Standard Operation Procedures (SOP) of Motor Unit Number Index (MUNIX)
In an European multi-centre project (SOPHIA)

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Objectives

Primary

To investigate the test-retest reliability of a novel neurophysiological measurement (Motor Unit Number Index, MUNIX) for estimation of functioning motor units, in ALS patients in a multi-centre European project (SOPHIA).

Secondary

To evaluate the feasibility and reliability of MUNIX measurements as a potential surrogate marker for multicenter ALS clinical trials.

Inclusion criteria for ALS patients

Informed consent must be signed by every patient.
ALS patients must fulfil the category for possible, probable-lab supported, probable or definite ALS regarding to the revised El Escorial criteria. Patients with pure upper motor neuron signs or “suspected ALS” will not be eligible for the study. Symptom onset, defined as onset of weakness, muscle wasting, fasciculations, dysarthria, dysphagia, dyspnoea, falls or disturbance of fine finger movements must be less than 18 months ago. In ALS patients follow-up visits will be performed approximately every 3 months.

Exclusion criteria

Any history of major neurological disorders that might influence MUNIX measurements (e.g. polyneuropathy, peripheral nerve damage, paresis of any cause, muscular atrophy) in ALS patients.

Investigational plan

In ALS patients the following clinical data will be collected: gender, age, bulbar or limb onset, time of symptom onset, level of certainty according to the revised El Escorial Criteria and disease duration till death if ALS patients decease. The revised ALS Functional Rating Scale (ALSFRS-R) will be applied. Before performing MUNIX measurements, manual muscle testing according to the Expanded Medical Research Council Scale for Manual Muscle Testing (MRC) will be performed in each investigated muscle, using the additional graduation M4- and M4+.

MUNIX will be recorded on the clinically less affected side of the body. If an ALS subjects has e.g. weakness and wasting in the left leg, all muscles are measured at the right side of the body in this subject throughout the study. Measurements will be performed from the abductor pollicis brevis, abductor digiti minimi, biceps brachii, tibialis anterior, extensor digitorum brevis and abductor hallucis muscles after supramaximal distal stimulation of the median, ulnar, musculocutaneus, peroneal and tibial nerves. Stimulation and recordings will be performed according to the attached guidelines.
If both sides are affected symmetrically or not affected (e.g. in bulbar onset subjects), the right side will be chosen. The chosen side in each individual will be kept throughout all follow-up examinations. A minimum of 12 ALS patients per centre is required to allow statistical analysis.
At each participating centre the same experienced investigator will perform all MUNIX studies. At visits with additional testing of the intra-rater reliability, examination will be performed twice with a break of minimum 30 minutes between each session. Electrodes will be completely removed and any traces of electrode placement will be deleted/avoided. In centres additionally investigating the inter-rater-reliability, a second experienced investigator will perform the investigation alternately with the first investigator. Between each session a minimum rest of 30 minutes must be allowed. All investigations will be performed on the same day.

Particular attention will be paid to electrode position and temperature (not less than 29 degrees dorsum of hands and not less than 27 degrees dorsum of feet). To ensure consistency between repeated measurements the placement of electrodes and distance between the active and reference electrode will be standardized (Appendix 1). The recording electrode position will be adjusted to achieve maximal amplitude and risetime and a sharp negative takeoff of the CMAP. [7] If CMAP amplitude is less than 0.5 mV, this muscle will be excluded and not be used for MUNIX calculation any more and MUNIX will be rated as zero.

Each centre will use own surface electrode equipment. The primary parameter of interest is decline from baseline in individual patients over time and therefore individuals serve as their own controls. This allows the use of different equipment. Instructions for Keypoint EMG Systems and Synergy are available on request.

Quality control
To ensure quality of measurements, each investigator of each centre will perform prior to study onset an in-person training session. This training session is mandatory. Each investigator has to study 4 normal subjects (e.g. staff members) twice at their centre corresponding to the protocol for test-retest in ALS patients. Raw data will be sent to the central MUNIX coordination site (Neuromuscular Diseases Unit/ ALS clinic, Kantonsspital St.Gallen). One reviewer (C.N.) will evaluate all data for quality and calculate the MUNIX values and test-retest variability of each investigator. Investigators will be able to continue with the study if average variability is less than 20%. This essential condition has already been

used in a recent MUNE longitudinal ALS-study. [14] Test-retest data will be monitored throughout the study by C.N. If quality of data is unsure, C.N. can request centres and raters for additional rest-retest data for recertification (see section “Use of data”).

Schedule

Follow-up examinations in ALS patients will be performed approximately every 3 months, (allowing a deviation of ± 2 weeks) for a designated time window of 18 months (7 measurements). If patients are willing to proceed after this time, data collection can be continued if there are no ethical or medical concerns.
In ALS patients a test retest examination will be performed by the same examiner (intra-rater-reliability) at the first visit (V0) and after 12 and 18 months (visit V4 and V6). In centres additionally investigating the inter-rater-reliability, a second experienced investigator will perform the investigation once (alternating with the first examiner [1-2-1] or after the second investigation of the first examiner).

Analysis

For each patient the amplitude (in mV) from each muscle (CMAP, peak to baseline) will be documented in the database PROGENY on a central European server (University of Utrecht, The Netherlands). Additionally the motor unit number index (MUNIX) and MUSIX (CMAP amplitude divided by MUNIX value) will be calculated for each muscle. For statistical analysis of intra-rater and inter-rater reliability the intraclass correlation coefficient (ICC) and coefficient of variation will be applied.
MUNIX results will also be correlated with the collected longitudinal clinical data and other validated measures of disease progression (ALSFRS-R, manual muscle testing). Statistical regression models will be applied to analyze if change of MUNIX is more sensitive to reflect disease progression than other measures. Variability of measurements between centres will also be assessed.

Use of data

Data are intellectual property of the contributing centres. Data will be distributed to other participants for further analysis after informed consent of the contributing centres. For quality control as a mandatory part of the procedure, Christoph Neuwirth (C.N.) will have access to MUNIX data of all centres. Raw data of test-retest examinations has to be placed at the disposal if requested by C.N. for quality control. Additionally test-retest examinations can be requested from each participating rater of each centre by C.N.

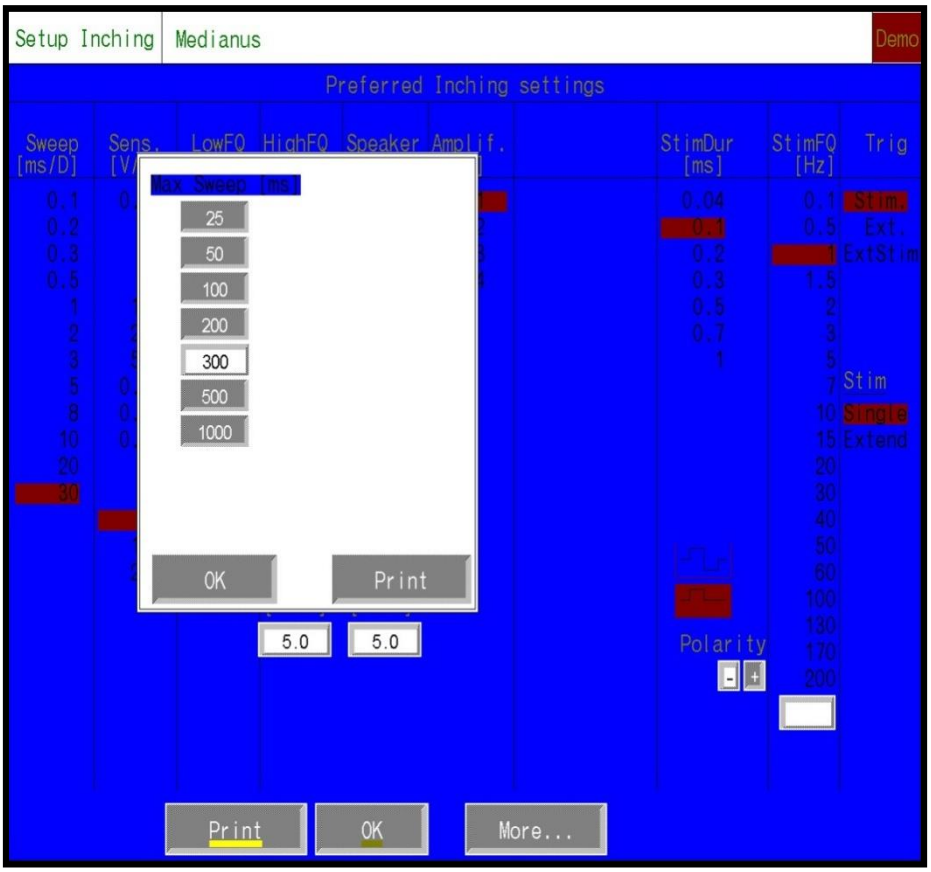
Analyzing the MUNIX recordings (Key Point classic)

- Before measurements, change filter settings and sweep for the inching program (see picture 1+2). You can permanently change these values in the pre-setting.
- This needs a PC with Windows XP or older system (e.g. Keypoint classic system). The MUNIXKP file, EGAVGA.BGI (graphic driver) should be in the same directory or the desktop. Run the MUNIX program.
- Input the text file name from the inching program including extension, e.g. 00042531.ich. If you are analyzing the data directly on the EMG machine, the path would be “c:/kp/patdata/xxxxxxxx.ich. The 8 digit number of the .ich file is the number of the examination (top right in keypoint classic or list if examination on the starting screen)
- Type the onset latency. This should be recorded when performing the study.
- Type Y for autoscale.
- Note the quick summary of measurements. MUNIX value is “Index” on the top right, on the left CMAP amplitude (peak to baseline, in yellow, picture 3). Hit any key to begin review. The keys are not case sensitive
  - ↓ and ↑Arrow keys: Change gain for SIP
  - N for Next SIP; P for Previous SIP epoch
  - R for reject; A for accept the SIP for analysis.
    - Reject if: quality index < 1.0 (IP/CAMP area), SIP Area < CMAP area, SIP area < 20 mVms, ICMUC > 100 or noise, interference, tremor, etc
  - Q for Quit review mode
  - H for hardcopy (HP 1100 Laser Printer only)

- There will appear a file called MUNIX.DAT. You can open it with e.g. with excel. Store it with an appropriate file name (e.g. APBleft.DAT). If you run the MUNIX.exe again with another .ICH file or other settings (e.g. latency), MUNIX.DAT will be overwritten.

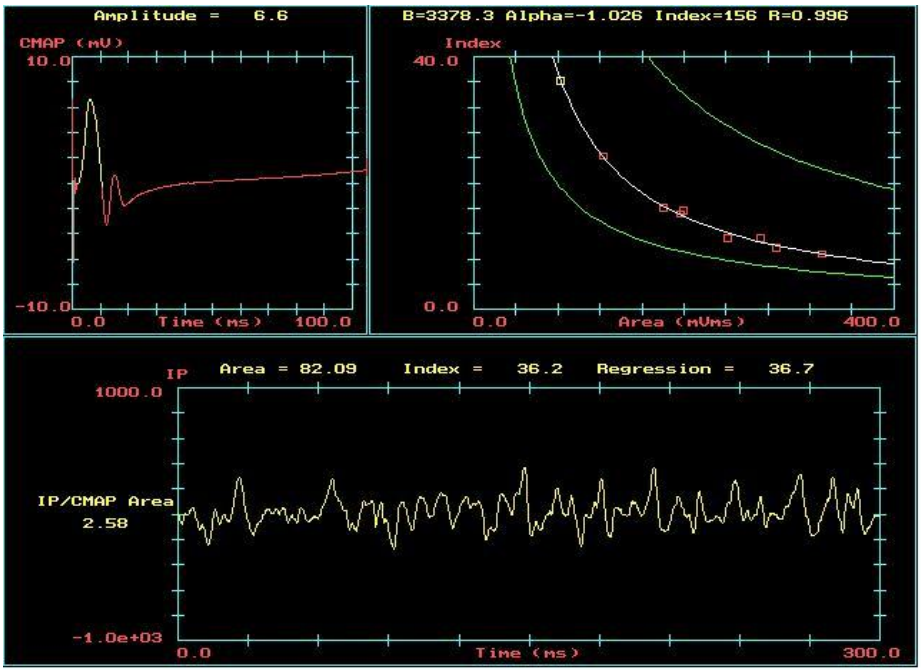
Hints

- Beware of stimulating artefacts and onset latency. If the yellow coloured CMAP amplitude is inappropriate, choose other latency (e.g. 2.3ms). Try as well if MUNIX.exe crashes while analyzing.
- If one SIP epoch is missing (e.g. 8 instead of 9), MUNIX.exe will not work. You can rescue the data. Do one measurement with no recording in the missing epoch and reject this flat row when running MUNIX.exe again.
- It is time-saving if you prepare the different examinations of different muscles for each patient in advance. If you recorded accidentally more than one muscle in one examination, you can rescue the data. MUNIX.exe will only analyse the first muscle. Copy the .ich-file containing more than one muscle measurement, rename it with the examination number of the missing muscle, open it with Keypoint classic and delete the CMAP and all SIP epoch of the first muscle. Run MUNIX.exe again and get the results from the second muscle.



Picture 1

Picture 2



Picture 3

Analyzing the MUNIX recordings (Keypoint.NET, Win 7)

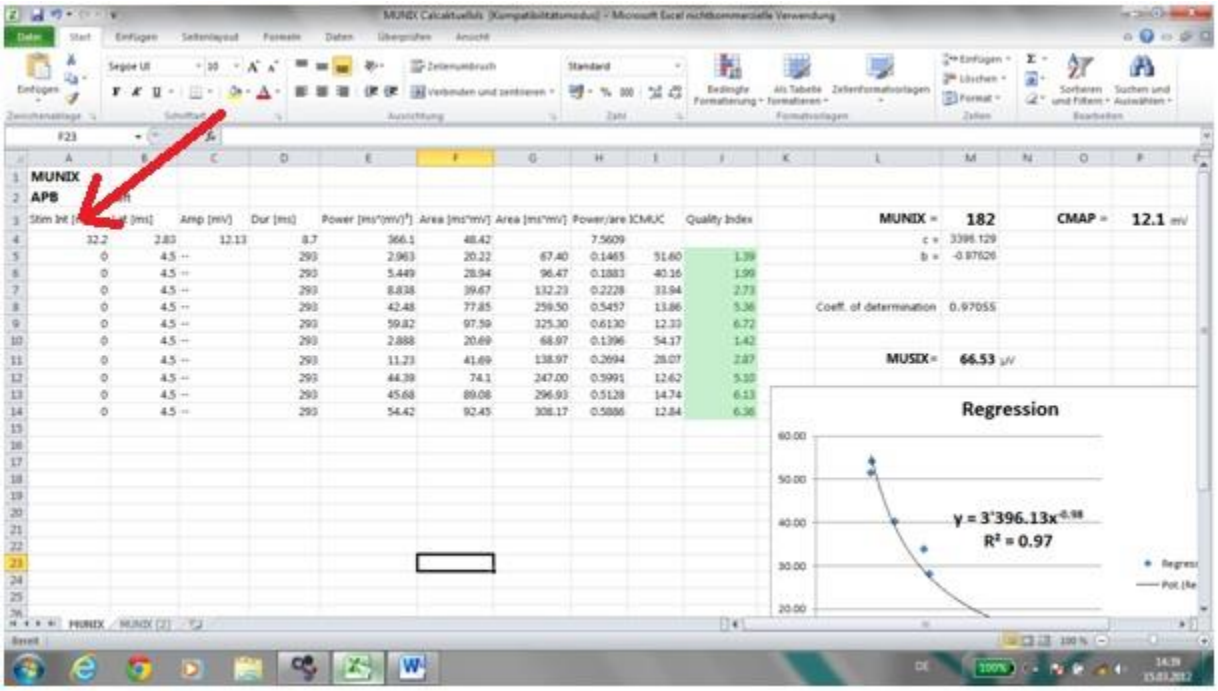
- Start keypoint.NET, enter Patient data
- Go to MUNE, choose MUNIX, choose your muscle
- Record your CMAP (Control, grey square button
- Set end latency marker, but at zero crossing line to get the negative amplitude and area (picture 1)!
- change to “test” (red square button), Reduce stim intensity to 0, stimulate repetitive
- make your measurements (10 rows) while increasing force with pressing “continue” (grey round button). For the last measurement just stop repetitive stimulation
- set the common M latency marker and common end latency marker (black lines trough all SIP rows) to the beginning and end of the 300ms recording time! (picture 2)
- Copy-paste the data in the table on the top right (all).
- Insert the data at the right position (Stim Int [mA)]). MUNIX value and graph will appear, as well as MUSIX and CMAP amplitude. (picture 3)
- Save data under separate file number, then use excel sheet again

Hints

- Do not forget to set the end latency marker from the CMAP at zero-crossing line and duration for SIP recordings
- If quality index is nearly for all SIPs < 1, go back and check the common beginning and end latency marker of the SIP recordings (Picture 2)
- Up to now, you cannot reject single measurements if quality index is low. You have to repeat these SIP recordings.

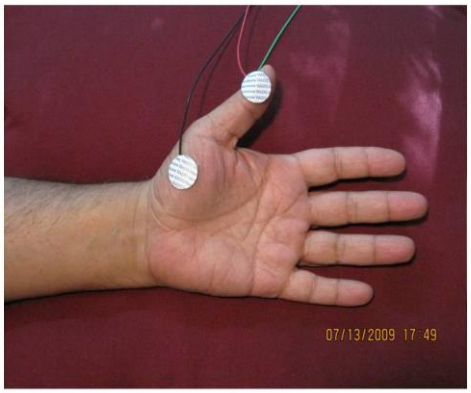
Picture 1: set end latency marker at zero-crossing line

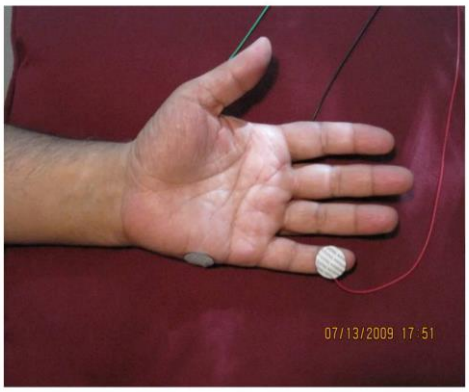
Picture 2: common M and end latency marker

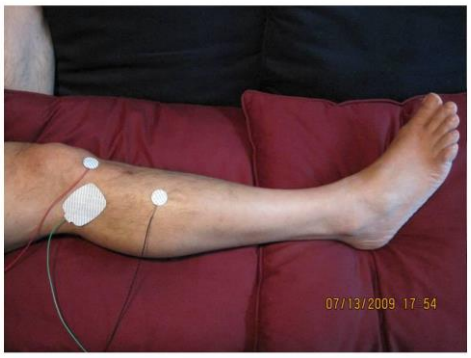


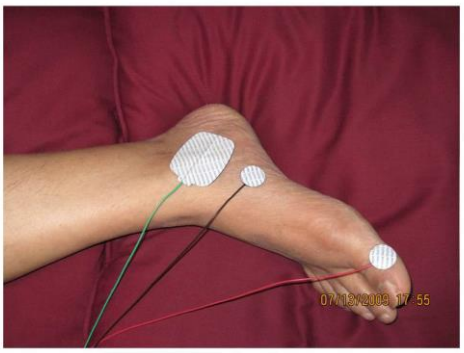
Picture 3: copy paste here

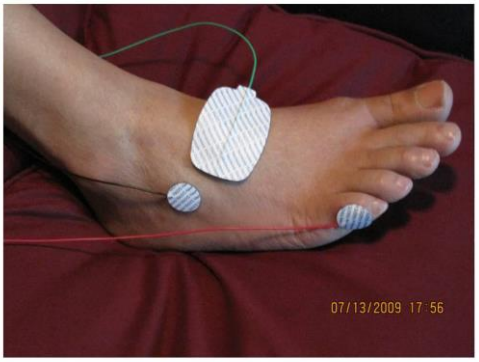
Electrode placements:
Place reference electrode as distally as possible, if applicable.
Replace recording electrode till maximal CMAP amplitude is detected.

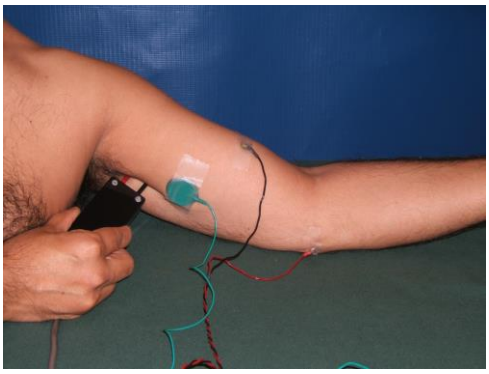






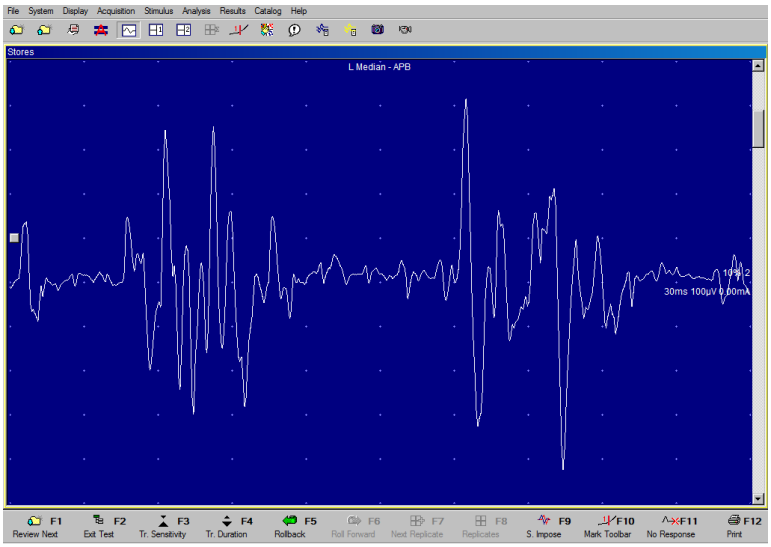




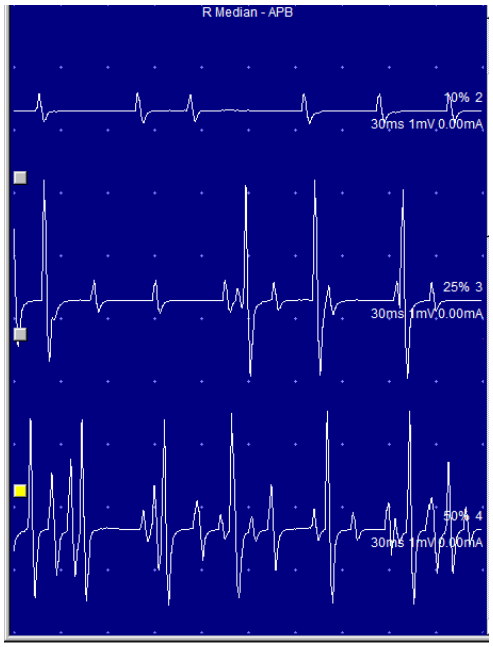


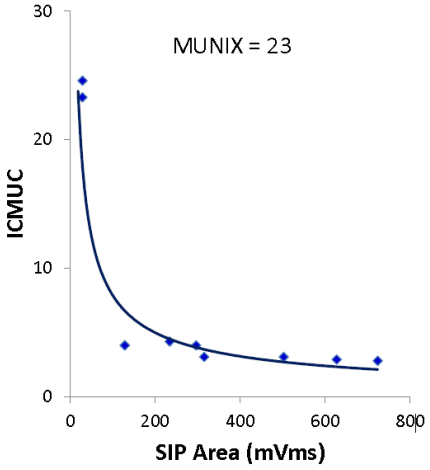
Recordings to avoid:

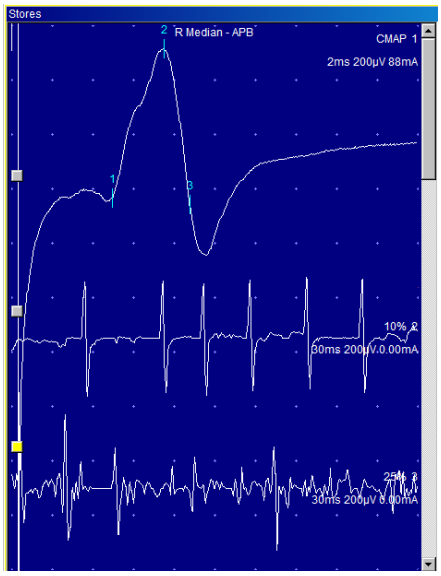
Tremor



Avoid SIP recordings with very low amplitude activity <0.2mV at low force levels
Bimodal pattern results in too high MUNIX values. Two measurements with SIP amplitude <0.2mV results in too high MUNIX in this ALS subject.







Top trace: The CMAP amplitude is 0.6 mV
Middle trace: At slight force, discharges of a fast firing MUAP are seen. Good configuration.
Bottom trace: At slightly higher force, the baseline is filled with low amplitude signals. Their amplitude is less than 200 uV. We have interpreted this as volume conducted signals. The MUAP in middle trace is not seen probably due to movement of electrodes with respect to muscle, as patient tried to make a contraction.