SHEFFIELD CHILDREN'S NHS FOUNDATION TRUST

CLINICAL CHEMISTRY
&
SHEFFIELD DIAGNOSTIC GENETICS SERVICE

USER'S HANDBOOK FOR METABOLIC INVESTIGATIONS

APRIL 2017

Do Not Use This Edition After March 2018

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CLINICAL CHEMISTRY
&
SHEFFIELD DIAGNOSTIC GENETICS SERVICE

SHEFFIELD CHILDREN'S NHS FOUNDATION TRUST
WESTERN BANK
SHEFFIELD S10 2TH

Clinical Chemistry

Website

http://www.sheffieldlab.org.uk

Telephone numbers & enquiries

Hospital switchboard: 0114 271 7000
Laboratory Fax: 0114 270 6121

	Direct Telephone
Professor J R Bonham, Director of Pharmacy, Diagnostics & Genetics & Consultant Clinical Scientist	2353831
Camilla Scott (Head of Department), Consultant Clinical Scientist)	2717404
Camilla Scott and Professor Bonham's PA – Lynne Darwin	2717318
Mr Philip Craddock – Laboratory Manager	2717444
Mr Craddock's secretary – Alison Lenthall	2717340
Duty Clinical Scientist	Bleep No 095

(From outside the hospital please dial the switchboard and request bleep 095)

Metabolic Section

Result enquiries	2717445
Dr Jane Dalley (Principal Clinical Scientist, Metabolic Lead)	2717307
Claire Hart (Clinical Scientist)	2717307
Jenny Watkinson (Lead Biomedical Scientist)	2717445
Louisa Ann Smith (Senior Biomedical Scientist)	2717405
Helen Chapman (Senior Biomedical Scientist)	

Tissue Culture Section

Joanne Croft (Principal Clinical Scientist, Tissue Culture and Enzyme Assay Lead)	2717267
Dr Simon Olpin (Tissue Culture and Enzyme Assay) + answer phone	2717267
Prenatal diagnosis enquiries	2717267

Newborn Screening Section

Dr Lynette Shakespeare (Screening Lead Scientist)	2717302
Catherine Dibden (Clinical Scientist)	2717346
Joyce Baston (Lead Biomedical Scientist)	2717500
Sheila Ellin (Senior Biomedical Scientist)	2717346
Ullas Cherachathoor (Senior Biomedical Scientist)	2717346
Newborn Screening Results (09:00-12:30) and Answering Machine	2717257
Newborn Screening Fax	2717263

Sheffield Diagnostic Genetics Service

Telephone numbers & enquiries

Ann Dalton (Director of Pharmacy, Diagnostics & Genetics & Head of Laboratory)	2717004
Richard Kirk (Lead Clinical Scientist – Inborn Errors of Metabolism)	3053641
Mandy Nesbitt (Clinical Scientist – Inborn Errors of Metabolism)	2260584
Nick Beauchamp (Clinical Scientist – Neurodegenerative/Mitochondrial Diseases	2260599
Laboratory fax	2750629
Email SDGS@sch.nhs.uk	

SERVICES PROVIDED

A Specialised service is provided as follows:

Inborn Errors of Metabolism

A regional service is provided by Clinical Chemistry for the investigation of suspected metabolic disorder. This service is available to the Sheffield Children's NHS Foundation Trust without cross charging and to other users on a cost per test basis (see pages 27-30).

Newborn Screening

Screening covers all babies born in the East Midlands SHA, South Yorkshire and South Humberside portion of the Yorkshire and Humber SHA (Derbyshire, Leicestershire, Lincolnshire, Northamptonshire, Nottinghamshire, Rutland, and South Yorkshire). They are tested for phenylketonuria (phenylalanine), congenital hypothyroidism (TSH), cystic fibrosis (immunoreactive trypsin), medium chain acyl CoA dehydrogenase (octanoylcarnitine), sickle cell disorders (haemoglobin profile), maple syrup urine disease (MSUD) (leucine), homocystinuria (methionine), isovaleric acidaemia (IVA) (isovalerylcarnitine) and glutaric aciduria type 1 (GA1) (glutarylcarnitine) using dried blood spots from the newborn screening cards.

Sheffield Diagnostic Genetics Service

Molecular Genetics is the analysis of the detailed structure of the Human genome. The aim is to establish the mutation or mutations that have given rise to the disorder in that individual or family. The laboratory investigates both familial and sporadic conditions, including molecular changes that give rise to malignant conditions.

NORMAL LABORATORY OPENING TIMES

Clinical Chemistry, Newborn Screening	Monday to Friday 9:00am- 5:00pm
Sheffield Diagnostic Genetics Service	Monday to Friday 9:00am- 5:30pm

REQUESTS FOR ANALYSES

Legible request forms (see below) must accompany all samples.
Every sample for which an analysis is required, other than those for routine newborn screening, must be accompanied by a FULLY COMPLETED laboratory request form signed by the doctor making the request and giving his/her bleep number. It is also important to include the ***time*** and ***date*** on which the sample was collected plus clinical details.

THE COMPLETION OF REQUEST FORMS

All samples including DBS samples must be accompanied by a request form (except DBS sample for Newborn Screening or DBS samples for monitoring amino acids.

All request forms *must* contain a minimum of the following essential information:

1. Full name (initials will be classed as missing information)
2. DoB (age only will be classed as missing information)
3. At least one of the following:
    - Hospital number
    - A/E or Majax number
    - NHS number
    - Clinical Genetics Family ID
4. Name of the requesting consultant (or referring laboratory)
5. Location where results are to be sent.
6. Test required.
7. Requesting hospital/location.
8. Sample type.

The following information is also *highly* desirable. Specific labelling requirements apply to Newborn Screening Samples. The appropriate national guideline should be consulted:
www.gov.uk/government/publications/newborn-blood-spot-screening-sampling-guidelines

9. Name of the person collecting/obtaining the sample.
10. Date & time sample(s) taken (particularly if more than one sample is likely to obtained on the same day)
11. Clinical details (full and appropriate clinical details including circumstances that may increase the risk of infection, provisional diagnosis and current drug therapy.
12. Patient's address including postcode.
13. Patient's sex.
14. Clinician's bleep number.

Clinical details and the patient's age are particularly important in requesting so that laboratory staff may:

1. Understand the reason for the request.
2. Interpret the results.
3. Consider the need for further investigations.
4. Advise and assist the clinical staff concerning the results obtained.

For urgent or telephoned requests, it is helpful to have the signature of the medical officer and the legible printed name for urgent or telephoned results.

SPECIMEN CONTAINERS

Please ensure that specimens are in suitable containers, otherwise they may be rejected for analysis.

Plasma samples – maximum **height** of the container should not exceed 55mm.
Urine samples – maximum **height** of the container should not exceed 100mm, width should not exceed 30mm.

PLEASE DO NOT SEND SAMPLES IN MICROCUPS OR TUBES WITH PUSH ON CAPS – THEY WILL NOT BE ACCEPTED.
These are a health and safety risk to us and also result in the loss of sample volume when we transfer them to an acceptable container.

Please do not use carrier tubes without the inner tube being labelled, we regard these as unlabelled samples and they will be rejected.

LABELLING OF PATHOLOGICAL SAMPLES

When collecting and labelling samples, the criteria for patient identification (outlined earlier) must be followed. **Sample and request form information *must* also be compatible**. Samples will only be accepted for analysis if minimum criteria are met. **This responsibility lies with the person collecting the sample.** Failure to meet these requirements may result in the sample being rejected.

Minimum Criteria
As defined by laboratory policy all pathological samples sent to the laboratory **must** contain a minimum of the following information:

1. Surname/family name.
2. Forename (or Baby, Twin One/Two, Triplet One/Two/Three etc, if forenames have not been given. Initials will be classed as missing information)
3. At least one of the following:

    • Date of birth (age only will be classed as missing information)
    • Hospital registration number
    • A/E or Majax number
    • NHS number

And ideally for samples being tested for patient monitoring purposes the following must also be included.

    • Date sample taken
    • Sample type

Sub sample e.g. plasma or serum

Must contain at least **two** of the following identifiers:

- ***NHS number (****Important identifier)*
- A/E number
- Hospital registration number
- Surname/family name
- Forename (or Baby, Twin 1 etc, if forenames have not been given)
- Date of birth

- Requesting laboratory number

Legal Responsibilities

In signing a request form the person making the request assumes responsibility under Section 7 of the Health and Safety at Work Act and will be assumed to be familiar with its requirements in relation to danger of infection.

To fulfil these regulations you must comply with the following:

1 All samples must be in a sealed plastic bag and the request form placed in the separate compartment provided.
2 Full and appropriate clinical details and danger of infection labels on both request form and sample are required from Category 3 risk patients.
3 Samples from patients with suspected and proven HIV infection must also be enclosed in a cardboard box.
4 Data on request form may be stored on laboratory computer files. It is assumed the person completing the form has done so in accordance with the requirements of the Data Protection Act 1984.

The attention of medical staff is drawn to the warning notice printed upon each laboratory request form concerning specimens which might carry a risk of infection. The doctor completing the request should also indicate on the form if the patient has a communicable disease such as rubella, for the protection of any laboratory staff who might attend the patient.

URGENT REQUESTS

Urgent requests must be arranged with the laboratory by telephone so that if there is any delay in receipt, steps can be taken to locate the sample. Urgent samples which arrive in the laboratory without prior arrangement run the risk of being delayed, as they will be analysed routinely.
Out of routine hours metabolic tests are not performed.
In extreme and urgent circumstances some tests may be performed out of hours, but only after discussing with the Consultant Clinical Scientist.

SPECIMEN TRANSPORT

Specimens must be sent to the laboratory contained in a transparent leak proof plastic bag. The request form must be separated from the specimen. Any label indicating a danger of infection must be shown on the request form.

Urgent samples must be arranged with this laboratory before dispatch and ***sent by courier***

Non-urgent samples
Suitable postal or other delivery arrangements must be made by the sending laboratory. Samples must be sent direct to the laboratory; we cannot undertake to collect samples from rail stations or other collection points. Contact the laboratory before dispatch if the request is unusual or urgent. Details of sample preservation and packaging are given below. Charges are detailed on pages 27-30.

Post Office regulations require that all pathological samples are sent by first class post. The use of second class letter or parcel post is specifically forbidden. Padded envelopes used alone without a suitable inner container are not permitted. The regulations (RML 12/87) are summarised below.

1. Hazard group 4 pathogens are prohibited, other pathological specimens may be sent provided that they comply with the regulations.

2. Specimens may be sent by qualified medical, dental or veterinary practitioners, a registered nurse, a recognised laboratory or institution.

3. Members of the public may not send such specimens unless requested to do so by one of the above who must supply them with the required packaging and instructions.

4. Only first class letter or Datapost may be used.

5. There is a range of acceptable packaging but the following must be observed.

6. Every specimen must be in a primary container hermetically sealed or otherwise securely closed. The capacity of the primary container must not exceed 50 mL unless specifically permitted. The primary container must be wrapped in enough absorbent material to absorb all possible leakage, and sealed in a leakproof plastic bag.

7. The container and its immediate packaging must be placed in one of the following:
    a) a polypropylene clip-down container
    b) a cylindrical light-metal container
    c) a strong cardboard box with a full-depth lid
    d) the appropriate groove in a two piece polystyrene box, empty spaces must be filled with absorbent material, the box must be secured with self-adhesive tape.

8. A padded outer bag is recommended.

9. Soft absorbent packaging must be used between samples to prevent contact.

10. Written agreement from the Post Office is required for non-standard packaging.

11. The outer packaging must be labelled 'PATHOLOGICAL SPECIMEN - FRAGILE WITH CARE' with the name and address of sender.

12. Therapeutic and diagnostic materials such as blood products are accepted under the same conditions.

13. Packets found in the post which contravene the regulations will be detained and may be destroyed. Any person who sends deleterious substances without conforming to the regulations may be liable to prosecution.

Please note. Infectious pathology samples may only be transported in packaging which meets the U.N. class 6.2 specifications and the 602 packaging requirements. These new packaging requirements are described below:

BASIC TRIPLE PACKAGING SYSTEM.

The system consists of three layers as follows:

Primary receptacle
A labelled primary watertight, leak-proof receptacle containing the sample. The receptacle is wrapped in enough absorbent material to absorb all fluid in case of breakage.

Secondary receptacle
A second durable, watertight, leak-proof receptacle to enclose and protect the primary receptacle(s). Several wrapped primary receptacles may be placed in one secondary receptacle. Sufficient additional absorbent material must be used to cushion multiple primary receptacles.

Outer shipping package
The secondary receptacle is placed in an outer shipping package which protects it and its contents from outside influences such as physical damage and water while in transit.

Information concerning the sample, such as data forms, letters and other types of information that identify or describe the sample and the identity of the shipper and receiver should be taped to the outside of the secondary receptacle.

Newborn Screening Dried Blood Spot (Guthrie) Cards

By common consent these regulations are deemed inappropriate for dried blood specimens on Newborn Screening (Guthrie) cards. The blood spots should be allowed to **dry thoroughly before packing** the card placed in the transparent paper (Glassine) envelope provided (not plastic as this may cause the specimen to "sweat") and sent, by first class post, in a stout envelope as if it were a normal letter or in a newborn screening pre paid envelope according to local arrangements.

Please accompany all dried blood spot samples other than those for routine newborn screening with a fully completed laboratory request form.

Tissue Culture

For skin biopsies sent from external hospitals within the Trent Inherited Metabolic Disease Group a request form with full clinical details and test request is required. Sample transport at room temperature, normal first class post to Clinical Chemistry Department to arrive ideally no later than 4.30pm Mon – Fri. Please contact laboratory if sample to arrive on the weekend (0114 271 7445 or 271 7267).

For skin biopsies sent from external hospitals outside the Trent Inherited Metabolic Disease Group, please contact the Tissue Culture laboratory prior to sample collection to discuss sample collection details and turnaround times. A request form with full clinical details and test request is required.

Please note turnaround times (TAT) are flexible when applied to cultured cell assays. As different patient cell lines grow at different rates. In general for most assays starting from a skin biopsy the TAT is 8-12 weeks

QUALITY ASSURANCE

The Department participates in national external quality assurance schemes to monitor the accuracy and precision of its analyses. Internal quality control is used to check the validity of results on a day to day basis. The laboratory computer also checks the credibility of individual results.

It is important that the laboratory be informed at once if results appear inconsistent with a patient's condition or are at variance with previous results.

SPECIALISED SERVICES

INVESTIGATION OF INBORN ERRORS OF METABOLISM

A service is provided for the detection, diagnosis and monitoring of patients with inborn errors of metabolism. Analyses performed include:

Acylcarnitine profile
Amino acids
Ammonia
Bile Salts
Biotinidase
Carnitine
Cholestanol
Collagen Cross Links
7-Dehydrocholesterol
Dimethylglycine
Ethylmalonic acid (quantitative)
Free fatty acids
Galactitol
Galactosaemia Screen
2OH glutaric acid (chirality)
Glutarate (quantitative)
Glycosaminoglycans (screen and electrophoresis)
Hexanoylglycine (quantitative)
Homocysteine (total)
Homocysteine (free)
HVA/VMA (quantitative)
3-hydroxybutyrate
Isovalerylglycine (quantitative)
Lactate
Methylmalonate (quantitative)
Organic acids
Orotic acid (quantitative)
Phenylalanine
Phytanic acid
Phytosterols
Pipecolic Acid
Plasmalogens (C_{16} and C_{18})
Pristanic Acid
Sulphocysteine
Sweat test
Trimethylamine and oxide
Very long chain fatty acids

Qualitative urine screening tests for glucose, reducing substances, cystine and homocystine, are also available.

It is important that requests for the investigation of inborn errors of metabolism are accompanied by adequate clinical information including drugs being taken at the time of sampling. If the relevant clinical information is detailed, the laboratory should be contacted by letter or telephone.

Further investigation of some disorders requires the use of cultured fibroblasts The following are routinely available:-

- Screen for disorders of long-or medium-chain fatty acid oxidation This screen will detect defects of carnitine transport and deficiency of carnitine-palmitoyltransferase types 1 and 2, carnitine acylcarnitine translocase deficiency, very-long- or medium-chain acyl-CoA dehydrogenases, long-chain 3-hydroxyacyl-CoA dehydrogenase and other disorders of the trifunctional enzyme complex and mild to severe multiple acyl-CoA dehydrogenation defects (ethylmalonic-adipic aciduria and glutaric aciduria type 2).

* Carnitine-acylcarnitine translocase
* Glutaryl-CoA dehydrogenase (for glutaric aciduria type 1)
* Palmitoyl carnitine transferase Type I and II
* Propionyl-CoA carboxylase (for propionic acidaemia)
* Pyruvate Carboxylase
* 3-Methylcrotonyl-CoA carboxylase
* Fumarate hydratase
* Release of $^{14}CO_2$ or ^{14}C-incorporation from various substrates for the detection of isovaleric acidaemia MSUD and other disorders
* Very long-chain fatty acids

Enquire for disorders not listed.

In general the laboratory will advise on the need for tissue based assays and make the necessary preliminary arrangements.

SHEFFIELD DIAGNOSTIC GENETIC SERVICES

All services from the diagnostic laboratories, including Molecular Genetics are described on our website at:

http://www.sheffieldchildrens.nhs.uk/our-services/sheffield-diagnostic-genetics-service/

PRE-NATAL DIAGNOSIS

Prenatal diagnosis may be performed in a variety of ways:

* Metabolite analysis on amniotic fluid (or occasionally chorionic villus or cultured fetal cells). We currently provide metabolite analyses for the diagnosis of methylmalonic aciduria and Smith Lemli Opitz Syndrome.

* Enzyme assay on chorionic villus (fresh or cultured) or cultured amniotic fluid cells. Most of the assays listed above for fibroblasts can be used with cultured amniotic fluid cells or chorionic villus material.

* DNA analysis

Prenatal diagnosis for other disorders is usually available in the UK but some conditions will require samples to be sent overseas.

Careful consideration of the technical aspects (timing, material and route, time of result and reliability) is an essential part of preparatory counselling and prenatal diagnosis should be arranged well in advance if possible. Reliable prenatal diagnoses requires that the initial diagnosis has been clearly established and it is important to appreciate the need for rigorous investigation even when the index case presents in a terminal phase with little hope of useful intervention.

NEWBORN SCREENING

Dried blood spot samples are collected between the 5th and 8th day of life (day of birth is day 0) to screen for phenylketonuria, congenital hypothyroidism, cystic fibrosis, sickle cell disorders, medium chain acyl CoA dehydrogenase deficiency, maple syrup urine disease, isovaleric acidaemia, glutaric aciduria type 1 and homocystinuria (pyridoxial unresponsive). Results are sent out to the appropriate Child Health Records Department for entry into the Child Health Records Computer and checking against birth lists. Positive cases are referred for further investigation and treatment directly to designated disorder specific clinicians. Individual negative results are NOT normally sent out to hospital doctors or Family Practitioners. If you wish to have a result returned to the ward or a particular doctor then the Newborn Screening dried blood spot specimen must be accompanied by a standard laboratory request form.

This service is largely separate from the routine analytical services offered in the hospital and in general it is NOT appropriate to enquire directly of the Newborn Screening Laboratory for a test result. If an abnormal result has been found then, as soon as it has been confirmed, the patient's Family Practitioner and designated clinician for that disorder will have been informed. If you have clinical suspicion of hypothyroidism it is better to initiate your own investigations since the neonatal test is only a screening assay and in any case will not detect secondary hypothyroidism. Similarly, suspicion of MCADD, MSUD GA1 and IVA should always be vigorously pursued in the newborn period and separate investigations (including urinary organic acid analysis and acyl carnitine profile) are indicated. Please bleep the duty biochemist if further advice on relevant investigations is required. Immunoreactive trypsin is not always abnormal in cystic fibrosis patients with meconium ileus.

TABLES OF ANALYSES

Analyses are listed alphabetically. If the required analysis is not listed, check for pseudonyms. If the request is unusual, consult the laboratory. This section begins on page 17.

Units

Most results are reported in molar SI units but some drugs, hormones and proteins may be quoted in other units. Assistance with the conversion of results into alternative units can be obtained from the Duty Clinical Scientist (Bleep No 095).

Reference (normal) ranges

Reference ranges are provided for guidance in clinical decision making, rather than for prescriptive use. They are conventionally set to give the range of values which would be found in approximately 95% of a 'normal' population. They are derived from results obtained by this Department and from other sources. Reference ranges for blood refer to serum or plasma samples unless stated otherwise.

Changes during growth and development create age-related reference ranges for most analytes. Detailed ranges are kept in the Department and information upon them may be obtained from one of the Duty Clinical Scientists.

For the day to day interpretation of results age-related reference ranges have been condensed to cover generally recognised stages of development. These are generally printed automatically by the laboratory computer when the result is generated.

Newborn: First 7 days of life for term baby.

Neonate: First month of life for a term baby. Ranges may not apply to pre-term or small-for-dates babies.

Infant: Normally from the second month to one year, neonates are included in these ranges if not separately quoted.

Child: Normally one year to adolescence, neonates and infants are included in these ranges if not separately quoted.

Adult: From the end of adolescence

Accuracy and Imprecision of Results (Clinical Chemistry)

These are monitored and controlled by our quality assurance procedures. When patients are being repeatedly tested the significance of any apparent change in their results depends upon many factors including biological variability (intrapersonal variation) and the imprecision with which an analysis is performed (analytical variation). To aid in the interpretation of consecutive results imprecision data can be generated from laboratory quality control data, which can then be used to determine whether two results are significantly different, or within the bounds of analytical variation.
As a first approximation a result has a 95% probability (using a level of $p < 0.05$) of being genuinely different from a previous result for the same patient if the results differ by more than the quoted imprecision (2.8 times the analytical standard deviation).

For example:

Plasma urea result on day 1 = 6.0 mmol/L

Plasma urea result on day 2 = 6.8 mmol/L

Difference (day 2- day 1) = 0.8 mmol/L

Imprecision estimate for Urea = 0.84 mmol/L

Because the difference between the two results is less than the imprecision estimate there is a less than 5% chance that these results are statistically different. Of course, even if there is a significant analytical change between consecutive results this may well be within expected biological variation and consequently have little significance for the patient. It should be noted that analytical imprecision is not constant over the reportable range. Should you wish to discuss the significance of results further please contact the Clinical Scientist (Bleep 095).

TURNAROUND TIMES

A	**B**
Ammonia Caffeine Free fatty acids GIPUT screen 3-Hydroxy butyrate Intermediary metabolites Lactate Methotrexate	Acylcarnitine profile Amino acid qualitative Amino acid quantitation Biotinidase Carnitine Ethylmalonate Galactitol HVA/VMA Methylmalonate Mucopolysaccharides Organic acid Orotic acid Sweat test Total homocysteine
Turn around time 1 week *(providing samples do not require repeat analysis)*	***Turn around times 5-14 days*** (But if requested urgently, these analyses can often be performed more quickly)

Turn around times for all other tests 4-6 weeks
Glycosaminoglycan electrophoresis and Trimethylamine 6-8 weeks

Occasionally TAT's may be delayed following a bank holiday or other exceptional circumstances. However, if the analysis is urgent please phone the laboratory and we will do our best to obtain a timely result.

<u>Post Mortem DBS and bile samples</u>

Please note that all samples taken at post mortem for dried blood spots and bile analysis, usually for acylcarnitines, will be returned to the originating laboratory along with the analytical report. Samples can then be stored or destroyed depending on the circumstances of the specific case. This change in protocol came into effect on 01.01.16.

A

Acylcarnitine profile

Lithium heparin plasma (not EDTA)(serum and fluoride possible) and/or dried bloodspot (Guthrie card) – at least 2 full circles.

NB. For most situations a plasma sample for acylcarnitines is likely to be more informative. In addition to interpretive comments regarding the profile we give a quantitative result for free carnitine with each sample.
Send sample with completed request form to Clinical Chemistry **not** Newborn Screening.
It is **not** acceptable to send a dried blood spot card without a request form.
Send first class post, Monday-Thursday.

Amino acids (screen)

urine: 10 mL aliquot of a random or 24 hour urine in a plain bottle; qualitative report given includes reducing substances, CNNP and dipstix tests.
Store sample at -20°C. Send by first class post Monday-Thursday with normal packaging. Include information on current therapy.

Amino acids (quantitative)

Plasma: µmol/L; Collect 1 mL blood into a lithium heparin tube, centrifuge as soon as possible (preferably at 4°C) separate plasma taking care not to disturb the buffy coat and store at -20°C. Age related reference ranges given with report.
Urine: µmol/mmol creatinine; Random or timed collection. Store at -20°C, send 10 mL aliquot; reference ranges given with report.
Send by first class post Monday-Thursday with normal packaging.
Include information on current therapy.

Amino acids in dried blood spot

Certain amino acids can be measured in dried blood spots for:

a) follow-up results from newborn screening programmes.
b) monitoring of selected patients receiving dietary treatment for inherited metabolic diseases. Contact the laboratory to arrange this.

Dried blood spot (Guthrie card) - at least two full circles.
This can be sent by first class post.

Amino acids in CSF

Serine and Glycine, Threonine, Alanine, can be measured in CSF when looking for specific conditions. Blood stained CSF is unsuitable. Must be accompanied by paired plasma Li heparin sample (fluoride acceptable).
Send by first class post.

Amino Acids in hair protein (*For the diagnosis of Trichothiodystrophy Syndrome only)*

50 mg of hair sample required (small lock of hair). Please contact laboratory prior to request.

Ammonia

µmol/L; Take at least 1.0 mL venous or arterial blood into a Li heparin tube standing in ice. Centrifuge at 4°C within 30 minutes. Store plasma at -70°C. Send to this laboratory still frozen (dry ice). Neonate up to 100, infant, child, adult up to 50 µmol/L.

B

Bile Salts, profile (For the diagnosis of bile acid biosynthesis disorders)

1 mL venous blood in Li heparin tube, separate and send 0.5 mL plasma, or 2 mL urine in a plain tube, contact the laboratory before collection. Send by first class post. Interpretation given with report.
NB. For the diagnosis of bile acid synthesis disorders (this is NOT the same as total bile acid/bile salts quantitation i.e. not for cholestasis in pregnancy).

Biotinidase

u/L; 1 mL venous blood in a Li heparin tube, separate and send 0.5 mL plasma, store at -20°C. Send by first class post Monday-Thursday with normal packaging.
Child, adult 2.5-10.5 u/L.

C

Caffeine

mg/L; 0.5 mL venous or capillary blood in a Li heparin tube, separate and send plasma. Therapeutic range 10-35 mg/L.

Carnitine

µmol/L; 1 mL blood in a Li heparin tube, separate and send plasma (serum and fluoride possible) store at -20°C. Send by first class post.
Total: 23-60 µmol/L
Free 15-53 µmol/L
Urine 5 mL plain sample **(must be accompanied by blood sample)**
Please note that for virtually all clinical purposes an acylcarnitine profile with free carnitine is a more thorough investigation and likely to provide better clinical information.

Catecholamine metabolites – See VMA & HVA.

Cholestanol

1 mL Li heparin venous blood, separate and send plasma. (Serum acceptable). For diagnosis of Cerebrotendinous Xanthomatosis. Send plasma by first class post. Normal range 3-16 µmol/L.

Collagen Cross-links

1mL urine (min). Fresh sample, protected from light. If delay in posting sample store at -20˚C and send 1st class post. For differential diagnosis of *PLOD1* defects (EDS Type VI) only.

D

7-Dehydrocholesterol (*For diagnosis of Smith Lemli Opitz Syndrome*)

1 mL Li heparin venous blood, separate and send plasma by first class post.
10 mL Amniotic fluid for prenatal diagnosis (contact laboratory prior to collection).
Cholesterol and full sterol profile included in the assay.
Abnormal 7-DHC > 5 µmol/L, Normal 7-DHC <2 µmol/L.

8-Dehydrocholesterol (*For diagnosis of X-linked Chondrodysplasia Punctata*)

Sample requirements as for 7DHC. Normal 8-DHC <3 µmol/L

Dimethylglycine

1 mL Li heparin venous blood, separate and send plasma. 2 mL urine in a plain tube. For the diagnosis of Dimethylglycine dehydrogenase deficiency.

E

Enzyme diagnosis of inherited metabolic diseases - see page 11

Ethylmalonic Acid

5mL random urine (no preservative)

F

Fish Odour Syndrome - see Trimethylamine (and Dimethylglycine).

Free Fatty Acids - see Intermediary metabolites.

Fumarate Hydratase (Fibroblasts)

Please contact laboratory prior to sending sample
Skin biopsy.

G

Galactitol

2 mL Plain Urine.
Interpretation given with the result.

Galactosaemia screen (Galactose -1-phosphate uridyl transferase in erythrocytes)

0.5 mL venous or capillary whole blood in a **lithium heparin** tube; send whole blood first class post, normal packaging. Interpretation given with report.
EDTA samples are unsuitable.

Glycine (CSF:Plasma ratio)

0.5 mL blood in a Li heparin tube separate and send plasma together with 0.5 mL CSF sample (no additive required) by first class post Monday-Thursday with normal packaging. Interpretation given with report.

Glycosaminoglycans (mucopolysaccharides, MPS)

mg/mmol creatinine; one random urine sample or a 20 mL aliquot from a 24 hour collection in a plain bottle, store urine at -20°C, send first class post, normal packaging. Urine must be adequately concentrated (creatinine over 1.0 mmol/L) for a valid result; 0-4wks, 22.1-40.8; 1m-3m, 9.2-38.8; 3-6m, 11.9-34.5; 6m-1y, 4.2-30.5; 1y-2y, 6.8-21.7; 2y-3y, 9.7-19.5; 3y-5y, 6.2-15.4; 5y-7y, 6.2-12.1; 7y-9y, 4.1-10.8; 9y-11y, 4.5-10.8, 11-13y, 2.8-10.4; 13y-15y, 2.0-7.6; >15y, 1.7-4.4.

H

Hair Protein Amino Acids - see Amino Acids in hair protein.

Hexanoylglycine

2 mL random urine (no preservative), store at –20°C send first class post; Used in the diagnosis of medium chain fatty acid oxidation defects. Normal range 0.1-1.1 µmol/mmol creatinine.
Laboratory ***MUST*** be contacted before sending sample.

HMMA – See VMA

Homocysteine (total)

µmol/L; Collect 3 mL blood into an EDTA or Lithium heparin tube separate within 1hour, store plasma at -20°C. Send first class post, normal packaging.
Patient should be fasting (overnight); child, adult 0-18 male, 0-16 female.

If urgent estimation of free homocysteine is required for diagnostic purposes please contact laboratory for sample requirements.

Homovanillic acid (HVA)

µmol/mmol creatinine; 10 mL aliquot of a 24 hour urine collected into 10 mL HCl 6 mol/L ***CARE*** store urine at -20°C. Send first class post, normal packaging. ; infant 4-25, 1-5y 2-15, >5y 2-13.

HVA - see Homovanillic acid.

2-Hydroxy Glutaric Acid (Chirality)

For the diagnosis of D-or L- 2- hydroxy glutaric aciduria - 5 mL urine in a plain tube. Please send evidence of increased excretion of 2- hydroxy glutarate.

3- Hydroxybutyrate – see Intermediary metabolites

I

Intermediary metabolites (part of fasting hypoglycaemia screen)

Glucose, lactate, free fatty acids, 3-hydroxybutyrate. 2 mL blood into fluoride heparin, fluoride EDTA or fluoride heparin; separate and send plasma by first class post, normal packaging.
Haemolysed samples unsuitable for FFAs

Emergency investigation protocol in cases of suspected hypoglycaemia;
Take 2 mL blood into fluoride heparin and **obtain the first urine sample passed** for organic acid analysis.

J

K

L

Lactate, fasting

mmol/L; 0.5 mL venous in a fluoride heparin tube, separate and send plasma first class post; neonate up to 3.0, child 0.9-1.8, adult 0.6-2.4;
CSF lactate 0.2 mL in a fluoride heparin tube, send first class post; neonate up to 3.0, child 0.9-1.8, adult 0.6-2.4 mmol/L.

Lipase

0.5ml venous or capillary Lithium heparin plasma or serum. Send first class post.

Long-chain fatty acids - see very long-chain fatty acids.

M

Methotrexate

µmol/L; 0.75 mL venous or capillary blood in a Li heparin tube; **contact laboratory before collection**; light protected.

Methylmalonate

5 mL random urine (no preservative). Child normal range 1-8 µmol/mmol creatinine. Adult range 0.2-2.4 µmol/mmol creatinine.
Send urine first class post, normal packaging.

5 mL amniotic fluid (for prenatal diagnosis of methylmalonic acidaemia) laboratory must be contacted before sending sample.

Molecular Genetics - see page 12

Mucopolysaccharides (MPS) - see Glycosaminoglycans.

Muscle Biopsy - Arrange with Clinical Chemistry **before sample collection**
Contact Duty Clinical Scientist (Bleep 095)

N

O

Organic acids, Profile

10 mL aliquot of a random or a 24 hour urine in a plain bottle, qualitative report given. Boric acid in urine makes analysis impossible**- do not use borate**. Send urine first class post.

Orotic acid

Urine µmol/mmol creatinine; 10 mL aliquot of a random or 24 hour urine in a plain bottle, infant/child/adult <3.5.
Send urine first class post.

P

Phenylalanine, fasting

µmol/L; 0.5 mL plasma from venous or capillary blood in a Li heparin tube; newborn 40-110, < 6mo 32-128, 6mo-2y 40-140, 2y-10y 20-130, 10y-17y 30-115, adult 40-100.

Dried blood spot (Guthrie card) At least 2 filled circles for screening and monitoring dietary control in PKU, adequate dietary control 200-400 (depending on age).

Phosphoethanolamine

µmol/mmol Cr; 10 mL urine in a plain container; child, adult <10 µmol/mmol Cr, for hypophosphatasia - heterozygote 3-8 x normal, homozygote 10-50 x normal.

Phytanic acid

1 mL Li heparin blood; separate and send plasma by first class post normal 0.2 - 19.3 µmol/L; interpretation given with the report.

Phytosterols

1 mL Li heparin venous blood, separate and send plasma (serum acceptable). Send by first class post. Quantitation of campesterol, stigmasterol and sitosterol included.

Pipecolic Acid

1 mL Li heparin blood/EDTA/Serum, 0.5 mL CSF, 5 mL urine in plain container. Separate blood and send plasma or serum, first class post. Interpretation given with report.

Plasmalogens in RBC

For the diagnosis of peroxisomal biogenesis disorder and rhizomolic chondrodysplasia punctata.
2 mL EDTA; red blood cells (RBC), wash RBC x 3 with saline, send by first class post. Please phone the laboratory before sending; interpretation given with the report.

Pristanic Acid

1 mL Li heparin, separate and send plasma by first class post. Included in Very Long Chain Fatty Acid Assay. Normal 0-1.88 µmol/L.

Pyridinoline/deoxypyridinoline

5 mL fresh urine for assessment of collagen/bone turnover.

Q

R

S

Sitosterol – see Phytosterols

Skin Biopsy – A protocol is available from the laboratory describing a suggested technique and transport arrangements (Tel 0114 2717267) Tissue culture medium and consent forms are available from the laboratory and samples should be arranged in advance to arrive not later than 4.30pm. A completed consent form should accompany all samples

Sterols, Profile – See 7-dehydrocholesterol (Included in 7-DHC Test)
See 8-dehydrocholesterol and Cholestanol

Sweat test – please contact the laboratory

Sulphocysteine

5mL urine (no preservative)

T

Trimethylamine (And Oxide)

For the diagnosis of primary and secondary trimethylaminuria (Fish Odour syndrome)
24 hour urine collected in HCl is optimal (10 mL of 6N HCl)
20 mL random urine is acceptable if fresh sample but please acidify to pH<2 with HCl before sending by first class post

Interpretation given on report.

Please note: Samples that test positive for nitrite will not be analysed.

U

V

Very long-chain fatty acids (peroxisomal disorders)

1 mL venous blood in a Li heparin tube or EDTA send plasma by first class post. (serum also accepted); µmol/L C_{22} 15-112, C_{24} 14-80, C_{26} 0.33-1.50, C_{24}/C_{22} 0.44-0.97, C_{26}/C_{22} 0.005-0.030; Assay includes pristanic and phytanic acid quantitation.
Fibroblast; Amniocyte; chorionic villus cell assays. Please contact laboratory.

VMA (HMMA, vanillylmandelic acid, VMA)

µmol/mmol creatinine; 10 mL aliquot of a 24 hour urine collected into plastic bottle with 10 mL HCl 6 mol/L ***CARE,*** store urine at -20°C. Send first class post, normal packaging; infant 2-12, 1-5y 2-9 > 5y 1-7

W

X

Y

Z

Charges to Laboratories Outside Sheffield
April 2017- March 2018

Test	Cost (£)	Cost to East Midlands and South Yorkshire
Acylcarnitine Profile	68.00	54.00
Amino acid in hair protein	190.50	95.00
Amino Acids: Full Quantitation Part Quantitation TLC of Urine (includes spot test)	82.00 58.00 62.50	82.00 58.00 62.50
Bile salts in plasma or urine (Quantitation of Taurocholic acid)	50.50	25.50
Biotinidase	53.00	53.00
Carnitine (Total and Free)	89.00	89.00
Cholestanol in plasma	93.00	46.50
Collagen Cross-links	97.00	97.00
7-dehydrocholesterol in plasma and (included) 8-dehydrocholesterol	93.00	46.50
Dimethylglycine	50.50	25.50
Ethylmalonic Acid (EMA)	72.00	36.50
Galactitol	91.50	46.50
Galactose 1 Phosphate uridyl transferase	36.00	36.00
Glutaric acid (in cerebrospinal fluid)	162.00	81.50
Hexanoylglycine in urine	162.00	81.50
HVA/VMA	78.00	78.00
Intermediary metabolites	53.00	53.00
Free Fatty Acids	17.50	17.50
β hydroxybutyrate	17.50	17.50
Methylmalonic acid in urine	72.00	36.50
Mucopolysaccharide electrophoresis	106.00	106.00
Organic Acid (GCMS) including extraction, assay and identification	91.00	45.50
Organic Acids (GCMS) (Peak identification only sent with a copy of the chromatogram)	42.00	21.00
Orotic Acid	77.50	39.00
Phytosterols	93.00	46.50
Pipecolic Acid (in plasma/serum/urine/CSF)	162.00	81.50
Plasmalogens	162.00	81.50
Sulphocysteine	72.00	72.00
Total Homocysteine	36.00	36.00
Trimethylamine (and oxide) in urine	137.00	68.00
Very long chain fatty acids and phytanic & pristanic acids (combined) in plasma	85.00	42.50
Additional charge for analyses out of hours	83.00	41.50

Test	Cost (£)	Cost to East Midlands and South Yorkshire
Quantitation in amniotic fluid for prenatal diagnosis		
7 Dehydrocholesterol	360.50	181.00
Methylmalonic acid	360.50	181.00
Determination of chirality (in urine)		
2-hydroxyglutaric acid	387.00	193.50

NOTES

a) Please give advance warning of prenatal diagnoses. These are priced on the assumption that the result is required urgently. Control amniotic fluid samples are run in parallel and should ideally be submitted by the requesting centre with the sample from the at-risk pregnancy.

Test	Cost (£)	Cost to East Midlands and South Yorkshire
Routine Chemistry	12.00	12.00
Ammonia	18.00	18.00
Lactate	18.00	18.00
Caffeine	28.50	28.50
Cyclosporin	59.00	59.00
Methotrexate	61.00	61.00
Tacrolimus	76.50	76.50
Lipase	15.00	15.00
Sweat test (collection and analysis)	56.50	56.50
Additional charge for analyses out of hours	83.00	41.50

The following tests are free of charge for patients living in districts covered by the East Midlands and South Yorkshire Newborn Screening Contracts

Test	Cost (£)
Tissue culture	
Establishing a primary culture from skin biopsy, growing on under sterile conditions (mycoplasma-free), cryopreservation and retention for at least 2 years	320.25
Growing on of fibroblast cell line for assay when received as cells from outside laboratory	202.65
Recovery of cryopreserved cultured cells and growing on	69.82
Onward dispatch of living culture (carriage extra at cost); Within the UK Abroad	 54.07 97.65
Assays on cultured cells	
$^{14}CO_2$ or ^{14}C-incorporation assays with cultured fibroblasts (propionic, isovaleric etc)	334.95
3-Methylcrotonyl-CoA Carboxylase	337.05
Carnitine Palmitoyl Transferase Type I	531.82
Carnitine Palmitoyl Transferase Type II	378.52
Carnitine Acylcarnitine Translocase (CATR)	486.67
Carnitine Transporter Assay (for primary carnitine deficiency)	259.87
Fatty acid oxidation screen (tritiated myristate palmitate and oleate in parallel on cultured fibroblasts)	298.20
Fumarate hydratase	497.70
Glutarate dehyrogenase (fibroblasts)	345.97
Incorporation of phenylalanine L[ring 2, 6-3H] and L-Ornitine [5-14C into – Cultured Fibroblasts for the detection of patients with hyperornithinaemias (HHH &OAT) Cultured Fibroblasts	394.80
Multiple Carboxyglases Cultured in low biotin medium. Please contact laboratory to discuss.	Price on request
Ornithine Oxo-Acid Aminotransferase assay (EC 2.6.1.13) for the confirmation of OAT – Cultured Fibroblasts	400.57
Propionyl CoA carboxylase (fibroblasts)	345.97

Test	
Pyruvate Carboxylase	337.05
Very long-chain fatty acids in cultured cells (fibroblasts, amniotic fluid cells or chorionic villus cells)	362.77
Very long-chain acyl-CoA dehydrogenase (ferricenium linked assay) (Research based).	432.60

NOTES

a) Cultured fibroblasts submitted for assay MUST be mycoplasma-free. If your local cytogenetics laboratory cannot ensure this then please send skin biopsy directly to us for culture. Cultures that are infected when received will be discarded.

b) The prices shown are indicative only. For some assays there are substantial reductions for multiple samples received at one time e.g. for family studies.

c) For prenatal diagnoses using cultured amniotic fluid cells or chorionic villus, reference material, cultured under the same conditions as the suspect sample, should be supplied by the referring centre. The above prices are increased by 40% for prenatal diagnosis or 70% if recovered cryopreserved material (e.g. the index case) is included as a positive control.

d) The prices shown do not include VAT, not usually applicable within the NHS. It may be applied to users outside the NHS dependent on current VAT rules