Addenbrooke's Hospital NHS
Cambridge University Hospitals NHS Foundation Trust

BIOCHEMICAL GENETICS UNIT
METABOLIC ASSAYS

USERS HANDBOOK
Version 5.0

PLEASE DO NOT USE AFTER JUNE 2011

CPA

CONTENTS

1 CONTACT DETAILS AND ENQUIRIES

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2 GENERAL INFORMATION

The Biochemical Genetics Unit (BGU), based at Addenbrooke's Hospital in Cambridge provides laboratory services for the investigation and monitoring of inborn errors of metabolism. The service includes newborn screening of approximately 27,000 babies born in East Anglia per annum. They are screened for phenylketonuria (PKU), congenital hypothyroidism (CHT), cystic fibrosis (CF), sickle cell disease (SCD) and medium chain acyl-CoA dehydrogenase deficiency (MCADD). The laboratory also provides core metabolic tests supporting the metabolic (adult and paediatric), genetic and neurology clinics both locally at Addenbrooke's and across the region. The laboratory has full CPA accreditation.
The biochemical basis of inherited disorders includes many metabolic pathways and there is a vast array of specialist assays available throughout the UK. Please contact one of the clinical scientists if you require information about assays or diseases not covered in this booklet.

Opening Times
The core opening hours of the BGU are 08:00 to 16:00 Monday to Friday. There is no formal out of hours service offered by this laboratory. If urgent assays / advice is required out of hours, please contact the on call biomedical scientist via switchboard (01223 245151, bleep 156-0383) who will forward the request to the Duty Biochemist.

3 SPECIMEN COLLECTION

In patients with episodic illness it is necessary to collect specimens at a time when they are symptomatic, therefore it is important that the date and time of sampling are included on the sample and request form. Diagnoses may be missed if the patient is well or if changes in treatment or diet are initiated prior to specimen collection. Drugs may interfere in the analytical processes or by *in vivo* alteration of metabolic pathways; details of any drug treatments or special diets should be included on the request form. Exchange transfusions / blood transfusions may affect analytes measured in blood, especially enzymes in erythrocytes.

Sample Identification & Policy on Unlabelled Specimens
Each specimen must be clearly labelled with the patient's demographic details. Unidentifiable samples are not suitable for analysis. All samples and request forms must have 3 points of identification.

Sample Volumes
Ideal sample volumes are quoted for each analyte. However it may be possible to use a smaller volume, for urine samples the volume is dependent on the creatinine concentration. It may also be possible to analyse samples on dilution where it is not possible to obtain another specimen. If in doubt please contact the laboratory.

Request Forms
A request form must accompany each sample (the exception to this rule is dried blood spot cards sent for routine newborn screening).
In addition to the demographic details, please provide:

1. Date and time of sample collection – particularly important where the patient is undergoing active (and changing) treatment.
2. Clinical details – important for selecting the method of analysis (eg qualitative vs quantitative urine amino acids) and interpretation of the results, particularly where there are mild or apparently non-specific abnormalities. Please include any suspected diagnosis so that the presence or absence of pathognomic metabolites and any further tests required can be included in the report.
3. Details of any drug therapy.
4. GP details and/or patient postcode – we are required to collate tests by PCT for the East Anglian work.

Transport of Samples

Urgent samples should be sent by courier but only after discussion with one of the clinical scientists. Non-urgent samples should be sent directly to the BGU by 1st class post or hospital transport. All samples should be packaged in accordance with UN3373 and Packaging Instruction 602.

Turnaround Times
The usual turnaround times are detailed with each test. If **urgent results** are required, please contact one of the clinical scientists to discuss.

Result Reporting
Reports are printed daily and dispatched by first class post.

4 ABBREVIATIONS

AA	Amino acids
AIP	Acute Intermittent Porphyria
BH4	Tetrahydrobiopterin
CDG	Congenital Disorders of Glycosylation
CF	Cystic Fibrosis
FAOD	Fatty Acid Oxidation Defect
GAGS	Glycosaminoglycans (mucopolysaccharides)
GC-MS	Gas Chromatography Mass Spectrometry
GE	Glycine Encephalopathy (Non-Ketotic Hyperglycinaemia)
HPLC	High Performance Liquid Chromatography
LCHADD	Long Chain Hydroxy Acyl CoA Dehydrogenase Deficiency
LSD	Lysosomal Storage Disorder
MCADD	Medium Chain Acyl CoA Dehydrogenase Deficiency
MMA	Methylmalonic Aciduria
MPS	Mucopolysaccharides
MSUD	Maple Syrup Urine Disease
NKH	Non Ketotic Hyperglycinaemia (Glycine encephalopathy)
OA	Organic Acids
PKU	Phenylketonuria
TLC	Thin Layer Chromatography
VLCADD	Very Long Chain Acyl CoA Dehydrogenase Deficiency

5 INDICATIONS FOR REQUESTING A METABOLIC SCREEN

It is difficult to provide an exhaustive list of the indications for screening a child (or adult) for an inborn error of metabolism. There are many diseases, most of which are very rare, associated with a variety of clinical signs and symptoms. These may be vague and non specific, and may not always be present. Other considerations include family history (e.g. infant deaths), parental consanguinity, previous unexplained episodes and regression. The presence or absence of other signs and symptoms and the age of the child should also always be taken into account. The presence of strange odours or coloured urine may provide important clues to the presence of metabolic disease; however these characteristic odours are **not** always present.

First-line (core) metabolic tests should usually include:
Urine organic acids
Blood spot acylcarnitines
Plasma amino acids

Urine amino acid screening has a fairly low yield but should be undertaken where a transport disorder (e.g. cystinuria, Hartnup disease) or renal tubular disorder is suspected. However urine organic acids and amino acids are undertaken on all samples on which a 'urine metabolic screen' has been requested.

Urine Metabolic Screen

Initially urine samples will be screened for the following:
Glucose, ketones, pH - Multistix
Amino Acids - desalted urine 2D TLC with ninhydrin detection
Organic Acids – GC-MS

In addition urine glycosaminoglycans may be added depending on the clinical details provided.

Indications: See section 5.1
Sample Type: 10 mL Urine (plain)
Turn Around Time: 5 working days
Depending upon the results of the qualitative amino acid screen, samples may be referred for quantitative amino acid analysis.

External laboratory information: Freeze urine on receipt and dispatch frozen (1st class post or hospital transport)

6 FREQUENT ENQUIRIES

6.1 GLUTARIC ACIDURIA TYPE 1

Glutaric aciduria type 1 (GA-1) is due to deficiency of the enzyme glutaryl-CoA dehydrogenase, which leads to the build up of glutarate and 3-hydroxyglutarate (excreted in the urine). Clinical features include macrocephaly, subdural haematoma, seizures and dystonia, often following an encephalopathic episode. It is an important diagnosis since GA-I can mimic non-accidental injury.
Tests to request: Urine organic acids (glutarate and 3-hydroxyglutarate) and blood spot acylcarnitines (glutarylcarnitine). It is stated in the literature that organic acids and acylcarnitines can be normal in GA-I.
If acylcarnitines and organic acids are normal but there is a strong clinical suspicion of this disorder fibroblast glutaryl-CoA dehydrogenase activity should be measured (skin biopsy), review by a paediatric neurologist is recommended. Please phone to discuss.

6.2 HELLP

Haemolysis, elevated liver enzymes and low platelets (HELLP) during the last trimester of pregnancy is associated with long chain hydroxyacyl-CoA dehydrogenase deficiency (LCHADD) in the infant. The incidence of HELLP in mothers whose babies have LCHADD is relatively high. Conversely, LCHADD is a rare cause of HELLP.
Tests to request: Blood spot acylcarnitines.

6.3 MEDIUM CHAIN ACYL-CoA DEHYDROGENASE DEFICIENCY

MCADD is the most common of the fatty acid oxidation defects and affects fatty acids of carbon chain length C6 to C10. Clinical features include hypoketotic hypoglycaemia, however ketosis does not exclude a fatty acid oxidation defect. Note: newborn screening started in East Anglia on 1st June 2008.

Tests to request: Blood spot acylcarnitine analysis (octanoylcarnitine) and urine organic acids (dicarboxylic aciduria, hexanoylglycine, suberylglycine). Mutation analysis is available as a confirmatory test (5-10 mL EDTA whole blood or a dried blood spot).

6.4 SULPHITE OXIDASE/MOLYBDENUM COFACTOR DEFICIENCIES

Sulphite oxidase deficiency is a cause of intractable seizures in infancy. Biochemical abnormalities include increased sulphite, thiosulphite, taurine and sulphocysteine with a low plasma total homocysteine. Molybdenum cofactor deficiency is clinically indistinguishable from sulphite oxidase deficiency. In addition to the biochemical abnormalities described above patients have raised urine xanthine and low uric acid in plasma and urine. Dip stick testing of urine sulphite is unreliable and is not recommended. Urine thiosulphate is also unsatisfactory with non-specific increases in acutely ill infants.

Tests to request: Sulphocysteine is a stable metabolite and a useful marker of sulphite oxidase deficiency. This compound can be detected at the beginning of the quantitative amino acid trace. Unfortunately interfering substances in plasma and urine can make interpretation difficult, however CSF often provides a cleaner sample.

6.5 TRIMETHYLAMINE

Patients with primary trimethylaminuria have defective trimethylamine N-oxide synthetase activity. This deficiency does not produce disease but the strong, unpleasant fishy odour can lead to social ostracism and psychological disorders. Secondary trimethylaminuria also occurs and is caused by enterobacterial overproduction of trimethylamine. A diet low in fish, liver and egg yolks usually improves the odour.
Tests to request: Urine samples for trimethylamine are referred to Sheffield Children's Hospital for analysis. Please phone to discuss.

6.6 INVESTIGATION FOR SUSPECTED MITOCHONDRIAL DISORDERS

Mitochondrial disorders are a clinically and genetically heterogeneous group of disorders which can present in any system, at any age with any pattern of inheritance. Investigation of these disorders is complex; Dr Alasdair Parker (Consultant Paediatric Neurologist) has developed investigation protocols for suspected mitochondrial disease. Included are a number of non-laboratory investigations (e.g. ophthalmology, audiology etc) as well as biochemistry, histopathology and genetic investigations. Biochemical investigations are mainly used to provide further clues to a possible mitochondrial disorder (e.g evidence of a tubulopathy) and to exclude other metabolic diseases which may mimic a mitochondrial disorder. The cardinal investigation for mitochondrial disease is measurement of plasma and CSF lactate, however it should be borne in mind that even in proven mitochondrial disease plasma lactate can be normal, slightly raised or only intermittently raised. CSF lactate may be more useful, a normal CSF lactate concentration in a fitting child is reassuring. Please phone one of the BGU clinical scientists or paediatric neurologists (01223 216662 / ext. 2662) to discuss.

6.7 INVESTIGATION FOR A METABOLIC CAUSE OF RHABDOMYOLYSIS

Once acquired causes of rhabdomyolysis have been excluded, metabolic causes should be considered. These include glycogen storage diseases and fatty acid oxidation defects. In the first instance acylcarnitines (blood spot and plasma) and urine organic acids should be analysed. For further information please contact one of the BGU Clinical Scientists.

6.8 WHEN TO MEASURE AMMONIA

Ammonia is primarily produced from the catabolism of amino acids. It is neurotoxic and is detoxified by conversion to urea in the liver via the urea cycle. Hyperammonaemia can present as an acute overwhelming crisis in the neonatal period or as a more insidious, episodic illness in children and adults.

Indications: any one of the following:

- Neonate
  unexplained neurological deterioration
- Infant
  unexplained illness, particularly if male,
  history of sibling death or parental consanguinity
- Infant or child
  failure to thrive, feeding problems, vomiting, unexplained seizures
  chronic neurological problems (including developmental delay or regression or ataxia)
- Child or adult
  unexplained episodic illness (lethargy, cyclical vomiting, ataxia, seizures) particularly if precipitated by protein intake
  unexplained encephalopathy
  'encephalitis', behavioural problems, psychosis
  unexplained progressive quadriplegia and learning disability

6.9 WHEN TO MEASURE LACTATE

Indications:

- Hypoglycaemia
- Hepatomegaly
- Neurodegeneration
- Encephalopathy
- Muscle disease
- Suspected mitochondrial disorder*
- Unexplained metabolic acidosis

*See section 6.6 above

6.10 WHICH SAMPLE TYPE FOR THE INVESTIGATION OF PORPHYRIA?

If in doubt please phone one of the BGU clinical scientists to discuss prior to collecting samples.

Porphyria is caused by deficiency of one of the 8 enzymes in the haem biosynthetic pathway. It is traditionally grouped into the acute and non-acute (cutaneous) porphyria.

The typical symptoms of the acute porphyrias include abdominal pain, vomiting and tachycardia. The screening test for these disorders is urine porphobilinogen. A negative result excludes acute porphyria as the cause of current acute symptoms, but does not exclude latent porphyria.

Where non-acute porphyria is suspected in a patient with skin lesions, the first line test is red cell and plasma porphyrins.

Where there is a family history of porphyria DNA analysis may be the most appropriate investigation, if the familial mutation is known.

If the screening test is positive further samples of urine, blood and/or faeces may be required. Where the first line test is negative, but there is a strong clinical suspicion of porphyria please phone one of the Clinical Scientists to discuss possible further investigations.

Note all samples for porphyrin investigations **must** be protected from light.

7 IN-HOUSE METABOLIC INVESTIGATIONS

7.1 ACYLCARNITINES

Analysis of the acylcarnitine profile is a powerful tool in the investigation of FAOD (eg MCADD, LCHADD, VLCADD) and classical organic acidurias. β-oxidation of long chain fatty acids has an important role in energy production, a process that becomes critical during prolonged fasting. The clinical presentation of FAOD is variable but they typically present in early childhood with hypoketotic hypoglycaemia. Analysis of plasma total and free carnitine may be indicated in the investigation of carnitine transporter defects and plasma acylcarnitines for carnitine palmitoyltransferase 2 deficiency (see section 8.5).
NB: Ketonuria does not exclude a FAOD
Diagnostic abnormalities may not be present in carnitine deficient individuals.

Indications:

- Hypoglycaemia (usually hypoketotic)
- Cardiomyopathy
- Hepatomegaly
- Hyperammonaemia
- Hypotonia
- Muscle weakness
- Rhabdomyolysis

Sample Type: Dried blood spot
Turn Around Time: 5 working days

Table 7.1: Blood Spot Acylcarnitine Reference Ranges

Acylcarnitine*	(μmol/L)	Acylcarnitine*	(μmol/L)
Free	8 – 35	Hexanoyl	< 0.2
Acetyl	5 – 27	Octanoyl	< 0.2
Propionyl	0.13 – 4.00	Decanoyl	< 0.2
Butyryl	< 0.5	Myristoyl	< 0.5
Isovaleryl	< 0.3	Palmitoyl	0.6 – 4.5

* Please note: the above acylcarnitines are quantitated. Any other acylcarnitines present in abnormal amounts will be reported qualitatively.

External laboratory information: Store at room temperature in glassine envelope. Send by 1st class post or hospital transport.
Method: Tandem Mass Spectrometry (underivatised)

7.2 AMINO ACIDS

7.2.a Plasma Amino Acids

Plasma amino acids may be abnormal in a variety of amino acid disorders, including urea cycle defects and some organic acidurias. Investigations should be carried out, as far as possible, on samples taken when the patient is symptomatic. Dietary restrictions may cause characteristic patterns to

disappear and result in false negative results. Plasma amino acids fluctuate depending on the protein intake and whether the patient is in a fed or fasted state. Patients receiving an intravenous amino acid mixture may have an abnormal amino acid pattern. Information on the type of diet and the timing of the sample in relation to meals will aid interpretation.
For the investigation of epileptic encephalopathy a paired plasma sample must accompany any CSF. For information about sulphocysteine see 6.4 Sulphite oxidase deficiency / molybdenum cofactor deficiency.

Indications:
- Hyperammonaemia
- Lethargy progressing to coma, overwhelming illness in first few days of life
- Unexplained seizures
- Episodic vomiting
- Microcephaly
- Epileptic encephalopathy

Sample Type: 0.5 mL Lithium heparin plasma
Turn Around Time: 5 working days

Table 7.2a: Plasma Amino Acid Reference Ranges
Please note the paediatric reference ranges relate to fasting samples

Amino Acid	µmol/L	Amino Acid	µmol/L
Taurine	0 – 232	Methionine	17 – 37
Aspartate	0 – 14	Isoleucine	41 – 93
Threonine	75 – 203	Leucine	85 – 169
Serine	70 – 178	Tyrosine	40 – 94
Glutamate	8 – 64	Phenylalanine	42 – 74
Glutamine	464 – 728	Ornithine	21 – 77
Proline	70 – 300	Lysine	142 – 198
Glycine	160 – 304	Histidine	65 – 105
Alanine	155 – 537	Arginine	49 – 129
Citrulline	8 – 47	Tryptophan	31 - 79
Valine	161 – 285		

External laboratory information: The sample should be separated within 1 to 2 hours of collection and the plasma stored frozen until dispatch. Dispatch frozen (1st class post or hospital transport)
Method: Cation exchange chromatography with post-column derivatisation (ninhydrin) and spectrophotometric detection

7.2.b Urine Amino Acids

Indications: Qualitative urine amino acid analysis is undertaken as part of the urine metabolic screen. Any samples showing abnormal patterns plus requests relating to specific disorders (e.g. ?homocystinuria) will undergo quantitative analysis. The most sensitive test for the investigation of suspected amino acidopathies is quantitation of *plasma* amino acids. However urine should be analysed if a transport defect is suspected (e.g. cystinuria or Hartnup disease) or where assessment of renal tubular function is required. In addition urine is also required for the measurement of phosphoethanolamine where

hypophosphatasia is suspected (skeletal problems with a low alkaline phosphatase activity). Please indicate on the request form that this is required as phosphoethanolamine is not normally reported.

Monitoring Cystinuria

In patients with cystinuria only cystine, ornithine, arginine and lysine will be reported. The cystine concentration will also be given in μmol/L, the solubility of cystine in urine is approximately 1000 μmol/L. At concentrations above this, the patient is at high risk of stone formation.

Sample Type: 2 mL Urine (plain)
Turn Around Time: 5 working days

Table 7.2b: Urine Amino Acid Reference Ranges

Amino Acid	μmol/mmol creatinine	Amino Acid	μmol/mmol creatinine
Threonine	< 300	Tyrosine	< 50
Serine	< 390	Phenylalanine	< 30
Proline	< 190	Ornithine	< 30
Glycine	< 1050	Lysine	< 190
Alanine	< 250	Histidine	< 310
Valine	< 30	Arginine	< 30
Cystine*	< 20	Homocystine	< 5
Leucine	< 20		

*NB Cystine reference range in those under a year of age is less than 50 μmol/mmol creatinine.

External laboratory information: freeze urine on receipt and dispatch frozen (1st class post or hospital transport)
Methods:
Qualitative Analysis – two dimensional thin layer chromatography
Quantitative Analysis – cation exchange chromatography with post-column derivatisation (ninhydrin) and spectrophotometric detection

7.2.c CSF Amino Acids

Indications: Intractable seizures. CSF amino acid analysis is required for the diagnosis of glycine encephalopathy (GE) (also known as non-ketotic hyperglycinaemia or NKH) and 3-phosphoglycerate dehydrogenase deficiency. It may also be useful in the investigation of sulphite oxidase deficiency. A paired plasma sample must always accompany the CSF.

Sample Type: 0.5 mL CSF (plain, fluoride oxalate may also be used) **with** a paired lithium heparin plasma sample (0.5 mL plasma). Note blood-stained CSF is not suitable for analysis.
Turn Around Time: 5 working days
CSF Amino Acid Reference Ranges

CSF serine	23 - 100 μmol/L
CSF glycine	< 10 μmol/L
CSF:plasma glycine ratio	< 0.04

External laboratory information: freeze CSF and plasma on receipt and dispatch frozen (1st class post or hospital transport)
Method: Quantitative analysis – cation exchange chromatography with post-column derivatisation (ninhydrin) and spectrophotometric detection

7.3 BIOTINIDASE

Biotin is a cofactor for multiple carboxylases and the recycling of biotin requires the activity of the enzyme biotinidase. Typically biotinidase deficiency presents between 3-6 months of life with a variety of symptoms. Treatment is with biotin replacement, which should be initiated prior to the result being available. Biotinidase is a relatively unstable enzyme; low results should be checked on a fresh sample if clinically indicated.

Indications:
- Seizures
- Ataxia
- Hypotonia
- Alopecia
- Skin rashes

Sample Type: 400 μL Lithium heparin plasma
Turn Around Time: 5 working days

Table 7.3 Plasma Biotinidase Reference Ranges

Biotinidase Activity (nmol/mL/min)	
Normal	4.4 – 12.0
Partial deficiency	0.7 – 2.1
Deficiency	< 0.7
Obligate heterozygote	2.2 – 5.2

External laboratory information: Separate and freeze plasma as soon as possible. Dispatch frozen (1st class post or hospital transport)
Method: Spectrophotometry

7.4 CHITOTRIOSIDASE

Gaucher disease is a lysosomal storage disorder resulting from an inherited deficiency of the enzyme β-glucosidase. This deficiency results in impaired breakdown of the lipid glucocerebrosidase and its subsequent accumulation in cells. Gaucher disease is characterised by markedly elevated chitotriosidase activity; symptomatic Gaucher patients typically exhibit concentrations 100 times greater than the reference range. However, chitotriosidase may be mildly increased in a number of other lysosomal storage disorders and other illnesses, such as sarcoidosis. Benign deficiency of chitotriosidase occurs in approximately 6% of Caucasians.

Indications: Diagnosis and monitoring of Gaucher disease
Sample Type: 100 μL Lithium heparin plasma or serum
Turn Around Time: 7 working days
Reference Range: 0 – 140 μmol/L/hour

External laboratory information: Store frozen, dispatch 1st class post or hospital transport
Method: Fluorimetric

7.5 CREATINE AND GUANIDINOACETATE

This relatively new group of disorders is characterised by cerebral creatine deficiency, the main symptoms of which are learning disability and speech delay, and, in some patients intractable seizures. Of the three disorders described; arginine:glycine amidinotransferase (AGAT) deficiency and guanidinoacetate methyltransferase (GAMT) deficiency show decreased creatine in the urine and plasma. In addition, in GAMT deficiency there is an increase in the excretion of guanidinoacetate. Defects in the creatine transporter (an X-linked disorder) result in an increase in the urine creatine/creatinine ratio. Guanidinoacetate is stable in urine and plasma. Whilst creatine is also stable in plasma, it is unstable in urine and concentrations increase within 1-2 hours of collection, leading to potentially false positive results for the creatine transporter defect or spuriously normal results in AGAT and GAMT.

Indications:
- Mental retardation
- Absent / delayed speech
- Seizures
- Movement disorder

Sample Type:
1 mL urine (plain) send to laboratory as soon as possible after collection (e.g. 1 to 2 hours)
100 µL Lithium heparin plasma (or serum)
Turn Around Time: 1 month

Table 7.5 Creatine and Guanidinoacetate Reference Ranges

	Age	Urine µmol/mmol creatinine	Plasma µmol/L
Creatine	All ages		10 - 100
	0 – 4 years	6 - 1200	
	4 – 12 years	17 – 720	
	Older than 12 years	11 – 240	
Guanidinoacetate	All ages		0.8 – 3.1
	0 – 15 years	4 – 220	
	Older than 15 years	3 - 78	

External laboratory information:
Urine: freeze as soon as possible (within 1 to 2 hours of collection) and transport on dry ice
Plasma/serum: store frozen, dispatch frozen by first class post
Method: Tandem mass spectrometry

7.6 GALACTOSE-1-PHOSPHATE URIDYLTRANSFERASE

Classical galactosaemia is caused by a deficiency of the enzyme galactose-1-phosphate uridyl transferase. Galactose is produced in the small intestine from the breakdown of dietary lactose into galactose and glucose. The presence of reducing substances in urine may be an important clue to diagnosis but equally can be misleading. False positives can occur in severe liver disease and false negatives can occur if lactose is not present in the diet. Carriers of galactosaemia cannot be detected by this screening method. Please note: results are invalid if the sample has been collected within 6 weeks of a blood transfusion. If galactosaemia is suspected in a child who has had a blood transfusion please discuss alternative testing with one of the BGU clinical scientists.

Indications:
- Hepatomegaly
- Prolonged jaundice with abnormal liver function tests
- Presence of reducing substances in urine (negative for glucose)

Sample Type: Dried blood spot
Turn Around Time: 5 working days
Reference Range: Qualitative result only

Normal results are reported as: 'Galactose-1-phosphate uridyl transferase activity measured by the Beutler screening test appeared to be within normal limits. Action taken on the strength of this result should recognise that it is a screening test.'

Deficient results are reported as: 'There was no detectable galactose-1-phosphate uridyl transferase activity when measured by the Beutler screening test. This enzyme is relatively unstable, particularly if the dried blood spot is subjected to hot or humid conditions. The screening test also requires the presence and activity of endogenous blood glucose-6-phosphate dehydrogenase. Action taken on the strength of this result should recognise that it is a screening test.'

Deficient results should be confirmed - the laboratory will contact you to arrange this.

External laboratory information: Store frozen, dispatch by first class post
Method: Beutler screening test

7.7 GLYCOSAMINOGLYCANS

The mucopolysaccharidoses are a group of inherited disorders characterised by the accumulation of glycosaminoglycans in the lysosomes. Children may appear normal at birth but later develop progressive skeletal abnormalities, coarse facies and hepatomegaly. Normal urine contains mostly chondroitin sulphate with traces of heparan and dermatan sulphates. Mucopolysaccharidoses are characterised by abnormal patterns of glycosaminoglycans in urine. Initially, samples are screened for total glycosaminoglycan concentration. False positive results are common, particularly in young infants. Positive results will be referred for typing by electrophoresis if clinical details suggestive of a mucopolysaccharidosis are given. False negative quantitative results may also be encountered, therefore if

there is a strong clinical suspicion of a mucopolysaccharidosis, please specifically request GAG typing. If urine glycosaminoglycan typing and white cell enzymes are normal and a storage disorder is still suspected clinically, urinary oligosaccharide and sialic acid analysis should be considered (see section 8.7).

Indications:

- Hepatomegaly
- Skeletal deformities
- Abnormal facies
- Behavioural problems
- Inguinal and umbilical hernias
- Loss of developmental skills

Sample Type: 5 mL Urine (plain)
Turn Around Time: 10 working days

Table 7.7: Urine GAG Reference Ranges

Age	Glycosaminoglycans (mg/mmol creatinine)
1 week – 2 months	< 70
2 months – 1 year	< 39
1 –2 years	< 29
2 –4 years	< 26
4 –8 years	< 24
8 - 12 years	< 21
> 12 years	< 13

All results within the reference range will be reported with the following comment: 'This assay is a screening test only. If there is strong clinical suspicion of a mucopolysaccharidosis, please contact Dr Jacqui Calvin or Sarah Hogg to discuss further investigations'.

External laboratory information: Store frozen, dispatch frozen (1st class post or hospital transport)
Method: GAG quantitation: dimethylmethylene blue dye binding method with spectrophotometric detection
GAG typing: two dimensional electrophoresis

7.8 HOMOCYSTEINE

Measurement of total homocysteine is offered for the diagnosis and monitoring of inherited defects in homocystine metabolism, such as classical homocystinuria and methionine synthase deficiency. Free homocystine is not recommended as it is only detectable in plasma when the binding capacity of plasma proteins has been exceeded. The binding of homocystine to plasma protein, mainly albumin, seems to be saturable with a maximal capacity of about 140 μmol/L total homocysteine. Likewise urine homocystine will only be detectable when the binding capacity is exceeded.

Indications:

- Marfanoid appearance
- Early onset vascular occlusive disease
- Lens dislocation (usually downward)
- Early onset osteoporosis

Sample Type: 0.5 mL Lithium heparin plasma, send to laboratory within one hour of collection
Turn around time: 1 month
Total homocysteine reference ranges: males: < 18 µmol/L
females: < 16 µmol/L

External laboratory information: separate plasma within one hour of collection and store frozen. Dispatch frozen by 1st class post.
Method: tandem mass spectrometry

7.9 ORGANIC ACIDS

Analysis of organic acids in urine can assist in the diagnosis of a number of disorders including those of amino acid metabolism (e.g. MSUD, urea cycle defects). Orotate is quantitated using d2-orotate internal standard – see below for more information. Methylmalonate is quantitated using d3-methylmalonate internal standard.

Indications: [Note: (+) indicates 'occurring with other features']
- Recurrent episodic ketosis, acidosis, vomiting and dehydration
- Reye-like syndrome
- Hypoglycaemia
- Hyperammonaemia
- Seizures (+)
- Seizures, ataxia, hypotonia
- Macrocephaly, dystonia, seizures, neurodegeneration
- Cardiomyopathy
- Unexplained lactic acidaemia
- Alopecia (+)
- Failure to thrive (+)
- Developmental Delay (+)

Table 7.9: Organic Acids Sometimes Requested Individually

Organic Acid	Disease
N-Acetylaspartate	Canavan Disease
Glutarate, 3-hydroxyglutarate	Glutaric aciduria type 1
Homogentisate	Alkaptonuria
4-Hydroxybutyrate	4-Hydroxybutyric aciduria
Orotate	Urea cycle defects
Methylmalonate	MMA
Mevalonate	Mevalonic aciduria
Suberylglycine, hexanoylglycine	MCADD
Succinylacetone	Tyrosinaemia type I

Sample Type: Urine (plain) - volume is dependent on the urine creatinine concentration (the more dilute the urine the larger the volume required). Usually 5 mL is sufficient for analysis.
Turn Around Time: 5 working days

Methylmalonate Reference range

0 - 1 yrs	< 20 μmol/mmol creatinine
1 yr to adult	< 10 μmol/mmol creatinine

Mild increases (up to 100 μmol/mmol creatinine) in methylmalonate are not uncommon, they are not believed to be significant unless the patient is a breast fed infant where there is maternal vitamin B12 deficiency. The advice is usually to repeat in one month, although an earlier repeat is recommended if there is evidence of acidosis, lethargy, hypotonia or developmental delay.

External laboratory information: Freeze urine on receipt and dispatch frozen (1st class post or hospital transport)
Method: Solvent extraction followed by GC-MS of silylesters (qualitative)

7.10 OROTATE

Orotic acid is an intermediate in the synthesis of pyrimidine nucleotides. In most defects of the urea cycle carbamoyl phosphate accumulates. This feeds into the pyrimidine biosynthetic pathway resulting in an excess of orotic acid. Mildly raised values have also been reported in mitochondrial disease. Note: samples collected following an allopurinol load test will be referred to Guy's Hospital for measurement of orotic acid and orotidine. (The Guy's protocol for this test is available from one of the BGU Clinical Scientists).

Indications:
- Differential diagnosis of urea cycle defects
- Disorders of pyrimidine metabolism

Sample Type: 5 mL Urine (plain)
Turn Around Time: 5 working days

Reference Range:

0 - 2 years:	0 – 6 μmol/mmol creatinine
Older than 2 years:	0 - 3 μmol/mmol creatinine

External laboratory information: Store frozen, dispatch frozen (1st class post or hospital transport)
Method: Solvent extraction followed by GC-MS of silylesters

7.11 VERY LONG CHAIN FATTY ACIDS, PRISTANATE & PHYTANATE

Peroxisomes are responsible for β–oxidation of very long chain fatty acids (fatty acids with a carbon length more than 22), bile acid metabolism and plasmalogen synthesis. Peroxisomal disorders can be classified into 2 categories; defects in peroxisomal biogenesis disorders (eg Zellweger syndrome, infantile Refsum disease) and defects in specific peroxisomal enzymes. Very long chain fatty acids are very sensitive for the diagnosis of X-linked adrenoleukodystrophy in males. However approximately 15% of symptomatic female carriers have normal very long chain fatty acids.

Phytanate and pristanate are assayed as part of the plasma very long chain fatty acid profile. They are useful in the diagnosis of Refsum disease, α-methyl-

acylCoA racemase deficiency and rhizomelic chondrodysplasia punctata (depending on the age of the patient). Pristanate and phytanate may be normal in young infants with peroxisomal biogenesis defects as both compounds are derived from exogenous, dietary sources.

Indications: [Note: (+) indicates 'occurring with other features']

- Idiopathic adrenal insufficiency in males
- Neurological abnormalities
- Leukodystrophy
- Ataxia
- Seizures (+)
- Hypotonia
- Ocular abnormalities
- Skeletal abnormalities
- Dysmorphic features
- Liver dysfunction (+)
- Hepatomegaly

Sample Type: 0.5 mL EDTA plasma, send to laboratory as soon as possible
Turn Around Time: 10 working days

Table 7.11: Plasma VLCFA Reference Ranges

VLCFA (μmol/L)	< 1 yr	1 – 10 yrs	> 10 yrs
C22 (docosanoate)	21 - 103	33 - 96	31 - 98
C24 (tetracosanoate)	22 - 87	25 - 71	24 - 66
C26 (hexacosanoate)	0.05 - 1.97	0.15 - 0.91	0.15 - 0.91
C24/C22 ratio	0 - 1.15	0 - 1.01	0 - 0.96
C26/C22 ratio	0 – 0.028	0 - 0.026	0 – 0.022
Phytanate	0 – 10	0 – 15	0 – 15
Pristanate	0 – 1	0 – 2	0 - 2

External laboratory information: separate plasma from cells within 2 hours of collection and store frozen. Dispatch samples by 1st class post or hospital transport
Method: GC-MS of methylesters

7.12 MONITORING TREATMENT OF PHENYLKETONURIA

Phenylketonuria is an autosomal recessive condition with an incidence of about 1 in 12,000. It is caused by a deficiency of phenylalanine hydroxylase which results in a marked increase in blood phenylalanine. Untreated, severe learning disability and spasticity ensues. Treatment is effective and consists of dietary phenylalanine restriction and supplementation of essential amino acids. Dried blood spot samples are used for monitoring and adjustment of the diet. The frequency of testing and the target concentration depends on the age of the patient.

Sample Type: Dried blood spot
Turn Around Time: 2 working days

External laboratory information: send by 1st class post
Method: tandem mass spectrometry

7.13 SWEAT TESTS

The determination of sweat chloride concentration is useful in the diagnosis of cystic fibrosis. Sweat testing can be performed after 2 weeks of age on infants greater than 3 kg that are normally hydrated and without significant systemic illness. If clinically important, sweat testing can be attempted after one week of age but will need repeating if insufficient sweat is collected. A repeat test is recommended when the result is abnormal or borderline and the genotype is not confirmatory.

Indications:
- Phenotype suggestive of CF (respiratory infection, exocrine pancreatic insufficiency)
- Positive newborn screening test

Sample Type: Sweat collected into a Wescor Macroduct tube. National guidelines state not less than 1g/m^2/min. A minimum sweat volume of 60 µL (approximately 185 mm) is required to enable duplicate analysis.
Turn Around Time: 1 working day

Table 7.13: Sweat chloride Reference Range

Sweat Chloride (mmol/L)	Interpretation
Less than 40	Low Probability of CF
40 – 60	Equivocal
Greater than 60	Supports diagnosis of CF

Method: Pilocarpine, transported by iontophoresis is used to induce sweating. Sweat is collected via the capillary 'macroduct' system. After collection the tube is sealed at both ends to prevent evaporation. The concentration of chloride ions is determined using an ion selective electrode. National guidelines for sweat collection are available.[2] For Addenbrooke's patients, sweat collection can be arranged with the CF Clinical Nurse Specialist.

8 REFERRED METABOLIC ASSAYS

8.1 3-HYDROXYBUTYRATE AND FREE FATTY ACIDS

These intermediary metabolites may be useful in the investigation of unexplained hypoglycaemia. (Please request a simultaneous lab glucose analysis.)
Interpretation depends on the fed or fasted state of the patient.
If hypoglycaemic, suppression of both 3OHB and FFA is consistent with hyperinsulinism whereas in FAOD the ratio of FFA to 3OHB is typically greater than 3. However these two diagnoses may be more easily made by analysing insulin at the time of hypoglycaemia and blood spot acylcarnitines.

Sample: 1 mL fluoride tube
Transport: Store frozen, dispatch frozen 1st class post
Referral laboratory: Chemical Pathology, Sheffield Children's Hospital

8.2 7-DEHYDROCHOLESTEROL

Smith-Lemli-Opitz syndrome (SLO) is an autosomal recessive disorder with multiple congenital malformations (microcephaly, 2,3 syndactyly, cleft palate, congenital heart defects). There is a deficiency of sterol delta-7-reductase that causes an increase in the cholesterol precursor 7-dehydrocholesterol (7DHC). In SLO the total cholesterol is typically below 1.5 mmol/L (measured by GC-MS), with an increase in the 7DHC:cholesterol ratio.

Sample Type: 0.5 mL lithium heparin or EDTA plasma, or serum
Transport: 1st Class post
Referral laboratory: Chemical Pathology, Sheffield Children's Hospital

8.3 BILE ACID METABOLITES

Inherited defects in bile acid biosynthesis cause cholestasis and malabsorption (due to bile acid deficiency), with progressive neurological dysfunction and xanthomas (due to deposition of precursors).
Bile acids are partly synthesised in the peroxisome. Analysis is also indicated in the workup of suspected peroxisomal biogenesis disorders where VLCFA are abnormal.

Sample type: 10 mL plain urine and/or 1 mL lithium heparin or EDTA plasma
Sample storage: Store frozen prior to dispatch first class post
Referral laboratory: Chemical Pathology, Sheffield Children's Hospital

8.4 BIOPTERINS AND DIHYDROPTERIDINE REDUCTASE

The conversion of phenylalanine to tyrosine by phenylalanine hydroxylase requires the cofactor tetrahydrobiopterin (BH4). This cofactor is also required for the synthesis of the neurotransmitters, serotonin and dopamine. Defects in the synthesis or metabolism of biopterin cause hyperphenylalaninaemia and are associated with severe learning disability and a neurodegenerative course.
All babies identified as presumptive positives on newborn PKU screening will be tested for biopterin defects. Interpretation depends on the phenylalanine

concentration at time of sampling. For newborn screening samples this is organised by the laboratory and does not need to be requested separately.
Please note that DHPR results are not valid within 6 weeks of a blood transfusion.

Sample type: at least 2 dried blood spots (6 & 8 mm in diameter)
Sample storage: Room temperature
Referral laboratory: Newborn Screening Laboratory, Birmingham Children's Hospital

8.5 CARNITINE (TOTAL AND FREE)

Plasma total and free carnitine is not recommended for the first line investigation of fatty acid oxidation defects as it gives less information than an acylcarnitine profile. However plasma carnitine and acylcarnitine analysis may be useful in the investigation of suspected carnitine transporter defects and carnitine palmitoyltransferase 2 deficiency, please phone one of the Clinical Scientists to discuss. Note: valproate treatment can cause low levels of free carnitine.

Sample Type: 0.5 mL lithium heparin plasma.
Sample storage: Store frozen if not sent immediately, 1st class post
Referral laboratory: BGU, Royal Victoria Infirmary, Newcastle

8.6 NEUROTRANSMITTERS

The following metabolites may be analysed in CSF, depending on the clinical details provided:
HVA, 5HIAA, 5-methyltetrahydrofolate (5MTHF), neopterin, dihydrobiopterin, tetrahydrobiopterin and pyridoxal phosphate.
Clinical indications include oculogyric crises, temperature instability, ptosis, parkinsonian features and dystonia. This test is usually requested after the child has been reviewed by a paediatric neurologist.

Sample Type: CSF collected into a set of three 1 mL tubes containing special preservatives and frozen on dry ice at the bedside. (Tubes and collection instructions are obtained from the Neurometabolic Unit, the National Hospital, Queen Square, London. Tel: 020 7837 3611 ext 3844).
Sample storage: Once collected CSF should be stored at –70°C and can be kept for more than a year
Transport: Courier - on dry ice
Referral laboratory: Neurometabolic Unit, National Hospital, London

8.7 OLIGOSACCHARIDES AND SIALIC ACID

Oligosaccharides are low molecular weight carbohydrate polymers made up of at least 3 monosaccharide subunits. The oligosaccharides in urine are derived from the incomplete breakdown of carbohydrate side chains of complex glycoproteins. Abnormal oligosaccharides accumulate in a range of lysosomal storage diseases.

Unfortunately this screening test is insensitive with poor specificity. The oligosaccharide excretion in glycoproteinoses may be variable and/or the abnormality subtle. Spurious results may be seen in patients infused with large amounts of complex carbohydrates (eg dextran). Neonates and breast fed infants show patterns which would be considered abnormal in older children. For these reasons **urine oligosaccharide chromatography is not offered as a first line test** by the BGU. If a lysosomal storage disorder is suspected clinically WCE analysis is recommended.

If the WCE analyses and urine GAGS are normal and a storage disease is still suspected urines will be referred to the Willink, Manchester for urine oligosaccharides and sialic acid, to investigate the possibility of sialidosis or galactosialidosis.

8.8 PLASMALOGENS

Plasmalogens are synthesized by peroxisomes and low values are encountered in a range of peroxisomal disorders. The plasmalogen assay is technically demanding and therefore its availability is limited. This assay is only available for cases of suspected Rhizomelic Chondrodysplasia Punctata (RCDP). Unlike Zellweger syndrome and other peroxisomal disorders plasma VLCFA are normal in patients with RCDP.

Sample: 1 mL EDTA whole blood (RBC's washed x3 with 0.9% saline)
Transport: Post special delivery
Referral laboratory: Chemical Pathology, Sheffield Children's Hospital

8.9 PORPHYRINS

Urine, blood and faecal porphyrin analyses are available for the investigation of the acute and non-acute porphyria; please see section 6.10 "Which sample for the investigation of porphyria?". Formal quantitation of porphobilinogen is available for monitoring patients with a diagnosis of porphyria, or following discussion with one of the BGU clinical scientists.

Samples:
Urine: 20 mL fresh, preferably early morning urine or crisis urine in plain white-topped universal
Faeces: about 1 g (Maltesers-sized) sample in faecal (blue) universal
Blood: 5-10 mL whole blood EDTA
Transport: First Class post
Referral laboratory: Porphyria Service, University Hospital of Wales, Cardiff

8.10 PURINES AND PYRIMIDINES

Over 20 different disorders of purine and pyrimidine metabolism are known, of which several cause significant clinical disease. Three organ systems are prominently affected: kidneys (renal stones), bone marrow (immunodeficiency ± megaloblastic anaemia) and brain (neurological problems, e.g. abnormal muscle tone, dystonia, autistic-like behavioural problems), some patients may have more than one organ system affected. This test is often undertaken in

children with unexplained neurological problems when first line investigations have drawn a blank.

Sample Type: 5 mL random urine, plain universal
Sample storage: Store frozen until sending to lab by 1st class post
Referral laboratory: Purine Research Laboratory, St Thomas' Hospital, London

8.11 TRANSFERRIN ISOELECTRIC FOCUSSING

The initial screening test for congenital defects of glycosylation (CDG) is isoelectric focusing of transferrin to detect abnormal patterns of glycosylation. Enzyme analysis is available for confirmatory testing of some subtypes. The molecular basis of more than 20 defects have been identified however CDG Type Ia is the most common, accounting for approximately 80% of cases.
Please note this test is unreliable in the first 3 weeks of life (reflects maternal transferrin).

Indications: [Note: (+) indicates 'occurring with other features']
- Psychomotor retardation
- Seizures (+)
- Strabismus
- Cerebellar hypoplasia
- Dysmorphy (fat pads, inverted nipples)
- Coagulopathy
- Protein-losing enteropathy (type 1b)

In view of the extremely broad clinical spectrum of CDG patients, it is recommended that transferrin glycoform analysis is considered in any unexplained multisystem disorder.

Sample Type: 1 mL serum or lithium heparin plasma
Storage: Store frozen prior to dispatch by 1st Class Post
Referral Laboratory: Neurometabolic Unit, National Hospital, London

8.12 WHITE CELL ENYZMES

Lysosomal storage disorders result from a deficiency of a specific lysosomal enzyme, activator or transport protein, causing an accumulation of un-degraded substrates within the lysosomes. Characteristically patients develop normally during the neonatal period but present with progressive deterioration in early childhood. Unlike 'small molecule' metabolic disorders, presentation tends to be slow and more insidious. Symptoms include bone deformities and short stature, heart and respiratory difficulties, coarse facial features, an enlarged head, tongue, liver and spleen, and, in many patients, neurological degeneration. There are no reliable screening tests for these disorders (apart from urine GAGS in suspected mucopolysaccharidoses) and white cell enzyme analysis is often the first line test when there is a strong clinical suspicion of an LSD.

Indications for analysis include hepatosplenomegaly, developmental regression, neurological deterioration, dysmorphic features, cherry red spot,

leukodystrophy and angiokeratoma. Please contact the laboratory to discuss before taking samples.

Sample Type: 10 mL EDTA whole blood
Sample storage: For Addenbrooke's patients please send whole blood (at room temperature) to the BGU urgently. Samples must be received before 2pm to enable same day despatch to Manchester. We are unable to accept samples on a Friday. **Please phone in advance to enable the laboratory to arrange transport.**
Referral laboratory: Willink Laboratory, Manchester Children's Hospital

Table 8.11: The following white cell enzymes (by disease) are offered as a battery of tests by the Willink laboratory.

Enzyme	Disease
Acid Esterase	Wolman disease
α-Fucosidase	Fucosidosis
α-Galactosidase	Fabry disease
α-Mannosidase	α-mannosidosis
α-N-acetylgalactosaminidase	Schindler disease
Arylsulphatase A	Metachromatic leukodystrophy
β-Galactosidase	GM1 gangliosidosis
β-Glucosidase	Gaucher disease
β–Glucuronidase	Sly disease
β-Hexosaminidase	Sandhoff Disease
β-Mannosidase	β-Mannosidosis
Chitotriosidase	Non-specific storage marker
Galactocerebrosidase	Krabbe leukodystrophy
Glycoasparaginase	Aspartylglucosaminuria
MUGS (methylumbelliferyl-N-acetylglucosamine-6-sulphate substrate)	Tay-Sachs
Sphingomyelinase	Niemann-Pick A&B

Other enzymes are available on an individual basis and include:

- α-glucosidase for Pompe disease: dried blood spot screen is available
- palmitoyl-protein thioesterase-1 and tripeptidyl peptidase 1 are enzymes deficient in two of the neuronal ceroid lipofuscinoses (Batten disease).
- enzyme analysis for Niemann-Pick type C is not available; instead cholesterol esterification studies and filipin staining are undertaken on fibroblasts.

Please phone to discuss, prior to sending samples.

9 QUALITY ASSURANCE SCHEMES

The BGU participates in the following QA schemes:

ERNDIM:	Bloodspot acylcarnitine
	Urine organic acids
	Quantitative organic acids (MMA)
	Urine proficiency testing
	Plasma amino acids
	Special assays serum
	Special assays urine
	Urine glycosaminoglycans (pilot scheme)
NEQAS:	Newborn screening
	Urine orotic acid
	Quantitative phenylalanine
	Sweat testing
	Metabolic cognitive scheme pilot
Willink:	Urine glycosaminoglycans
CDC:	Newborn screening schemes
IRTIQAS:	Newborn CF screening
WEQAS:	Urine dipstick scheme
DGKC:	Bloodspot TSH
Hamburg:	Newborn screening (by tandem mass spectrometry)

10 REFERENCES AND FURTHER INFORMATION

References

1. Vandemecum Metabolicum. Manual of Metabolic Paediatrics Zschocke & Hoffmann
2. National Guidelines for the Performance of the Sweat Test for the Investigation of Cystic Fibrosis, November 2003

Further Information

National Metabolic Biochemistry Network
http://metbio.net/

British Inherited Metabolic Disease Group
http://www.bimdg.org.uk/

Online Mendelian Inheritance in Man
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM

UK National Screening Committee
http://www.nsc.nhs.uk/

UK Newborn Screening Programme Centre
http://www.newbornscreening-bloodspot.org.uk/

Sweat Testing Guidelines (available via The Association for Clinical Biochemistry)
http://acb.org.uk/

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