Central Manchester University Hospitals NHS
NHS Foundation Trust

WILLINK BIOCHEMICAL GENETICS UNIT

A USER'S GUIDE TO THE SERVICE AND DIAGNOSTIC TESTS AVAILABLE

CONTENTS

1. INTRODUCTION

The Willink Biochemical Genetics Laboratory is now incorporated in the Genomic Diagnostics Laboratories within the Manchester Centre for Genomic Medicine at St Mary's Hospital, Manchester which is part of the Central Manchester University Hospitals Foundation Trust. Close integration of laboratory investigation and clinical management within the Willink Unit has led to the development of a unique service aimed towards the prevention of developmental delay by the early diagnosis and appropriate management of children and adults affected by inherited metabolic disorders. The Willink clinic area and office suites are situated just outside the laboratory area resulting in a close liaison between the clinicians and scientists responsible for the service.
The unit contains the laboratories responsible for the region's newborn screening programme as well as a wide range of biochemical investigations.

Clinical interpretation of results is essential when investigating rare disorders. Clinicians sending samples are contacted regarding their patients when positive or important negative diagnoses are made. Six consultant paediatricians provide a 24-hour on-call service for metabolic patients. Advice regarding investigations is available at all times by contacting the paediatricians or senior scientists in the laboratory.

There are a number of specialist metabolic clinics held each week, with outreach clinics also held in Bradford, Liverpool, Bristol, Cardiff, Belfast and Dublin. All clinics are consultant-led and patients are seen initially by the consultant staff, who are supported by specialist nurses and dieticians based within the Willink along with a senior Clinical Physiotherapist. In-patients are managed on Ward 85 of the Royal Manchester Children's Hospital, whilst patients receiving enzyme replacement therapy transfusions are managed on the Elective Treatment Centre (Ward 76).

The laboratory also provides a diagnostic service for the adult Lysosomal Storage Disease clinic situated at Salford Royal Hospital.

2. GENERAL INFORMATION

2.1 POSTAL ADDRESS

Willink Biochemical Genetics Laboratory
Genomic Diagnostics Laboratories
Manchester Centre for Genomic Medicine
Central Manchester University Hospitals NHS Foundation Trust
6th Floor, Pod 1
St Mary's Hospital
Oxford Road
Manchester
M13 9WL
Telephone: 0161 70 12137/8
Fax: 0161 70 12303

2.2 MANCHESTER GENETICS WEBSITE

http://www.mangen.co.uk/lab-services/WillinkBiochemicalGenetics.php

2.3 REQUEST FORM

Request form is available from the above website, go to Lab Services > Willink Biochemical Genetics > Request Form.
Alternatively, the request form can be downloaded using the following link:
http://www.mangen.co.uk/CubeCore/.uploads/Willink/doc512laboratoryrequest.pdf

2.4 WHERE TO FIND US

Willink Biochemical Genetics is situated on the sixth floor in Pod 1, Manchester Centre for Genomic Medicine at St Mary's Hospital, Oxford Road, Manchester. Access by foot can be made through the Children's Hospital main entrance on Hathersage Road or through the main entrance of St Mary's Hospital and is sign posted Genetic Medicine.

2.5 KEY PERSONNEL

The Willink Unit currently has six consultant paediatricians, Professor John Walter, Dr Simon Jones, Dr Andrew Morris, Dr Elisabeth Jameson, Dr Alexander Broomfield and Dr Bernd Schwahn who can be contacted through the unit office, tel. 0161-70 12137/8.

The Laboratory Director of the Genomic Diagnostics Laboratories is Dr Lorraine Gaunt.
The Laboratory Director of Willink Biochemical Genetics is Dr Mick Henderson.

Willink Biochemical Genetics Senior staff:

Teresa Wu	Newborn Screening & Metabolites	0161 701 2140/2	hoiyee.wu@cmft.nhs.uk
Alistair Horman	Newborn Screening & Metabolites	0161 701 2140/2	alistair.horman@cmft.nhs.uk
Heather Church	Lysosomal Storage Disorders	0161 701 2306/7	heather.church@cmft.nhs.uk
Karen Tylee	Lysosomal Storage Disorders	0161 701 2306/7	karen.tylee@cmft.nhs.uk
Robert Gibson	Chief Biomedical Scientist	0161 701 2143	robert.gibson@cmft.nhs.uk

Outside normal working hours the on-call metabolic paediatrician is available via the hospital switchboard (0161-276-1234).

Duty Scientist
The duty scientist can be reached at all times via a DECT phone (0161 701 8612) which is carried with them throughout the day (9am-5pm).

The Willink Laboratory generally works under two sections, Metabolites and Newborn Screening and Lysosomal Storage Disorders. The Duty Scientist will try to answer queries but if specialist knowledge is required they will pass the call to the most appropriate staff member to answer the query. Requests for urgent analysis would often be directed to and dealt with by the duty scientist, though not exclusively.

2.6 POPULATION SERVED

The laboratory performs the newborn screening service for Phenylketonuria, MCADD, Isovaleric Acidemia, Glutaric aciduria type I, Maple Syrup Urine Disease and Homocystinuria for the North West of England. The laboratory also serves as a reference laboratory for inherited metabolic disorders for the region, the UK and Internationally referred samples. It is a NCG designated national referral centre for lysosomal storage disorders, accepting samples from centres throughout the UK, EIRE and various other International centres.

2.7 LABORATORY HOURS

The laboratory is open Monday to Friday 9.00am to 5.00pm
The laboratory is closed at weekends and on official UK Public Holidays.

2.8 TEST PRICING STRUCTURE

In accordance with Central Manchester University Hospitals NHS Foundation Trust policy we have implemented a two tier pricing structure for NHS and non NHS referral work.
Samples referred to our laboratory for analysis from non NHS locations will have a 40% uplift in test cost compared to samples referred for analysis from other NHS laboratories. Please note, prior to this our test costs have not increased since 2009. Please contact the laboratory if current test costs are required.

3. USE OF THE LABORATORY

3.1 REQUESTS TO THE LABORATORY

Requests for tests performed by this laboratory should be sent from a referring doctor. Routine requests should be sent to the laboratory by the methods relevant to the test as stated in the handbook. Urgent and out of hours requests must be made by contacting the metabolic consultant on call via the hospital switchboard tel: 0161 276 1234.

The request form must be completed with all required information. The specimen container must also be fully identified with the patient name, date of birth, identification number and the date/time of sample collection. All requests must have 3 individual identifiers which will usually be name, date of birth and NHS/hospital number (first name and surname are classed as two identifiers). Requests without this information may be rejected.

The Willink Biochemical Genetics Laboratory has a request form (available online as detailed above) but will accept requests for tests written on other request forms or by letter from the referring doctor, provided that all the relevant information is given. The information given should include:

Patient name in full
Identification number e.g. hospital number or NHS number
Date of Birth
Gender
Consultant or referring doctor's name
Name and address to where reports should be sent
Date and time of specimen
Date and time of sending sample

Specimens sent from another laboratory must be identified with the referring laboratory number, which should also be on the request form.

3.2 PHLEBOTOMY SERVICE

The laboratory does not provide a phlebotomy service.

3.3 TRANSPORT TO THE LABORATORY

Samples are accepted by the laboratory from hospital porters (for hospital internal samples), by hand delivery, by external post and by courier services.
The hospital porters collect from each ward twice a day, morning and afternoon, and deliver samples to pathology sample reception where they are redistributed. It must be noted, however, that some samples will need to be delivered directly to the laboratory and cannot wait for the porter service (see appropriate test requirements).
All samples are delivered to Genetic Medicine sample reception, floor 6, pod 1, they are picked up from here by the laboratory staff. Urgent samples from outside the hospital should be delivered by taxi or courier to Genetic Medicine sample reception and not to elsewhere within the hospital. Samples must be sent direct to the laboratory, we cannot undertake to collect samples from rail stations, airports or other collection points.
Many external samples may be delivered by first class post (see relevant sample and test information as to whether this is appropriate for the test in question). Samples sent by post should follow the appropriate packaging requirements of the postal system (see below).

PACKAGING OF SAMPLES FOR TRANSPORT

Samples must be sent to the laboratory in a special closed polythene bag which allows the sample and the accompanying request form to be kept separated. Samples with a category 3 infection risk must be clearly marked with a yellow CATEGORY 3 RISK sticker on the request form.
Samples being delivered by post should follow the guidelines set down. 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 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 Data post 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 50mL unless specifically permitted. The primary container must be wrapped in enough absorbent material to absorb all possible leakage, and sealed in a leak-proof 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 section in a two piece polystyrene box, empty spaces must be filled with absorbent material, the box must be secured with adhesive tape.
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 'BIOLOGICAL SUBSTANCE, CATEGORY B' and show an open diamond with UN 3373 across its centre. The package should also show the name and address of the sender as well as the delivery address.
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.

Please note. Infectious pathology samples may only be transported in packaging which meets the U.N. class 6.2 specifications and the 650 packaging requirements. These new packaging requirements are described below.

BASIC TRIPLE PACKAGING SYSTEM

This 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 travel.
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.

Containers received with samples

As we receive a great number of samples for testing from outside the hospital, we also receive a great number of transport containers. It is now our laboratory policy that all re-usable sample transport containers received **with postage paid return labels** will be returned to the initiating laboratory. All other containers will either be reused by our laboratory or disposed of.

NEWBORN SCREENING CARDS

By common consent the above regulations are deemed inappropriate for dried blood specimens on Newborn Screening Cards. The blood spots should be allowed to dry thoroughly before packing, the card is placed into the transparent paper (Glassine) envelope provided (not plastic as this may cause the specimen to 'sweat') and sent in a stout envelope as if it were a normal letter, first class post.

3.4 RESULTS

Reports from samples taken within the hospital will be issued to the appropriate requesting clinician.
Reports from samples sent from another hospital will be sent to the referring hospital's pathology department, assuming this information has been provided when the sample is sent.
Reports from samples sent from abroad will be sent to the referring clinician, either by secure fax or encrypted email when possible, with a follow up written report. Reports by email must be encrypted unless they are being sent internally via Trust email; or via NHS.net email accounts if sent externally.
Reports are issued without delay, usually within 24 hours of results being obtained.
Positive diagnostic results are communicated to one of the Willink's consultant paediatricians by a registered scientist. The referring consultant is then contacted by telephone and the positive report is usually sent via secure fax. Hard copies of these reports are then sent out via the regular postal service.
Urgent results, such as for prenatal diagnoses, may be communicated by secure fax transmission. These will always be followed by a written report sent within 24 hours.
Results will not be communicated to patients or their relatives or to any unauthorised personnel.

4. OUT OF HOURS SERVICE

Urgent investigations will only be performed following discussion with one of our consultants. They may be contacted via the hospital switchboard, Tel 0161 276 1234 (see general information and notes on tests available).

5. QUALITY ASSURANCE

The department participates in national and international external quality assurance schemes to monitor the accuracy and precision of analyses performed. Internal quality control is used to check the validity of results on a daily basis or whenever an assay is performed.

6. GENERAL INFORMATION AND NOTES ON TESTS AVAILABLE

Urgent investigations - organic acid and amino acid disorders

Patients with suspected amino acid or organic acid disorders may require urgent studies in order to implement appropriate treatment. These patients often present in the neonatal period with failure to

thrive, vomiting, lethargy, hyperventilation, seizures and hypotonia. There may be metabolic acidosis, respiratory alkalosis, hypoglycaemia, hypocalcaemia and/or deranged liver function tests. Blood ammonia and lactate may be raised.
For organic and amino acid screen, as well as acylcarnitines, please send 10ml fresh urine and 2ml heparinised blood or a blood card with 4 spots of blood. Results should be available the same day assuming samples arrive in good time (before 11am) and the laboratory has been warned of the urgent sample. It is important that full clinical details are given including details of metabolic acidosis, jaundice, blood ammonia and drug history. For disorders of fat oxidation e.g. MCAD deficiency, it is important that urine is collected at the time of hypoglycaemic stress. Urgent investigations will normally only be performed following discussions with one of our consultants.

Galactosaemia screen

Patients with unexplained or prolonged jaundice should be screened for classical galactosaemia. The condition is often accompanied by septicaemia, has an incidence of around 1 in 45,000 births and is exacerbated by lactose containing milk. Reducing substances are not always found in urine and therefore a Beutler screening test should be carried out. Approx. 0.2 - 0.5ml heparinised blood should be sent directly to the laboratory. Note: the test is not valid if the patient has undergone a recent blood transfusion (within 4 months) and the test could also give a false positive result with G-6-PD deficiency. Transfused patients would require Gal-1-P analysis on whole heparinised blood (5ml). Since these samples require immediate processing it is important to warn the lab of any Gal-1-P analyses.

Sudden unexplained infant deaths

Some metabolic disorders may result in sudden infant death or SUDI. To investigate these disorders in SUD infants, please collect urine (5ml by supra pubic stab if necessary) or failing this CSF for organic acid analysis and cardiac blood (5ml EDTA) for DNA analysis. It is also recommended that a dried blood spot is taken, onto a standard newborn screening card, for tandem mass spectrometry of acylcarnitines. Tissue for culture should only be collected where there is a strong possibility of fat oxidation defect, i.e. fatty liver on gross examination. A small (approx. 2-3mm^3) piece of skin should be collected aseptically into sterile tissue culture medium.

Lysosomal disorders

The lysosomal enzyme screen covers 16 different disorders, mostly the sphingolipid and glycoprotein storage disorders but does not include the Mucopolysaccharide disorders. Mucopolysaccharidoses are initially screened for using two dimensional electrophoresis of glycosaminoglycans extracted from urine. Some disorders require specific tests which are not covered by the lysosomal enzyme screen or the MPS urine screen. These disorders include, but are not limited to, Pompe, Niemann-Pick type C, Sialic Acid Storage Disease and Sialidosis. Where the enzyme and MPS screens are negative but there is evidence of an underlying storage disorder (e.g. visceromegaly, vacuolated/foam cells in bone marrow or blood) further tests should be discussed with the laboratory.

Peroxisomal disorders

Plasma very long chain fatty acid analysis remains the most useful screening test for these conditions. VLCFA concentrations are significantly increased in general peroxisomal disorders such as Zellweger syndrome as well as in rare peroxisomal β-oxidation disorders. In X-linked adrenoleukodystrophy the C26/C22 ratio is less markedly raised. Where disorders such as Zellweger are strongly suspected it is important to also request plasmalogens on erythrocytes. Fibroblast assays may be required to confirm the diagnosis. Please note that phytanic acid levels will only be abnormally raised after sufficient dietary intake i.e. older patients.

Prenatal diagnosis

Prenatal diagnosis is available for a number of metabolic disorders. For all cases a firm biochemical diagnosis must be established in the proband, since a similar test is likely to be used for prenatal studies. Studies in the parents/obligate heterozygotes may also be necessary to exclude low enzyme activities or pseudo deficiencies which may compromise the interpretation of prenatal results. Advice should be sought from the laboratory on the type of sample best suited for diagnosis and optimum gestational age. Direct enzyme assay of CVS is usually the preferred approach but for some disorders amniocentesis may be more appropriate.

7. REPERTOIRE OF TESTS

The following pages list the various diagnostic tests available within Willink Biochemical Genetics section of the Genomic Diagnostic Laboratories. Details of samples from newborn infants for PKU, MCADD, IVA, GA1, MSUD and Homocystinuria screening are not listed but these tests are performed in our laboratory for the 'old North Western' Health Region and operates through the health visitors and midwives.

Additional tests can be added for patient samples previously received to the laboratory as long as there is sufficient viable sample remaining and the additional request is within our specified storage time for that sample type.

Please find listed below the codes used for different types of samples for various investigations. The table details sample requirements, turnaround times and any special precautions necessary for each test offered by the laboratory.

EDTA	= EDTA Whole Blood	HEP	= Lithium Heparin Whole Blood
P	= Plasma	U	= Urine
CC	= Cultured Skin Fibroblasts	WB	= Whole Blood
CSF	= Cerebrospinal fluid	AF	= Amniotic Fluid
AFC	= Cultured Amniotic Fluid Cells	CVS	= Chorionic Villus Sample
CCV	= Cultured Chorionic Villi	DBS	= Dried Blood Spots

If further details are required please do not hesitate to contact the laboratory. **Please note tissue culture costs are in addition to specific assay costs when cultured cells are required.**

ALL REQUESTS MUST DETAIL PATIENT AGE, SEX, CLINICAL DETAILS AND RELATED THERAPY

Test	Required specimen & volume	Special precautions	Turnaround time	Reference ranges	Section
CARBOHYDRATE DISORDERS					
Sugar Chromatography	5ml plain U	None	3 working weeks	Qualitative	Metabolites
α-glucosidase *Pompe (GSDII)*	5ml EDTA	Must reach laboratory within 48 hours of venepuncture	2 working weeks	3 – 20 µmol/g/h – acarbose 2 – 15 µmol/g/h + acarbose	Lysosomal
Cross Reactive Immunologic Material (CRIM) Analysis for Pompe disease	Contact laboratory for information	Contact laboratory	3 working days	Qualitative	Lysosomal
Beutler Test *Galactosaemia*	0.5 ml HEP	Must reach laboratory within 24 hours of venepuncture	3 working days	Qualitative	Metabolites
Galactose-1-phosphate *Galactosaemia monitoring*	5ml HEP	Must reach laboratory within 24 hours of venepuncture	3 working weeks	5 –10 µg/ml packed red cells	Metabolites
AMINO ACID DISORDERS					
Urine qualitative amino acid screen*	5ml plain U, no preservative	None	3 working weeks	Qualitative	Metabolites

Test	Required specimen & volume	Special precautions	Turnaround time	Reference ranges	Section
Quantitative amino acids	3ml HEP or 1ml plain CSF, blood not stained	Must be sent on ice or deproteinised with internal standard, contact lab for procedures. Avoid freezing whole blood.	3 working weeks	Quoted on report	Metabolites
White cell cystine *Cystinosis*	5ml HEP	Willink no longer offer this service. Please contact Metabolic Lab at St James' Hospital Leeds Tel: 0113 206 4256 CMFT Users: Please avoid collecting sample on a Friday. Next day delivery to Leeds cannot be guaranteed.	n/a	n/a	Metabolites
Total Homocyst(e)ine	3ml HEP P	Plasma must be sent frozen	4 working weeks	< 15 µmol/L	Metabolites
Orotic acid *Urea cycle defects*	2ml plain U	None	4 working weeks	< 5 µmol/mmol creatinine	Metabolites
14C-citrulline incorporation *Citrullinaemia and arginosuccinic aciduria*	CC, AFC, CCV	Contact lab prior to dispatch to discuss test	Dependent on culture time	Controls quoted	Metabolites
14C-leucine oxidation *Maple syrup urine disease*	CC, AFC	Contact lab prior to dispatch to discuss test.	Dependent on culture time	Controls quoted	Metabolites

Test	Required specimen & volume	Special precautions	Turnaround time	Reference ranges	Section
ORGANIC ACID DISORDERS					
Organic acids by GC-MS*	5ml plain U	Full drug history	3 working weeks	Qualitative	Metabolites
Pyruvate carboxylase	CC, AFC, CCV, CVS	Contact lab prior to dispatch to discuss test.	Dependent on culture time	Fibroblasts 6 - 40 nmol/h/mg	Metabolites
Methylmalonic acid Quantitation in plasma	2ml EDTA or HEP	Contact lab prior to dispatch test	4 working weeks	Quoted on report	Metabolites
Methylmalonic acid Quantitation in urine	2 ml Plain U	Contact lab prior to dispatch test	4 working weeks	Quoted on report	Metabolites
Succinylacetone – Qualitative screen on DBS *Tyrosinaemia Type I*	DBS	Contact lab prior to sampling; sample must reach lab within 24 hours.	4 working weeks	Absent in normal subjects	Metabolites
Propionyl-CoA carboxylase *Propionic aciduria*	CC, AFC, CCV, CVS	Contact lab prior to dispatch to discuss test.	Dependent on culture time	40 – 100 nmol/h/mg (fibroblasts)	Metabolites
Methylmalonic-CoA mutase *Methylmalonic aciduria*	CC, AFC, CCV, CVS	Contact lab prior to dispatch to discuss test.	Dependent on culture time	Fibroblasts 207 - 1730 pmol/min/mg	Metabolites

Test	Required specimen & volume	Special precautions	Turnaround time	Reference ranges	Section
^{14}C-propionate incorporation *Propionic and Methylmalonic aciduria defects in B12 Metabolism*	CC, AFC, CCV, CVS	Contact lab prior to dispatch to discuss test.	Dependent on culture time	Assay Controls quoted	Metabolites
Methylcrotonyl-CoA carboxylase	CC, AFC, CCV	Contact lab prior to dispatch to discuss test.	Dependent on culture time	2.5 – 12 nmol/h/mg (fibroblasts)	Metabolites
HMG-CoA lyase *3-hydroxy 3-methylglutaric aciduria*	CC, AFC, CCV	Contact laboratory prior to dispatch to discuss test.	Dependent on culture time	0.52 - 3.96 nmol/min/mg protein	Metabolites
Biotinidase *Multiple carboxylase deficiency*	2 - 3ml HEP, or P	To reach lab within 24 hours	2 working weeks	Plasma 4 - 12nmol/min/ml	Metabolites
Acyl carnitines (Includes free Carnitine)	1ml HEP or EDTA or DBS	None	2 working weeks	Free carnitine 20 - 40μM, values quoted for specific acyl carnitines	Metabolites
LYSOSOMAL STORAGE DISEASES					
Lysosomal enzyme screen *16 different lysosomal storage disorders (see page 21)*	5ml EDTA	To reach the laboratory within 72 hours of venepuncture	4 working weeks	See individual enzymes	Lysosomal

Test	Required specimen & volume	Special precautions	Turnaround time	Reference ranges	Section
MUCOPOLYSACCHARIDOSIS SCREEN					
2-D electrophoresis of GAGs* *Mucopolysaccharidoses*	10ml fresh plain U, 10ml AF	To reach the laboratory within 72 hours of sampling	3 working weeks	Qualitative	Lysosomal
Oligosaccharide screen	3ml plain U. Age of patient must be specified	To reach the laboratory within 72 hours of sampling	3 working weeks	Qualitative	Lysosomal
Quantitative sialic acid *Sialic acid storage disease, Sialidosis, Galactosialidosis*	2 - 3ml plain U, CC. Age of patient **must** be specified	To reach the laboratory within 72 hours of sampling	4 working weeks	Age-matched controls quoted	Lysosomal
MPS ENZYME ASSAYS					
α-iduronidase *MPS I, Hurler syndrome, Scheie syndrome, Hurler/Scheie syndrome*	5ml EDTA, CC, AFC, CCV,CVS	To reach the laboratory within 72 hours of venepuncture	2 working weeks	White cells 10 - 50 µmol/g/h Other tissues, assay controls quoted	Lysosomal
Iduronate sulphatase *MPS II, Hunter syndrome*	5ml EDTA, CC, AFC, CCV, CVS	To reach the laboratory within 72 hours of venepuncture	3 working weeks	Assay control values quoted	Lysosomal

Test	Required specimen & volume	Special precautions	Turnaround time	Reference ranges	Section
Heparan sulphamidase *MPS IIIA, Sanfilippo A syndrome*	5ml EDTA, CC, AFC, CCV,CVS	To reach the laboratory within 72 hours of venepuncture	3 working weeks	Assay control values quoted	Lysosomal
α-N-acetylglucosaminidase *MPS IIIB, Sanfilippo B syndrome*	5ml EDTA CC, AFC, CCV,CVS	To reach the laboratory within 72 hours of venepuncture	3 working weeks	Assay control values quoted	Lysosomal
Acetyl-CoA:α-glucosaminide N-acetyltransferase *MPS IIIC, Sanfilippo C syndrome*	5ml EDTA, CC, AFC, CCV,CVS	To reach the laboratory within 72 hours of venepuncture	3 working weeks	Assay control values quoted	Lysosomal
Galactose-6-sulphatase *MPS IVA, Morquio syndrome*	5ml EDTA, CC, AFC, CCV,CVS	To reach the laboratory within 72 hours of venepuncture	3 working weeks	Assay control values quoted	Lysosomal
β-galactosidase *MPS IVB, Morquio syndrome & GM1-gangliosidosis*	5ml EDTA, CC, AFC, CCV,CVS	To reach the laboratory within 72 hours of venepuncture	3 working weeks	White cells 100 - 400 μmol/g/h Other tissues, assay controls quoted	Lysosomal
Arylsulphatase B *MPS VI, Maroteaux-Lamy syndrome*	**10**ml EDTA, CC, AFC, CCV,CVS	To reach the laboratory within 72 hours of venepuncture	3 working weeks	Assay control values quoted	Lysosomal

Test	Required specimen & volume	Special precautions	Turnaround time	Reference ranges	Section
β-glucuronidase *MPS VII, Sly's syndrome*	5ml EDTA, CC, AFC, CCV,CVS	To reach the laboratory within 72 hours of venepuncture	3 working weeks	White cells 100 – 800 µmol/g/h Other tissues, assay controls quoted	Lysosomal
Multiple sulphatases *Multiple sulphatase deficiency*	5ml EDTA, CC, AFC, CCV,CVS	To reach the laboratory within 72 hours of venepuncture	3 working weeks	Assay control values quoted	Lysosomal
OTHER ENZYME ASSAYS					
Aspartylglucosaminidase *Aspartylglucosaminuria*	5ml EDTA, CC, AFC, CCV, CVS	To reach the laboratory within 72 hours of venepuncture	3 working weeks	Plasma 10 – 60 µmol/l/h Other tissues, assay controls quoted	Lysosomal
N-acetyl α-neuraminidase *Sialidosis*	CC, AFC, CCV	To reach the laboratory within 72 hours of venepuncture	3 working weeks following completion of culture	Assay controls quoted	Lysosomal
α-fucosidase *Fucosidosis*	5ml EDTA, CC, AFC, CCV,CVS	To reach the laboratory within 72 hours of venepuncture	3 working weeks	White cells 50 – 250 µmol/g/h Other tissues, assay controls quoted	Lysosomal
α-mannosidase *α-Mannosidosis*	5ml EDTA, CC, AFC, CCV,CVS	To reach the laboratory within 72 hours of venepuncture	3 working weeks	White cells 100 – 800 µmol/g/h Other tissues, assay controls quoted	Lysosomal
β-mannosidase *β-Mannosidosis*	5ml EDTA, CC, AFC, CCV, CVS	To reach the laboratory within 72 hours of venepuncture	3 working weeks	Plasma 150 - 1500 µmol/l/h Other tissues, assay controls quoted	Lysosomal

Test	Required specimen & volume	Special precautions	Turnaround time	Reference ranges	Section
Multiple hydrolases *ML II & III*	5ml EDTA, CC, AFC, CCV, CVS	To reach the laboratory within 72 hours of venepuncture	2 working weeks	Assay controls quoted	Lysosomal
β-hexosaminidase A (MUGS) *Tay-Sachs disease*	5ml EDTA, CC, AFC, CCV, CVS	To reach the laboratory within 72 hours of venepuncture	2 working weeks	Plasma 50 – 250 μmol/l/h Other tissues, assay controls quoted	Lysosomal
Hexosaminidase A & B *Sandhoff disease*	5ml EDTA, CC, AFC, CCV, CVS	To reach the laboratory within 72 hours of venepuncture	2 working weeks	Plasma 600 – 3500 μmol/l/h Other tissues, assay controls quoted	Lysosomal
Galactocerebrosidase (Natural Substrate) K*rabbe Leucodystrophy*	5ml EDTA, CC, AFC, CCV,CVS	To reach the laboratory within 72 hours of venepuncture	3 working weeks	White cells 0.4 – 4 μmol/g/h Other tissues, assay controls quoted	Lysosomal
Arylsulphatase A *Metachrom*atic *Leucodystrophy*	5ml EDTA, CC	To reach the laboratory within 72 hours of venepuncture	3 working weeks	White cells 50 – 250 μmol/g/h Other tissues, assay controls quoted	Lysosomal
α-galactosidase *Fabry disease*	5ml EDTA, CC	To reach the laboratory within 72 hours of venepuncture	1 working week	Plasma 3 – 20μmol/l/h White cells 10 – 50 μmol/g/h Other tissues, assay controls quoted	Lysosomal
β-glucosidase *Gaucher disease*	5ml EDTA, CC, AFC, CCV, CVS	To reach the laboratory within 72 hours of venepuncture	3 working weeks	White cells 1 - 5μmol/g/h Other tissues, assay controls quoted	Lysosomal

Test	Required specimen & volume	Special precautions	Turnaround time	Reference ranges	Section
Chitotriosidase *Marker for some lysosomal storage disorders, monitoring Gaucher patients on treatment*	2ml EDTA	To reach the laboratory within 72 hours of venepuncture	2 working weeks	4 – 120 µmol/l/h	Lysosomal
N-acetyl-alpha-galactosaminidase *Schindler disease*	5ml EDTA, CC	To reach the laboratory within 72 hours of venepuncture	3 working weeks	White cells 5 - 50 µmol/g/h Other tissues, assay controls quoted	Lysosomal
Sphingomyelinase *Niemann-Pick A and B*	5ml EDTA, CC, AFC, CCV,CVS	To reach the laboratory within 72 hours of venepuncture	3 working weeks	White cells 1 – 10 µmol/g/h Other tissues, assay controls quoted	Lysosomal
Acid esterase *Wolman disease and cholesteryl ester storage disease*	5ml EDTA, CC, AFC, CCV,CVS	To reach the laboratory within 72 hours of venepuncture	3 working weeks	White cells 350 – 2000 µmol/g/h Other tissues, assay controls quoted	Lysosomal

Test	Required specimen & volume	Special precautions	Turnaround time	Reference ranges	Section
NCL Screen Palmitoyl Protein Thioesterase 1 *Infantile Neuronal Ceroid Lipofucinoses (INCL)* *and* Tripeptidyl Peptidase 1 *Late infantile Neuronal Ceroid Lipofucinoses (LINCL)*	5ml EDTA	To reach the laboratory within 72 hours of venepuncture	3 working weeks	15 - 83 nmol/mg/hr 56 - 219 nmol/mg/hr	Lysosomal
Filipin Staining (Cholesterol esterification) *Niemann-Pick C*	CC	Cell cultures to reach the laboratory within 48 hours	12 working weeks after culture	Qualitative	Lysosomal
Oxysterol *Niemann-Pick C*	2ml EDTA	Separate plasma from whole blood on day sample taken, freeze and send to laboratory frozen.	4 working weeks	Normal: 8.1-37.7 ng/ml (95% CI 9.6-37.0) NPC1: 35.3-1170 ng/ml (95% CI 39.3-811.9)	Metabolites
PEROXISOMAL DISORDERS					

Test	Required specimen & volume	Special precautions	Turnaround time	Reference ranges	Section
Very Long Chain Fatty Acids *General peroxisomal disorders, VLCFA oxidation defects and X-linked ALD*	5ml EDTA or 2ml P	To reach the laboratory within 72 hours	4 working weeks	C26 / C22 < 0.033 C24 / C22 0.65 – 1.05	Metabolites
Phytanic and Pristinic acids *Refsum disease, RCDP and other peroxisomal disorders*	5ml EDTA or 2ml P	To reach the laboratory within 72 hours	4 working weeks	< 16umol/L	Metabolites
Plasmalogens *RCDP and general peroxisomal disorders*	5ml EDTA	To reach the laboratory within 72 hours	4 working weeks	Reference values quoted	Metabolites
OTHER DISORDERS					
Arylsulphatase C (Steroid sulphatase) *X-linked Ichthyosis*	5ml EDTA	To reach the laboratory within 72 hours of venepuncture	4 working weeks	In-assay controls quoted	Lysosomal
7-dehydrocholesterol *Smith-Lemli-Opitz syndrome*	2ml EDTA	None	4 working weeks	< 10 μmol/L	Metabolites

Test	Required specimen & volume	Special precautions	Turnaround time	Reference ranges	Section
Cholestanol *Cerebrotendinous Xanthamatosis (CTX)*	2ml EDTA	None	4 working weeks	< 16 μmol/L	Metabolites
TISSUE CULTURE					
Initiation of culture	Skin biopsy, amniocytes or CVS	To reach the laboratory within 48 hours	Dependent on cell growth	NA	Tissue Culture
Maintenance of cultures initiated elsewhere	CC, AFC, CCV	To reach the laboratory within 48 hours	NA	NA	Tissue Culture
FIRST TRIMESTER PRENATAL DIAGNOSIS*					
ALWAYS CONTACT LABORATORY PRIOR TO SAMPLING*	CVS, cell free amniotic fluid, cultured cells	To be transported in culture medium, at room temperature and to reach the laboratory within 72 hours of sampling	Dependent on assay needed. Up to 2 working weeks. Many assays will be within 5 working days of receipt. Check with lab for reporting time expected for individual assays.	Dependent on analysis. Control values included in the analysis are quoted.	Various

All samples **must be accompanied by relevant clinical details. This is particularly imperative for **urine amino acids, urine organic acids, urine mucopolysaccharides and all samples for prenatal diagnosis. Reports may be withheld where samples are received without clinical details** as an accurate interpretation may not be possible without them*

8. CONTACT NAMES AND NUMBERS FOR BIOCHEMICAL GENETICS LABORATORY SECTIONS

Genomic Diagnostics Laboratory Director	Lorraine Gaunt	0161 276 3269
Biochemical Genetics Laboratory Director	Mick Henderson	0161 701 1838

Newborn Screening & Metabolites

Principal Clinical Scientists	Teresa Wu/Alistair Horman	0161 701 2140/2
	hoiyee.wu@cmft.nhs.uk	
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Lysosomal Storage Disorders & Tissue Culture

Principal Clinical Scientists	Heather Church/Karen Tylee	0161 701 2306/7
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	karen.tylee@cmft.nhs.uk	

9. FURTHER INFORMATION

9.1 LYSOSOMAL STORAGE DISEASES

The following enzyme analyses are performed as a group lysosomal disorder screening test. The minimum sample required is **5mls** of whole blood in an EDTA specimen tube. Please post specimens early in the week to avoid samples being delayed over the weekend. All relevant clinical information should be provided with the sample. The following enzymes are routinely assayed:

Lysosomal enzyme screen – N.B. does not screen for MPS disorders

Plasma chitotriosidase	(non-specific marker for lysosomal storage disorders)
Plasma β-hexosaminidase	(Sandhoff disease, I-Cell disease)
Plasma β-mannosidase	(β-Mannosidosis, I-Cell disease)
Plasma β-hexosaminidase A [MUGS]	(Tay-Sachs disease)
Plasma aspartylglucosaminidase	(Aspartylglucosaminuria)
Leucocyte β-glucuronidase	(Sly disease, MPS VII)
Leucocyte β-galactosidase	(GM1-gangliosidosis)
Leucocyte α-mannosidase	(α-Mannosidosis)
Leucocyte α-galactosidase	(Fabry disease)
Leucocyte α-fucosidase	(Fucosidosis)
Leucocyte acid esterase	(Wolman/Cholesterol ester storage disease)
Leucocyte arylsulphatase A	(Metachromatic Leucodystrophy)
Leucocyte β-glucosidase	(Gaucher disease)
Leucocyte sphingomyelinase	(Niemann-Pick types A & B)
Leucocyte galactocerebrosidase	(Krabbe Leucodystrophy)
Leucocyte N-acetyl-α-galactosaminidase	(Schindler disease)

9.2 MUCOPOLYSACCHARIDE DISORDERS

Urine screen - Mucopolysaccharide 2-dimensional electrophoresis
This should be the first diagnostic test performed for MPS disorders. Urine should be sent prior to or with samples for enzyme analysis. Enzyme analysis will normally only be performed when an abnormal mucopolysaccharide pattern has been identified in urine. Routinely all urine samples are also tested for oligosaccharide and sialic acid containing conjugates by thin layer chromatography (TLC).

9.3 GLYCOPROTEIN AND SIALIC ACID STORAGE DISORDERS

Urinary oligosaccharide screen - Oligosaccharide TLC
Analysis of urinary oligosaccharides by TLC stained with orcinol and resorcinol for oligosaccharides and sialic acid containing conjugates. This test is not always easy to interpret but complements the lysosomal enzyme and urinary MPS screens and can be useful in detection of some oligosaccharide/glycoprotein disorders.

9.4 PEROXISOMAL DISORDERS

The following investigations can be carried out on the same 5ml EDTA sample.

Very Long Chain Fatty Acids
Screening for Zellweger, other general peroxisomal disorders, X-ALD and VLCFA oxidation defects by GC-MS

Phytanic acid and Pristanic acid
Refsum disease, rhizomelic chondrodysplasia punctata, other peroxisomal disorders

Plasmalogens
RCDP (especially) and general peroxisomal disorders e.g. Zellweger

9.5 PRENATAL DIAGNOSES

The laboratory should **always be contacted prior** to prenatal sampling. Direct analysis of uncultured chorionic villus is not universally appropriate, also, for some disorders, prenatal diagnosis is not yet available. It is important that biochemical diagnosis has been established in the proband and if this has not been done in this laboratory, it may be necessary to confirm the diagnosis on a fresh sample or to verify a diagnosis made elsewhere before accepting the sample. It may also be necessary to study enzyme activities in parents/obligate heterozygotes prior to prenatal studies. We would be happy to provide advice on appropriate samples for each condition, the amount required and the best gestational age for sampling. We **do not** charge for advice given over the telephone, so please contact us with any clinical or technical enquiries.

The charge for prenatal diagnosis by direct analysis of chorionic villi or cultured amniotic fluid cells is as quoted for each specific test plus an additional £55.14. Where cultured amniocytes or cultured chorionic villus cells are required, the appropriate culture charge must be added.

9.6 TISSUE CULTURE

For some conditions it is necessary to carry out enzyme or *in situ* radiochemical incorporation/oxidation studies on cultured cells. Skin biopsies for these studies must be taken with great care to avoid primary contamination of the fibroblast culture using aseptic technique with sterile instruments. The biopsy should not be full thickness or too large, but about 1mm by 3 - 4mm in area and dropped immediately into sterile tissue culture medium, making sure that the biopsy is well immersed. At post-mortem there is always a greater risk of contamination, particularly when a large biopsy is taken. An internal tissue such as fascia may be preferred.

Fibroblast cultures are initiated in the Cytogenetics Laboratory and cell lines are stored in the Cell Bank situated in the same laboratory. Sufficient cells for study should be available after about 2 - 5 weeks in culture. All cultures are subsequently banked in a cryogenic store.

10. RETENTION OF MATERIAL FOR FURTHER ANALYSIS

Genomic Diagnostics Laboratory including the Willink Biochemical Genetics Laboratory has a written policy for the retention of various clinical samples for future analysis should this be required (DOC 1464). Further analysis is obviously dependent on sufficient viable sample remaining after initial analysis. If sample remains from patients found to be affected on initial analysis these are retained whilst viable. This policy is based on the joint RCPath and IBMS guidelines on "The retention and storage of pathological records and specimens", 4th Edition, 2009.

NORMAL DIAGNOSTIC SAMPLES – TIME OF STORAGE PRIOR TO DISPOSAL

URINE	3 months after final report is issued.
WHOLE BLOOD (Beutler test)	up to 48 hours after final report is issued.
PLASMA/SERUM	6 months after final report is issued, however samples for quantitative amino acid analysis are deproteinised on arrival and are thus unsuitable for other analyses.
WHITE CELL PREPARATIONS	up to 48 hours after final report is issued, however, once cells are lysed, labile enzymes are rapidly degraded rendering samples inappropriate for further analysis.
CSF	6 months after final report is issued.
AMNIOTIC FLUID	up to 2 years after final report is issued.
BLOOD SPOTS	25 years after final report is issued for newborn screening samples, 1 year for Fabry & Pompe samples, 6 months for other samples. Post mortem blood spots are stored for 6 months followed by their return to histopathology or sensitive disposal. Also, for storage of post-mortem samples appropriate consent is required.
CULTURED FIBROBLASTS	Stored in the cell bank for a period of at least 1 year. Positive samples are stored for up to 30 years.

11. REFERRAL LABORATORIES USED BY THE WILLINK LABORATORY

When the Willink Biochemical Genetics Laboratory does not perform a particular test, samples can often be referred to other laboratories world wide. If a particular test is required, please contact the laboratory to discuss whether the test is covered by one of our approved referral laboratories or if preliminary testing needs to be performed by ourselves.

12. ALPHABETICAL LIST OF METABOLIC CONDITIONS FOR WHICH DIAGNOSTIC TESTS ARE AVAILABLE AT THIS LABORATORY

Sialidosis (Mucolipidosis I)	9, 16, 25
Sly disease (MPS VII)	9, 18, 25
Smith-Lemli-Opitz syndrome	22
Sudden infant death syndrome (SUDI)	9
Tay-Sachs disease	9, 19, 25
Urea cycle defects	13
Wolman disease	9, 20, 25
X-linked Ichthyosis	22
Zellweger syndrome, inc neonatal ALD and infantile Refsum	9, 26

13. ALPHABETICAL LIST OF TESTS PERFORMED WITHIN THE LABORATORY

Multiple Sulphatases	18
N-acetyl-α-galactosaminidase	20
α-N-acetylglucosaminidase	17
N-acetyl-α-neuraminidase	18
Oligosaccharide/Sialic acid screen	16, 25
Organic acids	8,9,14,23
Orotic acid	13
Oxysterol	21
Palmitoyl Protein Thioesterase 1	21
Phytanic acid and Pristanic acid	22, 26
Plasmalogens	10, 22, 26
Prenatal diagnosis	10, 23
^{14}C-Propionate incorporation	15
Propionyl-CoA carboxylase	14
Pyruvate carboxylase	14
Sialic acid, quantitative	16
Sphingomyelinase	20, 25
Steroid sulphatase – see Arylsulphatase C	22
Succinylacetone	14
Sugar Chromatography	12
Tissue culture: Initiation of cell culture from skin biopsy, amniocytes or chorionic villi and storage of cells in cryogenic cell bank	23, 26
Tissue culture: Maintenance of cultures initiated elsewhere until investigations are completed and cryogenic storage of cells	23, 26
Tripeptidyl Peptidase 1	21
Very Long Chain Fatty Acids	10, 22, 26
White Cell Cystine (analysis now performed at Leeds Metabolic lab)	13