

Diagnostic Service for the Primary Hyperoxalurias

The primary hyperoxalurias are rare inborn errors of metabolism characterised biochemically by increased production and excessive urinary excretion of oxalate. There are three distinct forms of primary hyperoxaluria that have been biochemically and genetically characterised:

- **Primary hyperoxaluria type 1 (PH1)**

The biochemical defect in PH1 is a deficiency of the liver specific enzyme alanine: glyoxylate aminotransferase (AGT), encoded by the *AGXT* gene. This enzyme is normally located in the peroxisomes of hepatic cells and is vitamin B6 dependent. In its absence the excess glyoxylate is converted to oxalate, and excess glycolate can also be produced. In some PH1 patients, notably those with the p.Gly170Arg missense mutation, the enzyme is mistargeted to the mitochondria where it is ineffective but still has catalytic activity.

- **Primary hyperoxaluria type 2 (PH2)**

The biochemical defect in PH2 is a deficiency of the enzyme glyoxylate reductase (GR), encoded by the *GRHPR* gene. This enzyme is found predominantly, but not exclusively, in the liver and it catalyses the conversion of glyoxylate to glycolate. In its absence oxalate is produced in a similar way to in PH1, and excess glycerate can also be produced.

- **Primary hyperoxaluria type 3 (PH3)**

PH3 has been described more recently and is due to deficiency of 4-hydroxy-2-oxoglutarate aldolase in liver and kidney, encoded by the *HOGA1* gene. The mechanism by which excess oxalate accumulates has not been definitively proven, but the level of hyperoxaluria can be similar to that in PH1 and PH2. In addition excess 4-hydroxy-2-oxoglutarate (HOG) and dihydroxyglutarate (DHG) can also be produced.

The department offers a complete laboratory service for the primary hyperoxalurias, from the measurement of metabolites and analysis of calculi, through to gene sequencing and enzyme activity analysis for definitive diagnosis.

Test repertoire and sample requirements

DNA ANALYSIS

Full gene sequencing, which includes all exons and intron-exon boundaries, is available for

- *AGXT*
- *GRHPR*
- *HOGA1*

The status (normal, affected or carrier) of other family members can be determined by mutation analysis on a DNA specimen.

Sample requirements: 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. Alternatively DNA can be prepared locally and sent to us by post. Please ensure that informed consent for such testing is obtained and enclose a copy with the samples.

PRENATAL DIAGNOSIS

First trimester prenatal diagnosis is available for all disorders where a mutation has been identified in the index case.

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

Please note: Only PH 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.

TISSUE DIAGNOSIS OF PH1 AND PH2 VIA LIVER BIOPSY

On receipt of a suitable liver biopsy the following analyses will be performed:

1. AGT and GR catalytic activities
2. AGT and GR protein immunoreactivity

Sample requirements: An absolute MINIMUM of 20 mg of liver should be removed by needle or open biopsy (16 gauge=2 cores; 18 gauge=3 cores). Immediately freeze the biopsy. The sample must be maintained FROZEN and transported as such.

RELEVANT URINE AND PLASMA METABOLITES

The first line screening test for primary hyperoxaluria is urine oxalate. The preferred sample is 10 mL urine from a 24 hour collection, collected into a bottle containing 40 mL of 25% HCl. Alternatively a random urine sample that has been acidified to pH 1 on receipt in the laboratory is acceptable.

We also offer quantitation of urine glycolate, glycerate, HOG and DHG to aid provisional diagnosis of PH1, PH2 or PH3 respectively. The sample requirement is the same as for urine oxalate.

Urine citrate (a stone formation inhibitor) is also available. Sample requirement is 10 mL urine, plain or acidified.

If the patient is anuric, plasma oxalate can be analysed. This test requires 5 mL venous plasma from an EDTA anti-coagulated specimen. The plasma must be separated ideally within 30 minutes of collection, immediately frozen and maintained frozen during transportation.

CALCULI

A minimum of 5 mg material is required sent in a plain container. Quantitative analysis is by Fourier transform infrared spectroscopy.

Terms Of Business

Fees: For current prices, please contact gill.rumsby@nhs.net or oliver.clifford-mobley@nhs.net

UK requests: An official order originating from the referral establishment 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 or credit card

AVAILABILITY OF RESULTS (working days from receipt of payment)

Liver biopsies	30 days
DNA analysis	
Sequencing of entire coding region (AGXT, GRHPR, HOGA1)	30 days
Prenatal diagnosis (urgent)	10 days
Urine oxalate	5 days
Urine glycolate, glycerate, HOG and DHG (Urine PHM)	14 days
Plasma oxalate	10 days
Calculus analysis	3 days

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

Requesting clinician and address for report Name:
Address
Tel:
Fax:

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

Patient details:

Surname:..... First name:..... Date of birth:

Ethnic origin: Sex: Male/Female

Age at presentation: Mode of presentation:.....

Current clinical findings:

Kidney stones: Yes/No	Has pyridoxine therapy been attempted? Yes/No
Nephrocalcinosis: Yes/No	
Systemic oxalosis: Yes/No	Is the patient currently on pyridoxine? Yes/No
Renal failure: Yes/No	If yes: Dose

Please provide the following data if available (including units):

Analyte	Result	Units	Analyte	Result	Units
Urine oxalate	_____	_____	Urine creatinine	_____	_____
Plasma oxalate	_____	_____	Plasma creatinine	_____	_____
Urine glycolate	_____	_____	Urine L-glycerate	_____	_____
Plasma glycolate	_____	_____			

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)

