Instructions for Shipping Samples for Porphyria Testing

This packet contains the following:

• Instructions for processing and shipping samples
• Order form for testing
• A primer on laboratory testing for porphyrias (updated 3/9/04)
Instructions for Sample Collection and Shipping

Keep all samples from the light during collection and storage

**Blood**

*Do not freeze blood in glass tubes.*

1. Draw blood into a heparinized (green top) tube. Mix well.
2. Obtain a simultaneous hematocrit. Send to a clinical laboratory. Record the result on the form or send us the result separately when it becomes available.
3. Transfer 0.5-1.0 ml of well-mixed whole blood into a small plastic tube with a stopper.
4. Label tube with patient’s name, the collection date and “Whole Blood”.
5. Freeze immediately after mixing. To avoid separation of red cells and plasma place the plastic tube on its side in a -20°C freezer, or on dry ice.
6. Centrifuge the remainder of the blood in the heparinized tube.
7. Separate the plasma.
8. Transfer 0.5-1.0 ml plasma to a small plastic tube with a stopper, label with patient’s name, the collection date and “Plasma” and then freeze.

**For serum porphobilinogen,** draw blood into a red top tube, allow clot to retract, centrifuge and save at least 1.0 ml serum in a plastic tube. Label with patient’s name, the collection date and “Serum” and then freeze.

**Urine**  A *random urine is sometimes adequate for acute porphyria screening.*

1. Obtain a 24-hour urine collection, using 5 grams sodium carbonate as preservative. (If this preservative is not available call us and we will send it by mail. If unable to wait for Na carbonate, it is better to use no preservative rather than an acid preservative.) Keep the sample refrigerated and shielded from light (or use a dark brown container) during and after the collection.
2. Record the total volume on the form.
3. Mix the sample thoroughly and then aliquot 50 ml to an unbreakable plastic container, and freeze. *(The container for the urine aliquot should not be filled more than 2/3 full, or it will leak or break during freezing).*
4. Label with patient’s name, the collection date, 24 hour urine volume and "Urine".

**Stool**

1. Obtain a random stool sample (at least 10 grams).
2. Freeze in an unbreakable, plastic, air-tight container. The container can be enclosed in a plastic bag before freezing.
3. Label with patient’s name, the collection date, and "Stool".

**For All Samples**

1. Fill out the Request Form and include it with the samples. Indicate the tests requested. Consult the laboratory by phone or letter if there are questions about which tests are appropriate for the patient.
2. Pack the samples with enough dry ice to last for 3 days (usually 5-10 pounds). Use a styrofoam insulated shipping container that is enclosed in a cardboard box. Samples in the container should be wrapped or padded to prevent damage in transit.
3. Ship prepaid by an overnight air transportation company that will deliver directly to The University of Texas Medical Branch. *(We cannot pick up at airports.)*
4. Ship to:
   Karl E. Anderson, M.D.
   Division of Human Nutrition
   Ewing Hall, Room 3.102
   700 Harborside Drive
   The University of Texas Medical Branch
   Galveston, Texas 77555-1109
   Write on shipping form: “Inside Delivery Only”
   Telephone: (409) 772-4661
   FAX: (409) 772-6287
5. Ship early in the week, to avoid weekend arrival. *Be sure to use the street address shown above. WE DO NOT PAY FOR SHIPPING*

**Interpretation of results** Test results are reported with an interpretation that takes into account any available clinical information and prior test results. 7/21/99
A Primer on Laboratory Testing for Porphyrias

Porphyrias result from deficiencies of enzymes of the heme biosynthetic pathway. This metabolic pathway is comprised of multiple enzymes and intermediates. The endproduct is heme, which is a component of many hemoproteins in the body, such as hemoglobin, myoglobin and cytochromes [1, 2].

The symptoms of porphyria are very nonspecific and can be mimicked by many other diseases. However, the excess intermediates that accumulate can be measured in blood (plasma and red blood cells), urine and feces, and these measurements are very useful for diagnosis, especially when a patient is experiencing symptoms that suggest porphyria. Diagnosis can be more difficult when symptoms have been absent for a long time.

Many tests are offered to practicing physicians for diagnosis of porphyrias, especially by large commercial laboratories [3-7]. Some tests for porphyria are highly specific and very sensitive. But some others are less specific and can be abnormal in other conditions. Therefore, experience is important in choosing the best test in particular situations and interpreting the results. Many are overused, which results in unnecessary expense, delay in diagnosis and even misdiagnosis of porphyrias. Misdiagnosis of porphyria in patients with nonspecific symptoms and some abnormalities in tests for porphyria has become a common problem and can lead to inappropriate management.

We and others recommend that physicians rely on a few first-line tests for screening of patients with symptoms that suggest porphyria, and reserve other tests for use only when a screening test is abnormal.

First-line testing for acute porphyrias

Urinary ALA and PBG are the preferred first-line tests for acute porphyrias. This should be done in two stages: 1) rapid screening of a spot urine for increased porphobilinogen (PBG), and 2) later measurement of δ-aminolevulinic acid (ALA, also known as 5-aminolevulinic acid) and PBG using the same spot urine sample.

ALA is an amino acid and PBG is a pyrrole (see adjacent figure). ALA and PBG are porphyrin precursors and NOT porphyrins. The term "porphyrin screen" should never be used, because it may specify a porphyrin test rather than PBG or ALA.

Porphyrians are tetrapyrroles. Shown below is a typical porphyrin in its reduced state (uroporphyrinogen III – one of the pathway intermediates) and its oxidized state (uroporphyrin III – the form of the intermediate that appears in urine).
Testing for urinary PBG should be available on an urgent basis in all hospitals. This enables physicians to make a diagnosis of acute porphyria promptly and institute specific and effective therapy. These are life-threatening disorders, and delay in diagnosis adversely affects outcome. In the past, the Watson-Schwartz [8] and the Hoesch tests [9] were widely used for screening spot urine samples, but now most hospitals send out samples for testing to commercial laboratories that require 24 hour urine collections and provide comprehensive and confusing reports of porphyrins as well as ALA and PBG, with a considerable delay before results are available.

The preferred method is to use a convenient test kit (Trace®) for PBG on a spot urine sample [10]. The kit is based on the standard Mauzerall-Granick method [11] and utilizes an anion exchange resin to separate PBG from interfering substances in urine. It also provides a color chart that permits semiquantitative estimation of high PBG levels. This method is much more reliable than the Watson-Schwartz Hoesch tests, and the color chart minimizes observer bias in interpreting the developed color. Because urinary PBG is generally strikingly increased during an attack of porphyria, a spot sample (rather than a 24 hour collection) is highly informative. Requiring a 24 hour urine collection can considerably delay the diagnosis. Furthermore, urinary PBG excretion may drop considerably (especially in HCP and VP) if there is a delay of several days in collecting a 24 hour urine, and especially if the patient is treated with intravenous heme.

After a urine sample is screened for increased PBG, it should be saved for further testing regardless of the initial results. ALA and PBG should be measured in the same spot sample by the Mauzerall-Granick method, which has been available for many years [11]. This will confirm a normal or high screening PBG result and also determine if ALA is markedly increased.

PBG and ALA can also be measured in serum or plasma, but the concentrations are less than in urine. Therefore, this is important primarily in patients with renal impairment.

We also measure total urinary porphyrins on all submitted urine specimens. While this may increase sensitivity, the results must be interpreted carefully, because increases in urinary porphyrins are not specific.

First-line testing for cutaneous porphyrias

Chronic blistering skin lesions occur in all the cutaneous porphyrias except erythropoietic protoporphyria (EPP). EPP usually presents with acute skin swelling, redness and pain soon after sun exposure.

Plasma total porphyrins is the preferred method for screening for cutaneous porphyrias. Circulating plasma levels are virtually always increased in patients with active, blistering skin lesions due to porphyria. Normal plasma porphyrin levels exclude porphyria as a cause of blistering skin lesions.

The preferred method for measuring plasma total porphyrins is a simple and direct fluorometric method [12-15]. This method includes determination of the fluorescence emission peak, after diluting plasma with buffer at neutral pH.

- For PCT, HCP and CEP, the fluorescence emission peak at neutral pH is at ~620 nm.
- The peak in VP (at ~626 nm) is different from all other cutaneous porphyrias, apparently because porphyrins in plasma in VP are mostly covalently linked to plasma proteins. These covalently bound proteins may not be detected by the HPLC methods offered by many laboratories.
- The peak for EPP (at ~634 nm) is also distinctive. Plasma porphyrins may be less elevated in EPP than in other cutaneous porphyrias, and are especially sensitive to light exposure. Therefore, when EPP is suspected, both plasma and erythrocyte porphyrins should be measured. However, because erythrocyte porphyrins (especially zinc protoporphyrin) are increased in many other medical conditions, an increase is not a specific finding.
• It needs to be kept in mind that the normal range for plasma porphyrins is higher in patients with end stage renal disease than in normals [16].

• Hemolysis of a blood sample invalidates a plasma porphyrin determination because normal erythrocytes contain much larger amounts of porphyrin (in the form of Zn protoporphyrin) than does normal plasma.

Second-line testing

Further testing may be indicated in the following situations.

• First-line testing is positive.

• A patient suspected to have porphyria but who has not had symptoms for a considerable time (weeks or months).

• Asymptomatic relatives of a patient with proven porphyria.

Much more extensive (and expensive) laboratory testing is usually justified in these situations because it is essential for management and genetic counseling to determine which type of porphyria is present. A full discussion of which tests are needed in these situations is beyond the scope of this primer. But many of the following may be needed. At this stage, a physician and laboratory with experience in testing for porphyrias and in the clinical management of these disorders should be consulted [3].

Urinary ALA, PBG and porphyrins. A 24 hour urine collection is generally indicated for more complete evaluation after a positive screening result or to enhance sensitivity in an asymptomatic individual.

Sodium carbonate (5 grams, added to a 24 hour urine bottle prior to collection) is widely recommended for urine specimens intended for measurement of ALA, PBG and porphyrins. Some laboratories require that acid be added to containers for collection of urine for ALA determination, because this substance is more stable in acid. But acid conditions enhance degradation of porphobilinogen. Therefore, it is preferable to use either sodium carbonate or no preservative rather than acid.

Increased in urinary porphyrins, especially coproporphyrin, are not specific, and are seen in many medical conditions, including liver and bone marrow diseases. Nonspecific increases are usually slight or moderate. The increases in active porphyrias are usually marked, and the pattern of porphyrins, as separated by high performance liquid chromatography (HPLC), can assist in determining the type of porphyria.

Fecal porphyrin determinations. Fecal porphyrins can be increased in several types of porphyrias. They are most strikingly increased and diagnostically useful in HCP (mostly coproporphyrin III) and VP (mostly coproporphyrin III and protoporphyrin IX).

The results can may be confounded by variations in fecal flow and by substances in the diet. A 24 hour collecting of feces is not a meaningful timed sample. It is best to use a spot fecal sample, since in general only striking elevations are diagnostically helpful.

Erythrocyte porphyrins. Normal erythrocytes contain a significant amount of zinc protoporphyrin. The amount is greatly increased in iron deficiency, lead poisoning and in virtually any erythrocyte disorder. Assays generally measure the total amount of porphyrins, but are usually expressed as protoporphyrin, since zinc protoporphyrin is almost always the type that is increased. However, free protoporphyrin rather than zinc protoporphyrin is primarily increased in EPP. In congenital erythropoietic porphyria (CEP), red cell porphyrins are markedly increased, and are mostly uroporphyrin I, coproporphyrin I, and sometimes zinc protoporphyrin.
Heme pathway enzymes. Assays for heme biosynthetic pathway enzymes in erythrocytes have become widely available through commercial laboratories. These assays should not be used as first-line tests for porphyrias when screening patients with symptoms. They are useful for family studies, when it is established that an index case has a particular enzyme deficiency. Difficulties with these assays in clinical practice include the following. (i) Ranges for a particular porphyria and normals may overlap. (ii) Some mutations may cause a particular enzyme to be deficient only in nonerythroid tissues. (iii) Falsely low values are common due to problems with collecting or transporting the sample. (iv) Some laboratories employ coupled enzyme assays that may lack specificity.

Laboratory testing of relatives and patients with subclinical porphyria. In all types of porphyrias there are striking increases in porphyrins and/or porphyrin precursors when these conditions are symptomatic. However, even in porphyrias that are inherited in an autosomal dominant pattern, there may be no such increases in many family members who carry the abnormal gene.

Before deciding which tests are appropriate for asymptomatic family members, the diagnosis of porphyria should be firmly established in the propositus, and the results of previous testing in the propositus should be reviewed. It may be necessary to retest either the propositus or another family member with confirmed porphyria before undertaking screening of family members.

DNA testing is ideal for detecting carriers of a known mutation in a particular enzyme, but requires that the mutation first be fully characterized in the propositus.

Porphyrias seldom become completely latent (such that all levels of porphyrins and porphyrin precursors become normal) within a short period of time. However, it does become more difficult to “rule out porphyria” if testing is delayed until after there is resolution of symptoms. If it is clinically important to exclude subclinical porphyria in a patient with past suggestive symptoms, and if definitive testing was not conducted near the time of symptoms, a specialist physician and laboratory should be consulted to advise on the choice and interpretation of laboratory tests.

Misinterpretation of porphryia test results. Diagnosis of porphyria is often delayed or missed, and physicians have been urged to maintain a high index of suspicion for these conditions. But it is less widely appreciated that incorrect diagnoses of porphyria are common in patients with symptoms due to other diseases. Therefore, in patients with a past history of porphyria it is important to review the laboratory data that were the basis for the original diagnosis.

Incorrect diagnoses of porphyria can occur in patients having minimal abnormalities in laboratory tests, such as small elevations in urinary porphyrins or porphyrin precursors that in fact have little or no diagnostic significance. Incorrect diagnoses are less likely if reliance is placed on a few first-line tests in most clinical situations, as described above. Overuse and overinterpretation of minor abnormalities in results of second-line tests account for many incorrect diagnoses of porphyria.

Patients with porphyria should be advised to obtain copies of the laboratory documentation for their diagnosis and retain them indefinitely. Further testing may be necessary if the diagnosis was not adequately documented, and this may be much less revealing if the patient has become asymptomatic since the original diagnosis was made.

**Patient Information**

Name: ______________________________________

Age: _______ DOB: _________ Sex: _______

Diagnosis ___________________________________

Hematocrit: _________ (Date ____________)

Hematocrit is required when erythrocyte tests are requested, because these tests are done on whole blood and the hematocrit is used to calculate results.

Reticulocytes: _________(%) (Date ____________)

Reticulocyte count is optional, but is suggested for the erythrocyte tests, because young erythrocytes can increase the results.

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**Clinical features:** (to facilitate interpretation of results)

- Abdominal pain
- Other pain
- Peripheral neuropathy
- Skin Blister
- Other skin lesions
- Other features: _______________________

Results are reported with an interpretation that includes consideration of any clinical information provided.

- Hematocrit and/or reticulocyte results will be sent later.

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**URINE:**

<table>
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<th>Test</th>
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<th>CPT Codes</th>
<th>Charges</th>
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<tbody>
<tr>
<td>* δ–Aminolevulinic acid (ALA)</td>
<td></td>
<td>82135</td>
<td>$85</td>
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<tr>
<td>Porphobilinogen (PBG)</td>
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<td>84110</td>
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<td>Total Porphyrin (Reflex: fractionation by HPLC if total porphyrin is increased)</td>
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<td>24 Hour urine (total volume)</td>
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<td>Spot urine</td>
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**PLASMA:**

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<tr>
<td>** Total Porphyrin (with neutral spectrum if increased)</td>
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**FECES:**

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<td>Total Porphyrin (Reflex: fractionation by HPLC if total porphyrin is increased)</td>
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**SERUM:**

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<td>Porphobilinogen (PBG)</td>
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**ERYTHROCYTE (Whole Blood):**

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<tr>
<td>Protoporphyrin (total porphyrin, expressed as protoporphyrin &amp; tested for free protoporphyrin vs. zinc protoporphyrin if increased)</td>
<td>84202</td>
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<tr>
<td>Porphobilinogen deaminase (PBGD)</td>
<td>82657</td>
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<td>Uroporphyrinogen decarboxylase (UROD)</td>
<td>84999</td>
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**Name and address of physician, laboratory or other individual where results should be sent:**

**Name & address to whom invoice should be sent**

(We do not bill insurance directly):

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**Recommended for initial screening for acute & cutaneous porphyries**

For further information about clinical indications for specific tests, see “Primer on Laboratory Testing for Porphyria”, or call number above for advice.

**See attached instructions regarding sample preparation and shipment**
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<tr>
<th></th>
<th>Physician Information</th>
<th>Non-Physician Information</th>
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