

**STUDY TITLE: Muscle surgery and blood samples from patients with facioscapulohumeral muscular dystrophy or other neuromuscular diseases to be used for cell culture and DNA and RNA analysis**

**I. INTRODUCTION**

Facioscapulohumeral muscular dystrophy (FSHD) is a unique disease. It is dominantly inherited when a DNA repeat array (D4Z4) on the end of the long arm of chromosome 4 is too short. Skeletal muscle samples from severely affected, moderately affected, and unaffected tissue of FSHD patients as well as disease controls are needed for research on FSHD. Surgery specimens are generally much larger than biopsy specimens and so should aid research on this enigmatic disease.

**II. PURPOSE:**

**To obtain three types of tissue samples for establishing cell cultures (myoblast cultures) for research and for isolation of RNA and DNA from muscle to study the genetic basis of the disease. They are as follows:**

1. muscle aliquots transferred sterilely to previously sent tubes containing transfer medium and shipped to participating research labs on cold packs (**not frozen**) the day of surgery for next morning delivery;
2. muscle aliquots that are snap-frozen (for example in liquid nitrogen) in cold-resistant cryovials on the day of the surgery, stored at -80C, and, when convenient, **shipped on dry ice** to participating research labs, where they will be used for analysis of RNA and DNA;
3. when possible, a 20-30 ml blood sample from the patient shipped **on a cold pack** for determination of the size of the FSHD-linked DNA array (D4Z4) by pulsed field gel electrophoresis.

**III. PATIENT CONFIDENTIALITY**

All information will be recorded in such a way that patient confidentiality is protected and subjects cannot be identified. The participating research laboratories will not need to ascertain the identity of these individuals in the future.

#### IV. PROCEDURES

##### PRIOR TO SURGERY

- (1) Within one month of surgery, obtain from each participating laboratory eight sterile plastic tubes (15-ml) filled to the top with transfer medium, sterile 20% FBS/F10 medium and five sterile cryovials (approximately 2-ml, empty). The tubes and vials will be labeled by the participating laboratory with the name of the laboratory and will have a place to put the code number assigned to the patient. The tubes will also have the date that the medium was sent and they will be stored at refrigerator temperature.
- (2) At least one week before surgery, send an email to each participating laboratory about what day the samples are expected to be sent and follow up with a phone message if there is no response to the email.
- (3) Obtain consent from patients for use of otherwise discarded surgical samples. If the patient agrees to give a blood sample, obtain consent from patient for drawing a blood for this research project.

##### SURGICAL SAMPLES TO BE HANDLED STERILELY AND SHIPPED WITHOUT FREEZING ON THE DAY OF SURGERY FOR CELL CULTURE

- (4) On the day of surgery and as soon as possible after the surgery, using sterile technique, put one aliquot (about 3-5 grams) of the otherwise discarded muscle surgical sample into each of three to six sterile tubes containing transfer medium for each participating laboratory.

If enough tissue is available, send two tubes with severely affected, two with moderately affected, and two with unaffected muscle to each participating laboratory.

If less tissue is available, send to each participating laboratory a total of only three tubes with severely affected or moderately affected and unaffected muscle.

- (5) Label each tube as follows:

**FSHD samples:** F followed an arbitrary number and a designation of severely affected, moderately affected or unaffected as well as the type of muscle and the location in the body.

**Disease control, non-FSHD patients:** DC followed by an arbitrary number and a designation of severely affected, moderately affected or unaffected as well as the type of muscle and the location in the body.

Muscle Surgery and Blood Samples: Cells for Culture and RNA and DNA analysis

- (6) Put all the tubes containing samples in a sealed bag,  
surround the bag with cold packs,  
and send to the participating laboratories by FedEx the same day in a Styrofoam box containing packing material.

The box for shipping the sample needs a "Do Not Freeze" label on the outside of the package.

Check NEXT MORNING delivery on the FedEx form.

- (7) On the day of surgery or early the next morning, notify participating laboratories by email of the upcoming delivery and include the tracking number.
- (8) Some time during that week, send an anonymous description of the age, gender, and a brief summary of the clinical and histological findings for the tissue samples labeled with the same code used on the sample tubes.

**SURGICAL SAMPLES TO BE SNAP FROZEN, STORED AT -80°C, AND SHIPPED LATER ON DRY ICE**

- (9) The remaining, otherwise discarded surgical sample should be cut and distributed among 2 - 4 cryovials per participating laboratory.

These cryovials should be labeled the same way as the tubes with medium.

After addition of the samples to the tubes, they should be snap-frozen and stored at -80°C.

- (10) They can be shipped on dry ice to the participating laboratories at a later date.

The laboratory contact person should be notified several days in advance of the shipment and then sent the tracking number on the day of shipment.

**BLOOD SAMPLES TO BE SENT WITHOUT FREEZING ON THE DAY OF COLLECTION**

- (11) If the patient consents in writing, obtain 20 - 30 ml of blood from the patient.

The blood sample should be collected in purple-capped blood tubes.

It need not be collected on the day of surgery.

- (12) The blood samples are to be shipped at ambient temperature on the day that they are obtained to the one participating lab that is determining the sizes of the FSHD-linked D4Z4 arrays.

The samples should be shipped in a Styrofoam box with packing material.

Check NEXT MORNING delivery on the FedEx form.

The box should have a large label with “Do Not Freeze” on the top.