SHEFFIELD CHILDREN'S NHS FOUNDATION TRUST

DEPARTMENT OF CLINICAL CHEMISTRY AND NEWBORN SCREENING

USER'S HANDBOOK FOR METABOLIC INVESTIGATIONS

June 2023

Do Not Use This Edition after April 2024

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Website

https://www.sheffieldchildrens.nhs.uk/laboratory-medicine/clinical-chemistry/metabolic-biochemistry/

Telephone numbers & enquiries

Hospital switchboard: 0114 271 7000

	Direct Telephone
Katherine Wright (Head of Department, Consultant Clinical Scientist, Director of Newborn Screening)	2717404
PA to HoD	2717318
Mr Philip Craddock – Laboratory Manager	2717444
PA to Mr P Craddock	2717340
Duty Clinical Scientist	Bleep No 095

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

Metabolic Section	
Result enquiries (telephone)	271 7445
Result enquiries (email)	Metabolic.sch@nhs.net
Claire Hart (Principal Clinical Scientist, Metabolic Lead Scientist)	271 7307
Sharon Colyer (Principal Clinical Scientist)	271 7307
Ben Nicholson (Senior Clinical Scientist)	271 7479
Louisa Ann Smith / Stephen McSweeney (Chief Biomedical Scientist)	271 7445

Tissue Culture Section	
Joanne Croft (Principal Clinical Scientist, Tissue Culture and Enzyme Assay Lead)	226 0972
General Enquiries	271 7267
Prenatal diagnosis enquiries	271 7267

Newborn Screening Section	
Dr Lynette Shakespeare (Screening Lead Scientist)	271 7302
Ben Sholademi (Senior Clinical Scientist)	271 7346
Ullas Joseph (Chief Biomedical Scientist)	271 7500
Jade Barber (Senior Biomedical Scientist)	271 7346
Sheila Ellin (Senior Biomedical Scientist)	271 7346
Newborn Screening Results (09:00-12:30) and Answering Machine	271 7257

NORMAL LABORATORY OPENING TIMES	Monday to Friday 9:00am- 5:00pm

Outside of these hours the on-call Clinical Biochemist can be reached via the hospital switchboard.

REQUESTS FOR ANALYSES

Legible request forms 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 as per ISO 15189 standards. It is particularly important for metabolic testing that CLINICAL DETAILS are included as this can significantly aid in interpretation of results and ensuring that the correct analysis is done in relation to the clinical question being asked.

The request form must give clear indication of where / how the results are to be returned and, if different, where the invoice should be sent to.

Tests can be added verbally by telephone if the sample and form have already been received in the laboratory and there is sufficient and suitable sample for the additional test remaining.

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 TEND TO LEAK AND 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.

URGENT / PRIORITY REQUESTS

We recognise two levels of urgency, "Urgent" and "Priority".

1. For "Urgent" samples we will aim to analyse the sample as soon as practicable after receipt in an urgent batch on its own. Depending on the test required and time of receipt this may be same or next day. This type of analysis is very disruptive of normal working patterns and can hold up the analysis of other samples therefore it is reserved for certain patients only e.g. acutely unwell child / high level of suspicion of an IEM / hyperammonaemia / acidosis / the need to make significant treatment decisions e.g. withdrawal of care
2. Samples that are not as urgent as "Urgent" but need prioritisation over routine analysis will be deemed "Priority" samples. These will be analysed on the next available batch and, where possible, consideration given to bringing the next batch forward if it is not scheduled within the necessary timeframe. Priority will be given to processing results and reporting results to the requestor as soon as practicable once analysis has been performed.

Urgent or priority requests must be discussed with a clinical scientist so that the appropriate action for the clinical circumstances can be planned and taken (0114 271 7307 / 7445). It also means that if there is any delay in receipt of the sample, steps can be taken to locate it. Any such samples which arrive in the laboratory without prior arrangement will be analysed routinely.

Metabolic tests are not performed out of routine hours.
In extreme and urgent circumstances some tests may be performed out of hours, but only after discussing with the Consultant Clinical Scientist who can be contacted via the hospital switchboard.

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 or taxi.***

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.

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 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 or special delivery may be used.

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

    - 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 leak-proof plastic bag.

6. 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.

7. A padded outer bag is recommended.

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

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

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

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

12. 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.

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 or courier, 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, for instance those for acylcarnitine analysis, 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 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

Sample Storage

With the exception of post mortem dried blood sample and bile samples, samples will only be stored for a maximum of 6 months before being discarded, unless (from the laboratory's perspective) the results from the sample have contributed to diagnosis.

Cultured skin fibroblasts are stored indefinitely in liquid nitrogen unless we are informed that the cells should be discarded or returned post analysis.

Post Mortem DBS and bile samples

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.

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 (full details are found in the table starting on page 13):

Acylcarnitine profile (includes free carnitine)
Amino acids
Bile Salts / Acids
Biotinidase
Cholestanol
Collagen Cross Links
7 and 8 Dehydrocholesterol (and other abnormal sterol species)
Dimethylglycine
Desmosterol
Ethylmalonic acid (quantitative)
Free fatty acids (part of intermediary metabolites profile)
Galactitol
Galactosaemia Screen
2-hydroxyglutaric acid chirality (D or L)
Glycosaminoglycans (screen and electrophoresis)
Hexanoylglycine (quantitative)
Homocysteine (total)
Homocystine (free)
HVA/VMA (quantitative)
3-hydroxybutyrate (part of intermediary metabolites profile)
Isovalerylglycine (quantitative)
Lactate (CSF)
Lathosterol
Methylmalonate (quantitative)
Organic acids
Orotic acid (quantitative)
Phenylalanine
Phytanic acid
Phytosterols
Pipecolic Acid
Plasmalogens (C_{16} and C_{18})
Pristanic Acid
Sulphocysteine
Trimethylamine and oxide (Fish Odour Syndrome)
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
* 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.

Pre-natal Diagnoses

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 Smith Lemli Opitz Syndrome.

- DNA analysis

- Enzyme assay on chorionic villus (fresh or cultured) or cultured amniotic fluid cells is, for most of the assays listed above for fibroblasts, possible. However, this would require discussion with the Lead Clinical Scientist in the Tissue Culture and enzyme assay section before we would agree to accepting these types of samples. Please also note that reference material should be supplied by the requesting centre.

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

The screening laboratory in Sheffield 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). Testing is for phenylketonuria (phenylalanine), congenital hypothyroidism (TSH), cystic fibrosis (immunoreactive trypsin), SCID (in evaluation phase), medium chain acyl CoA dehydrogenase deficiency (octanoylcarnitine), sickle cell disorders (haemoglobin profile), maple syrup urine disease (leucine), homocystinuria (methionine), isovaleric acidaemia (isovalerylcarnitine) and glutaric aciduria type 1 (glutarylcarnitine).

Dried blood spot samples are collected on day 5 of life (day of birth is day 0). Results are sent out to the appropriate Child Health Records Department for entry into the Child Health Records Computer and checking against birth lists screen.
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. Please note, immunoreactive trypsin is not always abnormal in cystic fibrosis patients with meconium ileus.

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 (Uncertainty of Measurement) of Results

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

Turnaround times for each assay are given in the table of analytes (page 13). Occasionally TATs may be delayed following a bank holiday or other exceptional circumstances but we aim for 95% of samples to be analysed within the stated TAT or less. This is monitored on a quarterly basis and remedial action taken when it is not achieved. While the TAT given represents the standard time taken to analyse a sample and produce a result we will always do our best to obtain a more timely result when analysis is urgent clinically or a specific diagnosis strongly suspected. Should this be the case please contact the Duty Biochemist or a Metabolic Section Clinical Scientist to discuss your needs.

If an assay is withdrawn or unavailable for any reason, or we expect any significant delay in the usual turnaround time, we will notify users of the service as soon as possible.

QUALITY ASSURANCE

The Department participates in national and international 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.

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.

External Quality Assurance Schemes:

The laboratory's policy is to participate in ISO 17043 accredited schemes wherever they are available. However the reality is that for most of the specialist metabolic assays no such scheme exists. The majority of our assays are covered by schemes offered by ERNDIM (European Research Network for evaluation and improvement of Diagnosis in IEMs) which is a European wide collaborative project to provide EQA material for esoteric assays. ERNDIM is internationally recognised as a quality provider of EQA schemes and is working towards ISO 17043 accreditation.

The Metabolic Section participates in the following EQA Schemes:

EQA Scheme	Assays Covered
UKNEQAS Urinary Catecholamines	VMA/HVA
UKNEQAS Quantitative Amino Acids	Phenylalanine,Tyrosine, Branched Chain Amino Acids
ERNDIM Quantitative Amino Acids	All amino acids
ERNDIM Special Assays in DBS	Free carnitine, phenylalanine, tyrosine, valine, leucine, isoleucine, methionine
ERNDIM Acylcarnitines in serum (quantitative)	Acylcarnitines including free carnitine
ERNDIM Acylcarnitines in DBS (qualitative)	Acylcarnitines with emphasis on diagnosis / interpretation
ERNDIM Quantitative Organic Acids in Urine	MMA, EMA, Isovalerylglycine, Hexanoylglycine
ERNDIM Special Assays in Urine	Free carnitine, orotic acid, GAGs, VMA / HVA, pipecolic acid, sulphocysteine, galactitol
ERNDIM Special Assays in Serum	FFA and 3-hydroxybutyrate, VLCFA, phytanate, pristanate, 7-dehydrocholesterol, total homocysteine, cholestanol, pipecolic acid, biotinidase
ERNDIM Qualitative Organic Acids	Qualitative Organic Acid profile
ERNDIM MPS Scheme	Quantitative / Qualitative analysis for MPS disorders (GAG/ creat ratio and GAG electrophoresis)
ERNDIM Diagnostic Proficiency Scheme	Test of laboratory's overall ability to apply the correct tests to a sample given clinical details and to correctly ascertain the diagnosis.

There are a number of assays for which no EQA scheme exists. The following assays are covered by sample exchange schemes with other laboratories in the UK or internationally.

Trimethylamine
Galactosaemia screen
Plasmalogens
Phytosterols
Urinary Collagen cross links

There is one assay which currently has no EQA scheme and no sample exchange scheme;

1. Bile Salts /Acids – no other accredited UK provider. Currently seeking other labs in Europe.

We assure quality by running regular iQC samples. Bile acid results can be compared with known affected patients and results can be correlated with the results of other complementary assays.

Where poor performance on an EQA scheme occurs we undertake to inform any affected users in a timely fashion.

A-Z TEST LIST (Metabolic Section)

Abbreviations:

CDPX2	Chondrodysplasia punctata (X-linked)
CTX	Cerebrotendinous Xanthomatosis
DBS	Dried blood spot
EMA	Ethylmalonic Acid
GAG	Glycosaminoglycan
GA1	Glutaric Aciduria Type 1
GA2	Glutaric Aciduria Type 2 (aka MADD)
HHH Syndrome	Hyperammonaemia, hyperornithinaemia and homocitrullinuria syndrome
HVA	Homovanillic acid
IVA	Isovaleric Acidaemia
IEM	Inborn error of metabolism
LiHep	Lithium heparin
MADD	Multiple AcylCoA dehydrogenase deficiency (aka GA2)
MCADD	Medium chain AcylCoA dehydrogenase deficiency
MMA	Methylmalonic aciduria (also methylmalonic acid)
MPS	Mucopolysaccharide disorder
NKH	Non-ketotic hyperglycinaemia
PA	Propionic Acidaemia
PDE	Pyridoxine Dependent Epilepsy
RCDP	Rhizomelic chondrodysplasia punctata
SCAD	Short chain AcylCoA dehydrogenase deficiency
SLO	Smith Lemli Opitz Syndrome
VMA	Vanilylmandelic acid
VLCFA	Very long chain fatty acids
X-ALD	X-linked adrenoleukodystrophy

Our UKAS Schedule of Accreditation can be found by entering our accreditation number (10139) on the search page of the UKAS website. The schedule includes information on methodology / equipment used.
https://www.ukas.com/search-accredited-organisations/

All tests listed below are within the scope of accreditation except for the following highly specialised and rarely performed assays;

- **2-hydroxyglutarate chirality**
- **Hair Amino Acids**
- **Methylmalonic acid in amniotic fluid**

Test	Clinical Utility	Sample Type and Volume (min in brackets)	Storage / Sending requirements	Reference Ranges	TAT	Comments / Notes	Price (Price for East Mids and South Yorks in brackets)
Acylcarnitine profile (including Free Carnitine) – Plasma	For the diagnosis of fatty acid oxidation disorders & some organic acidaemias including but not limited to; Primary Carnitine deficiency, SCADD, MCADD, MADD (GA2), VLCADD, CPT2, CPT1, LCHADD, PA, MMA, b-ketothiolase, IVA, 3HMGCoA-lyase deficiency, Biotinidase deficiency, GA1, Malonic aciduria	LiHep plasma 0.5ml (0.1) Serum and fluoride can also be used but NOT EDTA	Store at -20C, Send first class post	Free Carnitine = 15-53 µmol/L All other ranges given on report where relevant.	5-14 days	**PLASMA is the preferred sample type except for ?CPT1 when a DBS may be the best sample type**	£77.05 (£61.06)
Acylcarnitine profile (including Free Carnitine) -DBS	As above	Guthrie card, 2 full spots MUST be sent with a request form	Send by first class post	Free Carnitine = 5-35 µmol/L All other ranges given on report where relevant.	5-14 days	**ALWAYS treat Guthrie card as if it is a sample container and send a completed REQUEST FORM with it.** **DO NOT send to Newborn Screening lab**	£77.05 (£61.06)
Acylcarnitine profile - DBS (POST MORTEM)	As above	Guthrie card, 2 full spots	Send first class post	Reference Ranges and Interpretation given on report as relevant.	4-6 weeks		£77.05 (£61.06)

Test	Clinical Utility	Sample Type and Volume (min in brackets)	Storage / Sending requirements	Reference Ranges	TAT	Comments / Notes	Price (Price for East Mids and South Yorks in brackets)
Acylcarnitine profile - Bile (POST MORTEM)	As above	Guthrie card, 2 full spots	Send first class post	Reference Ranges and Interpretation given on report as relevant.	4-6 weeks		£77.05 (£61.06)
Amino Acids -Plasma	For the diagnosis of amino acid and urea cycle disorders	Lithium heparin plasma 0.5 ml (0.15)	Store at -20, Send by first class post.	Reference ranges vary by age and are provided with report – contact lab for separate summary document if required.	5-14 days		Full quantitation £92.84 Part quantitation £66.51
Amino Acids – Blood Spots	For monitoring of select patients receiving dietary treatment for specific IEMs. Contact lab.	Guthrie card, 2 full spots.	Send by first class post.	N/A	5-14 days		£66.51
Amino Acids – CSF (paired with plasma)	Diagnosis of NKH (non-ketotic hyperglycinaemia) and the Serine synthesis disorders.	CSF sample in plain tube 0.5ml (0.15) Unsuitable if bloodstained	Store at -20 Send by first class post.	Interpretation given on report	5-14 days	Requires a **PAIRED** plasma sample to be sent with the CSF sample (for these purposes a sample taken within a few hours is sufficient)	£92.84
Amino Acids- Hair	For the diagnosis of **Trichothiodystrophy** syndrome only	50mg of hair (a small lock)	Send by first class post	Interpretation provided with report	2 months	This assay is very rarely carried out – please contact laboratory before sending a sample	£215.71 (£107.64)

Test	Clinical Utility	Sample Type and Volume (min in brackets)	Storage / Sending requirements	Reference Ranges	TAT	Comments / Notes	Price (Price for East Mids and South Yorks in brackets)
Amino Acids – Urine (SCREEN)	Basic screen for a variety of amino acid disorders but not suitable if an amino acid / urea cycle disorder is being seriously considered.	10ml (2ml) random urine or aliquot from 24 hour urine collection (no preservative)	Store at -20 Send by first class post	Qualitative	3-6 weeks	Includes reducing substances, CNNP and dipstix tests. Please include information on current therapy	£70.74
Amino Acids – Urine (QUANTITATIVE)	Most amino acid disorders are better diagnosed by a plasma sample however urine analysis is essential for the diagnosis of Cystinuria, Hartnup's, HHH syndrome, Lysinuric Protein Intolerance and Prolidase deficiency.	5 ml aliquot in plain container Very dilute or deteriorated samples (Creat <0.8 mmol/L, nitrite positive) may not be analysed.	Store at -20 Send by first class post	Reference ranges vary by age and are provided with report – contact lab for separate summary document if required.	21 days	Diagnosis of **Prolidase Deficiency** requires analysis pre and post acid hydrolysis of a urine sample. Please contact lab to discuss if being considered as a diagnosis.	Full quantitation £92.84 Part quantitation £66.51
Bile Salts / Acids - Plasma	For the diagnosis of the primary bile acid biosynthesis disorders, for further investigations of probable Peroxisomal disorders, and for diagnosis of	LiHep plasma 0.5ml **PLEASE INDICATE IF PATIENT IS TAKING URSODEOXYCHOLIC ACID AT TIME OF SAMPLING**	Store at -20 Send by first class post	Interpretation provided with report	3-6 weeks	**NOT** for the diagnosis of **cholestasis of pregnancy** 4 primary bile salts are quantitatively measured with additional qualitative analysis looking for the	£77.05 (£61.06)

Test	Clinical Utility	Sample Type and Volume (min in brackets)	Storage / Sending requirements	Reference Ranges	TAT	Comments / Notes	Price (Price for East Mids and South Yorks in brackets)
	Cerebrotendinous Xanthomatosis (specific bile alcohols, see also entry for Cholestanol)					presence of abnormal bile acid / salt intermediates	
Bile Salts/Acids- Urine	As above	2ml urine	Store at -20 Send by first class post	Interpretation provided with report	3-6 weeks	As above	£77.05 (£61.06)
Biotinidase	Diagnosis of Biotinidase Deficiency	Lithium heparin plasma 0.5ml (0.1)	Store at -20 Send by first class post	u/L Child/adult 2.5 – 10.5	5-14 days	Patients with Biotinidase deficiency may also show abnormalities in organic acid and acylcarnitine profiles but this can be variable	£59.97
Carnitine (Free) – see acylcarnitine profile	-	-	-	-	-	-	-
Carnitine (Free) – Paired PLASMA and URINE	For the diagnosis of **Primary Carnitine Deficiency** (also known as Carnitine transporter deficiency) ONLY	1ml urine 0.5ml LiHep plasma (paired)	Store at -20 Send by first class post	Interpretation given with report (normal is typically >98%)	5-14 days	Fractional tubular reabsorption of free carnitine is calculated. Please take samples BEFORE giving the patient carnitine if at all possible.	£77.05 (£61.06)
Catecholamine metabolites - see VMA /HVA	-	-	-	-	-	-	-
Cholestanol	For the diagnosis of Cerebrotendinous Xanthomatosis	Lithium heparin plasma or serum 1ml (0.3)	Store at -20 Send by first class post	µmol/L 3-16	3-6 weeks	The bile alcohols associated with CTX (cholestane-tetrol-glucuronide,	£105.25 (£52.68)

Test	Clinical Utility	Sample Type and Volume (min in brackets)	Storage / Sending requirements	Reference Ranges	TAT	Comments / Notes	Price (Price for East Mids and South Yorks in brackets)
	(CTX)					cholestane-pentol-glucuronide, cholestane-hexol-glucuronide) can be detected by the Bile Acids/ Salts test	
Collagen Cross-links (pyridinoline / deoxypyridinoline)	For the diagnosis of PLOD 1 Defects / Ehlers-Danlos Type VI (also known as Kyphoscoliotic ED) only	Urine, fresh sample in plain container, **protect from light** 1ml (min)	Store at -20 Send by first class post	Interpretation given with report	6-8 weeks		£109.82
Cystine –Urine (includes lysine, ornithine, arginine)	For the diagnosis of Cystinuria (renal stones).	Urine 5 ml aliquot in plain container	Store at -20 Send by first class post	Interpretation given with report	21 days	Analysis is the same as urine quantitative amino acids	£66.51
7-Dehydrocholesterol	For the diagnosis of Smith-Lemli-Opitz syndrome (and other disorders of sterol metabolism via sterol profile)	Lithium heparin plasma 1 ml (0.3)	Store at -20 Send by first class post	µmol/L Reference range < 2 Affected range >5	3-6 weeks	Part of sterol profile that includes quantitative measurement of 7 and 8-dehydrocholesterol, lathosterol, desmosterol and cholesterol. Also includes a qualitative sterol profile which can detect the presence of**;** **Lanosterol, 8,(9)-cholestenol, and the 4-methyl-sterols**	£105.25 (£52.68)

Test	Clinical Utility	Sample Type and Volume (min in brackets)	Storage / Sending requirements	Reference Ranges	TAT	Comments / Notes	Price (Price for East Mids and South Yorks in brackets)
7-Dehydrocholesterol – Amniotic fluid	For prenatal diagnosis of Smith-Lemli-Opitz syndrome	10 ml Amniotic fluid for prenatal diagnosis- (contact lab prior to collection)	Contact laboratory prior to collection of sample	Interpretation given on report	N/A	**MUST** contact laboratory prior to taking a sample to arrange analysis. Please provide information on proband.	Amniotic fluid £408.14 (£204.93)
8-Dehydrocholesterol	Secondary metabolite in SLO, primary metabolite in diagnosis of Conradi-Hunermann-Happle (X-linked Chondrodysplasia punctata, CDPX2).	Lithium heparin plasma 1 ml (0.3)	Store at -20 Send by first class post	µmol/L Reference range <3	3-6 weeks	Part of sterol profile that includes quantitative measurement of 7 and 8-dehydrocholesterol, lathosterol, desmosterol and cholesterol. Also includes a qualitative sterol profile which can detect the presence of**;** **Lanosterol, 8,(9)-cholestenol, and the 4-methyl-sterols**	£105.25 (£52.68)
Desmosterol	Increased in Desmosterolosis (extremely rare disorder of cholesterol synthesis similar to Smith-Lemli-Opitz syndrome)	Lithium heparin plasma 1 ml (0.3)	Store at -20 Send by first class post	µmol/L Reference range <9	3-6 weeks	Part of sterol profile that includes quantitative measurement of 7 and 8-dehydrocholesterol, lathosterol, desmosterol and cholesterol. Also includes a qualitative sterol profile which can detect the presence of**;** **Lanosterol,**	£105.25 (£52.68)

Test	Clinical Utility	Sample Type and Volume (min in brackets)	Storage / Sending requirements	Reference Ranges	TAT	Comments / Notes	Price (Price for East Mids and South Yorks in brackets)
						8,(9)-cholestenol, and the 4-methyl-sterols	
Dimethylglycine	Diagnosis of Dimethylglycinuria (alternative but extremely rare cause of fish odour like syndrome). Also used for monitoring of treatment in Glutaric Aciduria Type 2 and betaine therapy.	Urine 2ml aliquot in a plain container.	Store at -20 Send by first class post	Interpretation given with report	3-6 weeks		£57.14 (£28.94)
Ethylmalonic acid	Confirmation of diagnosis / monitoring of SCAD deficiency or other disorders with elevated EMA.	Urine 5ml aliquot in a plain container	Store at -20 Send by first class post	µmol/mmol creatinine Reference range <15	5-14 days		£81.52 (£41.35)
Fish Odour Syndrome – see Trimethylaminuria	-	-	-	-	-	-	-
Free fatty acids	See Intermediary metabolites	-	-	-	-	-	-
Galactitol	For the diagnosis of Classical Galactosaemia,	Urine 2ml in plain	Store at -20 Send by first class post	Interpretation given with report	5-14 days	First line test for Galactokinase deficiency. Alternative	£103.6 (£52.68)

Test	Clinical Utility	Sample Type and Volume (min in brackets)	Storage / Sending requirements	Reference Ranges	TAT	Comments / Notes	Price (Price for East Mids and South Yorks in brackets)
	Galactosaemia due to UDP-galactose epimerase deficiency, and Galactokinase deficiency.	container				test for Galactosaemia when patient has been transfused in previous 3 months. Results will be less abnormal if patient on galactose free diet.	
Galactosaemia screen – Whole Blood	Screening test for diagnosis of Classical Galactosaemia (Galactose-1-phosphate uridyl transferase deficiency only)	LiHep whole blood 0.5ml (0.1) Do not separate (although the remaining red cell pellet left after plasma has been removed can be used) **(EDTA samples not accepted)**	Store in fridge, send by first class post	Qualitative. Normal samples fluoresce after 1 hr of incubation with substrate, failure to fluoresce after 4 hrs incubation indicates Galactosaemia (fluorescence at intermediate times can be due to sample deterioration or variant / mild forms).	1 week	The test is a quick screening rest for Galactosaemia, it is not fully diagnostic (which requires qualitative enzyme assay and galactose-1-phosphate measurement) **Test not valid if there has been a transfusion in the previous 3 months**. In this circumstance send urine for **GALACTITOL**	£40.81
Galactosaemia screen - DBS	As above	2 full spots on Guthrie card	Store at room temperature send by first class post.	As above	1 week	As above	£40.81
Galactose-1-phosphate uridyl transferase – see Galactosaemia Screen	-	-	-	-	-	-	-
Glycine – Plasma (see	-	-	-	-	-	-	-

Test	Clinical Utility	Sample Type and Volume (min in brackets)	Storage / Sending requirements	Reference Ranges	TAT	Comments / Notes	Price (Price for East Mids and South Yorks in brackets)
Plasma Amino acids)							
Glycine –CSF (see CSF amino acids)	-Diagnosis of NKH (non-ketotic hyperglycinaemia)	-	-	-	-	-	-
Glycosaminoglycans (GAG / creatinine ratio)	Screening test for Mucopolysaccharide disorders (Hurler, Hurler-Scheie, Hunter, Sanfilippo, Morquio, Maroteaux-Lamy, Sly)	Urine 10 mls random sample (no preservative) Dilute (creat <1.0mmol/L) or deteriorated samples (nitrite/protein positive) are not processed	Store at -20 Send by first class post	µmol/mmol creatinine (0-4 w 22.1-40.8) 1-3 m 9.2-38.8 3-6m 11.9-34.5 6-12m 4.2-30.5 1-2y 6.8-21.7 2-3y 9.7-19.5 3-5y 6.2-15.4 5-7y 6.2-12.1 7-9y. 4.1-10.8 9-11y. 4.5-10.8 11-13y 2.8-10.4 13-15y 2.0-7.6 >15 y 1.7-4.4	5-14 days	A normal result cannot rule out an MPS disorder. If an MPS disorder is a serious clinical consideration then GAG electrophoresis is required as a minimum. **GAG / creatinine ratio is not typically done on patients < 1 month of age as results can be unreliable. In the event of a neonate with a family history please contact lab to discuss appropriate testing.**	Cost included in organic acid / urine amino acid screen.
Hexanoylglycine	Primarily used for confirmation / rule out in MCAD screening. May have value in other circumstances.	Urine 2 mls random sample (no preservative)	Store at -20 Send by first class post	µmol/mmol creatinine 0.1-1.1	5-14 days	Lab **MUST** be contacted before sending sample	£183.39 (£92.29)
Homocysteine (Total)	For diagnosis and monitoring of	Lithium heparin or EDTA Plasma	Store at -20 Send by first	µmol/L	5-14 days	This is the most sensitive test for	£40.81

Test	Clinical Utility	Sample Type and Volume (min in brackets)	Storage / Sending requirements	Reference Ranges	TAT	Comments / Notes	Price (Price for East Mids and South Yorks in brackets)
	Classical Homocystinuria and other disorders of homocysteine metabolism such the remethylation defects and cobalamin metabolism disorders	Fasting sample (overnight) Collect 3mls blood spin and separate from cells within 60 minutes	class post	Child/adult Male 0-18 Female 0-16		"Homocystinuria" –urine testing is inadequate due to high renal threshold causing low sensitivity.	
Homocystine (free)	For diagnosis of Classical Homocystinuria – **HOWEVER this is not the recommended test- please send plasma sample for Total Homocysteine**	Urine 5 ml aliquot in plain container	Store at -20 Send by first class post	µmol/mmol creatinine Interpretation on report	21 days	In theory free homocysteine can be detected in urine (in the quantitative urine amino acid profile) – however because of a high renal threshold and a tendency to stick to protein the amount detectable in urine is very low in comparison to total body burden. It is a very insensitive test for hyperhomocystinaemia.	£66.51
HVA (see VMA / HVA)	-	-	-	-	-	-	-
3-Hydroxy Butyrate	See intermediary metabolites	-	-	-	-	-	-
2-Hydroxy Glutaric Acid Chirality (D or L)	To differentiate between L-2-	Urine 5 ml aliquot in	Store at -20 Send by first	Qualitative. Interpretation given	3-6 weeks	Please send sample with organic acid profile	£438.17 (£219.09)

Test	Clinical Utility	Sample Type and Volume (min in brackets)	Storage / Sending requirements	Reference Ranges	TAT	Comments / Notes	Price (Price for East Mids and South Yorks in brackets)
	hydroxyglutaric aciduria and D-2-hydroxyglutaric aciduria	plain container	class post	with report.		showing elevated excretion of 2-hydroxy glutaric acid (or request us to do OA analysis first)	
Intermediary Metabolites	Includes glucose, lactate, free fatty acids and 3-hydroxybutyrate.	Fluoride plasma sample 0.5ml	Send by first class post	Results for FFA and 3-OHbutyrate can only be interpreted in context of each other and glucose result. Therefore no ranges are provided for these parameters. Interpretation given on report	1 week	Part of fasting hypoglycaemia screen. Follow emergency protocol below: Take 2 ml blood in fluoride heparin and **obtain the first urine sample passed** for organic acid analysis.	£59.97 for full profile. Free fatty acids or 3-hydroxybutyrate individually are £19.81 each
Isovalerylglycine (IVG)	Diagnosis of Isovaleric Acidaemia	Urine 2 mls random sample (no preservative)	Store at -20 Send by first class post	< 2.6 µmol/mmol creatinine	5-14 days	Primarily used for confirmation of diagnosis for presumptive positive IVA on newborn screening	£183.39 (£92.29)
Lactate (CSF)		CSF Fluoride bottle, 0.2ml	Send by first class post	Neonate <3.0mmol/L Child 0.9-1.8 mmol/L Adult 0.6-2.4 mmol/L	1 week	CSF lactate may be clearly abnormal in some patients with mitochondrial disorders even when plasma is normal / borderline.	£20.35
Lathosterol	Increased in	Lithium heparin	Store at -20	µmol/L	3-6 weeks	Part of sterol profile that	£105.25

Test	Clinical Utility	Sample Type and Volume (min in brackets)	Storage / Sending requirements	Reference Ranges	TAT	Comments / Notes	Price (Price for East Mids and South Yorks in brackets)
	Lathosterolosis (extremely rare disorder of cholesterol synthesis similar to Smith-Lemli-Opitz syndrome)	plasma 1 ml (0.3)	Send by first class post	Reference range < 12		includes quantitative measurement of 7 and 8-dehydrocholesterol, lathosterol, desmosterol and cholesterol. Also includes a qualitative sterol profile which can detect the presence of**;** **Lanosterol, 8,(9)-cholestenol, and the 4-methyl-sterols**	(£52.68)
Methylmalonic Acid – Amniotic Fluid	For prenatal diagnosis later in pregnancy for the purposes of knowing that a baby is affected before birth so that appropriate plans can be put in place.	Amniotic Fluid-**CONTACT LAB TO DISCUSS BEFORE SENDING**	**CONTACT LAB TO DISCUSS**	Interpretation given with report	N/A	**MUST CONTACT LAB TO DISCUSS** Prenatal diagnosis for MMA by the measurement of MMA in amniotic fluid is now very rarely done and where a termination is being contemplated we would recommend mutational analysis.	£408.14 (£204.93)
Methylmalonic Acid - Urine	For diagnosis and monitoring of patients with methylmalonic aciduria, Cobalamin defects or Vitamin B12 deficiency	Urine 5-10ml (1.0) aliquot in plain container	Store at -20 Send by first class post	µmol/mmol creatinine Child 1-8 Adult 0.2-2.4	14-21 days		£81.52 (£41.35)

Test	Clinical Utility	Sample Type and Volume (min in brackets)	Storage / Sending requirements	Reference Ranges	TAT	Comments / Notes	Price (Price for East Mids and South Yorks in brackets)
Mucopolysaccharides – GAG electrophoresis	Screening test for Mucopolysaccharide disorders (Hurler, Huler-Scheie, Hunter, Sanfilippo, Morquio, Maroteaux-Lamy, Sly)	Urine 10ml aliquot in plain container **Very dilute (creatinine <0.8) and deteriorated samples (nitrite/protein positive) are not analysed.**	Store at -20 Send by first class post	Qualitative. Interpretation given with report.	6-8 weeks	Can identify vast majority of patients with MPS and narrow down which disorder but final confirmation requires enzyme assay	£120.05
Organic Acids	For the diagnosis of or as a pointer to diagnosis in a wide range of IEMs, including but not limited to classic organic acidurias (MMA, PA, IVA), b-ketothiolase, fumarate hydratase, malonic aciduria, mevalonate kinase def, GA1, 3-HMGCoA-lyase def, biotinidase deficiency, some amino acid disorders including Tyrosinaemia type1, fatty acid oxidation	Urine 10ml aliquot in a plain container. DO NOT USE BORIC ACID PRESERVATIVE	Store at -20 Send by first class post	Qualitative. Interpretation given with report.	5-14 days	Given the number of disorders that can be diagnosed by organic acid analysis it is particularly important that clinical details or information on which particular disorder is suspected (where relevant) is provided in order to aid interpretation, tailor report and include appropriate caveats. If you are not sure if the disorder under consideration is covered by OA analysis please contact that lab to discuss.	£103.06 (£51.59)

Test	Clinical Utility	Sample Type and Volume (min in brackets)	Storage / Sending requirements	Reference Ranges	TAT	Comments / Notes	Price (Price for East Mids and South Yorks in brackets)
	disorders, some urea cycle disorders (raised orotic acid), pyroglutamic aciduria (secondary and primary) etc						
Orotic Acid	For diagnosis and monitoring of patients with urea cycle disorders or hereditary orotic aciduria.	Urine 10ml aliquot in plain container	Store at -20 Send by first class post	µmol/mmol creatinine Infant/child/adult < 3.5	5-14 days	Orotic acid can be detected in urine organic acid profile but the quantitative test is more sensitive and allows comparison between samples.	£87.72 (£44.19)
Phenylalanine – Plasma (see Plasma Amino Acids)	-	-	-	-	-	-	-
Phenylalanine –Blood spot	For monitoring of known patients only – please contact lab.	-	-	-	-	-	-
Phosphoethanolamine	For the diagnosis of Hypophosphotasia	Urine 5 ml aliquot in plain container	Store at -20 Send by first class post	µmol/mmol creatinine "Normal range" is <10 but results above this are not necessarily significant depending on age /context. Interpretation given with report.	21 days		£66.51

Test	Clinical Utility	Sample Type and Volume (min in brackets)	Storage / Sending requirements	Reference Ranges	TAT	Comments / Notes	Price (Price for East Mids and South Yorks in brackets)
Phytanate – see VLCFA	-	-	-	-	-	-	-
Phytosterols	For the diagnosis of Phytosterolaemia / Sitosterolaemia. Includes the quantitative measurement of **campesterol, stigmasterol and sitosterol.** Fucosterol is detected qualitatively.	Lithium heparin plasma or serum 1ml (0.3)	Store at -20 Send by first class post	Campesterol: <22 µmol/L Stigmasterol: <4 µmol/L Sitosterol: <19 µmol/L	3-6 weeks		£105.25 (£52.68)
Pipecolic Acid –CSF	For the diagnosis of **Pyridoxine Dependent Epilepsy (Antiquitin deficiency).** Also used for further investigation of patients with probable **Peroxisomal disorders**	CSF 0.5ml (0.2)	Store at -20 Send by first class post	0.009 – 0.12 µmol/L	3-6 weeks	Please indicate on request form whether querying PDE or Peroxisomal disorder. For the diagnosis of PDE CSF is the best sample type, followed by plasma and then urine. For peroxisomal disorders plasma samples suffice.	£183.39 (£92.29)

Test	Clinical Utility	Sample Type and Volume (min in brackets)	Storage / Sending requirements	Reference Ranges	TAT	Comments / Notes	Price (Price for East Mids and South Yorks in brackets)
Pipecolic Acid - Plasma	As above	Lithium heparin/EDTA Plasma or serum 0.5ml (0.2)	Store at -20 Send by first class post	< 10.8 µmol/L (<1 week old) < 2.46 µmol/L (>1 week old)	3-6 weeks	As above	£183.39 (£92.29)
Pipecolic Acid -Urine	As above	Urine 5 ml (1ml)	Store at -20 Send by first class post	0.55 – 24.1 µmol/mmol creat (<6 months old) 0.01 – 1.54 µmol/mmol creat (>6 months old)	3-6 weeks	As above	£183.39 (£92.29)
Plasmalogens	For confirmation of diagnosis / further characterisation of patients with probable Peroxisomal Biogenesis disorders or Rhizomelic Chondrodysplasia Punctata (RCDP) or FAR1 defects	Red blood cell pellet 2 mls EDTA red blood cells, spin and take off plasma (including buffy coat), wash x3 with equal volume normal saline	Store at -20 (washed RBC only) and send frozen (dry ice). Can send by first class post if will arrive day after sample taken (in which case do not	C16/Palmitate: 0.082 - 0.14 C18/Stearate: 0.176- 0.28 (Please note that these are ratios and therefore there is no unit of measurement)	3-6 weeks	Please provide information on clinical context and information on VLCFA results in order to facilitate appropriate interpretation.	£183.39 (£92.29)

Test	Clinical Utility	Sample Type and Volume (min in brackets)	Storage / Sending requirements	Reference Ranges	TAT	Comments / Notes	Price (Price for East Mids and South Yorks in brackets)
			freeze)				
Pristanate – see VLCFA	-	-	-	-	-	-	-
Sitosterols – see Phytosterols	-	-	-	-	-	-	-
Sterols – see 7-dehydrocholesterol, 8-dehydrocholesterol, desmosterol, lathosterol, cholestanol or phytosterols as appropriate	-	-	-	-	-	-	-
Sulphocysteine (this replaces the urine sulphite test)	For the diagnosis of Sulphite Oxidase or Molybdenum Cofactor deficiency	Urine, no preservative, 5ml (1ml)	Store at -20 Send by first class post	µmol/mmol creatinine Ref range <10	3-6 weeks	If diagnosis is strongly suspected in a newborn please contact lab to request urgent analysis.	£81.52
Trimethylamine	Diagnosis of Fish Odour Syndrome, also known as Trimethylaminuria, (FMO3 gene defect)	Urine 10 aliquot of acidified (HCl) 24 hour collection or 10 ml random urine acidified to pH 2 with HCl prior to dispatch.	Store at -80 Send by first class post	Free TMA/ creat ratio: < 7.7 µmol/mmol crt TMA-N-Oxide / creat ratio: < 119 µmol/mmol crt % N-Oxidation: > 94% Additionally interpretation will be	6-8 weeks	TMAU is associated with ingestion of certain foods so it is important to collect this sample at the time of the odour. For this we recommend a dietary 'Choline Load' before sample collection using foods	£155.19 (£77.05)

Test	Clinical Utility	Sample Type and Volume (min in brackets)	Storage / Sending requirements	Reference Ranges	TAT	Comments / Notes	Price (Price for East Mids and South Yorks in brackets)
		Please note nitrite positive samples will not be analysed.		given with report		known to produce the odour eg beans, eggs, liver. **Suggested Procedure**: at 13:00 and 19:00 a high choline meal containing (eg 2 eggs + 400g baked beans or other beans – can reduce quantity for children). Then; 1. Start collecting with the first urine passed on the day after the choline load and collect urine until the end of the day i.e. empty bladder before going to bed (does not need to be a strict 24 collection). *or* 2. collect a single 20ml sample first thing in the morning after the choline load if 24hr collection is impractical (e.g. in young children)	
VMA / HVA	Primarily used for the investigation and monitoring of	Urine, 10ml aliquot of acidifed (HCl) 24	Store at -20 Send by first class post	µmol/mmol creatinine Infant:	5-14 days		£88.17

Test	Clinical Utility	Sample Type and Volume (min in brackets)	Storage / Sending requirements	Reference Ranges	TAT	Comments / Notes	Price (Price for East Mids and South Yorks in brackets)
	neuroblastoma	hour collection or 10ml random urine accepted		HVA 4 - 25 VMA 2 -12 1-5 yrs: HVA 2 -15 VMA 2 - 9 > 5 yrs: HVA 2 -13 VMA 1- 7			
Very Long Chain Fatty Acids (VLCFA) – includes C26, C24, C22, C26/C22, C24/C22, phytanate and pristanate	For investigation / diagnosis of peroxisomal disorders such X-ALD, Zellweger syndrome, Infantile Refsum's, Adult Refsum's, RCDP, D-bifunctional protein deficiency etc NB Diagnosis of **VLCAD deficiency** requires Acylcarnitine analysis as this is a fatty acid oxidation disorder NOT a peroxisomal disorder	Lithium or EDTA Plasma, or serum 0.5 ml (0.3)	Store at -20 Send by first class post	µmol/L C22 15-112 C24 14-80 C26 0.33-1.50 C24/C22 0.44-0.97 C26/C22 0.005-0.03 Pristanate 0 – 1.88 Phytanate 0.2-19.3	3-6 weeks	This is the first line test for the investigation of patients with suspected peroxisomal disorders. Almost all patients will show some abnormality with exception of some forms of RCDP which only have abnormal plasmalogens and some very rare variant / mild PEX gene defects.	£96.32 (£48.11)

Tissue Culture and Enzyme Assay

Most enzyme assays on cultured cells at Sheffield Children's NHS Foundation Trust are performed on cultured skin fibroblasts. These can be sent as either a skin biopsy or as cultured fibroblasts.

For skin biopsies sent from external hospitals 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 cultured fibroblasts, please send samples with the aim of them arriving Mon – Fri. 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.

Reference ranges are not provided in the table below. Patient reports will contain the relevant reference range.

Please note: there are no EQA schemes available for the enzyme assays performed by the laboratory.

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

Test	Clinical Utility	Comments / Notes	Price (£)
TISSUE CULTURE			

Test	Clinical Utility	Comments / Notes	Price (£)
Establishing a primary culture from skin biopsy, growing under sterile conditions (mycoplasma -free), cryopreservation and retention	To enable fatty acid oxidation flux assay, specific enzyme assay, cryopreservation of cells for future analysis, including molecular analysis. Cultured cells are not subject to secondary effects of post mortem.	Please send skin biopsy in a pot of sterile culture medium. Can be placed in the fridge overnight if posting straight away is not possible. **DO NOT freeze.**	£384.31
Growing on of fibroblast cell line for assay when received as cells from outside laboratory	As above.	Ensure flasks are filled completely with medium (no air bubbles) and are well insulated. Continue to grow cells at sending lab in case there are any issues. Cells MUST be mycoplasma free (see below).	£242.81
Recovery of cryopreserved cultured cells and growing on	For further studies.	Please contact the lab on 0114 2717267.	£83.81
Onward dispatch of living culture (carriage extra at cost): Within the UK: Abroad:	For studies not performed at Sheffield Children's NHS Foundation Trust	Please contact the lab on 0114 2717267.	 £64.87 £117.10
ASSAYS ON CULTURED CELLS			
$^{14}CO_2$ or ^{14}C-incorporation assays (isovaleric etc)	Isovaleric acid incorporation is used to confirm the diagnosis of isovaleric acidaemia. It can also be used to aid in the diagnosis of patients with 3-hydroxy-3methylglutaryl (HMG) CoA lyase deficiency.		£404.43
3-Methylcrotonyl-CoA Carboxylase	Specific enzyme assay for 3-Methylcrotonyl-CoA Carboxylase		£404.43
Carnitine Palmitoyl Transferase Type I	Specific enzyme assay for CPT1. Can be requested directly or added on following		£638.11

Test	Clinical Utility	Comments / Notes	Price (£)
	performance of the tritiated flux assay		
Carnitine Palmitoyl Transferase Type II	Specific enzyme assay for CPT2. Can be requested directly or added on following performance of the tritiated flux assay		£454.28
Carnitine Acylcarnitine Translocase (CATR)	Specific enzyme assay for Carnitine Acylcarnitine Translocase (CATR).		£584.01
Carnitine Transporter Assay	To aid in the diagnosis of primary carnitine deficiency		£311.86
Fatty acid oxidation flux assay (tritiated myristate, palmitate and oleate in parallel)	For diagnosis of fatty acid oxidation defects.		£357.86
Glutaryl CoA dehydrogenase	To aid in the diagnosis of Glutaryl CoA dehydrogenase (GA1) deficiency. To exclude GA1 in cases of suspected non-accidental injury.		£415.94
L-leucine $^{14}CO_2$ release	To aid in diagnosing/confirming maple syrup urine disease (MSUD)		£401.94
L-Valine $^{14}CO2$ release	Valine oxidation is a useful assay for detecting BCAT2 deficiency		£401.94
Propionyl CoA carboxylase	To aid with confirming diagnosis of Propionic Acidaemia.		£415.21
Pyruvate Carboxylase	Should be considered in any child presenting with lactic acidosis and neurological abnormalities.		£404.43
Very long-chain fatty acids in cultured cells (fibroblasts, amniotic fluid cells or chorionic villus cells)	To aid with the work up of patients with suspected peroxisomal defects.		£435.35

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) 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.

c) 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.