Diagnostics Directorate
Department of Biochemistry
QEUH & RHC
Metabolic Biochemistry
Investigation Supplement

The Metabolic Biochemistry Investigation Supplement is additional to the QEUH Biochemistry Laboratory Handbook

Core service: Monday - Friday 8:45am – 5:00pm
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1. GUIDANCE FOR REQUESTING METABOLIC INVESTIGATIONS

***Essential criteria for sample acceptance**:*

1. CHI number/unique identifier (date of birth where no unique identifier available)
2. Surname
3. Forename

FOR URGENT ANALYSIS IN A CRITICALLY UNWELL CHILD
CONTACT THE METABOLIC BIOCHEMIST ON 07511 154412 OR
THE METABOLIC CONSULTANT VIA QEUH SWITCHBOARD

- If a metabolic condition is suspected in a critical unwell patient, attempt to collect diagnostic samples during the acute illness. A wide range of samples should be obtained if the patient is unlikely to survive.
- Where an individual is critically ill and initial diagnostic tests will help decide early medical management, please contact the department to expedite relevant investigations.
- It is essential that the necessary pre-analytical handling and storage of samples be performed within the local laboratory prior to test referral (see Table 1 in section 2, page 5).
- Include clinical information to support result interpretation and ensure the appropriate metabolic investigations are performed (see below).

Relevant Clinical Information

***Presenting illness** e.g. diarrhoea and vomiting (with time/date of onset)*
***Clinical findings** e.g. hepatomegaly in hypoglycaemia or dysmorphic findings including corneal clouding in mucopolysaccharidosis*
***Relevant biochemical and haematological findings** e.g. acidosis and pancytopenia in methylmalonic acidaemia*
***Family history** e.g. foetal losses or neonatal deaths*
***Drug history** (may cause interference) e.g. paracetamol in urine amino acids*
***Nutritional details** e.g. type and amount of food or food aversions*
***History of blood transfusions** (recent transfusion (<3 months) may produce misleading results in red cell analytes)*

- Some investigations are complex in nature and results may take several days or weeks to complete. See Table 1 (section 2, page 5) for expected turnaround time.
- For discussion of metabolic investigations contact the metabolic biochemist on 07511154412 or email ggc.qeuhmetabolicbiochemist@ggc.scot.nhs.uk

2. METABOLIC INVESTIGATIONS

Table 1: Sample Requirements for Metabolic Investigations

Analyte	Sample Type	Sample Handling	TAT
Acylcarnitines	1 mL lithium heparin or DBS	Plasma separated within 7 hours collection. For DBS collection please refer to information on section 3, page 7.	7-10 days
Amino acids (plasma, urine, CSF) Urine stone screen	2 mL lithium heparin, 2 mL urine in white top universal, DBS or 3rd/4th collection of CSF	Separate and freeze plasma. Freeze urine and CSF immediately.	5 days
Ammonia	1 mL fluoride oxalate	Send to lab within 1 hour (on ice ideally) and separate	<2 hours
β-Hydroxybutyrate	0.5 mL lithium heparin	Separate and freeze	1 day
Biotinidase	1 mL EDTA	Separate and store at 4°C	14 days
Bromide	1 mL plasma or serum	Stable in blood	7 days
Chitotriosidase	1 mL EDTA	Separate and store at 4°C	14 days
G6PD activity in RBC	1 mL EDTA	Stable in whole blood for 1 week at 4°C	3 days
Galactosaemia screen Galactose-1-phosphate uridyl transferase in RBC (qualitative)	1 mL lithium heparin	Unseparated: age-matched control should be requested as a transport control	1 day
Galactose-1-phosphate uridyl transferase in RBC (Quantitative)	1 mL lithium heparin	Unseparated: age-matched control should be requested as a transport control	2 days
Hexanoylglycine	1 mL urine in white top universal	Stable at 4°C	14 days
Lactate	1 mL fluoride oxalate	Separate	<2 hours
Non-esterified (free) fatty acids	0.5 mL fluoride oxalate	Separate and freeze	1 day
Lysosomal enzymes (see Table 7 in section 8, page 11)	5-10 mL EDTA	Requires specialist handling – should arrive at QEUH before 12pm	15 -90 days (depending on enzyme)
Oligosaccharide screen	1 mL urine in white top universal	Store at 4°C	7 days
Organic acids	10 mL urine in white top universal	Freeze and send frozen	7 days

Orotic acid	0.5 mL urine in white top universal	Freeze and send frozen	7 days
Porphyrin investigations (urine) Porphobilinogen Total urine porphyrins	10 mL urine in white top universal	Protect from light and store at 4°C	7 days
Porphyrin investigations (blood) Plasma porphyrin scan Red cell porphyrins	5mL EDTA	Protect from light and store at 4°C	7 days
Sweat chloride	Minimum 20 uL sweat collected by the Wescor™ System	Store at 4°C	3 days
Urate (plasma, urine)	0.5 mL lithium heparin 1 mL urine in white top universal	Separate plasma. Freeze urine.	1 day
Urine creatine Urine guanidinoacetate	1 mL urine in white top universal	Freeze following collection and send frozen – affected by freeze thaw cycles	28 days
Urine glycosaminoglycan (screen and electrophoresis)	10 mL urine in white top universal	Store at 4°C	7 days
Monitoring branched chain amino acids *(in MSUD)*	DBS	For DBS collection please refer to information on section 3, page 7.	5 days
Monitoring phenylalanine and tyrosine *(in phenylketonuria)*			

3. DRIED BLOOD SPOT COLLECTION FOR MONITORING AND ENZYME ANALYSIS

Dried blood spot samples (DBS) are used for monitoring phenylketonuria and maple syrup urine disease patients. They are also used for investigation of lysosomal storage disorders and acylcarnitine analysis. The minimum acceptance criteria for home monitoring samples is *one circle >8 mm diameter, evenly saturated with a single drop of blood.*

Specific Guidance for DBS Sample Preparation

DBS Cards

- Whatman 903 paper is the preferred card for sample collection.
- Neonatal screening cards are acceptable and can be found in hospitals with a maternity department.
- Please contact ggc.qeuhmetabolicbiochemist@ggc.scot.nhs.uk if you require monitoring cards.

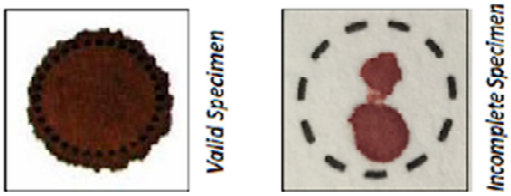
Filling the circles

- Each card contains four or five circles.
- A single filled circle is required for analysis.
- Use one large droplet of blood to fill one circle.
- 50 uL lithium heparin whole blood can be spotted onto each circle in the laboratory for acylcarnitine analysis.

Drying the sample

- Dry for a minimum 4 hours at room temperature once the blood is applied.
- Sample may be left overnight if necessary but avoid where ambient temperature is ≥ 30°C.
- The sample should not be heated or exposed to direct sunlight. Drying at high temperature and with hair dryers will lead to significant reduction in enzyme activity.
- Once dry, place the card in a sealable zip-lock plastic bag, ideally with a sachet of desiccant. Use of bags without an air-tight (zip-lock) seal should be avoided. Failure to seal the bag will lead to significant deterioration in enzyme activity.

Summary Guidance for DBS Sample Preparation



Do	Do Not
Fill one good circle	Layer on blood
Let it dry at room temp for 4+ hours	Compress sample
Seal in bag once dry (ideally with desiccant)	Expose to high temperatures

4. HYPOGLYCAEMIA

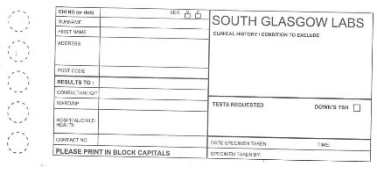
RESUSCITATION → DO NOT DELAY GLUCOSE THERAPY

Treatment of hypoglycaemia should not be delayed by waiting for the laboratory glucose result. However, where possible, appropriate samples should be collected prior to glucose therapy.

Hypoglycaemia: Glucose <2.6 mmol/L

This should be confirmed by laboratory analysis and the following investigations advised.

Table 2: Samples Required for the Investigation of Hypoglycaemia

Sample Type	Tests	Pre-treatment
1mL fluoride oxalate 	Glucose Lactate Free fatty acids	Separate and freeze
2x 5mL lithium heparin 	**Endocrine** Cortisol Insulin C-peptide **Metabolic** Ammonia β-hydroxybutyrate Acylcarnitines Amino acids	Separate and freeze
White top universal (first void urine; ≥5mL) 	Organic Acids	Freeze
Dried Blood Spot Card (2x good quality spots) 	Acylcarnitines Further enzyme analysis	2x 50 uL LIthium heparin blood spots or directly from finger, heel or toe. Dry fully. See Section 3, page 7 for additional information

For further information refer to MetBioNet Best Practice Guidelines for Hypoglycaemia

5. HYPERAMMONAEMIA

Ammonia samples should arrive within 1 hour of collection, ideally on ice where possible.

In any drowsy, confused patient, ammonia analysis should be performed as an URGENT request. Secondary causes of hyperammonaemia are more common and require consideration prior to that of primary metabolic causes. Ammonia concentrations greater than 200 µmol/L generally reflect a primary cause.

Pre-analytical management of the sample is crucial as artefactual increases in ammonia occur due to delay in receipt, haemolysis, difficult venepuncture and contamination from ammonium salts on the skin.

Table 3: Primary and Secondary Causes of Hyperammonaemia

Primary	Secondary
Urea cycle and amino acid disorders	Liver failure / impairment and reye-syndrome
Organic acid and tatty acid oxidation disorders	Infections; UTI, systemic herpes simplex and GI bacterial overgrowth
Hyperinsulinaemic hyperammonaemia	Medication and treatment e.g. valproate, chemotherapy and total parenteral nutrition
Mitochondrial respiratory chain disorders Pyruvate dehydrogenase deficiency Congenital lactic acidosis	Severe illness e.g. asphyxia, sepsis and respiratory distress syndrome associated with transient hyperammonaemia of the newborn

Table 4: Guidance on Plasma Ammonia Levels

Age	Ammonia Level
Premature neonate	< 150 umol/L
Neonates < 4 weeks	< 100 umol/L
Children > 4 weeks and adults	< 50 umol/L

Where a metabolic cause is suspected, collect plasma for amino acids and lactate, and urine for organic acids, orotic acid and amino acids.

For further information refer to MetBioNet Best Practice Guidelines for Hyperammonaemia

6. LACTIC ACIDOSIS

Primary causes of lactic acidosis should be investigated following exclusion of more common secondary causes.

Table 5: Primary and Secondary Causes of Lactic Acidosis

Primary	Secondary
Respiratory chain and krebs cycle disorders	Difficult venesection
Pyruvate dehydrogenase and pyruvate carboxylase deficiency	Intoxication e.g. ethanol (consider thiamine deficiency) Medication e.g. biguinides
Organic acid and fatty acid oxidation disorders	Severe systemic illness and infection including congenital syphilis and UTIs
Biotin metabolism disorders	Renal tubular syndrome
Glycogen storage and gluconeogenesis disorders	Seizures and assisted ventilation

7. URINE ORGANIC ACIDS

Collect 10 - 20 ml of urine in a white top universal container and freeze.
Please provide clinical information to aid interpretation and indicate if patient was symptomatic at time of sample collection

Urine organic acids are useful for metabolic investigation of children and adult patients presenting with:

1. Acute deterioration with encephalopathy, lethargy, hyperammonaemia, acid-base disturbances, ketosis, hypoglycaemia, unexplained liver dysfunction, rhabdomyolysis or cardiomyopathy.
2. Progressive neurological disease (especially if episodic), global developmental delay or developmental regression.

Samples collected during/immediately following an acute metabolic decompensation is likely to yield the most informative data. Urine should remain sealed and frozen until arrival at QEUH, due to instability of some metabolites and possible bacterial contamination of the sample.

8. LYSOSOMAL STORAGE DISORDERS

- More than 70 lysosomal storage disorders have been described which are characterised according to the type of material that accumulates within the lysosomes as a consequence of a specific enzyme deficiency.
- These storage materials build up within connective tissue, solid organs, bone and nervous tissues causing dysfunction that results in a broad range of clinical features.
- Several different investigations in urine (see Table 6) and in blood (see Table 7) are required to effectively screen for and diagnose these disorders.

Table 6: Urine Samples Required for the Investigation of Lysosomal Storage Disorders

Disorder	Test	Sample	Confirmation
Mucopolysaccharide disorders	Total GAG quantitation and electrophoresis	20 mL plain urine	Requires specific enzyme analysis to confirm diagnosis. Refer to Table 2 for details.
Oligosaccharide disorders	Oligosaccharide TLC		
Sialic acid storage disorders	Sialic acid TLC		
Lipidoses (screen for Gaucher, Niemann Pick, Krabbe & GM1-Gangliosidosis)	Chitotriosidase activity	Plasma (EDTA or lithium heparin)	

Table 7: Lysosomal Enzymes Analysed at QEUH

Disorder	Enzyme Deficiency	L	P	DBS
Lipidoses				
GM1 gangliosidosis	β-galactosidase			X
GM2 gangliosidosisl: **- Tay sachs** - Sandhoff	 Hexosaminidase A Total hexosaminidase			X
Galactosialidosis	β-galactosidase (+ neuraminidase – Manchester)			X
Metachromatic leucodystrophy	Arylsulphatase A	X		
Niemann-pick A/B	Sphingomyelinase			X
Gauchers	Gauchers	X		X
Krabbe	β-galactocerebrosidase	X		X
Fabry	α-galactosidase		X	X
Multiple sulphatase deficiency	Multiple sulphatases	X	X	X
Mucolipidosis type II (I-cell) and III (pseudo-hurler dystrophy)	Arylsulphatase A		X	
Lysosomal acid lipase deficiency (LAL-D) (Wolmans/Cesd)	Acid lipase			X
Screen for Gauchers, Niemann-pick type A/B/C, Krabbe & GM1-gangliosidosis	Chitotriosidase		X	

Mucopolysaccharidoses				
Disorder	**Enzyme Deficiency**	**L**	**P**	**DBS**
MPS I – Hurler/Scheie	α-l-Iduronidase	X		
MPS II - Hunter	Iduronate-2-sulphatase			X
MPS IIIA – Sanfilippo A	Heparan sulphatase	X		
MPS IIIB – Sanfilippo B	N-acetyl-α-d-glucosaminidase	X		
MPS IVA – Morquio A	Galactose-6-sulphatase	X		
MPS IVB – Morquio B	Beta-galactosidase			X
MPS VI – Maroteaux-Lamy	Arylsulphatase b	X		
MPS VII - Sly	Beta-glucuronidase			X
Oligosaccharidoses				
Fucosidosis	α-fucosidase	X	X	X
α-mannosidosis	α-mannosidase	X	X	
β-mannosidosis	β-mannosidosis		X	
Aspartylglucosaminuria	Aspartylglucosaminidase		X	
Schindler	N-acetyl-α-d-galactosaminidase		X	
This list does not include all lysosomal enzymes. Contact the metabolic biochemist on 07511 154412 to discuss investigations available from external laboratories				

Additional Enzyme Investigations				
Neuronal ceroid lipofuscinosis (Batten disease)				
Disorder	**Enzyme Deficiency**	**L**	**P**	**DBS**
Infantile (NCL1, CLN1)	Palmitoyl-protein Thioesterase			X
Late-infantile (NCL1, CLN2)	Tripeptidyl peptidase			X
Others				
Pompe (GSD type II)	α-glucosidase			X
Biotinidase deficiency	Biotinidase		X	

Key: L = leucocytes, P = plasma, DBS = dried blood spot

9. RED CELL ENZYMES

Please note, results of red cell enzyme assays are invalid in patients who have undergone recent red cell blood transfusion (within 90 days of sample collection).

Sample Requirements: 1 mL lithium heparin (unseparated). In addition, we ask external labs to send an unseparated age-matched lithium heparin control to exclude sample deterioration as possible cause of low results.

Classical Galactosaemia

Galactose-1-phosphate uridyl transferase (GAL-1-PUT) deficiency

- Classical galactosaemia can present acutely in the early neonatal period after starting milk feeds.
- Symptoms include vomiting, diarrhoea, jaundice, E. coli sepsis, liver dysfunction (low INR) and bilateral cataracts.
- Where galactosaemia is suspected, galactose containing feeds should be discontinued immediately without waiting for results of biochemical investigations.

If a blood transfusion has been given or a diagnosis of galactokinase / galactose-6-phosphate epimerase deficiency suspected, please contact the metabolic biochemist on 07511154412. Collection of alternative sample types will need to be arranged for referral to Bristol.

Glucose-6-Phosphate Dehydrogenase (G6PD) Deficiency

- G6PD deficiency is a common X-linked condition presenting with recurrent haemolysis and anaemia which can be triggered by drugs e.g. dapsone, infections and specific foods including fava/broad beans. The condition is prevalent in patients from malaria affected regions.

10. PORPHYRIAS

PROTECT ALL SAMPLES FROM LIGHT EXPOSURE
Use tin foil and/or a brown paper envelope for transport to the lab

Where there is a high suspicion of porphyria or urgent analysis is required please contact the metabolic biochemist on 07511154412.

For known porphyria patients whom require haem arginate please contact NAPS for prescription (see contact number below). This is stocked at QEUH, Glasgow.

Sample Requirements

- Acute porphyria: 10 mL urine in a plain universal, preferably early morning/during an acute attack.
- Cutaneous porphyria: 5 mL blood in a purple-top EDTA tube.
- Protect samples from light with tin foil and/or a brown paper bag.
- A clinical history is essential to aid in interpretation and appropriate biochemical investigations.
- Note, a negative result does not exclude a porphyria where the patient is asymptomatic.
- First line investigations are determined by the presenting clinical symptoms however a faecal sample may be required for confirmation of abnormal results identified in the first line investigations.

Acute Porphyria

Acute intermittent porphyria (AIP), variegate porphyria (VP), and hereditary coproporphyrinuria (HCP) can present with acute abdominal pain, vomiting, neurological convulsion, hyponatraemia and psychiatric symptoms. Approximately 75% of acute crises are precipitated by medication (including oral contraceptive). For information on medication that is contraindicated in porphyria patients see Drugs in Porphyria - Welsh Medicines Advice Service (wales.nhs.uk)

Cutaneous Porphyria

Cutaneous porphyrias include porphyria cutanea tarda (PCT), congenital erythropoietic porphyria (CEP), erythropoietic protoporphyria (EPP) and X-linked dominant protoporphyria (XLDPP). These porphyrias, as well as the acute porphyrias VP and HCP, can present with cutaneous blistering and skin fragility.

Table 8: Useful Contact Numbers when Investigating and Managing Patients with Porphyria

Useful Contacts	
National Acute Porphyria Service (NAPS) (24/7 Service)	029 2074 7747
Metabolic Biochemist (QEUH)	07511154412
Adult Metabolic Consultant	via QEUH hospital switchboard: 0141 201 1100
Cardiff Lab (office hours)	02921 846588
Kings College, London	0203 2995776
Welsh Medicines Information Centre (safe drug info)	02921 843877

11. CARDIOMYOPATHY

A wide spectrum of metabolic disorders including fatty acid oxidation and carnitine disorders, organic and amino acid disorders, lysosomal disorders, mitochondrial disorders and glycogen storage disorders can present with dilated and/or hypertrophic cardiomyopathy as part of the clinical phenotype. Around 5% of cases of cardiomyopathy can have an underlying inborn error of metabolism as the cause.

First Line Investigations:

- Plasma for lactate, CK, urate, cholesterol, TFT, Ferritin and iron studies
- Plasma and dried blood spot for acylcarnitines
- 10 mL urine for organic acids, amino acids and GAG electrophoresis
- Plasma for amino acids
- 5-10 mL EDTA blood for lysosomal enzymes

Investigation of glycosylation disorders and Barth syndrome using desialotransferrin isoforms and cardiolipin respectively requires referral of samples to external labs. These should be sent directly to the referral laboratory where possible.

MetBioNet Best Practice Guideline *"Investigation of Inherited Metabolic Cause of Cardiomyopathy"* Table 1 contains a comprehensive list of disorders that present with cardiomyopathy.

12. METABOLIC MYOPATHIES

Biochemical metabolic muscle disorders are generally caused by a deficient energy source for effective muscle function. These can be enzyme deficiencies affecting fatty acid or carnitine metabolism, glycogen metabolism or mitochondrial function within the muscle.

Biochemical metabolic muscle diseases in children may present clinically with muscle pain, proximal weakness, exercise intolerance and rhabdomyolysis because of inadequate energy production within the muscle cells. A clinical history will aid appropriate biochemical investigations as some disorders tend to be symptomatic when resting whilst others follow physical exertion.

First Line Investigations:

- Plasma for CK and lactate
- Urine for organic acids
- Plasma for acylcarnitines
- Dried blood spot for alpha glucosidase

These will aid diagnosis of possible CPT-2, Pompe disease (acid maltase deficiency, GSD Type II) and mitochondrial disease. More specific genetic or muscle biopsy investigations would be required for confirming a possible glycogen storage disease i.e. GSD V, GSD VII, GSD IXD.

For the investigation of adult patients with exercise related myalgia or for a single episode of rhabdomyolysis please refer to the Scottish Muscle Network – NHS Scotland National Network where investigation protocols are outlined (for review July 2026).
Investigation of Exercise Related Myalgia in Adults
Single Episode of Rhabdomyolysis in Adults

13. NEURODEVELOPMENTAL DISORDERS & GLOBAL DEVELOPMENTAL DELAY

Neurodevelopmental disorders involve deficits in cognitive functioning (IQ < 70) and adaptive skills. These children may have associated behavioural problems including hyperactivity, autism, aggressive and self-injurious behaviour, epilepsy and other neurological disabilities. Global developmental delay (GDD) refers to children < 5 years, who show deficits in two or more developmental domains.

There are currently over 100 treatable metabolic disorders that can present with these symptoms as a prominent feature. An algorithm app "Treatable ID" comprises up- to-date information on these disorders with relevant diagnostic tests and therapy.

Table 9: Primary and Secondary Investigations in Patients with Neurodevelopmental Disorders and GDD

Primary		Secondary
Blood	Urine	
Ammonia Lactate Urate Plasma amino acids Total homocysteine Acylcarnitines Copper and caeruloplasmin Biotinidase Blood lead Full blood count Genetic investigations *(DNA array, chromosomal studies and Fragile-X)*	Organic acids Urate Creatine & GAA Oligosaccharides Glycosaminoglycans	Disialotransferrin Very long chain fatty acids Urine purine and pyrimidines

14. PEROXISOMAL DISORDERS

Biochemical investigations for peroxisomal disorders are currently referred outside of Scotland incurring a charge for testing.

The peroxisomal disorders include Zellweger spectrum disorder, rhizomelic chondrodysplasia punctata, X-linked adrenoleukodystrophy, Refsums and multiple single enzyme deficiencies. The clinical spectrum is broad, including severe neonatal phenotypes and attenuated adult phenotypes manifesting with less severe signs and symptoms.

The organ systems affected include the central and peripheral nervous system, the eyes, and the auditory nerve, liver, adrenals and skeletal systems. This gives rise to the core clinical features which can include neonatal hypotonia, developmental regression, chronic spastic paresis, paediatric bilateral cataracts, sensorineural hearing loss with retinitis pigmentosa, adrenal insufficiency and rhizomelic shortening of limbs.

Investigations can include very long chain fatty acids (including phytanate and pristinate), plasma pipecolate, red cell plasmalogens and plasma and urine bile acid intermediates.

APPENDIX A - NEONATAL CLINICAL PRESENTATION

In neonates the vast majority of inherited metabolic disorders fit into four categories:

1. Encephalopathy without Acidosis

Disorder	Presentation	Biochemical Investigation
Urea cycle disorders	Present day 3-5 with respiratory alkalosis and hyperammonaemia	Plasma amino acids Urine orotic acid
Maple syrup urine disease	Present day 4-10 with progressive encephalopathy, raised ammonia and ketonuria. Seizures may occur later	Now included in NBS programme Plasma amino acids Urine organic acids
Pyridoxine-responsive seizures	Seizures respond to vitamin B6	Pipecolate*
Non-ketotic hyperglycinaemia	Intractable seizures characteristic EEG, hypotonia, apnoea and cortical blindness	Collect time matched CSF and plasma for amino acids
Sulphite oxidase/ molybdenum cofactor deficiency	Epileptic encephalopathy with severe microcephaly	Urate (low <0.1 mmol/L) Urine amino acids
Peroxisomal disorders	Mild facial dysmorphia and skeletal abnormalities	Very long chain fatty acids*

**Referred test*

2. Encephalopathy with Acidosis

Disorder	Presentation	Biochemical Investigation
Organic acid disorders	Pancytopenia and high ammonia	Urine organic acids
Dicarboxylic aciduria	Hypoglycaemia	MCADD – part of NBS
Lactic acidosis	See section 6, page 10	Exclude cardiorespiratory defects first

3. Ketoacidosis and Encephalopathy -/+ Hypoglycaemia

Idiopathic ketotic hypoglycaemia is the commonest, benign cause in neonates and is a diagnosis of exclusion.

Disorder	Presentation	Biochemical Investigation
Fatty acid oxidation disorders	MCADD: Reye-like with metabolic crises during ordinary illness, surgery or fasting. Glucose often normal. VLCADD: Hepatomegaly, gross ketonuria and raised CK	Plasma or DBS Acylcarnitine profile. MCAD – Now included in NBS programme
Maple syrup urine disease (MSUD)	Presents day 4-10 with progressive encephalopathy, raised ammonia and ketonuria. Seizures may occur later	Plasma amino acids Urine organic acids. Now included in NBS
Organic acid disorders	May be masked by gross ketonuria e.g. holocarboxylase	Repeat urine organic acids as encephalopathy resolves
Ketone utilisation defect	May be asymptomatic with ketosis	Urine organic acids
Endocrine disorders	Hypoglucocorticoid state	See section 4, page 8

4. Hepatic Presentations

Presentation	Biochemistry Other symptoms	Disorder	Biochemical Investigation
Jaundice	Unconjugated	Usually benign If >250 umol/L consider Crigler-Najar	N/A
	Conjugated	Biliary atresia A1AT deficiency Galactosaemia Tyrosinaemia Peroxisomal disorder Thyroid disorders	 A1AT level and phenotype Gal-1-PUT Urine organic acids and plasma amino acids Very long chain fatty acids* Thyroid function tests
Hepatic dysfunction	Raised AFP ALP >2000 U/L	Tyrosinaemia	Urine organic acids and plasma amino acids
	Low INR Bilateral cataracts	Galactosaemia	Gal-1-PUT
	Symptoms appear after weaning	Hereditary fructose intolerance	Aldolase*
	Raised AFP and ferritin with low transferrin and transaminases	Gestational alloimmune liver disease (formally known as neonatal hemochromatosis)	N/A
	Raised lactate, urate and triglycerides, acidosis and hypoglycaemia	Glycogen storage disease type I	N/A
	Grossly raised lactate and hepatomegaly with normal transaminases	Fructose-1,6-bisphosphonate	Urine organic acids
	Dysmorphic features	Smith-Lemli-Opitz Zellweger syndrome	7-dehydrocholesterol* Very long chain fatty acids*

**Referred test*

APPENDIX B - RECOMMENDED INVESTIGATIONS IN SUSPECTED LSD

Discussion with a metabolic clinician or metabolic clinical scientist is advised.

Presentation	Disorder	Biochemical Investigations
Dysmorphism Coarse features Skeletal dysplasia	Oligosaccharidoses: - α-mannosidosis - β-mannosidosis - Fucosidosis Aspartylglycosaminuria Gaucher disease (GSD type I) GM1 gangliosidosis Mucopolysaccharidoses Mucolipidosis type III I-cell disease Multiple sulphatase deficiency	2x 5 mL EDTA 20 mL urine in a white top universal DBS card
Leukodystrophy	Krabbe Metachromatic leukodystrophy Adrenoleukodystrophy	2x 5 mL EDTA Lithium heparin plasma
Hepatomegaly	Gaucher (GSD type I) Niemann Pick A, B or C Wolman/CESD I-cell disease GM1-gangliosidosis MPS type I and VII	2x 5 mL EDTA 20 mL urine in a white top universal DBS card
Seizures	Tay sach disease GM1-gangliosidosis Gaucher (GSD type I) Niemann Pick type C Krabbe NCL type I and II MPS disorders Oligosaccharidoses Biotinidase deficiency Non-ketotic hyperglycinaemia Molybdenum cofactor deficiency Creatine synthesis and transport defects Peroxisomal disorders Serine biosynthesis defects Cobalamin C deficiency Biopterin disorders* Hyperinsulinism-hyperammonaemia syndrome	2x 5 mL EDTA Lithium heparin plasma 20 mL urine in a white top universal DBS card
Seizures, ataxia and blindness	NCL type I/II Biotinidase deficiency	DBS card Lithium heparin plasma

**Referred test*

Ataxia	NCL type II Galactosialidosis (late onset) Sandhoff (late onset) MLD (late onset) Krabbe NPD type C α-mannosidosis	2x 5 mL EDTA
Behavioural disturbance Psychosis	Krabbe MLD Tay-Sach Niemann Pick type C α-mannosidosis β-mannosidosis NCL type I/II Sanfilippo MPS type III Peroxisomal disorders Wilson disease Hyperammonaemia - OTC deficiency	2x 5 mL EDTA Lithium heparin plasma 20 mL urine in a white top universal
Myopathy	See section 12, page 15	DBS card
Cardiomyopathy	See section 11, page 15	DBS card 5 mL EDTA 20 mL urine in a white top universal
Deafness	α-mannosidosis β-mannosidosis I-cell disease MPS type I, II or IV Biotinidase deficiency	5 mL EDTA 20 mL urine in a white top universal
Angiokeratoma	Fabry Fucosidosis β-mannosidosis Aspartylglycosaminuria Schindler disease Galactosialidosis Sialidosis (neuraminidase deficiency)	5 mL EDTA 20 mL urine in a white top universal
Arthritis Stiff joints	MPS type II or III Gaucher Oligosaccharidoses	DBS card 20 mL urine in a white top universal
Speech and language delay	Creatine synthesis defects	2-5 mL urine in a white top universal (frozen)
Foetal and neonatal hydrops	MPS type I, IVA or VII Sialidosis GM1-gangliosidosis Mucolipidosis type II Multiple sulphatase deficiency Gaucher NPD Type A and C Wolman	2x 5 mL EDTA 20 mL urine in a white top universal

APPENDIX C - LATE-ONSET NEUROENCEPHALOPATHIES

Careful clinical history and examination (e.g. peripheral neuropathy or cherry red spots), accompanied by imaging MRI for cerebral atrophy and nerve conduction studies will aid testing and diagnoses. Progressive neurological and mental deterioration between 10 and 70 years of age can be separated depending upon predominant features listed below.

1. Extra-pyramidal

Disorder	Biochemical Investigation
Ataxia-telangiectasia	Immunoglobulins (low IgA)
Wilson' disease	Urine and serum copper and caeruloplasmin
Leigh syndrome	Lactate
Purine disorders	Urate
Late onset OTC	Ammonia and orotic acid
Niemann-Pick type C	Plasma oxysterol and PPCS*
Niemann-Pick type A/B	Sphingomyelinase
GM2-gangliosidosis	β-Hexosaminidase
GM1-gangliosidosis	β-Galactosidase

**Referred test*

2. Peripheral Neuropathy

Disorder	Biochemical Investigation
Acute porphyria	Urine PBG and plasma porphyrins
Tyrosinaemia type I	Plasma amino acids
Vitamin E deficiency	Vitamin E
Refsum	Phytanic acid*
Krabbe	β-galactocerebrosidase
Metachromatic leukodystrophy	Leucocyte arylsulphatase A
Glycosylation disorders	Desialotransferrin*
Fatty acid oxidation disorder	Urine organic acids
Peroxisomal disorders	Very long chain fatty acids*
Abetalipoproteinaemia	Lipoprotein and apo-proteins
Fabry	Alpha-galactosidase

**Referred test*

3. Myoclonic Epilepsy

Disorder	Biochemical Investigation
Respiratory chain defects	Lactate
OTC deficiency	Ammonia and orotic acid
Gaucher	β-glucocerebrosidase
GM2-gangliosidosis	β-hexosaminidase
Sialidosis Type I	Neuraminidase

4. Cerebellar Ataxia

Disorders	Biochemical Investigation
Respiratory chain defects	Lactate
Ataxia-telangiectasia	Immunoglobulins (low IgA)
Abetalipoproteinaemia	Lipoprotein and apo-B
OTC deficiency	Ammonia and orotic acid
Refsums	Phytanic acid*
GM2-gangliosidosis	β-hexosaminidase
Gaucher	β-glucocerebrosidase
Metachromatic leukodystrophy	Leucocyte arylsulphatase A
Krabbe	β-galactocerebrosidase
GM1-gangliosidosis	β-galactosidase
Sialidosis type I	Neuraminidase
Cerebrotendinous xanthomatosis	Cholestanol

**Referred test*

5. Diffuse Leucodystrophy

Disorder	Biochemical Investigation
Adrenoleukodystrophy	Cortisol and ACTH
Metachromatic leukodystrophy	Leucocyte arylsulphatase A
Gaucher	β-glucocerebrosidase
Krabbe	β-galactocerebrosidase
GM1-gangliosidosis	β-galactosidase
GM2-gangliosidosis	β-hexosaminidase
Peroxisomal disorders	Very long chain fatty acids*

**Referred test*

Note, these lists are not exhaustive. Please discuss staged investigation with the laboratory following full clinical examination with possible neurophysiology studies and imaging.

APPENDIX D - EYE DISORDERS

Presentation	Disorder		Biochemical Investigation
Cataracts	Present at birth	Lowe syndrome	Urine amino acids
		Zellweger syndrome	Very long chain fatty acids*
		Rhizomelic chondrodysplasia punctata	
		Sorbitol dehydrogenase deficiency	
	After 5 days	Galactosaemia	GAL-1-PUT
	After 4 weeks	Galactokinase deficiency	Urine galactitiol*
		Oligosaccharide disorders	Urine oligosaccharides
		Mitochondrial myopathy	Lactate
	Infant/child	Diabetes mellitus	Glucose
		Wilson's disease	Plasma caeruloplasmin and urine copper
		Hypoparathyroidism	Plasma calcium and PTH
		Pseudohypoparathyrodism	Plasma calcium and PTH
		Fabry disease	a-galactosidase
Cherry-red spot	Lysosomal storage disorders e.g. Tay-Sachs and GM1-gangliosidosis		Lysosomal enzymes
Retinal degradation	Lipid metabolism disorders		Lipid investigations
	Peroxisomal disorders		Phenotype plus peroxisomal investigations*
	Lysosomal disorders		Lysosomal enzymes
	Kearns-Sayre syndrome		Genetics
	Menkes		Plasma caeruloplasmin and urine copper
	Gyrate atrophy		Plasma amino acids
	Cobalamin disorders		Urine organic acids and MMA
	Congenital disorders of glycosylation		Serum desialotransferrin*
	Fatty acid oxidation disorders		Acylcarnitines and urine organic acids
Ocular motor findings	Gauchers (type II and III)		β-glucosidase
	Niemann-Pick type C		Plasma oxysterol and PPCS*
	Tay-Sachs		Total hexaminosaminidase
	Neurotransmitter disorders		CSF neurotransmitters*
	Wilson's disease		Plasma caeruloplasmin and urine copper
	Respiratory chain defects		Genetics
Cornea defects e.g. corneal clouding	MPS		Urine glycosaminoglycans
	Fabry disease		a-galactosidase
	Cystinosis		White cell cysteine*
	Oligosaccharide disorders		Urine oligosaccharides
	Familial hypercholesterolaemia		Lipid profile
	Tyrosinaemia		Plasma amino and urine organic acids
	Wilson's Disease		Plasma Caeruloplasmin & Urine Copper

**Referred test*

APPENDIX E - PSYCHIATRIC DISORDERS

A wide variety of metabolic disorders have presented with behavioural disturbances, personality and character changes, mental regression, psychosis and schizophrenia-like syndrome.

Presentation	Disorder	Biochemical Investigation
Hyperactivity and behavioural disturbance	Sanfilippo	Urine glycosaminoglycan
Personality changes	Krabbe Metachromatic leukodystrophy	β-galactocerebrosidase Arylsulphatase A
Mental regression	Niemann-Pick type C Adrenoleukodystrophy	Filipin staining of fibroblast cultures and/or plasma PPCS* Very long chain fatty acids*
Schizophrenia-like syndrome	OTC deficiency	Ammonia, plasma amino acids, and urine orotic acid
	Wilson's disease	Urine copper Serum copper and caeruloplasmin
	Leigh syndrome	Plasma lactate
	Methylenetetrahydrofolate Reductase deficiency	Urine amino acids and total homocysteine
	Spielmegel-Vogt disease	Vacuolated lymphocytes
	Hallervorden spatz	Blood film; acanthocytosis with retinitis pigmentosa
	Cerebrotendinous xanthomatosis	Cholestanol*
	Acute porphyria	Urine porphobilinogen
	Niemann Pick type C	Plasma PPCS*

**Referred test*

APPENDIX F - ADDITIONAL USEFUL SOURCES OF INFORMATION

Clinical Guidelines

Scottish IMD Guidelines published by Scottish MCN for Inborn Errors of Metabolism
Best Practice Guidelines published by National Metabolic Biochemistry Networks
Emergency Guidelines published by British Inherited Metabolic Disease Group

Metabolic Websites

Vademecum Metabolicum
MetBioNet
IMD Scotland
Porphyria Network