

MITOCHONDRIAL ENZYME &
DNA ANALYSIS

SPECIMEN REQUIREMENTS JANUARY 2011

For diagnostic investigations in children, the preferred tissue is usually skeletal muscle when it is clinically involved or liver if the presentation is mainly hepatic. Patients with primary neurological involvement can have a defect expressed in muscle but not liver or liver but not muscle, and it may be ideal to obtain both tissues and assay one or both depending on results of pathology.

In order to confirm that the patient and samples are appropriate for investigation, all cases must be approved, prior to shipment, by the Metabolic Consultant either by phone (61 3 8341 6201) or by sending an email (mito.samples@mcri.edu.au) or faxed (61 3 8341 6212) summary.

N.B. Histology, enzyme histochemistry and electron microscopy of tissue samples are not done at the Murdoch Childrens Research Institute and must be organised separately. Use of thiopentone or bupivacaine during biopsies should be avoided as they can interfere with enzyme assays. Tissue samples should not be embedded in OCT or other media, as this may interfere with enzyme assays, and they should be wrapped in foil to prevent desiccation.

1. **Skeletal muscle** 2 pieces(each 0.5 cm cube), preferably quadriceps - wrap in alfoil, place in small screw-cap tube sitting in dry ice. Muscle needle biopsies can usually be assayed but often provide inadequate tissue for reliable histology and enzymology, so we recommend open biopsies.
2. **Liver biopsy** 2 cores from French Gauge 14 cannula or, if open biopsy, 2 x 0.5 cm cubes - wrap in alfoil, place in small screw-cap tube sitting in dry ice.
3. **Skin fibroblasts** 1 flask of cells at an early passage number, preferably grown in medium containing 50 ug/ml uridine and 1 mM pyruvate - DO NOT FREEZE.
4. **Blood (for DNA)** 4 ml EDTA blood (no mixing beads or separating gel) sent at room temperature if it will be received within 24 hours or alternatively frozen.
5. **Urine (for DNA)** 5-10 ml urine or pellet (frozen)
6. **Autopsy samples** **COLLECT AS SOON AFTER DEATH AS POSSIBLE, preferably within 2 hours.** Note the time between death and freezing of samples. Tissue samples should be no larger than 1.0 cm cubes; heart, kidney & brain samples may also be collected. Enzymes can be assayed in postmortem heart but endomyocardial microbiopsies are too small.

STORAGE & SHIPPING

Tissues should be stored at -70°C until transport on dry ice. Cultured cells should be sent at room temperature and must be in a container without dry ice. Trying to divide a single esky into half with dry ice for tissues and half without for cells **always results in freezing and death of the cell line.** The Mitochondrial Laboratory (Dr David Thorburn, phone 61 3 8341 6234 or at the email or fax address above) must be **notified in advance** of shipping details (date of arrival, courier, waybill number, sender's phone no.).

VCGS Laboratories	Prepared by: David Thorburn	Doc No.:BG-E-132
Laboratory: Biochemical Genetics	Authorised by: David Thorburn	Version: 6
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Mitochondrial Specimen Requirements (Current at January 2011)

Shipping address: **VCGS Pathology Specimen Reception**
9th Floor, Royal Children's Hospital,
David Thorburn/Mitochondrial Lab
Flemington Rd, Parkville VIC 3052
(03) 8341 6235

OTHER INFORMATION NEEDED FOR RELIABLE INTERPRETATION

1. Patient name, medical record no., date of birth, date of sample, delay between death and freezing if relevant
2. Paired plasma lactate & pyruvate levels; multiple estimations preferred.
3. Paired CSF lactate & pyruvate levels, preferably with blood levels obtained at about the same time.
4. Full clinical summary and family history with pedigree.
5. Copies of relevant investigations, as appropriate, including:
 - urine organic & amino acids
 - tissue histology (including electron microscopy & enzyme histochemistry)
 - summary of CT, MRI or MRS studies of brain.

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