

Document No.	UINF-3	Version No.	4
		Issue Date	31/01/24

PAEDIATRIC METABOLIC INVESTIGATIONS

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General investigations

(1) Metabolic screens

A wide range of biochemical assays may be useful in the investigation of patients presenting with a possible inherited metabolic disease. These range from routine analyses such as blood gases or glucose to specific enzyme analyses or DNA mutation studies.

Because of the vast number of inborn errors, unless specific features are present, investigation is usually staged, analyses capable of excluding a number of related disorders being carried out before specific enzyme or DNA analyses are considered.

- Symptoms such as vomiting or lethargy which may result from toxic levels of a metabolic intermediate should be investigated by collecting **blood for ammonia, amino acids and acylcarnitine profile, and urine for a metabolic screen in addition to routine biochemistry.** This detects urea cycle defects as well as many amino and organic acid disorders. The urine metabolic screen includes amino and organic acid profiles and mucopolysaccharides.
- Disorders of energy production or utilisation such as fatty acid oxidation defects, disorders of glycogenolysis or gluconeogenesis and mitochondrial myopathies due to electron transport chain defects may present with hypoglycaemia or severe hypotonia. In this case collect blood for **lactate, amino acids, acylcarnitine profile and urine for a metabolic screen.**

Include clinical details and drug therapy on the request form. Antibiotics cause gross interference in amino acid profiles and some anticonvulsants (especially valproate) interfere in organic acid and/or amino acid profiles.

A minority of inborn errors present with characteristic signs or symptoms that give a strong indication of a specific defect, e.g. characteristic facies in Hurler or Hunter disease, dislocated lenses in homocystinuria, and alopecia and skin rash in biotinidase deficiency.

Most patients with inborn errors have a relatively non-specific presentation, which may mimic more common disorders, such as sepsis or pyloric stenosis. Presentation may be with a first acute illness, repeated episodes of acute illness, or chronic symptoms. In acute admissions it is vitally important to collect samples for investigation as early as possible. If this is not done elevated levels of abnormal metabolites may rapidly fall on treatment and the diagnostic opportunity may be missed. Blood transfusion invalidates diagnostic enzyme analyses performed on red cells e.g. for galactosaemia. Early sample collection during the presenting illness may avoid the necessity of attempting to provoke a metabolic crisis by prolonged fasting or loading tests. For children who have repeated episodes but never in hospital, a urine container can be supplied for home collection of the first sample passed after an attack. Normal results on samples

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collected from an asymptomatic child at clinic attendance do not necessarily exclude a number of inborn errors with an episodic presentation.

(2) Emergency Sample Collection for Metabolic Investigations

In an acute life threatening situation or immediately post mortem the following samples allow a number of useful metabolic investigations to be undertaken. Discuss individual cases with the metabolic biochemist or the on-call consultant biochemist if at all possible before collecting samples, as samples for some investigations require special handling.

- **Blood:** Collect two full 5 mL lithium heparin tubes, and one fluoride oxalate tube, and send to the laboratory. The plasma and red cells will be stored while the most useful analyses are decided, and whole blood also stored unseparated at 4°C, for possible preparation of leucocytes or platelets for enzyme or DNA analysis.
- **Urine:** Collect as much urine as possible, and send to the laboratory.
- **CSF:** Collect CSF into fluoride oxalate and plain tubes and send to the laboratory.
- **Skin biopsy:** Fibroblasts for enzyme or DNA analysis can be grown from a skin biopsy taken up to 24 hours post mortem, provided the original biopsy is sterile. In an emergency, if transport medium is not available collect the biopsy into 0.9% sterile saline. Store at 4°C until transport to the laboratory is arranged.
- **Tissue Samples:** e.g. liver, muscle. These should only be taken if there is a very strong possibility or a provisional diagnosis of an inborn error requiring these samples for diagnosis. Wrap biopsies individually in aluminium foil and immediately snap freeze in liquid nitrogen/solid CO₂ (obtained from the laboratory).

As soon as possible after collecting these samples review the clinical presentation and previous investigations with the metabolic biochemist to allow selection of the most appropriate assays. Details of previous diet and drugs and blood products given are needed to select assays that will yield reliable information.

Specific investigations

(1) Galactosaemia Investigations

This is potentially a rapidly fatal condition that requires immediate investigation and treatment. Classical Galactosaemia is the result of Gal-1-PUT deficiency. Elevated galactose may result from three separate enzyme defects - Galactokinase, Galactose-1-phosphate Uridyl Transferase (Gal-1-PUT), and UDP-Galactose Epimerase, or from liver dysfunction.

Investigation of suspected galactosaemia may be required on clinical grounds, on newborn siblings of diagnosed galactosaemic children, or to follow up positive results from newborn PKU screening.

In a sick infant the first investigation is an enzymatic screening test for Gal-1-PUT deficiency. Quantitative Gal-1-PUT, and further metabolite testing must follow up an abnormal result. An equivocal Gal-1-PUT screen may be found following transfusion, as a result of G6PD deficiency, and in carriers and variants of the disorder.

A normal Gal-1-PUT screen may require further investigation eg blood galactose, depending on clinical assessment.

Red cell enzyme analysis only gives valid results if the infant has not had any transfusions, including top up transfusions, in the previous two months. Galactose analysis in blood and urine only give valid results if the infant is taking feeds of a lactose containing formula. Be aware that some special feeds, eg Caprilon, have a reduced galactose content. Ideally an initial 1 mL Lithium Heparin blood sample sent immediately on ice to the laboratory will allow a sick infant to stop

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galactose containing milk feeds and be transfused if necessary while providing sufficient sample for all confirmatory tests.

A well infant with prolonged jaundice is unlikely to have galactosaemia. Provided the baby has unconjugated jaundice and normal liver function tests, including clotting screen, enzyme testing for galactosaemia is not required. Note that following the withdrawal of Clinitest tablets by the manufacturer, urine reducing substances is no longer available.

(2) Lysosomal Enzymes

A number of lysosomal storage disorders may present similarly. Symptoms may be related to excessive storage of undegraded material in visceral organs, causing hepatomegaly and/or splenomegaly, e.g. Niemann Pick or Gaucher disease. Other disorders present with developmental delay and regression without organomegaly, e.g. Krabbe's disease and Metachromatic Leucodystrophy. Some disorders, e.g. Gaucher have different forms with or without neurological involvement. Mucopolysaccharidoses may present similarly, and have a separate investigation strategy (see separate section). Vacuolated lymphocytes or characteristic storage cells on bone marrow may suggest a specific storage disorder.

Where there is no clear clinical pointer one approach is to assay a number of lysosomal enzymes on the same sample of leucocytes extracted from a blood sample. This is sometimes referred to as a degenerative enzyme or leucocyte enzyme screen. The enzymes currently analysed in a group in this laboratory, and the disorders in which they are deficient are:

<i>Enzyme</i>	<i>Disorder</i>
a-Fucosidase	Fucosidosis
a-Mannosidase	a-Mannosidosis
Acid Esterase	Wolman disease, Cholesterol ester storage disease
Arylsulphatase A	Metachromatic Leucodystrophy
B-Galactosidase	GM1 gangliosidosis, MPS IVB (Morquio B)
B-Glucosidase	Gaucher disease
B-Mannosidase	B-Mannosidosis
Galactocerebrosidase	Krabbe disease, Globoid Cell Leucodystrophy
Hexosaminidase A	Tay-Sach Disease, GM2 Gangliosidosis B variant
Hexosaminidase, Total	Sandhoff Disease, GM2 Gangliosidosis 0 variant
Sphingomyelinase	Niemann Pick Disease Types A and B

A **minimum** of 5 mL of EDTA blood is required for the full lysosomal screen. If other biochemical investigations are requested leucocytes can be prepared from the lithium heparinised sample sent for these, although the yield may be poorer. Again a **minimum** of 5 mL blood is required. Samples taken in other hospitals may be sent by first class post. Delays of >1 day between sampling and leucocyte preparation lead to poor leucocyte yield and less reliable results.

Other metabolic enzymes are assayed individually, using appropriate samples. The investigational protocol depends on the clinical presentation of the patient. Individual assays are only carried out for patients with specific clinical or biochemical findings suggestive of a particular disorder, or for conditions where the enzyme analysis is the only reliable diagnostic test.

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Initially discuss patients with the metabolic biochemist to select the most appropriate enzyme analyses. A number of these assays are carried out in this department, while others are referred to specialist laboratories in the UK or abroad.

Arrangements can also be made with specialist laboratories offering prenatal diagnosis by analysis of chorionic villus samples. If prenatal diagnosis is being considered it is important to contact this laboratory as soon as possible to review the biochemical findings on the index case to assess what options are available.

(3) Mucopolysaccharidoses and MPS-like Disorders

Presentation of mucopolysaccharidoses varies with the specific enzyme deficiency. Hepatomegaly and coarse features may give a characteristic clinical presentation, as in Hurler and Hunter diseases. Several other disorders, such as I cell disease and fucosidosis may present similarly. MPS III (San Filippo disease) often presents with regression and neurological deterioration and without somatic features, while skeletal changes predominate in MPS IV (Morquio). A number of investigations may be required in children presenting with coarse features and other MPS like findings. The table below shows disorders diagnosed by urinary MPS or lysosomal enzyme screens, and those that require separate investigation. All urine samples referred for a metabolic screen have a quantitative MPS estimation performed as a first line screen, plus electrophoresis if elevated. Abnormal findings on mucopolysaccharide electrophoresis require enzymatic confirmation of the diagnosis, both to pinpoint the defect, and to provide a basis for any future request for prenatal diagnosis in that family.

Urinary oligosaccharide chromatography is reported as giving abnormal patterns in several storage disorders, and may be recommended in textbooks as a useful screening procedure. While it is certainly the case that abnormal patterns are seen in patients with established diagnoses, its use as a screen in infants and toddlers with a high milk intake is complicated by the presence of dietary oligosaccharides. This severely limits its usefulness in this population, although it can be a helpful investigation in older patients. In addition urinary oligosaccharides are extremely sensitive to bacterial degradation. We advise the appropriate enzyme assay is carried out on patients suspected of having any disorder causing oligosacchariduria.

<i>Disorder</i>	<i>MPS screen + Electrophoresis</i>	<i>Lysosomal Enzymes</i>	<i>Other Investigations</i>
Sample	Random Urine	Blood (5mL EDTA)	
MPS I, Hurler/Scheie	+	-	
MPS II, Hunter	+	-	
MPS III, San Filippo, Types A, B, C	+	-	
MPS IVA & B Morquio,	-	Type B only	Type A - Galactose6S sulphatase
MPS VI, Maroteaux-Lamy	+	-	
MPS VII, Sly	?	-	B-glucuronidase
Mucopolidosis II, I Cell Disease	+/-	-	plasma lysosomal enzymes
Mucopolidosis III, Pseudo Hurler	+/-	-	plasma lysosomal enzymes
GM ₁ Gangliosidosis	-	+	

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<i>Disorder</i>	<i>MPS screen + Electrophoresis</i>	<i>Lysosomal Enzymes</i>	<i>Other Investigations</i>
Sialidosis, Types I & II	-	galactosialidosis only	a-neuraminidase
a-Mannosidosis	-	+	
B-Mannosidosis	-	+	
Fucosidosis	-	+	
Aspartylglycosaminuria	-	-	Urine amino acids

(4) Peroxisomal Disorders

This group of disorders includes peroxisomal biogenesis defects, where peroxisomes are apparently absent and deficiencies are found in all measured peroxisomal functions. A second group shows deficiencies in targeting proteins to peroxisomes with defects in more than one of the peroxisomal metabolic pathways, and a third group correspond to single enzyme defects on specific peroxisomal metabolic pathways. The clinical presentation may be highly characteristic, as with the dysmorphia, hypotonia and renal cysts of classical Zellweger syndrome, or the rhizomelia and typical X-ray appearance of rhizomelic chondroplasia punctata. However, as the confusing nomenclature of this group of disorders demonstrates, similar clinical phenotypes may result from different biochemical defects.

Very Long Chain Fatty Acids (VLCFAs) is the first line investigation that will detect the widest range of peroxisomal disorders. 2 mL of EDTA or lithium heparin blood is required for VLCFAs, phytanic and pristanic acid. Further investigations should be discussed with the metabolic biochemist.

(5) Phenylketonuria (PKU)

The Scottish newborn screening laboratory reports an elevated blood phenylalanine (greater than 240umol/L) along with tyrosine, as "PKU is suspected" if the tyrosine is not elevated or "other disorder suspected" if the tyrosine is also elevated. The baby will be referred to the metabolic consultant. Before a low phenylalanine diet is started sufficient blood is taken for:

1. Basal phenylalanine and tyrosine concentrations, to confirm screening result.
2. Five blood spots on a newborn screening card are sent via this department to Birmingham Children's Hospital (19®) for centralised UK testing for bipterin defects that account for 1% of PKU cases. These are assayed for dihydrobiopterin reductase activity, and for total bipterin levels to detect defects in bipterin cofactor recycling and synthesis. Samples must be collected while phenylalanine levels are >200umol/L to interpret the results.
3. If "other disorder suspected" is reported, additional tests including screening for galactosaemia may be ordered by the metabolic consultant.

After initial confirmation of diagnosis, the frequency of repeat analyses is arranged in conjunction with the dietitian until stable phenylalanine levels are achieved.

For patients established on diet plasma phenylalanines are analysed every Wednesday, the same day as the regional PKU outpatient clinic. This department provides home sampling kits, and the phlebotomists teach capillary sampling to the family.

Considerable individual variation should be expected.

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(6) Specific enzyme and metabolite investigations

For biotinidase, homocysteine and a full departmental test repertoire please consult the test directory.

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