures 10 and 11). Choose another muscle for testing.

End of MUNIX Session

When finished, click on ‘Patient list’ icon to close the record. You will see the patient list on the screen.

3 Golden Rules:

1. Always carefully optimize the CMAP amplitude

2. Get a nice distribution of at least 20 included (!) SIP measurements of 500ms duration at different force levels from minimal to maximal on the regression curve, starting around quality values with 1, not “patchy”

3. Avoid inconstant SIP recordings with bursts or increasing/decreasing force levels during the 500ms epoch; do not acquire SIPs too fast < 500ms

Hints to Speed-up Success in the Qualification Process

- Please take your time and read this manual!
- Place the electrodes like demonstrated in the photos in this manual; do not use your routine electrode placement.
- Raters should try several measurements prior to the certification measurements to get familiar with the method.
- Please follow the above mentioned 3 golden rules.
- Waiting a long time between the training-course and first measurements at home labs should be avoided.
- Do not get frustrated with the biceps. We strongly suggest measuring your own biceps. Some raters found this experience extremely helpful! Once you get the shape of the CMAP, without tingling in fingers and wrist/finger flection, you will recognize what to go for.
- Do NOT exclude SIP recordings manually without urgent need (obvious interference, tremor or innervation bursts). If any SIPs are excluded separately, this has to be declared when sending the test-retest data. Additionally, you might be asked for sending the measurements again after re-including these SIP measurements or sending the raw data .mps files. Do not include system-rejected SIPs!
- Do not forget to import each CMAP to the word report by pressing F10 once after recording the final CMAP.
- Please be blinded to previous CMAP recordings when doing the retest. The procedure of proper CMAP recording is crucial for measurements in ALS patients!
Electrode Placement for Individual Muscles

Subjects should be lying comfortable on an exam table. This is especially relevant for positioning of the arm for the biceps measurements. However, patients may also be seated comfortably in a chair with table or chair with arms nearby for support of hands.

**Abductor Pollicis Brevis (APB)**
- Place hand upon flat surface, palm up.
- Place recording electrode on thenar eminence just lateral to mid-point of first metacarpal, aligned with first metacarpal.
- Place reference electrode distally at the thumb.
- Grounding electrode is placed on the dorsum of the hand.
- Place stimulator at wrist between flexor carpi radialis and palmaris longus tendons.
- Avoid partial abduction of the thumb and pronation of the forearm.
- Counter resistance: place your hand over the patient’s hand, with your thumb giving resistance to the patient’s thumb.

**Abductor Digiti Minimi (ADM)**
- Place hand upon flat surface, palm up
- Place recording electrode on ADM at midpoint fifth metacarpal
- Place reference electrode distally at the little finger
- Grounding electrode is placed on the dorsum of the hand
- Place stimulator at wrist adjacent to flexor carpi ulnaris tendon
- In some subjects, maximal CMAP is achieved with more proximal placement of the recording electrode
- Be aware of initial baseline shift due to electrode movement on the skin while increasing force levels
- Counter resistance: stabilize with your fingers/thumb. Do not allow abduction of digit V
First Dorsal Interosseous (FDI)

- Place hand on flat surface, for measurements palm upward, relaxed with no finger adduction/abduction
- Be aware, that position of the fingers/thumb abduction has an influence on CMAP
- Place recording electrode halfway between the first and second metacarpals, aligned with metacarpal. In some subjects, highest CMAP is obtained more proximal and next to the second metacarpal.
- Place reference electrode distally at the little finger to obtain highest CMAP (compared to the index finger) and to avoid positive take-off of CMAP.
- Grounding electrode is placed on the dorsum of the hand
- Place stimulator at wrist adjacent to flexor carpi ulnaris tendon
- Counter resistance: stabilize with your fingers/thumb. Do not allow abduction of digit II, avoid additional flexion of the index finger.

Right upper picture: correct CMAP with single biceps stimulation

Biceps

- Place arm such that biceps tendon and attachment to coracoid may be easily palpated, with elbow flexion about 90 degrees
- A flat positioning of the subject with arm abduction and elbow flexion (forearm on a pillow) might be most comfortable for subject and rater
- Place recording electrode on bulk of muscle between antecubital fossa and acromion, 1/3rd way up from antecubital fossa
- Place reference electrode over the medial epicondylus of the elbow
- Place stimulator just inferior to tendon of short head of biceps as the musculocutaneous nerve emerges from the axilla
- Isolated stimulation of the musculocutaneous nerve can be challenging. Verify visually that there is no additional wrist flexion by median nerve co-stimulation and no double peak shape of the CMAP (see recordings below)
- Counter resistance: place your hand, forearm or elbow at the patient’s wrist/distal forearm to avoid elbow flexion. SIP measurements should be performed with elbow positioned at 90 degrees

Right upper picture: correct CMAP with single biceps stimulation
Right lower picture: wrong CMAP with additional wrist flexor stimulation

Tibialis Anterior
- Lower leg is positioned naturally with sole of the foot on the floor, knee flexed approximately 90 degrees.
- Place recording electrode lateral to tibial crest, one-third of distance between ankle and knee (closer to knee).
- Place reference electrode over the patellar tendon.
- Grounding electrode should be placed at the level of the fibular head.
- Place stimulator one to two fingerbreadths inferior to fibular head.
- Counter resistance: use your hand to give resistance with the foot positioned at 90 degrees. Avoid pronation/supination of the foot.

Extensor Digitorum Brevis (EDB)
- Lower leg is positioned naturally on a bed.
- Place recording electrode on dorsum of foot two to three fingerbreadths from lateral malleolus.
- Place reference electrode distally over the little toe.
- Grounding electrode should be placed on the dorsum of the ankle.
- Place stimulator at ankle, just lateral to the tibialis anterior tendon.
- Counter resistance: place side of your hand or forearm on proximal phalanx of toes, foot in 90 degrees position.
Reference List


System manual for Synergy/Viking

The MUNIX program is available on the Natus EMG systems running version 21.1 software. In order to use this program, you may need to load the custom settings created for this project. The instructions are identical for Synergy and Viking users. They are summarized in Figure 7.

1. Click on ‘Edit’ in menu bar. From the drop down list, choose ‘User setup’, followed by ‘Test and Study configuration’.
2. You will see a window with many buttons on the right. Click on ‘Load Folder’ button.
3. Select the ‘MUNIX.mcs’ file that is supplied to you (if necessary).
4. Click on ‘Open’. The test settings will be loaded. Click on OK button to exit that screen.

![Figure 7](image)

Next you will need to identify the two important buttons on the control panels (Figure 8).

1. Locate the ‘Next’ button and ‘Acquire On/Off’ buttons on the Synergy control panel.
2. Locate the ‘Trace ▼’ and ‘Switch’ button on the Viking control panel.

The ‘Next’ or ‘Trace ▼’ buttons are used to select next trace for the surface EMG interference (SIP) epoch. The ‘Switch’ and ‘Acquire’ buttons are used to start and stop recording SIP.
The step by step process for MUNIX is now described below.

**Start a new investigation** (Figure 9)
1. Click on New Patient
2. Enter patient demographic information and click on OK.

Start the MUNIX program

**Synergy users** (Figure 10)
1. Open the ‘Biogen’ folder and start ‘MUNIX’ program.
2. Select the tested nerve/muscle.
3. Select the test side.
4. Click on OK.
Viking users (Figure 11)
1. Choose the tested side
2. Click on the appropriate area of the body where the tested nerve/muscle are found.
3. Selected the nerve.
4. Select the MUNIX test.