

Department of Clinical Biochemistry

Whitfield St Laboratories

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DIAGNOSTIC SERVICE FOR THE PRIMARY HYPEROXALURIAS

The primary hyperoxalurias are rare inborn errors of metabolism characterised biochemically by increased synthesis and excessive urinary excretion of oxalate.

The biochemical defect in primary hyperoxaluria type 1 (PH1) is a deficiency of the liver specific enzyme alanine: glyoxylate aminotransferase (AGT). This enzyme is normally located in the peroxisomes of hepatic cells and in its absence, excessive conversion of glyoxylate to oxalate occurs. In some PH1 patients, notably those with the Gly170Arg missense mutation, the enzyme is mistargeted to the mitochondria where it is ineffective but still has catalytic activity. Primary hyperoxaluria type 2 (PH2) results from a deficiency of the enzyme glyoxylate reductase (GR) which is found predominantly, but not exclusively, in the liver.

This laboratory offers definitive diagnosis of PH1 and PH2 via measurement of AGT and GR in liver biopsies. First trimester prenatal diagnosis, mutation analysis and family studies are also available for PH1 and PH2 using DNA analysis. The department offers a range of supporting investigations including relevant metabolites in plasma and urine and quantitative renal stone analysis.

TISSUE DIAGNOSIS OF PH1 AND PH2 VIA LIVER BIOPSY

On receipt of a suitable liver biopsy and EDTA blood sample the following analyses will be performed:

1. AGT and GR catalytic activities
2. AGT and GR protein immunoreactivity
3. DNA analysis to identify the AGT mitochondrial targeting sequence

PRENATAL DIAGNOSIS OF PH1

First trimester prenatal diagnosis is available for this disease. The technique utilises a combination of mutation and linkage analysis on DNA and has proven to be applicable in more than 99% of families studied. The procedure requires family studies to determine the phase of linkage of genetic markers.

CARRIER TESTING FOR PH1 AND PH2

The status (normal, affected or carrier) of other family members can be determined by linkage analysis. Please ensure that informed consent for such testing is obtained from family members and enclose a copy with the samples.

MUTATION ANALYSIS FOR PH1 AND PH2

A mutation screen is available for PH1 and PH2

For PH1 three mutations, c.33_34insC, c.508G>A (Gly170Arg) and c.731T>C (Ile244Thr), are analysed which will detect approx. 45% mutant alleles

For PH2, a single mutation c.103delG accounts for approx. 37% mutant alleles.

SAMPLE REQUIREMENTS AND COLLECTION PROCEDURES

Liver Biopsy

An absolute minimum of 20mg of liver should be removed by needle or open biopsy. Immediately freeze the biopsy. The sample must be maintained FROZEN and transported as such.

5 ml of EDTA whole blood is also required for DNA testing for the mitochondrial mistargeting mutation, Gly170Arg. Sample can be frozen and shipped in the same container as the liver biopsy or sent separately at ambient temperature.

Whole (EDTA) blood for mutation screening and/or family studies

Please collect 5 ml of whole blood from both parents and the index case in appropriately labelled plastic tubes containing K EDTA (not heparin) as an anticoagulant. Ensure that the tubes are not overfilled and are well mixed. The samples can be sent at ambient temperature.

Fetal tissue

20mg well dissected chorionic villus is required. The sample can be shipped at ambient temperature in media with antibiotics.

Please note: Only PH1 status is carried out in this laboratory. Fetal karyotype analysis must be performed locally. It is recommended that back up cultures of chorionic villus are established locally in case of loss in transit to this laboratory.

Oxalate (urine and plasma)

25 ml of urine from a 24 hour collection in HCl is required or alternatively, if patient is anuric, 5ml of venous plasma from an EDTA anti-coagulated specimen. The plasma must be separated within 15 minutes of collection, immediately frozen and maintained frozen during transportation.

DIAGNOSTIC SERVICE FOR THE PRIMARY HYPEROXALURIAS

Please photocopy and complete the following questionnaire and return with the sample (or before)

* Please note that despatch of samples to this laboratory indicates acceptance of our terms of business (see page 5)

Requesting clinician and address for report Name: PD Dr. Bernd Hoppe
Address University Children's Hospital Division of Pediatric Nephrology, Kerpenerstr. 62, 50924 Cologne, Germany
Tel: 0049 221 478 4391
Fax: 0049 221 478 5835

Address for invoice (if different)* Name:
Address:
Tel:
Fax:

Name of Patient: _____ Age/Date of Birth: _____ Male/Female: _____

Ethnic origin: _____ Consanguinity YES/NO Date of biopsy: _____

Age of first presentation: _____ Mode of presentation: _____

Are the following features present now?

Renal stones:	YES/NO	(If yes, recurrent YES/NO)
Nephrocalcinosis	YES/NO	
Systemic oxalosis	YES/NO	
Has pyridoxine therapy been attempted?	YES/NO	Response: YES/NO
Is patient currently taking pyridoxine?	YES/NO	(If YES dose _____ mg/day)

Please provide the following data (with units please)

Urine oxalate: _____	Urine creatinine: _____	Urine glycolate: _____
Plasma oxalate: _____	Plasma creatinine: _____	Plasma glycolate: _____
		Urine L-glycerate: _____

IMPORTANT: We reserve the right not to handle any samples which are known or likely to be infectious. Please contact the laboratory if in doubt. We carry out the analysis on the understanding that the following declaration is signed:

"I believe to the best of my knowledge that the above patient does not have hepatitis C, is not Hepatitis B Antigen or HIV positive".

..... (Signature of requesting clinician)

Blood & tissue samples obtained through the service may be used for further research into the nature of the primary hyperoxalurias. Please indicate, by signing below that this has been explained to the patient and/or family and that they agree with such use.

..... (Signature of requesting clinician)

ARRANGEMENTS FOR TRANSPORTATION

To enable us to make preparations for the reception of the frozen samples it is essential that, before sending any material, you telephone or fax one of the following contacts with the details shown below:

Tel: 44 (0)20 7636 8333 ext. 2955 Ask to speak to Dr Rumsby

Fax: 44 (0)20 7380 9584 Mark fax for the urgent attention of Dr Rumsby or Dr E Williams

Details required by fax or telephone are as follows:-

Name of Courier _____ Airwaybill Number _____

Date / Time of shipment _____

- Pack the **frozen samples** (see check list opposite) in an insulated container surrounded by dry ice (cardice). Liver biopsy, frozen EDTA whole blood
- Ensure that the container has sufficiently thick insulation walls and an adequate quantity of dry ice to maintain the samples frozen for **at least 5 days**. The package may spend several days in transit/customs.
- Packages should preferably be sent at the beginning of the week.
- For overseas samples send by air. Use an international courier (e.g. Federal Express, UPS, TNT). **You must arrange and pay for delivery to our door.** We do not accept liability for excess delivery charges. We cannot pick up packages from airports.
- The package should be addressed to: **Dr G Rumsby / Dr E Williams
Clinical Biochemistry
University College London Hospitals
60 Whitfield St
London W1T 4EU
UK**

YOU MUST CLEARLY LABEL THE PACKAGE AS FOLLOWS OR USE LABEL BELOW

VALUE LESS THAN \$10 (US)

CONTAINS FROZEN PERISHABLE NON-HAZARDOUS HUMAN TISSUE & FLUID SAMPLES FOR NON-COMMERCIAL MEDICAL/SCIENTIFIC PURPOSES.

PACKED IN DRY ICE - REQUIRES MAINTENANCE OF FROZEN STATE ON ARRIVAL.

TERMS OF BUSINESS

FEES (to be reviewed on 1.4.04):

LIVER BIOPSY		£329 per biopsy sample
DNA ANALYSIS	FAMILY STUDY	£329
	PRENATAL DIAGNOSIS	£329
	MUTATION ANALYSIS	£40 per genotype
URINE OXALATE		£25
PLASMA		£14
OXALATE		

Method of Payment

UK requests:

An official order originating from the referral establishment (preferably through the Chemical Pathology laboratory) must be sent with specimens. A separate invoice will be issued.

Overseas requests:

A proforma invoice will be issued on request.

An invoice will be issued on receipt of the patient samples if no proforma invoice has been issued.

Full payment of the proforma / invoice is required before results can be released.

Payment may be made by:

- Electronic bank transfer - BACS / Swift
- Bank draft
- Credit Card

AVAILABILITY OF RESULTS

Liver Biopsies

Assays will normally be completed within 30 days from the receipt of payment.

DNA analysis (Family Study)

Non urgent family studies and mutation analysis will be completed within three months from the receipt of payment.

Prenatal diagnoses

Prenatal diagnoses will be completed within 14 days.

Reminder : Despatch of samples to this laboratory indicates acceptance of the terms of business.

