



# ***Center for Inherited Disorders of Energy Metabolism***

University Hospitals Case Medical Center

The Center for Inherited Disorders of Energy Metabolism (CIDEM) at Case Western Reserve University School of Medicine, Cleveland, Ohio, is a group of inter-disciplinary, specialized laboratories which focus on disorders of mitochondrial function. These disorders include defects of pyruvate metabolism, fatty acid oxidation, the Krebs cycle, and the electron transport chain. Clinical conditions associated with these disorders include major disabilities affecting the central nervous system, skeletal muscle, heart and other organs. The goal of the CIDEM Laboratories is to provide comprehensive diagnostic laboratory services to facilitate diagnosis and treatment of patients affected with these disorders. These services are accompanied, upon request, with relevant consultation to health professionals. Consultations are provided by Douglas Kerr, MD, PhD, Shawn McCandless, MD, and Art Zinn, MD, PhD.

The CIDEM laboratories are located at University Hospital (directed by Dr. Douglas Kerr) and at the Case Western Reserve University School of Medicine (directed by Dr. Charles Hoppel). The CIDEM Laboratories are administered under the Childrens Research Foundation of Cleveland (Tax ID # 34-1438-215) and are certified under the quality control/assurance guidelines of the Clinical Laboratory Improvement Act (CLIA Certificate #36D0680824, 36D0925804, 36D0923239).

The enclosed information packet provides detailed information about each of the tests offered. This packet includes:

- General Test Information
- Specimen Collection and Shipment
- Test and Price List
- Laboratory Requisition

Please review all of the provided information prior to shipping samples. The CIDEM laboratory is open Monday through Friday, 8:30 am to 5:00 pm, and is closed weekends and holidays.

These forms are periodically updated; for the most recent version, contact our laboratory by phone: 216-844-1286, fax 216-844-8005, or email: [cidem@case.edu](mailto:cidem@case.edu), or download forms from our Home page: [www.case.edu/med/CIDEM/](http://www.case.edu/med/CIDEM/)



**General Test Information**  
(See *Test and Price List* for Details)

**Metabolites in Body Fluids:**

- **Acylcarnitine Profile (Quantitative):** This test is useful for diagnosis and monitoring treatment of patients with strongly suspected or known metabolic disorders. The analysis, developed in Dr. Hoppel's laboratory, includes a quantitative HPLC/ESI/MS/MS determination of over 35 different acylcarnitines in plasma, urine, and tissue samples, including short-, medium-, and long-chain derivatives. The acylcarnitine profile includes quantitation of free and total carnitine. Turnaround time: 2 weeks
- **Carnitine Screening (Free and Total):** This test is recommended for detection of primary or secondary carnitine deficiency and monitoring of patients being treated with carnitine. Candidates include patients with failure to thrive, cardiomyopathy, weakness, or possible metabolic disorders. Turnaround time: 5 days
- **$\beta$ -Hydroxybutyrate, Acetoacetate, and Free Fatty Acids:** These compounds are measured in plasma as parameters of lipolysis and fatty acid oxidation (e.g.: for diagnostic testing of fasting hypoglycemia or after an oral fat tolerance test) or to monitor efficacy of ketogenic diets. The ratio of  $\beta$ -hydroxybutyrate/acetoacetate reflects mitochondrial NAD/NADH, and may be a useful parameter for diagnosis of defects of the Krebs cycle. Turnaround time: 5 days
- **Lactate and Pyruvate:** Measured enzymatically in blood or CSF as an index of impaired pyruvate metabolism due to defects of glucose oxidation (fed state) or gluconeogenesis (fasted). The ratio of lactate to pyruvate reflects the NAD/NADH ratio and is useful in distinguishing primary defects of pyruvate metabolism from defects of electron transport (or oxidation). Deproteinize **immediately** (in perchloric acid) to avoid artifacts of lactate formation in red cells or loss of pyruvate. Turnaround time: 3 days
- **Urine Organic Acids:** Semi-quantitative determination of over 50 non-volatile organic acids as TMS derivatives by capillary dual column gas chromatography with confirmation by mass spectrometry. Useful for newborn screening followup, and diagnosis and monitoring of a large variety of metabolic disorders, including defects of pyruvate metabolism, the Krebs cycle, amino acid and fatty acid oxidation, and the electron transport chain. Turnaround time: 10 days

**Enzyme Assays:**

- **Pyruvate metabolism:** Pyruvate dehydrogenase complex deficiency, typically associated with post-prandial lactic acidemia with a normal lactate/pyruvate ratio, can be assayed in freshly isolated blood lymphocytes, cultured skin fibroblasts, or frozen tissues. The most common defects affect the X-linked E<sub>1</sub>-alpha subunit, which may be variably expressed especially in females. Measurement in more than one cell type or tissue is recommended because of variable cell/tissue expression with males and females. If low PDC activity is found, the activities of components E<sub>1</sub>, E<sub>2</sub>, and E<sub>3</sub> are assayed. Gluconeogenic defects of pyruvate carboxylase (more common) and phosphoenolpyruvate carboxykinase (rare) are associated with fasting hypoglycemia and lactic acidosis, and can be assayed in skin fibroblasts or liver.
- **Other Biotin Carboxylases:** Assays of propionyl-CoA carboxylase and  $\beta$ -methylcrotonyl-CoA carboxylase are available in cultured fibroblasts and/or liver. These assays are useful for diagnostic confirmation of specific defects of these enzymes (usually recognized through organic acid or acylcarnitine analyses), for distinction from general defects of biotin sufficiency/metabolism, or for detection of heterozygosity. These assays can be performed on cultured amniocytes for prenatal diagnosis. Assay of pyruvate carboxylase (another biotin enzyme) is included as an internal control.



## General Test Information

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- **Krebs Cycle:** Defects of the Krebs cycle are usually associated with increased excretion of the respective intermediates in the urine and/or increased blood lactate. Activities of several of these enzymes are available in cultured skin fibroblasts or tissues: the  $\alpha$ -ketoglutarate complex (including the E<sub>3</sub> component), succinate dehydrogenase, and fumarase. Citrate synthase is included with several assays of other mitochondrial enzymes as an internal marker of mitochondrial content.
- **Fatty Acid Oxidation:** Defects of fatty acid oxidation may be suspected in non-ketotic fasting hypoglycemia associated with dicarboxylic aciduria or in unexplained cardiomyopathy or rhabdomyolysis. Certain defects (e.g.: medium-chain acyl-CoA dehydrogenase deficiency) in many cases (but not all) can be diagnosed directly by DNA mutational analysis. Chain length specific acyl-CoA dehydrogenases, enoyl-CoA hydratases, 3-hydroxy-acyl-CoA dehydrogenases, and 3-ketothiolases can be assayed in cultured skin fibroblasts or certain tissues.
- **Carnitine Metabolism:** Defects of carnitine metabolism may present with many of the same clinical features as other disorders of fatty acid oxidation, associated with either increased plasma long chain acylcarnitines, and/or increased or decreased free carnitine. Rhabdomyolysis is commonly associated with CPT II deficiency. Carnitine palmitoyltransferases (CPT I and II), carnitine acetyltransferase, and carnitine acylcarnitine translocase can be assayed in cultured fibroblasts and/or certain tissues).
- **Electron Transport Chain:** There is a large variety of clinical manifestations associated with defects of the electron transport ("respiratory") chain, as the components are encoded by many nuclear and mitochondrial DNA genes. Blood and/or CSF lactate may be increased with a high lactate/pyruvate ratio, and urine organic acid or acylcarnitine analyses may show evidence of impaired fatty acid and amino acid oxidation. Muscle biopsies may show proliferation and/or abnormally shaped mitochondria. Available assays include, initially, rotenone-sensitive NADH-cytochrome *c* reductase (complexes I + III), antimycin-sensitive succinate cytochrome *c* reductase (complexes II + III), decylubiquinol cytochrome *c* reductase (complex III), NADH ferricyanide reductase (part of complex I), succinate dehydrogenase (part of complex II), and cytochrome *c* oxidase (complex IV). If these results are abnormal, follow-up testing includes complex II and, in isolated mitochondria, complex I. These defects can, in some cases, be detected by assays in cultured skin fibroblasts (complexes II-IV), but assays of skeletal muscle or heart, if available, are more valuable (Complexes I-IV). Mitochondrial DNA analysis should be considered as part of the evaluation of these defects.

### **Mitochondrial Oxidative Phosphorylation:**

- **Isolation of fresh mitochondria:** Comprehensive analysis of disorders of mitochondrial energy metabolism is greatly facilitated by performing a surgical muscle (and/or liver) biopsy, obtaining sufficient tissue for both neuropathology (histochemistry and electron microscopy) and isolation of intact mitochondria from the fresh specimen. The muscle biopsy must be performed in Cleveland.
- **Oxidative phosphorylation:** The fresh intact mitochondria are incubated with a variety of substrates which donate electrons at different sites along the electron transport chain. Oxygen uptake is measured polarographically in the presence of high/low amounts of ADP and in the uncoupled state. If an abnormality of rate or control is detected, additional appropriate assays are included. The remaining mitochondria are frozen for further analysis of components of the electron transport chain and assay of specific relevant enzymes.



## **Sample Collection and Shipment**

(see also *Test and Price List*)

**Collection and Shipment of Whole Blood for Lymphocyte Assays:** It is *necessary to schedule and confirm the date of collection of whole blood sample(s) in advance* by calling our laboratory. The lymphocytes must be isolated within 48 hours of collection and prior arrangements must be made to reserve laboratory time to complete the assays.

- Shipment of sterile anti-coagulated whole blood must be arranged and scheduled ahead of time.
- Blood must be collected in ACD\* or CPD-A1\* (0.15 ml/ml blood) using sterile technique.
- Volume of blood required for each patient for each assay:
  - 5 ml for patient(s) < 6 months old
  - 10 ml for patient(s) 6 months to 6 years old
  - 20 ml for patients(s) > 6 years old
- Volume of blood required from a normal unrelated adult volunteer in the same way at the same time: 20 ml.
- Ship tubes in styrofoam box at room temperature on scheduled day with enough packing material so the tubes do not crack or break during shipment. Indicate clearly on container: Room Temperature - Do Not Freeze. Use the Overnight Priority/Next Morning service offered by your courier (preferably Federal Express, Airborne Express, or UPS).
- Please notify us if for any reason you are not able to ship on the scheduled day.

\*ACD is available in yellow stoppered Vacutainer tubes. CPD-A1 is an anticoagulant/preservative mixture used routinely in blood banks. It is also possible to obtain CPD-A1 in a blood pack unit (from the blood bank), and to then transfer the CPD-A1 to plain sterile Vacutainer tubes. If neither of these is available, use whatever equivalent mixture is in your blood bank and note this on the sample. Use an equivalent proportion of the mixture/blood sample as would be used routinely in collecting blood. Please Note: Yellow stoppered Vacutainer tubes containing SPS, for use in microbiology, are not suitable for enzyme assays.

**Shipment of Cultured Skin Fibroblasts:** Shipment of fibroblasts *must* be arranged and ***scheduled in advance*** by calling our laboratory. Certification must be provided with the sample(s) indicating recent testing showing absence of mycoplasma. Mycoplasma testing services are available through Bionique Testing Laboratories, Inc., RR 1, Box 196, Fay Brook Drive, Saranac Lake, NY 12983, Phone: (518) 891-2356, Fax: (518) 891-5753. Bionique offers various services by which you can ship fibroblasts for mycoplasma testing; the most commonly used by our referring laboratories is the Cell Shipper M-100 Coated Slide.

- Shipment of cultured skin fibroblasts must be arranged and scheduled in advance.
- Documentation must be provided with the sample(s) indicating recent testing showing absence of mycoplasma contamination. Cultured skin fibroblasts can not be accepted without this documentation.
- Please make sure that there are no visual signs of fungal, bacterial, or yeast contamination before the shipment of these samples. Also confirm the absence of cracks or leaks in the flasks.
- We request two confluent small flasks (T25) from the patient, and also from a control (if available). However, if several assays are being requested, the shipment of additional T25s is recommended. Fill the flasks up to the neck (not into) with media and tape/parafilm shut. Do not attempt to screw the caps on so tightly as to cause them to crack. We recommend that you ship skin fibroblasts in non-vented flasks; phenolic-style and plug seal caps are ideal. If you must ship in vented flasks, please make sure the vented opening is adequately covered with tape/parafilm.
- We recommend that you save actively dividing back-up flasks of the skin fibroblast culture(s) in your laboratory until you have received reports from us. This will guarantee the availability of the cell line(s) in the event of breakage of flasks or contamination during transport. We also recommend that you store indefinitely several frozen plugs of the cell line in liquid nitrogen, for possible future use.
- Ship flask(s) in a styrofoam box at room temperature on the scheduled day with enough packing material so that the flasks do not crack or break during shipment. Indicate clearly on the container: Room Temperature - Do Not Freeze. Use the Overnight Priority/Next Morning service offered by your courier.
- Please notify us if for any reason you are not able to ship on the scheduled day.



## **Sample Collection and Shipment**

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**Collection and Shipment of Frozen Tissues:** Frozen tissues (muscle, heart, or liver) should be quick-frozen in liquid nitrogen immediately after biopsy/autopsy. Shipment of tissues does not need to be scheduled in advance, but please notify our laboratory of the shipment.

- Amounts requested for biopsies: 100 - 500 mg.
- Amounts requested for autopsies: 1 - 3 g.
- Quick-freeze tissues in liquid nitrogen.
- Store at -70°C until shipment.
- Ship tissue(s) in a styrofoam box in enough dry ice to maintain them during shipment. Ship samples early in the week; shipments on Thursdays and Fridays are not recommended. Use the Overnight Priority/Next Morning service offered by your courier (preferably Federal Express, Airborne Express, or UPS).
- Please notify the laboratory of the shipment.

**Collection and Shipment of Blood or CSF for Lactate and Pyruvate Assays:** Collect venous or arterial blood without prolonged stress to the patient with brief use of the tourniquet, if needed. If collecting blood for several purposes/tests, quickly draw all the blood needed and place initially in a plain tube. As soon as the blood or CSF is obtained, measure exactly 1.0 ml (with a tuberculin syringe or a pipette), and transfer immediately into a tightly stoppered tube containing 2.0 ml of 8% w/v perchloric acid\*. Stopper the tube and shake vigorously for at least 15 seconds. The sample is now stable for local transport to a laboratory where it can be centrifuged. Two centrifugations may be required to obtain a clear supernatant. The supernatant should be removed with a Pasteur pipette and transferred to a tightly stoppered polypropylene tube. Freeze the supernatant, and pack in sufficient dry ice for shipment. Ship within 4 days of collection. Shipment of these samples does not need to be scheduled in advance, but ship samples early in the week; shipments on Thursdays and Fridays are not recommended. Use the Overnight Priority/Next Morning service offered by your courier.

\*Perchloric acid is prepared by mixing 7 ml of 70% perchloric acid and distilled water to make 100 ml total volume. Refrigerate until ready to use.

**Collection and Shipment of Plasma for Carnitine,  $\beta$ -Hydroxybutyrate/Acetoacetate, and Free Fatty Acid Assays:** Collect 0.5-1 ml blood (see *Test and Price List*) in an EDTA tube (lavender top), mix gently, and centrifuge as soon as possible after collection. Transfer plasma into a polypropylene tube, and freeze. Pack in sufficient dry ice. Shipment of these samples does not need to be scheduled in advance, but please notify our laboratory of the shipment. Ship samples early in the week; shipments on Thursdays and Fridays are not recommended. Use the Overnight Priority/Next Morning service offered by your courier.

**Collection and Shipment of Urine for Carnitine or Organic Acid Assays:** The patient's perineal area should be thoroughly washed and rinsed with water to remove all dirt, oils, and soap. Collect urine in a thoroughly clean container. Transfer 5 ml of urine (minimum 1 ml) to a tightly stoppered polypropylene tube. Freeze and pack in sufficient dry ice for shipment to the address below. Shipment of these samples does not need to be scheduled in advance, but please notify our laboratory of the shipment. Ship samples early in the week; shipments on Thursdays and Fridays are not recommended. Use the Overnight Priority/Next Morning service offered by your courier.

The referring physician, send-out laboratory, and/or responsible/billing party indicated on the CIDEM laboratory requisition will receive written copies of the report in the mail (a copy is not sent to the patient's family when they are responsible for payment). If you require that another party also receive written notification, please indicate their name and address on the requisition. Final reports are available by FAX transmission if a FAX number is provided on the requisition.