1. Type of Specimen

Serum Amount: mg  Not known

DNA Amount: mg  Not known

Muscle Amount: mg  Not known

Nerve Amount: mg  Not known

CSF Amount: mg  Not known

Fat aspirate Amount: mg  Not known

Urine Amount: mg  Not known

1. If a biopsy, what was the location?

Muscle:

Nerve:

Other, specify:

1. Date collected? / /
2. How collected?

Open biopsy

Needle biopsy

Punch biopsy

Other, specify:

1. If a biopsy, how processed?

Flash frozen

Frozen in isopentane

Formalin fixed

Glutaraldehyde fixed

Paraformaldehyde fixed

1. How stored?

-160⭘C -80⭘C -20⭘C 4⭘C Room temperature

1. Bio-bank name?
2. Consented length of storage?

Months

Years

1. Has the sample been freeze/thawed prior to use in the study?

If so, how many times?

1. Other comments:

Biomarkers emcompass a potentially large range of specimen and data generated from them. The NIH NINDS neuromuscular CDE group has a general summary document regarding the use and application of biomarkers for neuromuscular diseases research. This document emphases the fact that, while no biomarkers are specifically validated or required for NMD research, it is critically important that biomarker collection, derivation and application be done in as uniform manner as possible. Therefore, the following steps are recommended when either developing a biomarker or else using a biomarker in a neuromuscular disorders clinical research study.

1. Proper specimen collection- It is essential that specimens are obtained in the proper fashion to assure uniformity and broad applicability of results. Technical recommendations for obtaining and processing a muscle biopsy can be found here[[1]](#footnote-1). Recommendations for nerve biopsy can be found below In addition, there are existing CDEs that cover the collection of fluid specimens including blood, CSF, plasma and urine.
2. Recording of specimen characteristics- It is recommended that characteristics of the biologic specimen are recorded at the time that the specimen is obtained and processed. We recommend using the existing CDEs for fluids mentioned above and using the demographic CDEs for muscle and nerve biopsy. In addition, we recommend using the Biomarker Sample CDE to record the basics of collection and processing data (see below).
3. Processing and storage- It is critically important for all processing and storage of the specimen and the biomarker to be recorded. In that vein, we have created the Biomarker Sample CDE, to be used to record basic elements of the specimen and the biomarker- date of collection, any processing, date and length of storage, and instances of freezing/thawing.

Instructions for obtaining and processing muscle and nerve biopsies

\*\* A segment of sural nerve up to 6 cm in length is removed by a surgeon or experienced physician under sterile conditions. Techniques for performing the biopsy are beyond the scope of this summary, but published guidelines are available ([Dyck *et al.* , 2005](#_ENREF_1)). Biopsies are then typically processed for light and electron microscopy (EM) and for teased fiber analysis. Alternatively, a portion of the biopsy may be processed for immunohistochemistry (IHC) including teased fiber IHC. Typically, for light and EM studies, biopsies are fixed in isosmolar glutaraldehyde (2.5%), osmicated and embedded in epon blocks. Sections of epon-embedded nerve (typically cross sections) are cut at 1 m thickness and stained with toluidine blue for light microscopic examination (plastic sections). Ultrathin sections are cut from the same blocks for electron microscopy. For teased fiber studies, glutaraldehyde fixation and osmication are performed as above. But instead of processing into epon, segments of nerve are softened in glycerin, then teased into individual fibers using pins or fine forceps. The technique is described in detail in ([Dyck *et al.* , 2005](#_ENREF_1)). For IHC, a portion of the nerve biopsy is oriented in OCT medium and frozen as fresh tissue or after brief fixation in 4% paraformaldehyde (e.g. 30 minutes, but time can be varied depending on the antibody to be used). Cryosections are then cut for performing IHC. For teased fiber IHC, a segment of nerve is teased into individual fibers (or small clusters of fibers) on glass slides after up to 30 minute fixation in 4% paraformaldehyde. Slides are then immunostained according to individual protocols. For IHC studies, nerve tissue in OCT or glass slides (unstained cryosections or unstained teased fibers) can be stored at -80o C. Immunostained material may also be stored in the freezer, but the intensity of fluorescence will likely fade over time.

Dyck P, Dyck P, J E. Pathologic Alterations of Nerves. In: Ddyck PJ TP, editor. Peripheral Neuropathy. 4th ed. Philadelphia, PA: Elsevier; 2005. p. 733-829.

1. [University of Iowa Diagnostic Laboratories Muscle Biopsy Instructions](https://www.healthcare.uiowa.edu/path_handbook/Appendix/AnatomicPath/Muscle_Biopsy_General_Instructions.pdf) [↑](#footnote-ref-1)