

| Biochemistry South Glasgow Sector | | |
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| LI_M_021 | South Glasgow Biochemistry Department Metabolic Handbook | Version:1.16 |
| Document Owner: Alana Burns | Authorised by: Jane McNeilly | Date of Issue: 13/10/21 |



**Diagnostics Directorate
Department of Biochemistry, QEUH (RHC)**

Notes for Guidance of Staff Using the Specialised Metabolic Investigations

Normal hours: 8.45 a.m. - 5 p.m. Monday - Friday
 8.45 a.m. - 12 p.m. Saturday

Outwith normal hours contact Consultant Staff and/or On Call BMS
via switchboard.

0141-201 1100

Specific Metabolic enquires 07505 255241

General Enquiries (0141 354) 9060 (*option4*)
(Reporting Room)

Dr Jane McNeilly (0141 354) 9047
Consultant Clinical Scientist

Dr Rajeev Srivastava (0141 354) 9030
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Queen Elizabeth University Hospital,
1345 Govan Road,
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These notes are for guidance to supplement the principle handbook for the department. They indicate the range of investigations undertaken at QEIH. We hope the section on specific clinical disorders will encourage exchange of clinical information with the laboratories and provide some initial guidance in the approach to a particular problem. The three textbooks referenced at the end offer more extensive and specific coverage of the wide field of inherited metabolic disorders.

In non-acute situations, samples should ideally arrive before 3.30 p.m. and those involving significant preparation, e.g. leukocyte enzymes, before 2 p.m. Where a child has a possible metabolic crisis, samples should be delivered promptly to the department. Please immediately inform the Reporting Room in normal working hours, and 'on call' BMS via Switchboard out of hours. Senior consultant level staff are available to discuss appropriate investigations and their interpretation at all times.

Each year, new diagnoses are described. The pathophysiological processes underlying well recognised conditions / syndromes are increasingly being identified as having an underlying metabolic condition. In approaching the metabolic investigation of an individual, it is important to recognise the effects of drugs, blood transfusions, intercurrent illnesses, and nutritional intake (calories, protein, fat, carbohydrates, trace elements and vitamins). All request forms should include accurate and appropriate information to allow full interpretative comments to be included with results.

Analytes Available as Emergency

An initial range of blood analyses can be performed at all times at QEIH (within 1 hour), and give significant clues to the underlying pathophysiological process:-

| <u>Analyte</u> | <u>Sample</u> | <u>Special considerations</u> |
|--|-----------------------------|---|
| U&Es, LFTs, Bone profile and Bicarbonate | 1 ml Lithium-heparin | |
| Glucose | 0.5 ml Fluoride Oxalate | |
| Blood Gases | Capillary or Arterial Blood | |
| Ammonia | 0.5 ml Lithium-heparin | Must arrive within 30 mins to lab for accurate result |
| Lactate | 0.5 ml Lithium-heparin | |

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Suitable samples for more wide ranging investigations MUST be collected in the acute phase, if the diagnostic window is not to be missed. When further samples, e.g. hypoglycaemia investigations, are collected during the acute presentation, it is essential that any necessary acute pre-analytical handling and appropriate storage are performed within the local laboratory.

The QEUH Biochemistry Laboratory aims to produce results in a timely manner. Due to the complex nature of many analytical methods, some results may take several days/weeks. Where an individual is critically ill, the initial diagnostic tests will help decide early medical management. A dialogue with the department is encouraged and may expedite more complex investigations.

General laboratory requirements are covered in main laboratory handbook; which includes general information on how to complete request forms, etc. **It is imperative that CHI number is given for all external requests.**

Add on complex tests:

- Requests for add-ons within 12 hours of receipt should be made to extension 89060 (*option 1*) or by emailing SouthGlasgow.BiochemistryAddOn@ggc.scot.nhs.uk
- Longer term (<1 month) should be discussed with the duty biochemist on extension 89060 (*option 4*). Some metabolic samples are available for significantly longer and should be discussed with a biochemistry consultant.

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1. General Guidance

A number of metabolites are only present during metabolic decompensation. It is critical that appropriate samples are obtained and handled correctly at QEUH and in your local laboratory.

| Analyte | Sample | Handling | Max. Turnaround (Working Days) |
|--|--|---|--|
| BLOOD | | | |
| Amino Acids | 2 ml Lithium-heparin blood | Deproteinise plasma immediately + freeze (See Appendix A) | 5 |
| β -Hydroxy butyrate | 0.5 ml Lithium-heparin blood | Separate + freeze (See Appendix A) | 3 |
| Blood Spot or plasma for Acylcarnitines (two, if possible)/ | 75 μ l whole blood per spot onto neonatal screening blood spot card filter paper | Dry thoroughly in air | 12 |
| Free Fatty Acids/NEFA | Or 0.5 ml Lithium-heparin blood | Separate + freeze | 12 |
| | 0.5 ml Fluoride oxalate | Separate + freeze | 3 |
| Leukocyte & plasma enzymes | 5-10 ml EDTA blood or blood spot assays | Requires specialist handling - discuss with laboratory | 15 -90 (depending on enzyme) <i>(RBC Assays 2 working days)</i> |
| Urate | 0.5 ml Lithium-heparin blood | Separate | 0.2 |
| Samples for other investigations should be treated according to instructions in individual sections. | | | |
| URINE | | | |
| Urine creatinine should be >1.0 mmol/L. Samples with creatinine <1.0 mmol/L would require extensively large volumes to be extracted & could give misleading results. | | | |
| Amino acids | Casual urine (thymol preservative) 25 mls | Store at 4°C Timed collection in less acute cases | 5 |
| | Or Casual urine (no preservative) | Freeze (-20°C) | 5 |
| Glycosaminoglycans, oligosaccharides | Casual urine (thymol preservative) 20 mls | Store at 4°C | 10 |
| Organic acids | Casual urine (no preservative) 20 mls with no preservative | Freeze and store at -20°C (-20°C) | 5 |

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| Analyte | Sample | Handling | Max. Turnaround (Days) |
|---|--|--|------------------------|
| CSF | | | |
| Amino acids | At least 500 µl plain container | Store frozen | 5 |
| Glucose | At least 200 µl Fluoride oxalate | Store frozen | 0.2 |
| <u>Tissue Samples</u> | - no preservative | Store frozen at -70°C | |
| <u>Skin for fibroblast culture</u> | - | Discuss with Clinical Genetics, QEUH. Emergency samples into culture medium. | |
| SWEAT | | | |
| Sweat Chloride | At least 20uL of sweat collected by the Wescor™ System | Samples are stored refrigerated prior to analysis and at room temperature post analysis. | 3 |

Not all analytes/enzymes are present in one fluid, or every tissue. A wide range of samples should be obtained from a child who is unlikely to survive, for future diagnosis and appropriate family counselling. When a child is not critically ill, samples should be obtained within the normal working day. If critically ill, appropriate samples **MUST** be collected and a senior member of the Metabolic Section, Biochemistry Department is always available to discuss requirements.

To avoid unnecessary, lengthy and costly laboratory investigations, close co-operation between the attending clinicians and the Metabolic Section is **NECESSARY**. All request forms **should** include accurate and appropriate information to allow full interpretative comments to be included with results; and allow us to extend/alter the analyses to give the requester the most appropriate service.

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APPROPRIATE INFORMATION may include:

1. Presenting illness e.g. diarrhoea and vomiting, time/date of onset of symptoms.
2. Family history e.g. Sudden Infant Death Syndrome, fetal losses, neonatal deaths.
3. Clinical findings, e.g. hepatomegaly in hypoglycaemia, dysmorphic findings including corneal clouding in mucopolysaccharidosis.
4. Previous biochemical and haematological findings done locally, e.g. acidosis and pancytopenia in methylmalonic acidaemia.
5. Drug history - may cause interference, e.g. paracetamol in urine amino acids.
6. Nutritional details - type and amount of food. ? adequate protein intake.
7. History of blood transfusions - a recent transfusion (<3 months) may produce misleading results in red cell analytes.

IN CHILDREN GIVING CONCERN, FOLLOWING A DISCUSSION WITH ONE OF THE CONSULTANTS, THE SAMPLE WILL BE PRIORITISED.

A metabolism Request Form is included in Appendix E, and we encourage requesters to use the form

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2. Range of investigations undertaken at QEUH

“Metabolic Screening” tests have variable diagnostic efficiency and may vary from centre to centre. A highly sensitive test is critical for routine use. In addition, as our knowledge of metabolic conditions increases, it is important to perform up to date investigations (e.g. in peroxisomal disorders in addition to VLCFA, pristanic/phytanic acid, bile acids and plasmalogens may require measurement if a disorder is to be diagnosed). Care is needed in referring samples within the UK and international framework of laboratories capable of diagnosing conditions. Considered advice is available from senior staff with close links to many laboratories. Help with interpretation and planning of investigations is encouraged.

Other guidance notes/protocols can be obtained from the Scottish MCN for Inborn Errors of Metabolism (<http://www.imd.scot.nhs.uk/guidelines.html>), and from the National Metabolic Biochemistry Networks (www.metbio.net) or from the British Inherited Metabolic Disease Group (www.bimdg.org.uk).

Useful investigative website included OMIM (www.ncbi.nlm.nih.gov/omim).

NHSGGC biochemistry laboratories provide a wide range of screening tests and diagnostic enzymatic tests in addition to the ‘routine’ tests listed on page 2. The principal tests performed are (*in plasma unless stated*):

- Free Carnitine and Acylcarnitine profiles
- Metabolites of Intermediate Metabolism: NEFA, β -Hydroxy-butyrate
- Aminoacids in Plasma, Urine and CSF
- Urine Orotic and Organic acids
- Urine Glycosaminoglycan and Oligosaccharide Screen
- RBC Galactose-1-phosphate, Gal-1-P Uridyl transferase
- RBC G6PD
- Biotinidase
- Bloodspot Phenylalanine/tyrosine and Branch-chain aminoacids (monitoring)
- Copper and caeruloplasmin
- Cellular enzymes (Table 1 pg 9)

The department also performs a very wide range of routine analyses and additionally supports sweat testing, endocrine, nutrition and gastroenterology teams with a comprehensive panel of tests.

PRENATAL DIAGNOSES are undertaken for a wide variety of conditions. The diagnostic basis of the proband is critical and individual cases must be discussed beforehand with the department.

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The Biochemistry Department is a member of an increasing range of external quality schemes to cover all analytes possible.

Table 1 Cellular enzymes available at QEUH

| Department of Biochemistry, Queen Elizabeth University Hospital | | | | | |
|---|---|--------------|------------|--------|-----|
| ENZYME DEFICIENCY | DISEASE | ERYTHROCYTES | LEUCOCYTES | PLASMA | DBS |
| GLUCOSE – 6- DEHYDROGENASE | G6PD DEFICIENCY | X | | | |
| α-GLUCOSIDASE | POMPE | | | | X |
| α-FUCOSIDASE | FUCOSIDOSIS | | X | X | X |
| GLYCOSYLASPARAGINASE | ASPARTYLGLYCOSAMINUIRA | | | X | |
| α-MANNOSIDASE | α-MANNOSIDOSIS | | X | X | |
| B-MANNOSIDASE | B-MANNOSIDOSIS | | | X | |
| N-ACETYL-α-D-GALACTOSAMINIDASE | SCHINDLER | | | X | |
| ACID HYDROLASE | I-CELL | | | X | |
| ACID LIPASE | WOLMANS CESD | | | | X |
| ARYLSULPHATASE A | METACHROMATIC LEUCODYSTROPHY | | X | | |
| B-GALACTOCEREBROSIDASE | KRABBE | | X | | X |
| α-GALACTOSIDASE | FABRY | | | X | X |
| B-GALACTOSIDASE | GM1 GANGLIOSIDASE | | | | X |
| B-GLUCOSIDASE | GAUCHERS | | X | | X |
| B-HEXOSAMINIDASE A | TAYSACHS | | | | X |
| TOTAL HEXOSAMINIDASE | GM2 GANGLIOSIDOSIS | | | | X |
| SPHINGOMYELINASE | NIEMANN-PICK | | | | X |
| α-L-IDURONIDASE | MPS I | | X | | |
| HEPARAN SULPHAMIDASE | MPS IIIA | | X | | |
| N-ACETYL-α-D-GLUCOSAMINIDASE | MPS IIIB | | X | | |
| GALACTOSE-6-SULPHATASE | MORQUIO MPS IVA | | X | | |
| ARYLSULPHATASE B | MPSVI | | X | | |
| B-GLUCURONIDASE | MPSVII | | | | X |
| GALACTOSE-1-PHOSPHATE URIDYL TRANSFERASE | GALACTOSAEMIA | X | | | |
| BIOTINIDASE | BIOTINIDASE DEFICIECNY | | | X | |
| Palmitoyl-Protein Thioesterase (PPT) | Infantile Neuronal Ceroid Lipofucinoses (NCL) | | | | X |
| Tripeptidyl Peptidase (TPP) | Late-onset NCL | | | | X |

Details of sample requirements and methods of preservation and transportation can be obtained by telephoning the metabolic biochemist on 07505 255 241

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3. Glucose-6-phosphatase deficiency (G6PD)

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is an X-linked disorder that may result in reduced erythrocyte viability and haemolytic anaemia.

G6PD is measured in erythrocytes therefore EDTA (purple top) samples are preferred. Minimum volume is 20uL.

G6PD is stable stored as whole blood for at least one week at 4°C.

Reference ranges

Neonates (\leq 1 month) - 12.0 – 24.0 IU / g Hb (mean = 18) (n = 109) EDTA

Adult (> 1 month) - 7.0 – 15.0 IU / g Hb (mean = 10) (n = 128) EDTA

Obligate Heterozygotes - to be determined IU / g Hb (n=x; adult females)

Lithium heparin sample received – G6PD activity is ~20% higher using lithium heparin samples compared to EDTA. Quoted reference range is for EDTA samples.

Classes of enzyme variants

| Class | Level of deficiency | Percentage mean normal | Activity (IU/g Hb) | |
|--------|---------------------|------------------------|--------------------|---------|
| | | | Neonate | Adult |
| I & II | Severe deficiency | <10% mean normal | <1.8 | <1.0 |
| III | Moderate deficiency | 10-60% mean normal | 1.8-10.8 | 1.0-6.0 |
| IV | Not deficient | >60% mean normal | >10.8 | >6.0 |

4. Sweat Chloride analysis

Sweat tests are used to diagnosis cystic fibrosis as it causes abnormally high levels of sodium and chloride in sweat.

Pilocarpine iontophoresis stimulates the production of sweat that is collected as liquid via the Wescor system. Sweat samples for chloride analysis **must** be collected by Wescor method. A minimum of 20ul of sample is required for analysis.

Samples are stored refrigerated prior to analysis and at room temperature post analysis.

Reference range <6 months of age

Chloride <30 mmol/L CF unlikely
 Chloride 30 – 60 mmol/L Intermediate result – requires further investigation
 Chloride >60 mmol/L Supports the diagnosis of CF

Reference range >6 months of age

Chloride <40 mmol/L CF unlikely
 Chloride 40 – 60 mmol/L Intermediate result – requires further investigation
 Chloride >60 mmol/L Supports the diagnosis of CF

Reference ranges are based upon guidance stated in guidelines for the performance of the Sweat Test for the investigation of Cystic Fibrosis in the UK version 2 (an evidence based guideline - March 2014).

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5. Investigation of Hypoglycaemia

Resuscitation → Do **NOT** delay glucose therapy.

Glucose should be given intravenously 0.2 g/kg (i.e. 2 ml/kg 10% w/v solution) followed by infusion of 10% glucose at normal fluid maintenance rates. If hyponatraemia, and a clinical suspicion of hypopituitarism/hypoadrenalism, then give hydrocortisone IV.

The causes of hypoglycaemia vary from hyperinsulism, hormone insufficiency (e.g. GH, cortisol), poisoning with alcohol, liver disorders (e.g. tyrosinaemia, viral), to inborn errors of glycogenesis, gluconeogenesis, fatty acid oxidation disorders, ketolytic defects, and organic acidurias. This vast range requires a **SYSTEMATIC APPROACH** within the laboratory or diagnoses such as cortisol deficiency can be overlooked. **Hypoglycaemia should be confirmed by laboratory analysis (GLUCOSE <2.8 mmol/L);** 6-10 mls of blood should be obtained, if possible before initial resuscitation and sent immediately to the laboratory. This should be handled as:

1. **2 x 1 ml Fluoride Oxalate** - plasma glucose + free fatty acids (separate + freeze).
2. **LITHIUM HEPARIN tube** – separate and freeze the plasma in three aliquots.
These can be analysed for:
 - i) **ENDOCRINE** - Cortisol (ACTH), (GH), Insulin, (C-peptide).
 - ii) **METABOLIC** - β -OH Butyrate, Lactate, Ammonium, acylcarnitine, and plasma amino acids.
3. Obtain **dried blood spot cards**, or ask laboratory to spot out from lithium heparin sample. **Dry** in air.

The first voided URINE should be collected into a plain universal and frozen for organic acid analysis. If inadequate volume (<5mL), freeze and add next urine.

Clinical features such as length of fasting, or hepatomegaly may target analyses. The full emergency profile is appropriate (see page 2), and may indicate hyponatraemia (suggestive of adrenal failure), lactic acidosis (present if shocked, in disorders of gluconeogenesis, glycogenesis and respiratory chain disorders) and hyperammonaemia (suggestive of build up of acyl CoA metabolites).

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Raised CK may suggest Fatty Acid Oxidation Defect. Any patient with Encephalopathic features not rapidly resolving (> 20 minutes) following glucose therapy should be discussed with senior biochemistry staff to obtain optimal service.

Identification of Disorders:

1. **Increased Glucose Utilisation** -
Requiring > 12 mg/kg/min glucose infusion to maintain euglycaemia, free fatty acids (<1 mmol/L). Check insulin (+/- C-peptide) and IGF II if large tumour present.
2. **Impaired Ketogenesis** –
Increased free fatty acids with poor β -OH butyrate rise (Ratio >1). [Very low birth weight babies have a naturally impaired ketogenic response.] Suggestive of fatty acid oxidation disorder, ketogenic disorder or carnitine deficiency. Check urine organic acids, acyl carnitines, and carnitine.
3. **Elevated Free Fatty Acids + β -OH Butyrate** –

If hepatomegaly, grossly abnormal liver function or clotting tests, consider fructose biphosphatase deficiency (hyperlactacidaemia), glucose-6 phosphatase deficiency, or neonatal haemochromatosis.

If hyperlactacidaemia, exclude septicaemia (CRP), respiratory chain defects (other organs affected and lactate ratio) and hereditary fructose intolerance (dietary history preceding event). Remember commonest cause for hyperlactacidaemia is cardiac, so must make sure coarctation or other critical outflow obstruction are excluded in neonate.

Otherwise, exclude cortisol and growth hormone deficiency, consider toxicological causes [plasma osmolality, ethanol and salicylate concentration], tyrosinaemia, maple syrup urine disease (plasma and urine amino acids) and other organic acidaemias.

Notes: FAO Defects can have hyperketosis:

Hyperketosis and hypoglycaemia especially with encephalopathy can occur in: (1) FAO Defects; (2) Ketone utilisation defects; (3) can be obscuring underlying organic acidosis. Specific unexplained features hepatomegaly, raised CK or ENCEPHALOPATHY are important clues.

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6. Mucopolysaccharidoses and Oligosaccharidoses

Mucopolysaccharides or glycosaminoglycans (GAGs) are complex heterosaccharides attached to specific proteins. They are degraded inside lysosomes. If a genetic defect exists, resulting in loss of a specific lysosomal enzyme, then there is chronic progressive storage of the metabolite.

The screening tests for these involve measuring the total GAG output/mmol creatinine (which is compared to age related reference ranges) and the glycosaminoglycans are separated by electrophoresis to identify an abnormal pattern.

There are three patterns of GAG excretion:

- 1) Increased dermatan sulphate and heparan sulphate (Types I/II/VI and VII)
- 2) Increased heparan sulphate in Type III
- 3) Increased keratan sulphate in Type IV

Additionally, thin-layer chromatography is performed to identify the oligosaccharidoses (including mannosidosis and fucosidosis). Oligosaccharidoses are rarer than GAG disorders (~ 1/5 less common). If specific features present, then oligosaccharide investigation is appropriate.

Presence of:

- 1) ORGANOMEGALY;
- 2) COARSE FACIES;
- 3) CATARACT;
- 4) DEAFNESS (especially mannosidosis);
- 5) X-Ray changes; and
- 6) ANGIOKERATOMA.

An alternative approach is to submit 10 mls EDTA whole blood for plasma oligosaccharide enzyme analysis.

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Confirming MPS and oligosaccharide disorders

The confirmation of the individual disorder requires specific enzyme analysis. The clinical separation of milder cases of Types I/II/VI and the sub-types of III is often impossible on examination of the individual. The clinical features below will help identify the exact diagnosis and limit unnecessary, expensive, and time consuming enzyme analysis:–

Type I (Hurler, Scheie, Hurler-Scheie)/II (Hunter)/VI (Maroteaux-Lamy) and VII (Sly) – all have similar excretion patterns. Those presenting shortly after birth are more likely to have Hurler’s disease with its classical features. Corneal opacities are more common in Types I and VI. Deafness and cardiac problems are more common in Type II. Type VI usually only appears beyond age 4.

Type III A/B/C/D Sanfilippo – is usually recognised after 2 years of age (often 4-5) with impaired mental development and/or hyperactivity spectrum. Thick eyebrows may be present, as well as hepatomegaly and impaired hearing. Other dysmorphic features are rare.

Type IV A/B Morquio – is not associated with mental retardation but impaired growth, bone dysplasia and joint contractures. Corneal opacity and facial dysmorphism may be present. There may only be hip abnormalities in IVB and these are late in onset. Radiology may be helpful.

These tests will not identify the full range of possible disorders. Another possible diagnosis is I-cell disease which often produces a normal GAG screen and is diagnosed by demonstrating increases in plasma acid hydrolase enzyme activities [2^o defect in incorporation of enzymes into lysosomes].

Discussion of these cases with a medical doctor or clinical scientist member of the Metabolic Section is advisable.

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7. Organic Acid Analysis

20 mls of urine should be collected into a plain universal container and frozen. It should remain sealed and frozen till arrival at QEUH Biochemistry. All samples routinely undergo both organic acid, urate and orotic acid analysis (if sufficient). A sample collected during/immediately following an acute metabolic decompensation is likely to yield the most informative data.

Samples collected in less acutely ill situations should have a creatinine concentration >1 mmol/L [i.e. they should not be colourless].

It is important to be aware of the instability of some metabolites (hence freezing), bacterial contamination of the sample or the effects from diet especially medium chain triglyceride supplemented feeds, or drugs ingested.

The three clinical presentations where organic acids are useful are:

1. Acute encephalopathy/acidosis/ketosis/hypoglycaemia, see also section 12, page 27.
2. Progressive neurological disease (especially if episodic)
3. Specific features such as:
Vomiting; self-imposed protein restriction; haematological abnormality.

Some key diagnostic compounds may be present in relatively small quantities even in asymptomatic patients – this reinforces the importance of including relevant clinical information with the request.

The interpretation of the chromatogram often requires a fine judgement of the significance of a small peak in a wealth of other peaks.

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8. Hyperlactacidaemia

Increased lactate is present from a wide variety of conditions. It is caused by tissue hypoperfusion +/- hypoxia (e.g. congenital Heart Disease in neonates-most common cause in this age group), drug/toxin ingestion (e.g. salicylates and ethanol), as well as a wide variety of inherited metabolic disorders.

9. Hyperammonaemia

Raised ammonia levels can be easily measured by certain analysers. In any drowsy, confused child and adult, it must be performed promptly, as an emergency request.

Raised ammonia levels may reflect liver disease (with abnormal clotting and transaminases) such as liver failure from paracetamol overdose, idiosyncratic response to valproate, hepatitis such as herpes simplex in neonates, or a urinary tract infection with a urea splitting organism where there is an obstructed uropathy. It may also be caused by a primary inherited metabolic disorder. Any disorder which results in build up of Propionyl CoA metabolites will result in hyperammonaemia due to inhibition of N-Acetyl glutamate synthetase which is a promoter of carbamyl phosphate synthetase (CPS). Thus, organic acid disorders (e.g. propionic acidaemia) or defects of fatty acid oxidation will be accompanied by raised ammonia levels during the acute episode of metabolic decompensation. Collection of urine for organic acids, blood spot for acyl carnitines and amino acid analysis should allow their identification.

Other causes of hyperammonaemia are defects of the urea cycle enzymes, or transporter defects of di-basic amino acids resulting in inadequate mitochondrial arginine (lysinuric protein intolerance and hyperornithinaemia-hyperammonaemia-homocitrullinaemia). These two disorders and all urea cycle disorders (except CPS and NAGs deficiencies) result in **raised orotic acid** formed from the alternative metabolism of carbamylphosphate.

Normal levels of orotic acid measured as part of a request for organic acids are found in carbamyl phosphate synthetase deficiency and N-acetylglutamate synthetase deficiencies. The laboratory should be informed about any case with high ammonia levels, to expedite

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amino acid, organic acid and orotic acid analyses.

Investigations

- Plasma aminoacids
- Urine organic acids including orotate

10. Late-onset Genetic Metabolic Encephaloneuropathies

Alois Alzheimer's original report in 1907 of a 51 year old female with jealousy towards her husband, increasing memory impairment and disorientation, who died bed-ridden, had the pathological features now described as metachromatic leukodystrophy. While no clear pathophysiological understanding of Alzheimer's Disease (as now described) is available, increasing number of conditions have been identified resulting from a single genetic mutation. Whilst most are untreatable (except very rare causes such as cerebrotendinous xanthomatosis pharmacologically), early diagnosis offers the possibility for careful prenatal counselling for other family members.

There is a vast array of disorders which may present in a variety of ways. 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 diagnoses.

Laboratory analyses can range from autoantibodies in SLE, drug screen for amphetamine and LSD, liver function tests to more complex metabolic studies. Progressive neurological and mental deterioration between 10 and 70 years of age can be separated depending upon predominant features of EXTRA PYRAMIDAL SIGNS, MYOCLONUS EPILEPSY, CEREBELLAR ATAXIA, POLYNEUROPATHY, PSYCHIATRIC, AND DIFFUSE CNS DISORDERS WITH LEUKODYSTROPHIC CHANGES ON CT.

A useful guide to identify the range of disorders is Chapter 65 (Clinical Phenotypes – Diagnosis/Algorithms in Scriver's Inherited Metabolic Diseases, Ed. 8). There are NO (biochemical) screening tests which are very sensitive for neurometabolic disorders as a group.

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While screening profiles do not exist, further initial help can be obtained from basic biochemistry; followed by more selective specialised biochemistry, leukocyte morphology and possibly molecular genetics.

A. EXTRA PYRAMIDAL

- Basic** Immunoglobulins (\downarrow IgA Ataxia-Telangiectasia),
Urine Copper, Serum Copper/Caeruloplasmin (Wilson's Disease),
Oligosaccharides (Gangliosidoses),
Lactate (Leigh's Syndrome – mitochondrial disorder),
Urate (Purine metabolic disorder)
Ammonia (Late-onset OCT)
Storage cells in bone marrow biopsy and in peripheral lymphocytes
(Niemann-Pick C)
Acanthocytes (Hallervarden-Spatz)
- Selective** Organic acids (may identify conditions normally presenting under 5)
Enzymes in purine metabolism
Mitochondrial DNA/enzymes
Sphingomyelinase (Niemann-Pick)
 β -Hexosaminidase (GM₂ Gangliosidosis)
 β -Galactosidase (GM₁ Gangliosidosis)

B. PERIPHERAL NEUROPATHY

- Basic** Acute presentation consider porphyrias (PBG) and Tyrosinaemia Type I
(Plasma amino acids)
Immunoglobulins, cortisol, vitamin E (tocopherol), lipoprotein and
apoproteins (Abetalipoproteinaemia), lactate (Leigh's
syndrome/respiratory chain disorder/Pyruvate dehydrogenase deficiency).
Storage cells in bone marrow and peripheral lymphocytes
- Selective** Phytanic acid (Refsum's disease)
 β -Galactocerebrosidase (Krabbe)
Aryl sulphatase A (Metachromatic leucodystrophy)
Desialo transferrin (Carbohydrate-Deficient Glycoprotein Syndrome)
Organic acids (3-hydroxy carboxylic aciduria)

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Very Long Chain Fatty Acids (peroxisomal disorder)

Nerve biopsy

α -Galactosidase (Fabry)

C. MYOCLONIC EPILEPSY

Basic

Lactate (respiratory chain disorder e.g. MERRF),

Ammonia (OCT deficiency)

Storage cells in bone marrow biopsy and peripheral lymphocyte
(Spielmeyer-Vogt-Vacuolated lymphocytes)

Selective

β -Glucocerebrosidase (Gaucher Disease Type 3)

β -Hexosaminidase (GM₂ Gangliosidosis)

Neuraminidase (Sialidosis Type 1)

D. CEREBELLAR ATAXIA

Basic

Lactate (respiratory chain disorders),

Immunoglobulins (low IgA – Ataxia Telangiectasia),

Lipoproteins (Abetalipoproteinaemia),

Ammonia

Storage cells in bone marrow and peripheral lymphocytes

Selective

Phytanic acid (Refsum's disease)

β -Hexosaminidase (GM₂ Gangliosidosis)

β -Glucocerebrosidase (Gaucher Disease)

Aryl sulphatase A (Metachromatic Leukodystrophy)

β -Galactocerebrosidase (Krabbe)

β -Galactosidase (GM₁ Gangliosidosis)

α -Neuraminidase (Sialidosis)

Cholesterol (Cerebrotendinous Xanthomatosis)

Apo B (abetalipoproteinaemia)

E. DIFFUSE LEUKODYSTROPHY

Basic

Cortisol/ACTH (Adrenoleukodystrophy),

CSF protein and lactate (Metachromatic leucodystrophy and respiratory chain disorders)

Storage cells in bone marrow or peripheral lymphocytes

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- Specific** β -Glucocerebrosidase (Gaucher III)
Aryl sulphatase A (Metachromatic leucodystrophy)
 β -Galacterocebrosidase (Krabbe Disease)
 β -Galactosidase (GM₁ Gangliosidosis)
- β -Hexosaminidase (GM₂ Types 1 and 2 Gangliosidosis)
Mitochondrial studies (Respiratory chain disorders)
Very Long Chain Fatty Acids (peroxisomal disorder)

These lists are not all inclusive but are the more common investigations. Please discuss with the laboratory about staged investigations following full clinical examination and possible neurophysiology studies and imaging.

10. Psychiatric Disorders

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

| <u>Problems</u> | <u>Possible Diagnoses</u> | <u>Biochemical Test</u> |
|--|--|---|
| Hyperactivity/ Behavioural disturbance | Sanfilippo | Urine glycosaminoglycans |
| Personality changes | Krabbe Metachromatic leukodystrophy | β -Galactocerebrosidase Aryl sulphatase A |
| Mental regression | Niemann-Pick C Adrenoleukodystrophy | Fibrillin staining of fibroblast cultures Very Long Chain Fatty Acids |
| Schizophrenia-like | OCT Deficiency | Ammonia & plasma amino acids and urine orotate |
| | Wilson's Disease | Urine copper Serum copper/ceruloplasmin |
| | Leigh Syndrome | Plasma lactate |
| | Methylene tetrahydrofate reductase deficiency | Urine amino acids, total homocysteine |
| | Spielmeigel-Vogt disease | Vacuolated lymphocytes |
| | Hallervorden Spatz | Acanthocytosis with retinitis pigmentosa |
| | Cerebrotendinous Xanthomatosis | Cholesterol |
| | Porphyria (AIP) | Urine Porphobilinogen |
| | Niemann Pick C | Fibrillin staining of fibroblast cultures |

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11. Peroxisomal disorders

Peroxisomal enzymes are involved in a number of essential steps in both degradation (particularly of very long chain fatty acids, pristanate, L-pipecolate, glyoxalate) and synthesis (e.g. bile acids, etherphospholipids and isoprenoids).

An increasing range of defects are now recognised. Peroxisomal disorders should be considered in children showing one or more:-

Craniofacial abnormalities +/- other dysmorphic features

Skeletal abnormalities including shortened proximal limbs and calcific stippling

Neurologic abnormalities including encephalopathy, hypotonia, seizures, hearing loss and cerebral abnormalities (neocortical dysgenesis/myelination problems).

Ocular abnormalities including retinopathy, cataract, optic nerve dysplasia and abnormalised electroretinogram +/- VEP.

Hepatic abnormalities including hepatomegaly, liver dysfunction, cholestasis and fibrosis/cirrhosis.

Most have no routine metabolic abnormalities on organic acid, urine and plasma amino acid analysis, or emergency profile. Their diagnosis depends on identifying the clinical phenotype. However, even where this is very typical, the biochemical phenotype may be atypical.

As a rule, those with neurological abnormalities will have abnormal neurophysiological studies. 9 of the 17 peroxisomal defects with neurological dysfunction have elevated Very Long Chain Fatty Acids, but phytanic acid, pristanic acid, urine bile acids and plasmalogens are also measured to try and identify the other missed defects. Initially consider:

- Plasma Very Long Chain Fatty Acids
- Plasma Bile Acids (GC-MS) ± urine bile acids,
- Plasmalogens

then:

- Specific Enzymes

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12. Metabolic Causes of Cataracts

In less than 10% of cases of congenital cataract, can an underlying metabolic cause be demonstrated. The following are the major first line causes:

| <u>Time when Cataract first visible</u> | <u>Investigations</u> |
|---|--|
| <u>At birth</u> Lowe's Syndrome Zellweger Syndrome Rhizomelic chondrodysplasia punctata Sorbitol dehydrogenase deficiency | Phenotype, EEG, Urine Aminoacids ± GAGs. Phenotype plus Peroxisomal Investigations Phenotype plus Peroxisomal Investigations |
| <u>After 5 days</u> Galactosaemia Marginal maternal galactokinase deficiency Hyperglycaemia | GAL-1 PUT Maternal Urine Sugar Chromatography ± Enzymology. |
| <u>After 4 weeks</u> Galactokinase deficiency Partial galactokinase deficiency Oligosaccharide disorders Mitochondrial myopathy | Galactitol (urine) Galactitol Urine oligosaccharides (GAG request + features) Plasma Lactate ± DNA mutations. |
| <u>In Child</u> Diabetes mellitus Wilson's Disease Hypoparathyroidism Pseudohypoparathyroidism Fabry's Disease | Plasma Glucose Plasma Caeruloplasmin and copper Plasma Calcium, PTH Hands, Plasma Calcium α-Galactosidase |

13. Cardiomyopathy

Two distinct echocardiographic pictures (Hypertrophy and Dilation) are found in individuals with cardiac enlargement. Echocardiography also excludes hypertrophic changes associated with aortic stenosis and coarctation of the aorta.

DILATED CARDIOMYOPATHY

The majority are idiopathic. Some are familial; from viral myocarditis; related to connective tissue disorders; neurological disorders, hyperthyroidism, nutritional deficiencies of thiamine, selenium and vitamin E; or are reaction to drugs such as antibiotics, dexamethasone or anthracycline.

While most metabolic diseases have infiltrative (hypertrophic) changes, the distinction may be difficult. Disorders to consider following exclusion of non-metabolic causes are 1^o or 2^o carnitine deficiency, disorders of oxidative phosphorylation, mitochondrial cytopathies, childhood haemochromatosis and 3-methylglutaconic aciduria.

HYPERTROPHIC/INFILTRATIVE CARDIOMYOPATHY

The AD hypertrophic obstructive cardiomyopathy and non metabolic causes such as Noonan's Syndrome, hyperthyroidism and lipodystrophy need considered.

Conditions where infiltrative cardiomyopathy may be presenting feature with others:-

- | | |
|--|--|
| Pompe (GSD II) | - large tongue, muscle weakness - fibroblast enzyme |
| GSD IV | - liver failure |
| Disorder of oxidative phosphorylation Respiratory Chain Disorder Mitochondrial Cytopathies | } - lactic acidosis, liver disease, muscle disease, cataract and developmental delay |
| 3-methylglutaconic aciduria | - severe developmental delay |
| Carbohydrate deficient Glycoprotein Syndrome | - Cardiac and pleural effusion, dysmorphism, FTT and skin anomalies |
| 1 ^o /2 ^o Carnitine Defects | - Myopathy, hepatomegaly +/- organic aciduria +/- hypoglycaemia |

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Conditions where other pathological processes dominate

Nesidioblastosis

GSD III - hypoglycaemia and hepatomegaly

Organic aciduria - recurrent ketosis and encephalopathy

I cell Disease

MPS I/II/VI

Oligosaccharidosis

Gaucher's Disease

Type 1 Tyrosinaemia

Inborn errors of B₁₂ metabolism- developmental delay, haemolytic uraemic syndrome

Isolated Cardiomyopathy

Rarely respiratory chain disorders and phosphorylase kinase deficiency limited to cardiac muscle occur.

Useful first time investigations:-

Plasma amino acids, lactate (2 hours after meal with carbohydrate), plasma carnitine, Ferritin and iron, thyroid function tests, creatine kinase, blood film for vacuolated lymphocytes and urine for organic acids and glycosaminoglycans. Consider carbohydrate deficient transferrin.

Further specialist investigations:- (obtain correct specimen, particularly if rapidly dying)

Skeletal muscle and cardiac muscle biopsies, DNA studies, e.g. dystrophin gene, specific fibroblast or leucocyte enzyme and liver biopsy, and enzymology for iron stores, GSDs and mitochondrial disorders.

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14. Neonatal Presentations

Please inform laboratory whether the child is ingesting adequate protein; maternal health (HELLP → LCHADD in fetus); previous neonatal sibling deaths; organomegaly; acidosis + pancytopenia in the fetus. The vast majority of inherited metabolic disorders in neonates fit into the following simple system:

ENCEPHALOPATHY without ACIDOSIS –

Maple Syrup Urine Disease – usually day 4 – 10. Progressive encephalopathy. Raised ammonia. Ketones in urine. Seizures may occur later.

Urea Cycle Disorders – usually day 3 – 5.
- respiratory alkalosis and hyperammonaemia.

Seizures in First 4 Days Only ~ 7% of all infantile seizures are metabolic in origin, most onset later after encephalopathy.

-**Pyridoxine – Responsive seizures** - responds to pyridoxine.

-**Non-ketotic Hyperglycinaemia** – please inform lab of concerns.

Routine biochemistry normal. Collect plasma + CSF for glycine analysis.

-**Sulphite oxidase** – usually associated with low/undetectable urate < 0.1 mmol/l

-**Peroxisomal disorder** – often mild facial dysmorphism.

-elevated VLCFA's /Phytanic acid.

ENCEPHALOPATHY with ACIDOSIS

Organic Acidosis – associated with pancytopenia + hyperammonaemia especially PA / MMA/ Holocarboxylase.

Dicarboxylic aciduria – MCAD may present very early as can long chain hydroxyl acyl + very long chain acyl dehydrogenase deficiency associated with hypoglycaemia.

Lactic acidemia – exclude cardio respiratory defects first.

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HEPATIC PRESENTATIONS

Jaundice

- Unconjugated: usually benign, consider haemolysis and non-IEM causes
 - unless > 250 $\mu\text{mol/l}$ at 2 weeks in Crigler-Najjar.
- Conjugated + FTT: remember non-IEM causes e.g. biliary atresia
 - α_1 antitrypsin (check phenotype), CF (IRT by blood spot),
 - peroxisomal disorders + rarer causes.

HEPATIC Dysfunction (Gross clotting problems)

- **Tyrosinaemia**
 - associated with increased plasma tyrosine and urine tyrosuria.
 - AFP raised (NB age+gestation related reference ranges) and alkaline phosphatase often > 2000 u/l.
 - Check plasma/urine amino acids first. Then succinylacetone or 7-oxo-heptanoate.
- **Galactosaemia**
 - Gal-1-P Uridyl transferase
- **Hereditary Fructose Intolerance**
 - not from UK milks; symptoms will only appear after weaning
- **Neonatal Haemochromatosis**
- **Respiratory chain/Mitochondrial Disorder**
- **Hypoglycaemia**
 - without acidosis, hyperammonaemia or hepato cellular dysfunction.
- **GSD I** (raised urate, Lactate, Trigs),
- **Fructose 1, 6 bis Phosphatase** (Grossly raised lactate).
 - with encephalopathy remember FAO defects.
 - adrenal insufficiency (? sex ambiguity or micropenis).
- **Dysmorphic / Storage Disorders**
 - Smith - Lemi – Opitz → measure 7 dehydrocholesterol.
 - Zellweger Syndrome → measure VLCFA's.
- **Hydrops fetalis**
 -
 - Consider I-cell disease.

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- MPS VII.

Ketoacidosis and Encephalopathy+/- Hypoglycaemia :-

Idiopathic Ketotic hypoglycaemia is the commonest (benign) cause. It is a disorder arrived at by exclusion. Certain clinical features should demand further investigation:

- 1) Delayed response (> 15 minutes) to glucose therapy – any slowness in response requires further investigations.
- 2) Hepatomegaly.
- 3) Raised CK.
- 4) Low carnitine.

Ketoacidosis and Encephalopathy with or without hypoglycaemia.

Consider

- 1) FAO Defect – MCAD – Ratio of β OH Butyrate / NEFA < 0.7.
 - VLCAD - low free carnitine.
 - gross Ketosis possible.
 - hepatomegaly and raised CK.
 - acylcarnitine profile required.
- 2) MSUD
- 3) Organic acid disorder masked by gross ketonuria e.g. holocarboxylase.
 Repeat organic acids as encephalopathy resolving.
- 4) Ketone utilisation defect - may be asymptomatic with ketosis.
 Check ketones disappear after meals.
- 5) Endocrine disorders especially hypogluccorticoid states.

Intellectual Disability

Biochemical screening for causes of intellectual disability has a **very limited** yield. Surveys have revealed the following incidences – %

| | |
|------------------------------|--------------|
| Chromosomal abnormalities | 4 – 28 |
| Recognisable syndrome | 3 – 7 |
| Structural CNS abnormalities | 7 – 17 |
| Complications of prematurity | 2 – 11 |
| Metabolic / endocrine | 1 – 5 |
| Unknown | 30 – 50 |

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With time specific features may evolve. The use of random screening urine organic / amino acids results in high frequency of non-specific, non-diagnostic abnormalities.

Suggested first line screening –

(i.e. 3-4mls Li Hep, 1ml EDTA (FBC), 4mls Li Hep to genetics)

CK Duchenne’s, Carnitine Palmitoyl Transferase 2

Calcium di George, Williams, Pseudohypoparathyroidism

Chromosomes

Fragile X Screen

TFTs (free T4, TSH)

Urate

UE - ? Acidosis – suggesting further investigations with organic acidosis.

FBC

Blood Lead

Biotinidase

Features which may indicate the need for further metabolic investigations:

- Parental consanguinity or family history
- Developmental Regression
- Failure of appropriate growth
- Recurrent unexplained illness
- Seizures e.g. CSF and plasma glucose / amino acids
- Ataxia see pages 26/27
- Loss of psychomotor skills – GAG’s
- Hypotonia
- Coarse appearance – GAG’s/oligosaccharides
- Eye abnormalities - VLCFA and oligosaccharide analysis
- Recurrent somnolence/ coma – Ammonia and orotate
- Abnormal sexual differentiation – urine steroid profile
- Arachnodactyly
- Hepatosplenomegaly
- Unexplained deafness – Oligo’s, (? β -mannosidosis)

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- Bone abnormalities – GAG's, Copper, VLCFA
- Skin abnormalities – GAG's, plasma amino acids

A suggested second-line screen in a child with no specific features, including those with behavioural problems, includes:

NEUROIMAGING : consider if abnormal head size, focal neurology (e.g. spasticity, dystonia, seizures, increase or loss of reflexes)

UGAGS by DMB quantitation – Sanfilippo (MPS 111)

Oligosaccharides – where specific features: deafness, coarse facies, bony abnormalities,

eye signs, hepatosplenomegaly, angiokeratoma

Urine organic acids and orotate

Plasma Lactate

Plasma free Carnitine

Plasma amino acids

Plasma Very Long Chain Fatty Acids

Plasma Ammonia

Plasma Total Homocysteine

Urine Creatine and guanidinoacetate – to exclude creatine synthesis defects.

Current Lab Investigations on Frozen/Rapidly submitted urine samples with developmental delay.

- **Urine organic acids**
- **Urine amino acids**
- **DMB to exclude Sanfillipo Disease**
- **Urine Creatine & GAA**

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Appendix A

REQUIREMENTS AND TREATMENT OF SAMPLES FOR AMINO ACID ANALYSIS

Urine

Timed specimens of urine are collected, over 24 or 12 hours if practicable, into bottles containing one or two large crystals of thymol as preservative. Entire 24 hour collections may be tendered directly to QEUH Biochemistry for further processing, though it is often more convenient for the referring laboratory to forward only a portion of the collection. Two 20 ml aliquots in securely capped universal containers are usually enough to complete the full profile of screening tests.

Casual specimens of urine may be submitted whenever collection of timed specimens is impracticable, providing that the volume of urine collected is not less than 25 ml and that the specimen is not obtained by forced diuresis. One large thymol crystal may be added to each fresh casual specimen on receipt.

Storage of urine specimens must be minimised. If the aliquot(s) cannot be forwarded to QEUH shortly after collection, then it may be frozen overnight before prompt despatch on the following working day.

Blood

3 ml fasting or pre-feed venous blood is collected into a lithium heparin tube and must be received for pre-treatment by the referring laboratory within three hours of the time of collection.

Pre-treatment:

Spin tube at once in cooled centrifuge for minimum of 10 minutes at 2500g (~3500 → 4000 rpm)

Separate plasma without delay into plain 2ml tube(s)

Deproteinise at least 1 ml plasma immediately

Add 50 mg AnalaR 5-sulphosalicylic acid for each 1 ml plasma being treated

Agitate capped tube on vortex mixer to dissolve acid crystals

Uncap tube slowly to reduce effervescence from release of CO₂

Recap tube and wait for minimum of 10 minutes to denature plasma proteins

Spin tube for 20 minutes in cooled centrifuge at 2500g

Separate clear supernatant into labelled plain 2 ml tube marked 'SUPERNATANT'

Avoid transfer of protein particles but, if present, re-spin and re-separate

Transfer any untreated plasma into labelled plain 2 ml tube marked 'PLASMA'

Storage of treated supernatant must be minimised. If the supernatant(s) cannot be forwarded to QEUH shortly after treatment, then it may be stored frozen overnight before prompt despatch on the following working day.

Appendix B

Common Metabolic Investigations and their sample types

| Blood | |
|---|--|
| Hypoglycaemia | Both required for full range of investigations in suspected hypoglycaemia (see Section 2) Specifically for neonates under 3 months. |
| Alpha 1 antitrypsin phenotype | 1 ml heparinised |
| Amino acids | 2 ml fasting heparinised blood |
| Ammonia | 0.5 ml heparinised - rapid delivery |
| β -Hydroxy butyrate | 1 ml heparinised - rapid delivery |
| Bile acids for peroxisomal disorders | 2 ml Heparinised – separate and protect from light |
| Carnitine | 2 ml heparinised |
| Cellular enzymes & Cystine (white cell) | Contact laboratory before taking 5ml heparinised blood |
| Desialo transferrin | 2 ml heparin |
| Free Fatty Acids/NEFA | 1 ml fluoride/heparin |
| G6PD | 1ml EDTA (purple top) |
| Galactose | 1 ml fluoride (grey top) |
| Galactosaemia screen (transferase) | 1 ml heparinised blood – RBCs or whole blood |
| Gal-1-phosphate | 3 ml heparin – RBC on whole blood |
| Homocysteine | 1 ml heparin – rapid delivery |
| Lactate | 0.5 ml heparin - rapid delivery |
| Porphyria | 2 mls EDTA blood Fresh sample faeces and urine Protect from light |
| Very Long Chain Fatty Acid (Peroxisomal disorders) | 2 mls heparin |
| Blood Spots | |
| Acylcarnitine | Bloodspot card (All 4 spots please) |
| Biopterin | 3 or more bloodspots |
| Maple Syrup urine disease (leucine, isoleucine, valine) | Monitoring only. 1 (or ideally 2) }bloodspots |
| Phenylalanine/Tyrosine | |
| α Galactosidase (Fabry) | Ensure blood spots dried for 4 hours at room temperature, ideally overnight |
| α Glucosidase (Pompe) | Ensure blood spots dried for 4 hours at room temperature, ideally overnight |
| CSF | |
| Amino acids | 1 ml plain |
| Glucose | 0.5 ml Fluoride |
| Urine | |
| Amino acids | 20 mls plain |
| Bile acids | 5 - 20 ml plain |
| Glycosaminoglycans and oligosaccharides | 20 mls plain |
| Organic acids | 20 mls freeze while collecting or rapid delivery |

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Appendix C

POST MORTEM PROTOCOLS

Unfortunately, a number of children die from an acute illness without its pathophysiological causes being identified. Where a metabolic cause is suspected, a number of tissues should be collected and frozen for further diagnostic assessment.

A dried bloodspot card with blood and another with bile, plasma, urine (~10 ml) and CSF (~4 ml) should be collected and frozen - 20°C (ideally before death). Similarly, 10-20 ml EDTA blood frozen at -70°C can subsequently be used for chromosome and DNA analysis.

Enzyme diagnosis can be made on liver (min. 10 - 20 mg net weight) and muscle (min. 20 - 50 mg wet weight) which can be collected either by needle biopsy or open incision. There are advantages to multiple biopsies at different sites in case an enzyme defect is expressed in a tissue-specific pattern. The tissue samples are immediately frozen - ideally with liquid nitrogen. Please discuss, in case fresh material is required. The liver and muscle biopsy should also be fixed for histology and electron microscopy.

Collection of whole blood for leucocyte preparation is useful pre-mortem. Similarly, two skin fibroblast biopsies should be placed in culture medium or alternatively in sterile saline overnight, prior to culture preparation.

Difficulties in interpreting post mortem samples

Unfortunately, rapid tissue lysis is common just before death in a group of acutely ill children. Thus, free carnitine, ammonia, lactate and amino acids increase rapidly without any specific significance.

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Appendix D

Useful Reference Texts

1. The Metabolic and Molecular Bases for Inherited Disease. Edⁿ8 2001.
 Editors: Scriver, Beaudet, Valle, Sly
 ISBN: 0-07-116336-0
Principal textbook on subject in 4 volumes. Chapter 65 - Clinical Phenotypes: Diagnosis/Algorithms is particularly useful for identifying likely diagnoses for a clinical symptom/sign.
2. Physician's Guide to the Diagnosis of Metabolic Disease.
 2nd Edition 2003
 Editors: Blau, Duran, Blaskovics
 ISBN: 3-540-42542-X
Helpful in interpretation of metabolites identified in the wide variety of conditions.
3. Inborn metabolic disease. Diagnosis and Treatment. 5th Edition 2012
 Editors: Saudubray, van den Berghe, Walter
 ISBN: 978-3-642-15719-6
Readable textbook with good clinical management guidelines.

Appendix E

CLINICAL SYNOPSIS FOR METABOLIC REQUESTS

CLINICAL SYNOPSIS FOR METABOLIC REQUESTS

(amino acids, organic acids, carnitine, glycosaminoglycans, cellular enzymes and other complex tests)

**

NAME: _____
HOSP NO: _____
CHI: _____
DOB: _____
SEX: _____
WARD: _____
HOSPITAL: _____
CONSULTANT: _____

**** - Label (s) may be used provided all fields are present**

Referring Laboratory Number: _____

Tests required: _____

Date of specimen: ___ / ___ / ___
Time of specimen: ___ : ___
Date & time of admission/acute symptoms: ___ / ___ / ___ ___ : ___

History: _____

Family history: Consanguinous Yes / No
 Previous sibling death or affected case? _____

Nutrition:

Feeding: Breast fed
 Formula feeds state type: _____
 Normal diet
 TPN
 Confirm protein >2g/kg Yes/No
 Any supplements (e.g. MCT) _____

Drugs

| | N | Y | if 'Y', give details |
|--------------------------------|--------------------------|--------------------------|-------------------------|
| Hypoglycaemia | <input type="checkbox"/> | <input type="checkbox"/> | _____ |
| Hepatomegaly | <input type="checkbox"/> | <input type="checkbox"/> | _____ |
| Dysmorphic features | <input type="checkbox"/> | <input type="checkbox"/> | _____ |
| Failure to thrive | <input type="checkbox"/> | <input type="checkbox"/> | _____ |
| Developmental delay | <input type="checkbox"/> | <input type="checkbox"/> | _____ |
| Regression | <input type="checkbox"/> | <input type="checkbox"/> | _____ |
| Abnormal neurological findings | <input type="checkbox"/> | <input type="checkbox"/> | _____ _____ _____ |
| Imaging abnormalities | <input type="checkbox"/> | <input type="checkbox"/> | _____ _____ _____ |

Significant Laboratory Results (if known/relevant)

| | N | Y | |
|----------------------|--------------------------|--------------------------|--------------|
| Acidosis | <input type="checkbox"/> | <input type="checkbox"/> | |
| Neutropenia | <input type="checkbox"/> | <input type="checkbox"/> | |
| Glucose | <input type="checkbox"/> | <input type="checkbox"/> | _____ mmol/l |
| Lactate | <input type="checkbox"/> | <input type="checkbox"/> | _____ mmol/l |
| Ammonia | <input type="checkbox"/> | <input type="checkbox"/> | _____ umol/l |
| Cortisol | <input type="checkbox"/> | <input type="checkbox"/> | _____ nmol/l |
| Liver Function Tests | _____ _____ | | |

Further advice is available from:
 Dr Galloway (07711731559)
 and metabolic biochemist (07505255241).