# NHS North Thames Genomic Laboratory Hub

Service Pack

NHS Great Ormond Street Hospital for Children NHS Foundation Trust

Version 12.3

The NHS North Thames Genomic Laboratory Hub is commissioned by the NHS England Genomics Medicine Unit to deliver comprehensive genomic testing services for patients across North London and serves a population of approximately 10 million people. This pack outlines the testing services delivered by the Rare and Inherited Disease Laboratory at Great Ormond Street Hospital on behalf of the NHS North Thames GLH.

The laboratory provides an extensive range of diagnostic testing services and is accredited to the ISO 15189:2022 standard (UKAS accredited medical laboratory No. 7883). The laboratory accreditation schedule and list of accredited services can be viewed via the UKAS website: https://www.ukas.com/download-schedule/7883/Medical/

We have a staff of approximately 170 including consultant and HCPC registered clinical scientists, registered bioinformaticians, genetic technologists, administrative and support staff. The service processes approximately 50,000 samples per year and issues over 30,000 analytical reports. Laboratory services are delivered in accordance with the NHS England National Genomic Test Directory for Rare and Inherited Disease: https://www.england.nhs.uk/publication/national-genomic-test-directories/

Molecular Genetics diagnostic services are offered primarily through multi-gene next generation sequencing (NGS) panels targeted to particular diseases or phenotypes. Where a genetic diagnosis has been made, carrier, confirmatory, predictive and, where appropriate, prenatal testing is available. Non-Invasive Prenatal Diagnosis and Fetal Exome Sequencing using NGS technology are delivered as part of the prenatal service. The laboratory is also nationally commissioned to provide highly specialised services for Bardet-Biedl syndrome, craniosynostoses, lysosomal storage disorders and severe combined immunodeficiencies.

Cytogenetic service work uses SNP microarray analysis as the first line test for postnatal and prenatal samples where appropriate. Targeted microarray, Quantitative polymerase chain reaction (qPCR) and Fluorescence *in situ* Hybridisation (FISH) tests are used for follow-up of array studies. Karyotyping, targeted chromosome analysis and FISH are used to identify chromosomal structural changes in adults with subfertility/infertility and for exclusion of structural chromosome abnormalities associated with specific syndromes. Samples from pregnancy losses are studied using microarray.

Translational research to develop the next generation of genomic services is a key objective with dedicated scientific, bioinformatic and technical support embedded in the service. The service has a strong commitment to education and training and public & patient engagement and participates in clinician, scientist and technologist training programmes. A number of staff are also actively involved at a regional and national level in policy development, training and examination.

Details of the genomic testing services available through the commissioned NHS England Genomic Medicine Service are provided in this pack. We also accept private, UK Devolved Nations and overseas referrals. Please contact the laboratory for further information.

## Director

**Deborah Morrogh** *FRCPath*

## Cytogenetics Service

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**Dr Ajith Kumar**
Lead Clinician

## Other contacts

**Beth Kimpton**
General Manager

**Elinor Lamrick**
Associate Director of Operations

**Nicole Elkins**
Service Manager

## Laboratory Opening Hours

The laboratory is staffed Monday - Friday, 9.00am – 5.30pm excluding bank holidays.

Sample reception is open from 9.00am to 5.30pm.
Specimens arriving outside these hours are refrigerated / frozen prior to processing. There is no out-of-hours service.

Please send samples to Specimen Reception, Level 5 Barclay House at the address shown in the right-hand panel.

## Discussion with patients and family about genomic testing

**It is the responsibility of the referring clinician to discuss genomic testing with their patient prior to submitting a sample and to retain a record of discussion:**

- An appropriate discussion of the genomic test and possible implications should take place according to the Consent and Confidentiality in Genomic Medicine guidelines (https://bit.ly/2XkBtMu ).
- The patient should be advised that the sample may be used anonymously for quality assurance, research and training purposes, please advise of any restrictions.
- A record of discussion should be retained within the patient's record. A recommended record of discussion is provided on our website.
- Consent is not required for DNA storage.

## Sample Requirements

For sample requirements, please refer to our request cards which can be found on the North Thames GLH website.

It is the responsibility of the patient's clinician to ensure that all requests meet testing criteria, that samples are correctly labelled and request forms are completed to a minimum standard.

### Postnatal Samples

5 mls venous blood in plastic EDTA bottles (>1 ml from neonates)

5 mls venous blood in plastic Lithium Heparin bottles (1-2 ml from neonates)

*Lithium Heparin blood samples must be received in the lab within 24 hours (refrigerate overnight at 4° C if necessary).*

For DNA samples, it is requested that the referral laboratory provides sufficient DNA for the analysis being requested.

**For any other sample type (e.g. buccal swab, muscle) please contact the laboratory for advice. For prenatal samples, see below.**

**Please note that blood samples taken after HSCT (bone marrow transplant) or after recent blood transfusion are not suitable for genetic testing.**

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**Sample Labelling:** The sample tube and referral card must have three matching identifiers to be accepted for testing.

- Patient's full name (surname/family name and first/given name)
- Date of birth; NHS number; Referring hospital number
- The date and time sample was taken

## Prenatal Diagnosis

Please contact the laboratory in advance of arranging a prenatal sample.

**Cytogenetic analysis** of prenatal samples is routinely available. The type of test offered is dependent on criteria such as the patient having a serum screen risk only, or having abnormalities detected on ultrasound scan.

**Molecular genetic** prenatal diagnosis can only be offered by prior arrangement where the genetic diagnosis has been confirmed by molecular means and parental samples are fully informative. It is standard practice for the laboratory to exclude maternal cell contamination of all fetal samples; a maternal blood sample is required for this analysis.

**Fetal Exome Sequencing** where abnormality has been identified on fetal ultrasound scan. Testing is by prior arrangement only for cases that meet nationally agreed eligibility criteria.

**Non-Invasive Prenatal Diagnosis** for analysis of cell free fetal DNA circulating in maternal blood is available for specific clinical indications only. Bespoke analysis may also be possible. Please contact the laboratory in advance to discuss testing.

## Prenatal Samples

CVS / Amniocentesis: Tissue type and date of biopsy should be clearly documented on the referral information. In the case of twins, special attention must be given to the identity of each sample.

For non-invasive prenatal diagnosis (NIPD) / free fetal DNA analysis please send 20ml blood in Streck or PAXgene ccfDNA cell- stabilising tubes. Sample date & gestation as confirmed by ultrasound scan must be provided along with a valid clinical indication for early sex determination or single gene testing. This test does not apply to twin pregnancies.

Please contact the laboratory in advance of arranging a prenatal sample for molecular testing, NIPD or exome analysis.

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## Alternative referral documentation

If the laboratory referral form cannot be used, alternative referral cards / letters are acceptable; it is preferable that any referral card is fully completed. However, referral documents must provide the minimum criteria of:

- Patient's full name and date of birth
- NHS number (essential) and hospital number
- Full name and address of referring clinician/consultant
- Patient's postcode
- Patient's GP name and address
- Clearly mark if referral is for a non-NHS patient
- Analysis can only be carried out if specific disease gene or test(s) is requested
- For family / targeted mutation tests, a mutation report or GOS genetics family ID is required along with the relationship of your patient to family members previously tested.

## Sending samples

Samples sent by Royal Mail or courier must comply with PI 650 for category B substances.

- This is a triple layer system which comprises a primary leak-proof receptacle within a secondary leak-proof receptacle contained in a rigid outer package. The packaging should be strong enough to withstand a 95 kPA pressure differential and a drop of 1.2 m.
- There should be sufficient absorbent material between the primary and secondary packaging to absorb any spillage. The primary container and absorbent material must be placed into a single bag with the request form in the pouch.
- The package should be clearly labelled 'diagnostic specimen UN3373'.

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## Testing via the National Genomic Test Directory

Seven Genomic Laboratory Hubs (GLH) provide testing to designated geographical areas of England. Every GLH offers testing for a core set of common clinical indications to their local population; each GLH is also commissioned to deliver a designated subset of specialist tests. The test directory categorises tests by clinical indication, each being given an 'R' code. Referring clinicians should send all samples that require genomic testing to their local GLH who will route them as appropriate. It is a requirement that all patients meet NHSE eligibility criteria for testing and that relevant clinical information is provided for the laboratory to assess referrals; non-eligible test requests may be rejected.

Rare and inherited disease eligibility criteria are available on the NHS England National Genomic Test Directory web site (https://www.england.nhs.uk/publication/national-genomic-test-directories/).

## Core Services

Core services are for commonly occurring genetic test requests and are delivered by every GLH for referrals originating in their own geographical area. These include

- All cytogenetic tests
- All acquired cancer tests
- Inherited cancer predisposition for breast, ovarian and colorectal cancers
- Familial hypercholesterolaemia
- Cystic fibrosis
- *FGFR3* related skeletal dysplasia
- Myotonic dystrophy
- Spinal Muscular Atrophy
- Huntington Disease
- Hereditary neuropathy (*PMP22* copy number)
- Y-chromosome microdeletions
- Hereditary Haemochromatosis
- Prader Willi and Angelman syndromes
- Family testing for known mutations (e.g. predictive, prenatal, carrier and familial diagnostic testing).
- Validation of research results

## Specialist Testing

Specialist tests are delivered by designated GLH in accordance with agreed routing rules. Clinical specialisms are as follows: Cardiology, **Dermatology,** Endocrinology, Gastrohepatology, Haematology, **Hearing Loss, Immunology, Metabolic, Mitochondrial, Musculoskeletal, Neurology, NIPD, Ophthalmology, Rare Inherited Cancer, Renal,** and Respiratory.

The North Thames GLH is a designated provider for the clinical specialties shown in bold text. Details for each are provided in this pack. For specialties that are routed outside North Thames, samples should be referred to GOSH as the 'home' GLH who will export them as appropriate for the test requested.

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Specialist testing services include diagnostic testing to establish a diagnosis and, although designated as core, any family follow up testing will be carried out by the designated specialist laboratory.

### Neurology

Neurogenetic testing in the North Thames GLH is provided by the UCLH Neurogenetics Laboratory. This service, part of the National Hospital for Neurology and Neurosurgery relocated from the Queen Square Institute of Neurology in August 2020, and now works alongside the Great Ormond Street Molecular Genetics and Cytogenetics laboratories as part of the North Thames Rare Disease lab. More details of the tests offered through this service can be found on the Neurogenetics website:

https://www.uclh.nhs.uk/our-services/find-service/neurology-and-neurosurgery/neurogenetics/neurogenetics-laboratory

### Fetal Exome Sequencing (R21)

Fetal exome sequencing is carried out for England by two GLHs, North Thames (GOSH) and Central & South (Birmingham). Testing is available for pregnancies where structural abnormalities have been identified on ultrasound scan and an expert panel agree there is a potential monogenic cause. Trio (fetus and parents) analysis is usually undertaken. Guidance documentation can be found via the North Thames GLH website.

### Rapid Paediatric Genome Sequencing (R14)

Rapid Paediatric Genome Sequencing is carried out by the South West GLH (Exeter). Trio analysis is carried out where the child is critically ill and a genetic diagnosis would impact management of the patient. Pre-approval is required to access testing. Emma Wakeling (Consultant Clinical Geneticist) advises on referrals for North Thames. Guidance documentation is published on the SW GLH website:

https://www.exeterlaboratory.com/wp-content/uploads/Guidance-Document-NICU-PICU-Referrals-v4.0-20200622.pdf

### Whole Genome Sequencing

Born out of the 100K Genomes project, whole genome sequencing has been rolled out nationally for some Test Directory Clinical Indications. Where possible, trio analysis is carried out, with samples sent to the home GLH for processing and export to a central sequencing facility. Sequence data is then returned to the designated specialist GLH for analysis and reporting. Specific consent is required for WGS. Details can be found on the North Thames Genomic Laboratory Hub site:

https://www.norththamesglh.nhs.uk/healthcare-professionals/ordering-whole-genome-sequencing/

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### Non-Test Directory Testing

Molecular genetic screening for some disorders may not currently be available within the NHSE Genomic Medicine Service laboratories. These tests may be available at other diagnostic laboratories within and outside the UK and in some cases samples can be forwarded provided funding is available. Please contact the laboratory for further information. The current accreditation status of UK laboratories registered with UKAS can be checked at http://www.ukas.com/

### Updating the Test Directory

An evaluation process has been put in place whereby new tests can be proposed for inclusion in the national test directory; amendments to existing tests, including updating gene content of panels may also be requested. Proposals to amend eligibility criteria for testing may also be submitted via this route. Guidance on submitting applications for test directory updates can be found: https://www.england.nhs.uk/publication/national-genomic-test-directory-supporting-material/

## Pricing

### Commissioned Testing

The North Thames GLH is funded to provide testing for English patients; no invoices will be raised as long as the following criteria are met:

- The test is included in the NHSE National Genomic Test Directory
- The patient clinical presentation / family history meets the required NHSE eligibility criteria for testing
- The patient is eligible for NHS care, resident in England and referred from an appropriate English healthcare provider

This applies whether the testing is undertaken locally at GOSH or outsourced to another GLH.

### NHS Provider to Provider and Private / Overseas test prices

Testing undertaken outside of the NHSE criteria will be invoiced in accordance with our current price list which is available on request. NHS prices apply for the UK devolved nations, with a separate tariff for private and overseas referrals. Testing may be undertaken on an *ad hoc* basis for occasional requests or within the scope of a Service Level Agreement.

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# Angelman Syndrome R47

## Contact details

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## Samples required

- 5ml venous blood in plastic EDTA bottles (>1ml from neonates)
- Prenatal testing must be arranged in advance, through a Clinical Genetics department if possible.
- Amniotic fluid or CV samples should be sent to Cytogenetics for dissecting and culturing, with instructions to forward the sample to the Regional Molecular Genetics laboratory for analysis
- A completed DNA request card should accompany all samples

## Patient details

To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician

## Introduction

Angelman syndrome (MIM 105830) occurs in 1/15000 - 1/20000 individuals. It is characterised by severe motor and intellectual retardation, seizures associated with characteristic EEG traces, microcephaly, ataxia, frequent jerky limb movements and flapping of the arms and hands, hypotonia, hyperactivity, hypopigmentation (39%), absence of speech, characteristic face shape, and episodes of paroxysmal laughter. The AS phenotype results from the lack of a maternal contribution at chromosome 15q11-q13. This can be caused by deletion (~75%), paternal uniparental disomy (UPD) (~2%) or pathogenic variants in the imprinting centre (IC) (~5%) that cause abnormal methylation at exon alpha of the *SNRPN* gene at 15q11-13. These are all detected by disrupted methylation. About 20% of AS patients have a normal methylation pattern and are believed to have a pathogenic variant in *UBE3A*. Deletions and UPD are usually *de novo* events, associated with low recurrence risks, although it is important to determine whether either parent of an affected child has a predisposing chromosomal translocation. There is a recurrence risk of up to 50% in families with confirmed AS who do not show maternal deletion or UPD.

## Referrals

- Confirmation of clinically suspected AS in children/adults.
- Investigation of the molecular defect in genetically confirmed AS cases (parental samples required).
- Carrier testing in adult relatives of confirmed (genetic) AS patients who are suspected of having an IC mutation (samples from appropriate family members are required).

## Prenatal testing

Prenatal diagnosis is available to couples where AS has been confirmed in the family and to couples at risk of having a child affected with AS due to a balanced chromosomal rearrangement involving chromosome 15 in one of the parents. Please contact the laboratory to discuss, prior to sending prenatal samples.

## Service offered

Confirmation of AS by methylation analysis and microsatellite analysis to determine the underlying cause in confirmed cases and carrier testing for adults (requires samples from appropriate family members). *UBE3A* testing is via the appropriate WGS panel.

## Technical

For diagnostic referrals, the initial test is to determine the methylation status of exon alpha of the *SNRPN* gene. Methylation analysis is performed by methylation-specific PCR following bisulphite modification of genomic DNA. Normal individuals yield a 313bp maternally-derived fragment and a 221bp paternally-derived fragment. Patients with AS show a single 221bp paternal fragment only. Positive results are confirmed by either MS-MLPA or aCGH analysis. Chromosome 15 microsatellite markers from within and flanking the commonly deleted region can also be used to characterise the mechanism in patients shown to have abnormal methylation. Cytogenetic analysis is also helpful in identifying deletions and predisposing parental translocations**. NB: A similar testing process is undertaken for Prader-Willi syndrome.**

## Target reporting time

Routine analysis - the initial methylation test takes up to 6 weeks. Microsatellite marker analysis takes 6 weeks from receipt of parental samples. Please contact the laboratory for urgent cases.

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# Breast / ovarian / prostate cancer and Li Fraumeni syndrome (LFS) – R207 R208 R430 R444 R216

## Contact details


Rare & Inherited Disease Laboratory

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**T** +44 (0) 20 7762 6888

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## Samples required

- 5ml venous blood in plastic EDTA bottles (>1ml from neonates)
- Prenatal testing must be arranged in advance, through a Clinical Genetics department.
- Amniotic fluid or CV samples should be sent to Cytogenetics for dissecting and culturing, with instructions to forward the sample to the Regional Molecular Genetics laboratory for analysis
- A completed DNA request card should accompany all samples

## Patient details

To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician

## Introduction

Hereditary predisposition to breast, ovarian and prostate cancer has been linked to pathogenic variants in several different genes, most notably *BRCA1* and *BRCA2* which account for the majority of cases. Other genes implicated in these hereditary cancers include *PALB2, RAD51C, RAD51D, BRIP1, ATM* and *CHEK2*. Specific genes tested vary dependent on the panel being analysed (based on type of cancer detected and patient age / presentation, in line with NHS England test directory criteria).

Li-Fraumeni syndrome (*TP53* gene) and *POT1* tumour predisposition syndrome are rare hereditary cancer predisposition conditions. LFS is associated with early onset (including paediatric) tumours such as sarcomas, breast, brain and adrenal cancers. *POT1* is more commonly associated with melanoma, however a range of cancer types have now been reported. Both *TP53* and *POT1* are tested via the R216 panel.

## Referrals

Referrals are accepted from the NHS North Thames Genomic Laboratory Hub.
Referrals for Li Fraumeni syndrome testing are also accepted from the NHS South Thames Genomic Laboratory Hub
Patients must meet the referral criteria for the specific test indication as outlined in the National Genomic Test Directory rare and inherited disease eligibility criteria document. https://www.england.nhs.uk/publication/national-genomic-test-directories/

## Prenatal testing

Prenatal diagnosis is not routinely offered for breast and ovarian cancer susceptibility genes. It may be offered for Li Fraumeni syndrome where there is a known pathogenic variant, but referrals can only be accepted from Clinical Genetics following appropriate counselling.

## Service offered

**R207 Inherited ovarian cancer (without breast cancer)**
**R208 Inherited breast cancer and ovarian cancer**
**R430 Inherited prostate cancer**
Details of genes analysed for each test indication above can be found on the NHS Genomic Medicine Service (GMS) Signed Off Panels Resource webpage https://nhsgms-panelapp.genomicsengland.co.uk/entities

**R444 NICE approved PARP inhibitor treatment**
R444.1 – Breast cancer. Testing of *BRCA1, BRCA2, PALB2* only.
R444.2 – Prostate cancer.*BRCA1, BRCA2* genes only
**R216 Li Fraumeni Syndrome - *TP53* and *POT1* genes.**

All diagnostic panels are tested via next generation sequencing, including dosage analysis.

Predictive testing is offered to individuals who have a known familial pathogenic variant in the above genes. A familial control is required for analysis.

## Technical

In-house variant screening is carried out by next generation sequencing with library preparation using a custom TWIST target enrichment followed by sequencing on the Illumina platforms. Read alignment and variant calling is performed by the Illumina Dragen platform followed by variant annotation and copy number analysis using an in-house pipeline.

## Target reporting time

42 days for next generation sequencing screening in a routine index case, 21 days for urgent R444.1 referrals and 14 days for familial or targeted variant testing.

Please contact the laboratory for urgent cases.

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# Colorectal cancer/polyposis and Lynch syndrome R210, R211, R212, R213, R414

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## Samples required

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- A completed DNA request card should accompany all samples

## Patient details

To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician

## Introduction

Lynch syndrome is an inherited cancer syndrome which accounts for 5-10% of cases of inherited colon cancer. It is characterised by a family history of colon cancer at an early age, but can also present with other tumours including endometrial, small bowel, pancreatic, biliary tract, stomach, ovarian, urinary tract and brain.

It is caused by defective DNA mismatch repair as a result of pathogenic variants in one of the mismatch repair genes: *MLH1, MSH2, MSH6* or *PMS2*.

Lynch syndrome may be indicated in patients with a tumour showing loss of expression of one or more of the MLH1, MSH2, MSH6 or PMS2 proteins and/or microsatellite instability. Loss of MLH1/PMS2 protein expression may also arise sporadically due to somatic hypermethylation of the MLH1 gene.

Colorectal polyposis is not usually seen in Lynch syndrome, but this can also be associated with an increased risk of colorectal cancer. There are a number of different inherited cancer syndromes including Familial adenomatous polyposis (FAP), MUTYH-associated polyposis (MAP), Juvenile Polyposis Syndrome (JPS), Peutz-Jeghers syndrome (PJS), PTEN-Hamartoma Tumour syndrome (PHTS). These often have characteristic polyp types and may also be associated with an increased risk of other cancers.

## Referrals

Referrals are accepted from the NHS North Thames Genomic Laboratory Hub and according to the agreed routing rules.
Patients must meet the referral criteria for the specific test indication as outlined in the National Genomic Test Directory rare and inherited disease eligibility criteria document. https://www.england.nhs.uk/publication/national-genomic-test-directories/

## Prenatal testing

Prenatal diagnosis may be offered where there is a known pathogenic variant, but referrals can only be accepted from Clinical Genetics following appropriate counselling.

## Service offered

**R210 Inherited MMR deficiency (Lynch syndrome)**
R210.2 - *MLH1, MSH2, MSH6, PMS2* and *EPCAM* (copy number only) gene testing via next generation sequencing, including dosage analysis.
R210.4 - germline *MLH1* promoter methylation testing, where appropriate, is offered via the Royal Marsden Molecular Diagnostics laboratory.
R210.6 PMS2 CNV analysis – testing by MLPA and Long range PCR where appropriate

**R211 Inherited polyposis - germline test**
Details of genes analysed can be found on the NHS Genomic Medicine Service (GMS) Signed Off Panels Resource webpage https://nhsgms-panelapp.genomicsengland.co.uk/entities

**R414 APC Associated Polyposis**
*APC* single gene testing via next generation sequencing, including dosage analysis.

**R212 Peutz Jegher Syndrome**
*STK11* single gene testing via next generation sequencing, including dosage analysis.

**R213 PTEN Hamartoma Tumor Syndrome**
*PTEN* single gene testing via next generation sequencing, including dosage analysis.

Predictive testing is offered to individuals who have a known familial pathogenic variant in the above genes. A familial control is required for analysis.

*MUTYH* carrier testing can be offered as per UKCGG recommendations -

# Colorectal cancer/polyposis and Lynch syndrome R210, R211, R212, R213, R414

Full *MUTYH* gene analysis is offered for partners of individuals affected with MAP

Full *MUTYH* gene analysis may also be offered for children of individuals affected with MAP in the event that their other parent is not available for carrier testing.

Carrier testing is NOT offered for partners of *MUTYH* heterozygotes in the absence of consanguinity or a personal or family history of polyposis or colorectal cancer, particularly if reproductive decision-making will not be influenced.

UKCGG statement regarding carrier testing for partners of carriers of (likely) pathogenic variants in MUTYH - Cancer Genetics Group

## Technical

In-house variant screening is carried out by next generation sequencing with library preparation using a custom TWIST target enrichment followed by sequencing on the Illumina platforms. Read alignment and variant calling is performed by the Illumina Dragen platform followed by variant annotation and copy number analysis using an in-house pipeline.

## Target reporting time

42 days for next generation sequencing screening in an index case and 14 days for familial or targeted variant testing.

Please contact the laboratory for urgent cases.

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Version 12.3

# Cystic Fibrosis R184

## Contact details

Rare & Inherited Disease Laboratory
North Thames Genomic Laboratory Hub
Levels 4-6, Barclay House
37 Queen Square
London, WC1N 3BH
T +44 (0) 20 7762 6888
E gos-tr.norththamesgenomics@nhs.net

## Samples required

- 5ml venous blood in plastic EDTA bottles (>1ml from neonates)
- Prenatal testing must be arranged in advance, through a Clinical Genetics department if possible.
- Amniotic fluid or CV samples should be sent to Cytogenetics for dissecting and culturing, with instructions to forward the sample to the Regional Molecular Genetics laboratory for analysis
- A completed DNA request card should accompany all samples

## Patient details

To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician

## Introduction

Cystic fibrosis (MIM 219700) is an autosomal recessive condition caused by pathogenic variants in the cystic fibrosis transmembrane regulator (CFTR) gene. To date, over 2000 pathogenic variants with varying frequency have been identified in this gene. The ethnic origin of the patient influences the incidence of CF in the population and the pathogenic variants most commonly identified.

## Referrals

- Confirmation of diagnosis in individuals clinically suspected of having CF. A sweat test should be undertaken prior to molecular genetic analysis wherever possible.
- Testing in individuals who may have a mild variant form of CF (CFTR-related disease), e.g. congenital bilateral absence of the vas deferens (CBAVD), bronchiectasis and pancreatitis.
- Carrier testing in pregnant couples with fetal echogenic bowel.
- Carrier testing in individuals at increased risk (above the population risk) of having an affected pregnancy, for example a family history of CF, a partner shown to be a carrier or first cousin partnerships. Accurate carrier testing in CF families ideally requires either a sample from an affected family member or information regarding the pathogenic variants carried in the family. Without this information, the extent to which we can reduce an individual's carrier risk is less than if information on familial pathogenic variants is available.
- In accordance with UK genetic testing guidelines carrier testing is only exceptionally undertaken in minors.

## Prenatal testing

Prenatal testing is available for couples in whom pathogenic variants have been identified. Non-invasive prenatal diagnosis may be available - please contact the laboratory to discuss.

## Service offered

Common variant analysis is carried out using the CFEU2v1 kit from Yourgene which tests for 50 common pathogenic variants and the partially penetrant intronic polyT variant in cases referred for CFTR-related disease where the p.(Arg117His) variant has been detected.

Next generation sequencing and copy number variant analysis is available to individuals with a clinically confirmed diagnosis of cystic fibrosis, who have an unidentifiable pathogenic variant after testing using the CFEU2v1 kit.

## Technical

- The detection system in use in this laboratory is the CFEU2v1 kit from Yourgene. As only 50 of the most commonly identified pathogenic variants are covered by this analysis failure to identify a pathogenic variant cannot exclude affected/carrier status, a residual risk to the individual is therefore calculated and reported wherever possible.
- Full gene analysis is performed using custom TWIST target enrichment and sequencing on the Illumina NextSeq550. Copy number analysis is undertaken and CNVs confirmed using MLPA analysis (P091 kit from MRC-Holland).
- Detection of known pathogenic variants in relatives of patients with confirmed pathogenic variants by Sanger sequencing and/or MLPA analysis, as appropriate.

## Target reporting time

Two weeks for routine analysis or routine testing of known familial pathogenic variants. 42 days for NGS analysis. Please contact the laboratory if urgent or prenatal testing is required.

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# Cystic Fibrosis Newborn Screening follow up R253

## Contact details

Rare & Inherited Disease Laboratory
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Levels 4-6, Barclay House
37 Queen Square
London, WC1N 3BH
T +44 (0) 20 7762 6888
E gos-tr.norththamesgenomics@nhs.net

## Samples required

- 1 x dried blood spot
- A completed DNA request card should accompany all samples

## Patient details

To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician

## Introduction

Cystic fibrosis (MIM 219700) is an autosomal recessive condition caused by pathogenic variants in the cystic fibrosis transmembrane regulator (CFTR) gene. To date over 2000 pathogenic variants with varying frequency have been identified in this gene. The ethnic origin of the patient influences the incidence of CF in the population and the pathogenic variants most commonly identified.

## Referrals

Testing is performed for all newborns with a raised IRT following referral from the newborn screening laboratory.

This laboratory accepts referrals from three newborn screening laboratories:

- North Thames – based at Great Ormond Street Hospital for Children
- South East Thames – based at St Thomas' Hospital
- South West Thames – based in St Helier Hospital

## Service offered

Initial testing involves screening for the four most common pathogenic variants using the CF4v2 kit from Yourgene: c.489+1G>T, c.1521_1523del p.(Phe508del), c.1624G>T p.(Gly542*) and c.1652G>A p.(Gly551Asp)

Any cases where one of more pathogenic variants are detected using this kit are tested with the CFEU2v1 kit (also from Yourgene), which tests for 50 most common pathogenic variants. The kit also detects the presence of the partially penetrant intronic polyT variant, which is only analysed when the p.(Arg117His) variant is detected.

## Technical

The detection system in use in this laboratory is the CF4v2 kit and the CFEU2v1 kit. As only 4 or 50 of the most commonly identified pathogenic variants are covered by this analysis, respectively, failure to identify a pathogenic variant cannot exclude affected status. The results are reported back to the screening laboratories, who produce a final report including all biochemical and genetic analyses.

## Target reporting time

The nationally agreed turnaround time is 6 days. The screening laboratories are notified if this will be exceeded, and are also immediately notified of any failed samples or unusual results, as repeat samples will be required to complete analysis.

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# Duchenne and Becker Muscular Dystrophies R73 R378

## Contact details

Rare & Inherited Disease Laboratory
North Thames Genomic Laboratory Hub
Levels 4-6, Barclay House
37 Queen Square
London, WC1N 3BH
T +44 (0) 20 7762 6888
E gos-tr.norththamesgenomics@nhs.net

## Samples required

- 5ml venous blood in plastic EDTA bottles (>1ml from neonates)
- Prenatal testing must be arranged in advance, through a Clinical Genetics department if possible.
- Amniotic fluid or CV samples should be sent to Cytogenetics for dissecting and culturing, with instructions to forward the sample to the Regional Molecular Genetics laboratory for analysis
- A completed DNA request card should accompany all samples

## Patient details

To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician

## Introduction

Duchenne muscular dystrophy (DMD) and Becker muscular dystrophy (BMD) are X-linked disorders caused by pathogenic variants in the dystrophin gene.

DMD is characterised by progressive muscular weakness. The age of onset is 3-5 years and manifests as delayed motor skills, awkward gait, and the Gower sign. Affected males are usually wheelchair bound by adolescence and disease progression leads to onset of dilated cardiomyopathy and respiratory failure, leading to death in late teens or early twenties.

BMD has a more variable age of onset and disease progression. It is characterised by later onset of skeletal muscle weakness and slower progression than DMD. Individuals affected with DMD and BMD are expected to have significantly elevated serum creatine kinase (CK) levels.

## Referrals

- Referrals are accepted from individuals with clinical features strongly suggestive of Duchenne or Becker muscular dystrophy.
- Requesting specialisms include: Clinical Genetics, community paediatrics, neurology, and paediatrics.

## Prenatal testing

Prenatal diagnosis is available to male pregnancies of women who are carriers of a pathogenic variant or who have had a previously affected child. Non-invasive prenatal diagnosis may be available – please contact the laboratory to discuss.

## Service offered

MLPA analysis carried out to detect deletions and duplications in the dystrophin gene which account for ~70-80% of disease-causing variants. Sequence analysis of the dystrophin gene can be offered to patients in whom no variants are found; samples will be exported according to agreed routing rules.

## Technical

MLPA analysis is undertaken using the P034 DMD-1 and P035 DMD-2 kits from MRC-Holland.

## Target reporting time

Routine analysis – 42 days. Prenatal diagnosis 3 days.

Please contact the laboratory for urgent cases.

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# Familial Hypercholesterolaemia R134

## Contact details

Rare & Inherited Disease Laboratory
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Levels 4-6, Barclay House
37 Queen Square
London, WC1N 3BH
T +44 (0) 20 7762 6888
E gos-tr.norththamesgenomics@nhs.net

## Samples required

- 5ml venous blood in plastic EDTA bottles (>1ml from neonates)
- Prenatal testing must be arranged in advance, through a Clinical Genetics department if possible.
- Amniotic fluid or CV samples should be sent to Cytogenetics for dissecting and culturing, with instructions to forward the sample to the Regional Molecular Genetics laboratory for analysis
- A completed DNA request card should accompany all samples

## Patient details

To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician

## Introduction

Familial hypercholesterolaemia (FH) (MIM 143890) is a relatively frequent autosomal dominant condition, characterised clinically by elevations in low-density lipoprotein cholesterol (LDL-C), tendon xanthomata (TX) and premature coronary heart disease (CHD). Heterozygous FH has an incidence of around 1/200-250, and severe homozygous FH affects 1/1000 000 individuals. FH is genetically heterogeneous; however, the primary genetic defect in FH is a pathogenic variant in the gene encoding the LDL-receptor (LDLR). *LDLR* has 18 exons and family specific pathogenic variants are found throughout the gene, although some recurrent pathogenic variants are reported. Large deletions or duplications encompassing one or more exons accounts for 5% of pathogenic variants in *LDLR*. A clinically indistinguishable disorder, familial defective apolipoprotein B100 (FDB), is due to a pathogenic variant in the gene encoding apolipoprotein B (*APOB*), which is one of the ligands of the LDL-receptor. The majority of FDB cases (2-5% of hypercholesterolaemic individuals) have a single pathogenic variant: p.(Arg3527Gln). Pathogenic variants causing FH have also been identified in the *PCSK9, APOE* and *LDLRAP1* genes that account for a small proportion of cases.

## Referrals

- Dutch (or Welsh) lipid score >5, OR
- Simon Broome Criteria indicating possible FH (following assessment in a specialist Lipid Clinic of Familial Hypercholesterolaemia service)
- Requesting specialities include cardiology, chemical pathology, Clinical Genetics, Metabolic Medicine and Paediatrics

## Service offered

Analysis of the R134 Familial hypercholesterolaemia panel, which includes the genes: *LDLR*, *APOB*, *PCSK9, APOE* and *LDLRAP1*.

## Technical

Analysis is carried out by next generation sequencing (TWIST and Illumina NextSeq) for the entire coding regions and intron-exon boundaries of the *LDLR, APOB, PCSK9, LDLRAP1* and *APOE* genes to minimum read depth coverage of 30 reads. The NGS panel has been validated to detect large exon deletions or duplications (copy number variants).
Testing for previously identified pathogenic variants is available to other family members and is carried out by sanger sequencing or MLPA analysis.

## Target reporting time

42 days for a full screen in an index case and 42 days for familial testing.

Please contact the laboratory for urgent cases.

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# Fragile X

## Contact details

Rare & Inherited Disease Laboratory
North Thames Genomic Laboratory Hub
Levels 4-6, Barclay House
37 Queen Square
London, WC1N 3BH
T +44 (0) 20 7762 6888
E gos-tr.norththamesgenomics@nhs.net

## Samples required

- 5ml venous blood in plastic EDTA bottles (>1ml from neonates)
- Prenatal testing must be arranged in advance, through a Clinical Genetics department if possible.
- Amniotic fluid or CV samples should be sent to Cytogenetics for dissecting and culturing, with instructions to forward the sample to the Regional Molecular Genetics laboratory for analysis
- A completed DNA request card should accompany all samples

## Patient details

To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician

## Introduction

Fragile X syndrome (MIM 309550) is an X-linked mental retardation syndrome associated with dysmorphic features in a proportion of cases. Around 1 in 5000 of the population is affected with fragile X, they are predominantly male but females can also be affected. The majority of fragile X cases are caused by expansion of the (CGG)n repeat in the promoter region of the *FMR1* gene on chromosome Xq27.3 (FRAX A cases). Expansion of the (CGG)n repeat sequence to >200 repeats accompanied by methylation of the adjacent CpG island extinguishes the *FMR1* gene expression (full mutation expansion). Premutation alleles with 59-200 (CGG)n repeats are unstable at meiosis and can lead to full expansion mutations in subsequent generations. Intermediate alleles (46-58 repeats) are not believed to be associated with fragile X syndrome, but may display size instability in future generations. *FMR1* point mutations and deletions are rare causes of the syndrome. Premutation allele carriers can display additional phenotypes such as premature ovarian failure (POF) and a neurodegenerative disorder of older adults, fragile X associated tremor/ataxia syndrome (FXTAS).

## Referrals

- Clinical features characteristic of fragile X syndrome or other FMR1-related disorder
- Typical fragile X syndrome manifestation in females: learning difficulty (usually mild, IQ often 80-85 but can be moderate or severe LD)
- Typical fragile X syndrome manifestation in males: moderate to severe developmental delay/learning difficulty (IQ if measured would be 35-70)

R27 paediatric disorders (trio whole genome sequencing)

R54 hereditary ataxia with onset in adulthood should be used in preference in adults with ataxia given the broad range of possible causes.

R402 premature ovarian insufficiency should be used where this is the relevant clinical context.

## Prenatal testing

Prenatal testing is available for confirmed fragile X carriers - analysis can be carried out on prenatal samples by direct analysis of the *FMR1* (CGG)n repeat and/or by linked marker analysis if samples from the relevant family members are available.

## Service offered

The targeted test for the FMR1 expansion associated with Fragile X syndrome has been withdrawn from the test directory. FMR1 repeat expansions can be detected using whole Genome Sequencing (WGS). If the patient fulfils the eligibility criteria for R27 Paediatric Disorders (by WGS) consider ordering this test. Please note that WGS orders require a fully completed WGS Test Order Form, a Record of Discussion (consent), and blood samples from child and both parents where possible. Targeted testing of the *FMR1* (CGG)n repeat is available in families where an expansion has previously been identified. Linked marker analysis is available in families where we are unable to identify a mutation in a clinically affected individual; this relies on the clinical diagnosis being correct and sample availability from the affected individual and appropriate family members.

## Technical

FMR1 repeat expansions can be detected using whole Genome Sequencing (WGS). The AmplideX™ FMR1 PCR kit is used for familial testing but does not detect point mutations or deletions within the *FMR1* gene, and is unable to exclude mosaicism. The AmplideX™ assay is not methylation sensitive and provides no information on the methylation status of the FMR1 promoter.

## Target reporting time

Routine analysis – 42 days. Please contact the laboratory for urgent cases.

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# Rare inherited cancer syndromes

## Contact details

Rare & Inherited Disease Laboratory
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Levels 4-6, Barclay House
37 Queen Square
London, WC1N 3BH
T +44 (0) 20 7762 6888
E gos-tr.norththamesgenomics@nhs.net

## Samples required

- 5ml venous blood in plastic EDTA bottles (>1ml from neonates)
- Prenatal testing must be arranged in advance, through a Clinical Genetics department.
- Amniotic fluid or CV samples should be sent to Cytogenetics for dissecting and culturing, with instructions to forward the sample to the Regional Molecular Genetics laboratory for analysis
- A completed DNA request card should accompany all samples

## Patient details

To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician

## Introduction

Inherited cancer syndromes are rare genetic predisposition syndromes where individuals have a significantly increased risk of developing certain cancers. Each syndrome has an association with a particular cancer type(s), dependent on the genes involved and the pathways affected. Cancer risk is variable for most conditions and is often not fully penetrant. Age of onset can range from childhood through to adulthood and again can be variable. Most are autosomal dominant conditions, but there are also some autosomal recessive cancer predisposition syndromes.

Breast, ovarian and prostate cancer, Li Fraumeni syndrome and colorectal cancer/polyposis predisposition gene testing (including *TP53, POT1, APC, STK11* and *PTEN*) are detailed separately.

## Referrals

Referrals are accepted from the NHS North Thames Genomic Laboratory Hub and according to the agreed routing rules.

Patients must meet the referral criteria for the specific test indication as outlined in the National Genomic Test Directory rare and inherited disease eligibility criteria document. https://www.england.nhs.uk/publication/national-genomic-test-directories/

## Prenatal testing

Prenatal diagnosis may be offered for some conditions where there is a known pathogenic variant, but referrals can only be accepted from Clinical Genetics following appropriate counselling.

## Service offered

**R214 Nevoid Basal Cell Carcinoma Syndrome or Gorlin syndrome**
*PTCH1* and *SUFU* gene testing via next generation sequencing, including dosage analysis.

**R215 Hereditary diffuse gastric cancer**
*CDH1* and *CTNNA1* single gene testing via next generation sequencing, including dosage analysis.

**R219 Retinoblastoma**
*RB1* gene testing via next generation sequencing, North Thames referrals exported to Barts Retinoblastoma screening unit.

**R220 Wilms tumour with features suggestive of predisposition**
WT1 gene testing via next generation sequencing / 11p15 methylation testing, North Thames referrals exported to Birmingham.

**R358 Familial rhabdoid tumours**
*SMARCA4* and *SMARCB1* gene testing via next generation sequencing, including dosage analysis

**R359 Childhood solid tumours**
Gene panel testing via next generation sequencing, North Thames referrals exported to Birmingham

**R224 Inherited renal cancer**
Testing via next generation sequencing, including dosage analysis. Details of genes analysed can be found on the NHS Genomic Medicine Service (GMS) Signed Off Panels Resource webpage https://nhsgms-panelapp.genomicsengland.co.uk/entities

**R225 Von Hippel Lindau syndrome**
*VHL* single gene testing via next generation sequencing, including dosage analysis

**R254 Familial melanoma**
Gene panel testing via next generation sequencing, North Thames referrals exported to Birmingham

**R422 BAP1 associated tumour predisposition syndrome**
Gene panel testing via next generation sequencing, North Thames referrals exported to Birmingham

# Rare inherited cancer syndromes

**R363 Inherited predisposition to GIST**
Gene panel testing via next generation sequencing, North Thames referrals exported to Birmingham

**R364 DICER1-related cancer predisposition**
Gene panel testing via next generation sequencing, North Thames referrals exported to Birmingham

**R365 Fumarate hydratase-related tumour syndromes**
Gene panel testing via next generation sequencing, North Thames referrals exported to Birmingham

**R367 Inherited pancreatic cancer**
Gene panel testing via next generation sequencing, North Thames referrals exported to Birmingham

**R404 Testing of unaffected individuals for inherited cancer predisposition syndromes**
Gene panel testing via one of the inherited cancer test panels in a patient who has no personal history of cancer, but meets relevant testing criteria.

Predictive testing is offered to individuals who have a known familial pathogenic variant in the above genes. A familial control is required for analysis.

## Technical

In-house variant screening is carried out by next generation sequencing with library preparation using a custom TWIST target enrichment followed by sequencing on the Illumina platforms. Read alignment and variant calling is performed by the Illumina Dragen platform followed by variant annotation and copy number analysis using an in-house pipeline.

## Target reporting time

42 days for next generation sequencing screening in an index case and 14 days for familial or targeted variant testing.

Please contact the laboratory for urgent cases.

# Aminoglycoside exposure posing risk to hearing R65

## Contact details

Rare & Inherited Disease Laboratory
North Thames Genomic Laboratory Hub
Levels 4-6, Barclay House
37 Queen Square
London, WC1N 3BH
T +44 (0) 20 7762 6888
E gos-tr.norththamesgenomics@nhs.net

## Samples required

- 5ml venous blood in plastic EDTA bottles (>1ml from neonates)
- Prenatal testing must be arranged in advance, through a Clinical Genetics department if possible.
- Amniotic fluid or CV samples should be sent to Cytogenetics for dissecting and culturing, with instructions to forward the sample to the Regional Molecular Genetics laboratory for analysis
- A completed DNA request card should accompany all samples

## Patient details

To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician

## Introduction

Mitochondrial variants, m.1095T>C, m.1494C>T and m.1555A>G, in the mitochondrial MT-RNR1 (12S rRNA) gene have been associated with aminoglycoside-induced hearing loss (AIHL). The use of aminoglycosides should be avoided in individuals with an MT-RNR1¬ variant associated with an increased risk of AIHL unless the risk of permanent hearing loss is outweighed by the severity of infection and safe or effective therapies are not available (PMID: 34032273). The m.1555A>G variant has also been associated with non-aminoglycoside related sensorineural hearing loss (PMID: 9490575).

The risk of deafness is dependent upon the level of heteroplasmy, with a risk of deafness of zero for individuals with heteroplasmy <50% (Zhu et al, BMC Genetic 15:26, 2014). These variants have been detected in families with maternally transmitted deafness and seem to have an age dependent penetrance for deafness, which is enhanced by treatment with aminoglycosides.

## Referrals

- Patients with hearing loss for m.1555A>G, m.1095T>C and m.1494C>T analysis.
- Patients who may require aminoglycosides.
- Maternal relatives of patients with the m.1555A>G, m.1095T>C and m.1494C>T variants.

## Service offered

Analysis for the MT-RNR1 m.1555A>G, m.1095T>C and m.1494C>T variants.

## Technical

Internal data shows that PCR and Sanger sequencing can detect a minimum level of heteroplasmy of ~25%. Sanger sequence analysis has a sensitivity of approximately 99% (Ellard et al Genet Test & Mol Biomark 13,3 381-386 (2009)).

## Target reporting time

Routine testing of variant in index case – 42 days.

Urgent testing of m.1555A>G, m.1095T>C and m.1494C>T variants in index case where treatment with aminoglycosides is imminent – 2 weeks

Testing for maternal relatives of patients with the m.1555A>G, m.1095T>C and m.1494C>T variants – 42 days.

Please contact the laboratory for urgent cases.

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# Hereditary haemochromatosis

## Contact details

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37 Queen Square
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T +44 (0) 20 7762 6888
E gos-tr.norththamesgenomics@nhs.net

## Samples required

- 5ml venous blood in plastic EDTA bottles (>1ml from neonates)
- A completed DNA request card should accompany all samples

## Patient details

To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician

## Introduction

*HFE*-related hereditary haemochromatosis (HH) is an autosomal recessive condition characterized by excessive absorption of dietary iron. Approximately 90% of affected individuals are homozygous for the p.(Cys282Tyr) pathogenic variant, however penetrance is low, with only 15-25% of homozygotes developing disease.

Compound heterozygotes for p.(Cys282Tyr) and p.(His63Asp) may accumulate iron, but the risk of developing clinical haemochromatosis is lower than that of p.(Cys282Tyr) homozygotes.

Homozygosity for p.(His63Asp) is rarely associated with HH and is not considered a disease-associated genotype.

## Referrals

Diagnostic testing is available for individuals with unexplained iron overload (with raised transferrin saturation and/or serum ferritin).

Females: TSAT >45% and ferritin >200 lg/L

Males and postmenopausal women: TSAT >50% and ferritin >300 lg/L

First degree adult relatives (parents, siblings and children) of an affected person or C282Y carrier may also be tested. Testing of more distant healthy relatives is not generally recommended.

Haemochromatosis is an adult onset condition, therefore carrier testing is not recommended in healthy children.

## Service offered

Testing for the c.845G>A p.(Cys282Tyr) and c.187C>G p.(His63Asp), also known as C282Y and H63D,

## Technical

Analysis is carried out by multiplex fluorescent ARMS PCR. This is a targeted assay designed to detect only the c.845G>A p.(Cys282Tyr) and c.187C>G p.(His63Asp) variants.

## Target reporting time

Routine analysis – 42 days. Please contact the laboratory for urgent cases.

# Myotonic Dystrophy Type 1 R72

## Contact details

Rare & Inherited Disease Laboratory
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Levels 4-6, Barclay House
37 Queen Square
London, WC1N 3BH
**T** +44 (0) 20 7762 6888
**E** gos-tr.norththamesgenomics@nhs.net

## Samples required

- 5ml venous blood in plastic EDTA bottles (>1ml from neonates)
- Prenatal testing must be arranged in advance, through a Clinical Genetics department if possible.
- Amniotic fluid or CV samples should be sent to Cytogenetics for dissecting and culturing, with instructions to forward the sample to the Regional Molecular Genetics laboratory for analysis
- A completed DNA request card should accompany all samples

## Patient details

To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician

## Introduction

Myotonic dystrophy type 1 (OMIM #160900) is an autosomal dominant multisystem disorder, characterised by muscle weakness, myotonia, cataracts and cardiac arrhythmia. Myotonic dystrophy type 1 may also be known as dystrophia myotonica type 1, or abbreviated to DM1. DM1 is caused by a (CTG)n expanded repeat in the 3'untranslated region of the dystrophia myotonica protein kinase (*DMPK*) gene on chromosome 19.

Disease severity varies with the number of repeats: normal individuals have 5 to 35 repeats, mildly affected persons have 50 to 150 repeats, patients with classic DM1 have 100 to 1,000 repeats, and those with congenital onset can have more than 2,000 repeats.

## Referrals

- Children and adults with clinical features strongly suggestive of myotonic dystrophy type 1
- Hypotonic infants with a likely central cause

## Prenatal testing

Prenatal testing is available for individuals with a confirmed family history of DM1. Please note, it is not possible to provide details of the number of repeats in prenatal testing.

## Service offered

Direct analysis of the *DMPK* (CTG)n repeat to identify alleles in the normal (5 to 35 repeats), intermediate (36 to 50 repeats) and affected ( >50 repeats) expansion ranges. Linked marker analysis is not currently available.

## Technical

DNA is analysed by three different PCR methods. The first PCR amplifies the (CTG)n repeat region, and can be used to size alleles in the normal and intermediate ranges, and small pathogenic expansions up to approximately 80 repeats. The (CTG)n repeat is also analysed in both directions by two triplet-primed PCRs, in order to detect larger expanded alleles. Triplet-primed PCR will not precisely determine the CTG repeat number, and instead samples are reported as within the normal, intermediate or pathogenic ranges. This testing strategy will not detect point mutations or deletions of the *DMPK* gene, and will be unable to exclude mosaicism.

## Target reporting time

Routine analysis- 6 weeks for the PCR-based expansion screen.

Hypotonic infants – 2 weeks for the PCR-based expansion screen.

For urgent samples please contact the laboratory.

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# Prader-Willi Syndrome R48

## Contact details

Rare & Inherited Disease Laboratory
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London, WC1N 3BH
T +44 (0) 20 7762 6888
E gos-tr.norththamesgenomics@nhs.net

## Samples required

- 5ml venous blood in plastic EDTA bottles (>1ml from neonates)
- Prenatal testing must be arranged in advance, through a Clinical Genetics department if possible.
- Amniotic fluid or CV samples should be sent to Cytogenetics for dissecting and culturing, with instructions to forward the sample to the Regional Molecular Genetics laboratory for analysis
- A completed DNA request card should accompany all samples

## Patient details

To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician

## Introduction

Prader-Willi syndrome (PWS) (MIM 176270), occurring in 1/15000 - 1/20000 individuals, is characterised by diminished fetal activity, obesity, muscular hypotonia, developmental delay, short stature, hypogonadotropic hypogonadism, and small hands and feet. The PWS phenotype results from the lack of a paternal contribution at 15q11-q13. This can be caused by a deletion (~70%), maternal uniparental disomy (UPD) (25-30%) and rarely due to mutations in the imprinting centre (IC) that cause abnormal methylation at exon alpha of the SNRPN locus. These are all detected by disrupted methylation. Deletions and UPD are usually de novo events, associated with low recurrence risks, although it is important to determine whether either parent of an affected child has a predisposing chromosomal translocation. There is a recurrence risk of up to 50% in families with **confirmed** PWS who do not have a deletion or UPD and are therefore likely to have an IC mutation.

## Referrals

- Confirmation of clinically suspected PWS in children/adults.
- Investigation of the molecular defect in confirmed PWS cases, distinguishing between UPD, deletion and IC mutations (parental samples required).
- Carrier testing in adult relatives of confirmed PWS patients who are suspected of having an IC mutation (samples from appropriate family members are required).

## Prenatal testing

Prenatal diagnosis is available to couples where PWS has been confirmed in the family and to couples at risk of having a child affected with PWS due to a balanced chromosomal rearrangement involving chromosome 15 in one of the parents. Please contact the laboratory to discuss each case prior to sending prenatal samples to the laboratory.

## Service offered

Confirmation of a PWS diagnosis by methylation analysis and microsatellite analysis to determinate the molecular defect in confirmed cases (requires samples from appropriate family members).

## Technical

For diagnostic referrals, the initial test is to determine the methylation status of exon alpha of the SNRPN gene. Methylation analysis is undertaken by methylation specific PCR following bisulphite modification of genomic DNA. Normal individuals yield a 313bp maternally derived fragment and a 221bp paternally derived fragment. Patients with Prader-Willi syndrome show a single 313bp maternal fragment only.

Positive results are confirmed by either MS-MLPA or aCGH analysis. Chromosome 15 microsatellite markers from within and flanking the commonly deleted region can also be used to characterise the mechanism in patients shown to have abnormal methylation. Cytogenetic analysis is also helpful in identifying deletions and predisposing parental translocations. NB: A similar testing process is undertaken for Angelman syndrome.

## Target reporting time

Routine analysis - the initial methylation test takes up to 6 weeks. Microsatellite marker analysis takes 6 weeks from receipt of parental samples. Please contact the laboratory for urgent cases.

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# RNA analysis of variants R296

## Contact details

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## Samples required

- 1 tube venous blood in PAXgene blood RNA tube
- Must reach laboratory within 72 hours of sampling to preserve the RNA
- Testing should be agreed with the laboratory in advance
- A completed DNA request card should accompany all samples

## Patient details

To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician

## Introduction

RNA analysis of specific variants suspected to affect splicing can be used to detect abnormal splicing. If detected, abnormal splicing may be used as evidence to aid variant classification, potentially allowing for the upgrading of certain variants of uncertain clinical significance to likely pathogenic/ pathogenic.

Suitable variants for RNA analysis include those where the gene in question is expressed in blood and fits with the phenotype of the patient, and existing evidence supports pathogenicity of the variant but it remains a variant of uncertain clinical significance. The variant should also be located within a region that is possible to design appropriate primers. Where appropriate, RNA analysis may be offered as possible further testing on a molecular genetics report issued by the laboratory. Historical cases may be discussed with Clinical Genetics and the laboratory as to whether RNA analysis may be appropriate.

## Referrals

R296 RNA analysis of variants:

- Variant(s) requiring RNA analysis to aid interpretation where a molecular diagnosis will guide management or alter advice through reclassification of a variant from ACMG class 3 to class 4 or class 5
- Testing should be discussed in advance with the laboratory to determine if analysis for the gene/ variant in question is technically possible eg. expressed in blood

## Service offered

- RNA extraction and storage from blood in PAXgene blood RNA tube
- cDNA synthesis from RNA using RT-PCR
- PCR amplification of cDNA region flanking variant of interest, followed by analysis of products by agarose gel electrophoresis and sanger sequencing to look for abnormal splicing

## Technical

Analysis is carried out by sanger sequencing and gel electrophoresis of the cDNA region of interest to detect abnormal splicing. This technique may not detect splicing changes that result in very large insertions or deletions or cases where the mutant allele has been subject to very efficient nonsense mediated decay. Analysis is carried out on RNA extracted from blood as a proxy for clinically relevant tissues; in some cases splicing may differ in clinically relevant tissues.

## Target reporting time

Routine analysis – 42 days from when working primers available (primers are designed bespoke for each case and may require redesign).

Please contact the laboratory for urgent cases.

# Spinal Muscular Atrophy R70

## Contact details

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## Samples required

- 5ml venous blood in plastic EDTA bottles (>1ml from neonates)
- Prenatal testing must be arranged in advance, through a Clinical Genetics department if possible.
- Amniotic fluid or CV samples should be sent to Cytogenetics for dissecting and culturing, with instructions to forward the sample to the Regional Molecular Genetics laboratory for analysis
- A completed DNA request card should accompany all samples

## Patient details

To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician

## Introduction

Spinal muscular atrophy type 1 (MIM #253300) is an autosomal recessive neuromuscular disorder, characterized by degeneration of the anterior horn cells of the spinal cord, leading to symmetrical muscle weakness and atrophy. SMA type 1 is caused by a homozygous loss of *SMN1* exon 7 (~96% cases) and in rarer cases, by small sequence variants in the *SMN1* gene (~4%).

The carrier frequency of SMA varies depending upon ethnicity, and has been reported as high as 1 in 40 individuals from some ethnic groups.

## Referrals

- Children and adults with clinical features strongly suggestive of SMA
- Hypotonic infants with a likely central cause
- Carrier testing in individuals at increased risk (above the population risk) of having an affected pregnancy, for example a family history of SMA, a partner shown to be a carrier or first cousin partnerships. Accurate carrier testing in SMA families ideally requires either a sample from an affected family member or information regarding the pathogenic variants carried in the family. Without this information, the extent to which we can reduce an individual's carrier risk is less than if information on familial pathogenic variants is available. Please provide the ethnicity of individuals undergoing carrier testing.
- In accordance with UK genetic testing guidelines carrier testing is only exceptionally undertaken in minors.

## Prenatal testing

Prenatal testing is available for couples in whom pathogenic variants have been identified. Non-invasive prenatal diagnosis may be available - please contact the laboratory to discuss.

## Service offered

MLPA analysis of *SMN1* exons 7 and 8, and *SMN2* exons 7 and 8, using MRC Holland kit P060. Copy number of *SMN2* is provided when an individual is found to have no copies of *SMN1* exon 7.

Individuals in whom a rare mutation in the SMN1 gene is likely can be referred for R71 once R70 has been carried out and no diagnosis made.

## Technical

Samples are analysed using MLPA. This method will not detect rare sequence variants in the *SMN1* gene and does not test other genes associated with spinal muscular atrophy phenotypes.

## Target reporting time

Routine analysis- 6 weeks. Hypotonic infants – 2 weeks.

For urgent samples please contact the laboratory.

# X-Inactivation R111

## Contact details

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## Samples required

- 5ml venous blood in plastic EDTA bottles (>1ml from neonates)
- Prenatal testing must be arranged in advance, through a Clinical Genetics department if possible.
- Amniotic fluid or CV samples should be sent to Cytogenetics for dissecting and culturing, with instructions to forward the sample to the Regional Molecular Genetics laboratory for analysis
- A completed DNA request card should accompany all samples

## Patient details

To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician

## Introduction

The X-inactivation status of females may be determined using X-linked methylation sensitive polymorphic markers. This information may be useful to explain the manifestation of X-linked recessive conditions in females or to indicate carrier status for certain X-linked disorders.

In females, random X-inactivation/lyonisation occurs where one of the two X chromosomes is randomly inactivated in every somatic cell. Hence the expression levels of most genes on the X chromosome are similar in males and females. However, 5-20% of the normal female population appear to have non-random or skewed X-inactivation. Non-random X-inactivation is also thought to increase with age. In certain conditions, if a female has a mutation in a given gene on one X chromosome then non-random X-inactivation can occur, but this can be tissue dependent and therefore care must be taken to ensure the most appropriate tissue is analysed.

The technique can be applied to any appropriate condition, however in this laboratory X-inactivation studies are most commonly used to indicate carrier status for the immunodeficiency conditions Wiskott Aldrich syndrome (WAS), X-linked severe combined immunodeficiency (XSCID) and X-linked agammaglobulinaemia (XLA). In these conditions carrier females have unilateral X-inactivation patterns in their whole blood, T cells only, or B cells only, respectively (separated cells will be required for this analysis, please see information below).

## Referrals

- Testing is available to individuals where X-inactivation testing will alter clinical management and/or assist reclassification of variant using the ACMG guidelines.
- To indicate carrier status of females with a suspected family history of the immunodeficiency disorders, WAS, XSCID and XLA, where no sample is available from the affected male or where no mutation has been identified.
- For studies in other X-linked recessive conditions, please contact the laboratory to discuss.

## Service offered

X-inactivation status at the androgen receptor (AR) gene, Xq11-q12 (MIM 313700).

## Technical

A methylation sensitive restriction enzyme is used to detect differential methylation patterns between the inactive and active X chromosomes. The methylation sensitive sites are in close proximity to the polymorphic site allowing the two X chromosomes to be distinguished. The androgen receptor is very informative and has a heterozygosity of 90%.

## Target reporting time

Routine analysis - 6 weeks. For urgent samples please contact the laboratory.

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# Y chromosome microdeletions

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## Samples required

- 5ml venous blood in plastic EDTA bottles (>1ml from neonates)
- A completed DNA request card should accompany all samples

## Patient details

To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician

## Introduction

Testing for azoospermia factor (AZF) deletions on the Y chromosome forms part of the diagnostic work-up for males with non-obstructive azoospermia or severe oligozoospermia. After Klinefelter syndrome (47,XXY), Y chromosome AZF deletions are the second most common genetic cause of male infertility. Detection of AZF deletions may explain the patient's azoospermia or oligozoospermia, and identification of the specific deletion type can provide prognostic information regarding the likelihood of successful sperm retrieval by testicular sperm extraction (TESE) for use in assisted reproduction. TESE is not recommended for patients with complete AZFa or AZFb deletions, but it may be successful in those with an AZFc deletion.

## Referrals

Diagnostic testing is available for individuals with non-obstructive azoospermia or severe oligospermia where testicular sperm extraction (TESE/microdissection TESE) is considered and the outcome of testing will inform eligibility for (m)TESE and success of sperm retrieval.

Testing for Y chromosome microdeletions should not routinely be performed before ICSI.

## Service offered

Testing for the c.845G>A p.(Cys282Tyr) and c.187C>G p.(His63Asp), also known as C282Y and H63D,

## Technical

First line analysis is carried out using the Devyser AZF v2 kit for chromosome Y microdeletion detection and detects the following markers: AZFa: sY86, sY84; AZFb: sY127, sY134; AZFc: sY255, sY254; Controls: ZFY/ZFX, sY14 (SRY).

Extension analysis is carried out using the Devyser AZF extension kit and detects the following markers: AZFa: sY82, sY84,sY86, sY1064, sY1065, sY88; AZFb: sY105, sY121, sY127, sY134, sY1192, sY153; AZFc: sY1191, sY1291, sY254, sY255, sY160; Controls: ZFY/ZFX, sY14 (SRY).

Partial deletions, mosaic deletions, structural rearrangements, sex chromosome aneuploidies and single nucleotide variants in specific genes will not be detected.

## Target reporting time

Routine analysis – 42 days. Please contact the laboratory for urgent cases.

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# Non-invasive prenatal sexing R251

## Contact details

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## Samples required

- **Pregnant Patient**
  2 x 10ml* venous blood in cell stabilising bottles (PAXgene Blood ccfDNA or Streck Cell-Free DNA)
- **Testing must be arranged in advance,** through your local Clinical Genetics department or Fetal Medicine Unit.
- A completed DNA request card should accompany all samples

## Patient details

To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician

## Introduction

Free fetal DNA may be detected in maternal plasma from early in gestation and used for determination of fetal gender. The sex of the fetus is determined by the presence of Y-specific sequence for a male fetus and the absence of Y specific material in the cell free DNA extract in the case of a female fetus.
The analytical sensitivity and specificity of the Real Time PCR assay was measured in 189 pregnancies (394 tests) over a period of 2 years from April 2007 to March 2009. When audited against pregnancy outcome there were 145 cases with a known outcome and in these cases the test demonstrated 100% (95% CI 97.5-99.9) concordance with no false positives or false negatives.
This is achieved by testing two separate maternal samples for the presence of SRY and by stipulating that the fetus is at least 7 weeks gestation at the time of sampling.

## Referrals

All referrals should be made via a Clinical Genetics Department or Fetal Medicine Unit, please contact the laboratory in advance of sending a sample.
Samples are accepted from patients from 7 weeks gestation (confirmed by scan) at which time there should be a sufficient concentration of free fetal DNA in the circulation.
*From 7 to 9 weeks gestation, 2 x 10ml samples are required, taken one week apart. At 9+ weeks gestation 2 x 10ml samples may be taken at the same time.

Referrals for testing will be triaged by the Genomic Laboratory; testing should be targeted at those where a genetic or genomic diagnosis will guide management for the proband or family.

Referrals are accepted from English patients according to the agreed test routing rules. Referrals may be accepted from the devolved nations and from paying overseas/private referral centres.

## Service offered

Non-invasive prenatal sex determination is offered to inform management in pregnancies at risk of severe sex-linked disorders, those affecting one sex in particular or where genitalia are ambiguous. It is not available for non-medical indications.
Testing may not be possible in multiple pregnancies including those with a possible vanishing twin. In such cases contact the laboratory for discussion.

## Technical

Maternal blood is separated after collection. Cell free DNA is then extracted from the plasma. Molecular analysis is performed using real time PCR and Taqman assays for the SRY marker and a CCR5 control marker. Results of the duplicate analysis will be released following analysis of the second sample.
A male fetus is detected by the presence of SRY-specific sequence. The assay cannot distinguish between a lack of SRY indicative of a female fetus and a failure to extract sufficient free fetal DNA for analysis. A second sample ideally at later date but dependent on the gestation age is therefore required to repeat the analysis.
Consistent absence of SRY in the presence of the control marker is taken as evidence that the fetus is female.

## Target reporting time

The nationally agreed turnaround time is 21 days

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# Non-invasive prenatal diagnosis

## Contact details

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## Samples required

- **Pregnant Patient**
  2 x 10ml venous blood in cell stabilising bottles (PAXgene Blood ccfDNA or Streck Cell-Free DNA)
- The minimum gestation (by scan) is 9wks for accepting a sample.
- Control samples may include:
- **Paternal** blood (5ml EDTA) or DNA
- **Proband** (or confirmed non-carrier child) blood (1ml EDTA or DNA)
- **Testing must be arranged in advance,** through your local Clinical Genetics department or Fetal Medicine Unit.
- A completed DNA request card should accompany all samples

## Patient details

To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician

## Introduction

Cell-free fetal DNA (cffDNA) may be detected in maternal plasma from early in gestation and used for detection of *de novo* / paternally inherited pathogenic variants. Total cell-free DNA reflecting both maternal and fetal material is extracted from maternal plasma. The presence of fetal DNA in maternal plasma can be established by chromosomal sex analysis in males or by the presence of a paternal HLA type on Next Generation Sequencing (NGS). When this is uninformative a separate NGS assay is used containing 40 heterogeneous SNPs to identify paternally inherited alleles.

Definitive non-invasive prenatal diagnosis (NIPD) by relative haplotype dosage analysis (RHDO) is also possible using cffDNA in pregnancies at risk of cystic fibrosis, spinal muscular atrophy or congenital adrenal hyperplasia for confirmed carrier couples (including couples who are carriers of the same variant)
This test is only applicable to couples
1) who are known carriers AND
2) DNA is available from the affected proband or a confirmed unaffected child
Relative haplotype dosage analysis will be used to determine if the fetus has inherited the high risk allele from both parents.

NIPD by RHDO can also be offered in male pregnancies at risk of DMD/BMD. Control DNA must be available from an affected child or other male family member.

## Referrals

Paternal exclusion testing can be offered in families at risk of a recessive disorder when parents carry different mutations or where the father has an autosomal dominant mutation or is known mosaic for a mutation. Pre-pregnancy work up (R389) is required to enable confirmation that NIPD is possible and to allow timely delivery in pregnancy.

NIPD for *FGFR2* and *FGFR3* related disorders can also be offered based on abnormal ultrasound findings or to exclude recurrence due to germline mosaicism.

NIPD should only be offered for conditions where invasive testing would otherwise be offered and following discussion with the testing laboratory. Referrals for testing will be triaged by the Genomic Laboratory; testing should be targeted at those where a genetic or genomic diagnosis will guide management for the proband or family.

Testing should be discussed in advance with the testing laboratory to ensure that necessary samples are available and validation work has been performed.

NIPD by RHDO is not currently possible for consanguineous couples.

Testing may not be possible in multiple pregnancies including vanishing twin. In such cases contact the laboratory for discussion

Patients must meet the referral criteria for the specific test indication as outlined in the National Genomic Test Directory rare and inherited disease eligibility criteria document. https://www.england.nhs.uk/publication/national-genomic-test-directories/

Referrals are accepted from the English patients according to the agreed test routing rules, including a national service for NIPD for *FGFR2* and *FGFR3* related disorders. Referrals may also be accepted from the devolved nations and from paying overseas/private referral centres.

# Non-invasive prenatal diagnosis

## Service offered

**R249 NIPD using paternal exclusion testing for very rare conditions where familial mutation is known**

Targeted NGS for variant of interest. Pre-pregnancy work up (R389) is required to enable confirmation that NIPD is possible and to allow timely delivery in pregnancy

**R250 NIPD for congenital adrenal hyperplasia - CYP21A2 haplotype testing**

Currently exported to Birmingham

**R304 NIPD for cystic fibrosis - haplotype testing**

Relative haplotype dosage analysis by targeted NGS

**R305 NIPD for cystic fibrosis - mutation testing**

Targeted NGS for one of the following paternal CFTR variants p.(Phe508del), c.489+1G>T, p.(Gly542*), p.(Gly551Asp), p.(Trp1282*) p.(Arg553*), p.(Ile507del), p.(Arg560Thr), p.(Ser549Asn), p.(Ser549Arg)

**R306 NIPD for Apert syndrome - mutation testing**

Targeted NGS for c.755C>G p.(Ser252Trp), c.758C>G p.(Pro253Arg) and c.755_756delinsTT p.(Ser252Phe) mutations in *FGFR2*.

**R307 NIPD for Crouzon syndrome with acanthosis nigricans - mutation testing**

Targeted NGS for *FGFR3* c.1172C>A p.(Ala391Glu).

**R308 NIPD for *FGFR2*-related craniosynostosis syndromes - mutation testing**

NGS for 28 *FGFR2*-related craniosynostosis syndrome mutations

**R309 NIPD for *FGFR3*-related skeletal dysplasias - mutation testing**

NGS for *FGFR3* variants associated with skeletal dysplasia

**R310 NIPD for Duchenne and Becker muscular dystrophy - haplotype testing**

Currently exported to Birmingham

**R311 NIPD for spinal muscular atrophy - mutation testing**

Relative haplotype dosage analysis by targeted NGS

**R389 NIPD - pre-pregnancy test work-up**

Targeted NGS for variant of interest

## Technical

Maternal blood is separated after collection, then cffDNA is extracted from plasma. Molecular analysis is performed by PCR, followed by NGS (Illumina MiSeq). Amplification of fetal DNA will be confirmed using chromosomal sex analysis in males or by the presence of paternal HLA sequences. When this is uninformative a separate NGS assay is used containing 40 heterogeneous SNPs to identify paternally inherited alleles.

We cannot rule out the possibility of false negative results due to the complex architecture of fetal cell free DNA confounding this analysis. To date, we have carried out over 500 tests on 93 different amplicons and identified one true false negative case (1.1%) that may be attributable to non-random shearing of the fetal cell free DNA.

## Target reporting time

The nationally agreed turnaround time is 21 days for non-invasive prenatal diagnosis and 42 days for pre-pregnancy test work up.

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# Fetal anomalies with a likely genetic cause – whole exome sequencing R21 R412

## Contact details

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London, WC1N 3BH
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## Samples required

- DNA extracted from CVS or amniocytes OR 20ml amniotic fluid or 20mg of chorionic villi.
- 5ml parental venous blood in plastic EDTA bottles OR DNA
- Prenatal testing must be arranged in advance, through a Clinical Genetics department
- A completed DNA request card should accompany all samples

## Patient details

To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician

## Introduction

Whole exome sequencing (WES) performed with interpretation based on analysis of a fetal anomalies panel comprising genes in which variants may result in structural changes in the fetus detectable by prenatal imaging.

## Referrals

Referrals will only be accepted for testing following discussions between the local Clinical Genetics department, the mother's local clinical management team and the testing laboratory. These referrals must be from pregnancies with multiple major structural abnormalities detected on fetal imaging where multidisciplinary team review considers a monogenic malformation disorder is likely and a molecular diagnosis may influence pregnancy or early neonatal management in the index pregnancy.

Trio testing (both parents and the fetus) is the preferred option to aid rapid interpretation. Duo testing is accepted in exceptional circumstances (e.g. where one parent is not available or in instances of ovum or sperm donation), and these referrals should be discussed with the Testing GLH and parents need to be advised of the potential decreased diagnostic yield in this circumstance.

Patients must meet the referral criteria for the specific test indication as outlined in the National Genomic Test Directory rare and inherited disease eligibility criteria document. https://www.england.nhs.uk/publication/national-genomic-test-directories/

Referrals are accepted from English patients according to the agreed routing rules. Referrals may be accepted from the devolved nations.

## Service offered

### R21 Fetal anomalies with a likely genetic cause

Fetus with multiple major structural abnormalities detected on fetal ultrasound where multidisciplinary review to include clinical genetics, tertiary fetal medicine specialists, clinical scientists and, where appropriate, relevant paediatric specialists considers a monogenic malformation disorder is likely.

This indication is relevant in ongoing pregnancies where a genetic diagnosis may influence management of the ongoing pregnancy and NOT where there is imminent fetal loss or termination of pregnancy, or miscarriage has already occurred.

### R412 Fetal anomalies with a likely genetic cause – non urgent

Fetus from a demised/non-continued pregnancy, with multiple major structural abnormalities detected on fetal ultrasound or post-mortem examination (by autopsy, imaging, metabolic and/or histological tests) and where multidisciplinary review (clinical genetics, tertiary fetal medicine specialists, clinical scientists and, where appropriate, relevant paediatric specialists) consider a monogenic malformation disorder is likely.

Only for cases where it is not possible to test by WGS via R27 (e.g. when there is insufficient DNA for WGS). Testing should be primarily targeted to those families for which this test may influence future pregnancies.

# Fetal anomalies with a likely genetic cause – whole exome sequencing R21 R412

## Technical

Whole exome sequencing (TWIST, Illumina NextSeq) followed by analysis of a panel of nationally-agreed genes known to cause disorders which can present in the fetal context called the “Fetal anomalies gene panel”. Read alignment and variant calling is performed by the Illumina Dragen platform followed by variant annotation using Alamut Batch, copy number analysis is undertaken using the ExomeDepth tool.

Variants of interest confirmed by Sanger sequencing for SNVs/ indels/ frameshifts, or microarray/qPCR for CNVs

Only Class 4 or 5 variants that are thought likely to explain the fetal phenotype will be reported. Class 3 variants will not be reported unless agreed in multidisciplinary team discussions e.g. class 3 variant *in trans* with a class 5 in a gene linked to a phenotype that is thought likely to explain the fetal ultrasound findings

## Target reporting time

The nationally agreed turnaround time is 21 days for R21 and 42 days for R412.

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# Cytogenetics – (R244.1/R242.1/R240/R375.1/R465)


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Samples required

- 2ml Blood in EDTA
- 2ml Blood in Lithium Heparin
- A completed request card should accompany all samples

Patient details

To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician

## Introduction

Cytogenetic tests can be requested in line with NHS England test directory criteria.

When a cytogenetic abnormality has been identified, family members at risk of carrying the abnormality may be tested by an appropriate targeted method e.g. targeted chromosome analysis, karyotype, FISH, targeted microarray, quantitative (real-time) PCR.

When a cytogenetic variant of uncertain significance is identified, parental testing may be required to aid interpretation. Testing will be by an appropriate targeted method e.g. targeted chromosome analysis, karyotype, FISH, targeted microarray, quantitative (real-time) PCR.

## Referrals

**R244.1 Carrier resting for known familial mutation**

**R375.1 Family follow up testing to aid variant interpretation**

**R242.1 Predictive testing for known familial mutation**

**R240.1 Diagnostic testing for known mutation**

**R465.1 Familial cytogenetic rearrangement**

## Service offered

Targeted testing by targeted chromosome analysis, karyotype, targeted microarray, qPCR or FISH or a combination of methods will be used to identify the presence of the index patient's pathogenic variant and exclude a predisposing chromosome abnormality when applicable.

## Technical

Chromosomal microarray analysis is carried out using the Illumina CytoSNP-850K microarray and Bluefuse Multi v4.5 (Illumina) software. The resolution for CNV will be checked to ensure that it is sufficient to identify the familial CNV.

Targeted chromosome analysis/karyotype will be carried out if there is a risk of balanced chromosomal structural rearrangement (e.g. translocation/inversion). FISH using custom probes will be carried out to exclude submicroscopic chromosome structural rearrangement (e.g. insertion).

Quantitative real time PCR (qPCR) will be carried out to determine the presence/absence of a familial CNV.

## Target reporting time

Routine analysis – 42 days

Urgent analysis – 14 days

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# Cytogenetics – Targeted chromosome analysis/Karyotyping (R463.1/R464.1/R466.1/R467.1/R468.1/R265.1/R402.1)

## Contact details


Rare & Inherited Disease Laboratory

North Thames Genomic Laboratory Hub

Levels 4-6, Barclay House

37 Queen Square

London, WC1N 3BH

T +44 (0) 20 7762 6888

E gos-tr.norththamesgenomics@nhs.net


## Samples required

- 1-2ml venous blood in plastic lithium heparin tube
- 20ml amniotic fluid or 20mg of chorionic villi (for R463.1 only)
- A completed request card should accompany all samples

## Patient details

To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician

## Introduction

Cytogenetic testing by karyotype can be requested in line with NHS England test directory criteria.

## Referrals

**R463.1 Cytogenetic characterisation of a genomic abnormality**

Proband with possible structural chromosomal rearrangement requiring karyotype or targeted chromosome analysis (TCA), such as Robertsonian translocation, reciprocal translocation, ring chromosome, or other microscopically visible structural rearrangement, either indicated by, or alongside, findings from aneuploidy testing, microarray, WGS or other laboratory techniques. [TCA/Karyotype as appropriate]

**R464.1 Recurrent miscarriage, products of conception not available – parental karyotype**

Recurrent miscarriage (defined as three or more miscarriages): • where testing of the pregnancy loss has not been possible due to an unsuitable/failed sample e.g. no fetal material/MCC/fixed in formalin, and no previous losses have been successfully tested and reported.

Note that although parental karyotype analysis is available following a failed test, this is of limited utility and the most informative pathway is to test any subsequent pregnancy loss.

Recurrent miscarriage (five or more pregnancy losses): Where none of the previous losses have been successfully tested and reported e.g. biochemical pregnancies, no products available for testing

**R466.1 Unexplained infertility**

Unexplained infertility prior to infertility treatment

**R467.1 Gamete donors**

Egg/sperm donor requiring a karyotype prior to acceptance

**R468.1 Possible sex chromosome aneuploidy or structural rearrangement**

Clinical suspicion of a sex chromosome abnormality e.g. Turner syndrome, sex chromosome mosaicism, sex chromosome structural abnormality [TCA].

**R265.1 Chromosomal mosaicism**

Individuals with possible mosaic chromosome abnormality requiring extended count karyotype including:

1. possible mosaic chromosome abnormality indicated by findings from conventional karyotype, microarray, WGS or other laboratory technique, OR

2.clinical features strongly suggestive of a specific chromosomal phenotype, for example Down syndrome, in whom conventional testing is negative

**R402.1 Premature Ovarian Insufficiency**

1. Four consecutive months of unexplained amenorrhoea (primary or secondary), AND
2. Elevated serum FSH of >30IU/L on two separate occasions at least 6 weeks apart, AND
3. Age of onset is <30 years, AND
4. Non-genetic causes have been excluded including presence of thyroid and adrenal auto-antibodies

## Service offered

The format of the service offered will vary depending on the patient's clinical features. The testing is performed by karyotyping or targeted chromosome analysis , and additional

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# Cytogenetics – Postnatal Chromosomal Microarray (R343.1/R28.1/R137.1/R199.1/R146.1)


Contact details

Rare & Inherited Disease Laboratory

North Thames Genomic Laboratory Hub

Levels 4-6, Barclay House

37 Queen Square

London, WC1N 3BH

**T** +44 (0) 20 7762 6888

**E** gos-tr.norththamesgenomics@nhs.net


Samples required

- 2ml Blood in EDTA
- A completed request card should accompany all samples

Patient details

To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician

## Introduction

Cytogenetic tests can be requested in line with NHS England test directory criteria.

Chromosomal microarray is performed for a number of clinical phenotypes please check the NHS genomic test directory for all eligibility criteria.

## Referrals

Note: some referral reasons should be carried out alongside sequencing panels/WGS – see NHS genomic test eligibility criteria for more details.

**R28 Congenital malformation and dysmorphism syndromes**

**R137 Congenital heart disease**

**R146 Disorders of sex development**

**R343 Chromosomal mosaicism**

**R199 Congenital anomalies of the kidney and urinary tract**

## Service offered

DNA is extracted directly from the sample and used for Chromosomal microarray.

## Technical

Chromosomal microarray analysis is carried out using the Illumina CytoSNP-850K microarray and Bluefuse Multi v4.5 (Illumina) software. The expected resolution for copy number variants is 300kb. The SNP microarray can also detect absence of heterozygosity (AOH). AOH consistent with a shared parental genetic heritage will not be reported. AOH consistent with potential Uniparental Disomy will be reported.

## Target reporting time

Routine analysis – 42 days

Urgent analysis – 14 days

# Cytogenetics – Postnatal Common Aneuploidy (R26) – Send test.

Contact details

Rare & Inherited Disease Laboratory

North Thames Genomic Laboratory Hub

Levels 4-6, Barclay House

37 Queen Square

London, WC1N 3BH

**T** +44 (0) 20 7762 6888

**E** gos-tr.norththamesgenomics@nhs.net

Samples required

- 1-2ml venous blood in lithium heparin
- A completed request card should accompany all samples

Patient details

To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician

## Introduction

Cytogenetic tests can be requested in line with NHS England test directory criteria. Exclusion of the common aneuploidies – trisomy 13,18 and 21 - is carried out using QF PCR when clinical features strong suggest Patau, Edwards or Down syndrome. Investigation of sex chromosome aneuploidies is carried out if clinical indications of Turner syndrome or ambiguous genitalia are present. Blood cultures will be established in order to confirm positive results using targeted chromosome analysis. If trisomy is not present, the pathway may reflex to microarray testing.

## Referrals

**R26 Likely common aneuploidy**

1. Clinical features strongly suggestive of trisomy 13, 18 or 21, Turner syndrome or other sex chromosome aneuploidy.

## Service offered

Samples are sent to the London South Genomics Laboratory at Guy's hospital for QF-PCR testing. Test reports are sent direct from the lab at Guys to the referring centre. Any follow up testing e.g. karyotype confirmation of trisomy is carried out at the RIDL laboratory at GOSH.

## Technical

The QF PCR has markers for chromosomes 13, 18, and 21 which will identify if trisomy is present. If there is clinical indication of monosomy X or ambiguous genitalia, then the X and Y markers will also be reported.

QF PCR can also be used to determine if uniparental disomy is present when a parent has a chromosome translocation which may predispose to UPD e.g. a Robertsonian translocation involving chromosome 14. If this is required then parental blood samples (2ml in EDTA) must accompany the sample.

## Target reporting time

Routine analysis – 3 days from receipt at London South GLH.

# Cytogenetics – Pregnancy loss/Fetal demise Chromosomal Microarray (R22.2/R318.2)

Contact details

Rare & Inherited Disease Laboratory

North Thames Genomic Laboratory Hub

Levels 4-6, Barclay House

37 Queen Square

London, WC1N 3BH

**T** +44 (0) 20 7762 6888

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Samples required

- 2ml fetal blood in EDTA or Products of conception/fetal skin/fetal tissue biopsy in sterile container. Note: Formalin fixed tissue and frozen POC cannot be accepted.
- A completed request card should accompany all samples

Patient details

To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician

## Introduction

Chromosomal microarray testing can be requested in line with NHS England test directory criteria.

## Referrals

Note: some referral reasons should be carried out alongside sequencing panels/WGS – see NHS genomic test eligibility criteria for more details.

**R22.2 Fetus with likely chromosomal abnormality** Fetus must have anomalies/abnormalities consistent with a chromosome abnormality or unexplained fetal demise after 16 weeks gestation.

**R318.2 Recurrent miscarriage with products of conception available for analysis** At least three consecutive unexplained miscarriages.

## Service offered

DNA is extracted directly from the sample and used for Chromosomal microarray. Chromosomal microarray identified copy number variants across the genome. Copy number variants are assessed for pathogenicity and relevance to phenotype. Pathogenic and likely pathogenic variants are reported. Variants of uncertain clinical significance are reported only if parental studies may inform the interpretation.

## Technical

Chromosomal microarray analysis is carried out using the Illumina CytoSNP-850K microarray and Bluefuse Multi v4.5 (Illumina) software. The expected resolution for copy number variants using DNA from POC or fetal tissue is 1Mb however smaller CNV will be reported if clearly visible. If quality is reduced due to DNA quality then a reduced resolution report may be issued. The SNP microarray can also detect absence of heterozygosity (AOH). AOH consistent with a shared parental genetic heritage will not be reported. AOH consistent with potential Uniparental Disomy will be reported.

## Target reporting time

Routine analysis – 42 days

# Cytogenetics – Prenatal Chromosomal Microarray (R22)

Contact details

Rare & Inherited Disease Laboratory

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37 Queen Square

London, WC1N 3BH

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Samples required

- 20ml amniotic fluid or 20mg of chorionic villi.
- Maternal EDTA blood sample to accompany all chorionic villi samples.
- A completed request card should accompany all samples

Patient details

To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician

## Introduction

Cytogenetic tests can be requested in line with NHS England test directory criteria. Invasive prenatal samples from pregnancies where there are indications on ultrasound scan of fetal anomaly/abnormalities consistent with a chromosomal abnormality are tested by chromosomal microarray. Note that prenatal chromosomal microarray is not applicable if there are no scan anomalies present, if scan anomalies are not consistent with a chromosomal cause and no family history of chromosome abnormality. Analysis may be limited to specific chromosomes if there are no scan anomalies and the reason for testing is due to a family history of chromosome abnormality.

Exclusion of the common aneuploidies – trisomy 13,18 and 21 by QF PCR will be carried out in parallel.

## Referrals

**R22.2 Fetus with a likely chromosomal abnormality**

1. Ultrasound scan anomalies/abnormalities consistent with chromosomal abnormality. (Note: fetal anomalies e.g. absent nasal bone, are insufficient to qualify for chromosomal microarray).
2. Family history of a fully penetrant chromosome abnormality when referred from Clinical Genetics.

## Service offered

If sample size is sufficient, cell cultures are established. DNA is extracted directly from the sample and used for Chromosomal microarray. If the samples are compromised due to maternal bloodstaining, DNA is extracted from cultured cells and tested. If chromosomal microarray indicates a chromosome abnormality is present then a preliminary report is issued and the result confirmed by either repeating the microarray or carrying out alternative testing e.g. karyotyping. Where uncultured CVS has been used for the original testing then confirmation testing is carried out on cultured cells. If mosaicism is identified in uncultured CVS, cultured cells are tested before a report is issued.

## Technical

Chromosomal microarray analysis is carried out using the Illumina CytoSNP-850K microarray and Bluefuse Multi v4.5 (Illumina) software. The expected resolution for copy number variants is 300 kb. The SNP microarray can also detect absence of heterozygosity (AOH). AOH consistent with a shared parental genetic heritage will not be reported. AOH consistent with potential Uniparental Disomy will be reported.

## Target reporting time

Routine analysis – 14 days if uncultured DNA used / 21 days if cultured DNA used

# Cytogenetics – Prenatal Common Aneuploidy (R401) – Send test

Contact details
Rare & Inherited Disease Laboratory
North Thames Genomic Laboratory Hub
Levels 4-6, Barclay House
37 Queen Square
London, WC1N 3BH
**T** +44 (0) 20 7762 6888
**E** gos-tr.norththamesgenomics@nhs.net

Samples required

- 12-20ml amniotic fluid or 12-20mg of chorionic villi.
- A completed request card should accompany all samples

Patient details

To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician

## Introduction

Cytogenetic tests can be requested in line with NHS England test directory criteria. Exclusion of the common aneuploidies – trisomy 13,18 and 21 is carried out on all invasive prenatal samples. Investigation of sex chromosome aneuploidies is carried out on invasive prenatal samples if clinical indications are present. Prenatal cultures will be initiated for samples where additional genetic testing is indicated or for samples where aneuploidy testing is positive.

## Referrals

**R401 Common aneuploidy testing - prenatal**

Prenatal findings requiring common aneuploidy testing including:

1. high risk screening result
2. characteristic findings of a common aneuploidy on ultrasound scan

## Service offered

Samples are sent to the London South Genomics Laboratory at Guy's hospital for QF-PCR testing. Test reports are sent direct from the lab at Guys to the referring FMU. Any follow up testing e.g. karyotype confirmation of trisomy is carried out at the RIDL laboratory at GOSH.

## Technical

The QF PCR has markers for chromosomes 13, 18, and 21 which will identify if trisomy is present. If there is clinical indication of monosomy X or sex-specific monogenic disorder, the X and Y markers will also be reported.

QF PCR can also be used to determine if uniparental disomy is present when a parent has a chromosome translocation which may predispose to UPD e.g. a Robertsonian translocation involving chromosome 14. If this is required then parental blood samples (2ml in EDTA) must accompany the prenatal sample.

## Target reporting time

Routine analysis – 3 days from receipt at London South GLH.

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# Paediatric Neurology

## Contact details

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Levels 4-6, Barclay House
37 Queen Square
London, WC1N 3BH
T +44 (0) 20 7762 6888
E gos-tr.norththamesgenomics@nhs.net

## Samples required

- 5ml venous blood in plastic EDTA bottles (>1ml from neonates)
- Prenatal testing must be arranged in advance, through a Clinical Genetics department if possible.
- Amniotic fluid or CV samples should be sent to Cytogenetics for dissecting and culturing, with instructions to forward the sample to the Regional Molecular Genetics laboratory for analysis
- A completed DNA request card should accompany all samples

## Patient details

To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician

## Introduction

The Paediatric Neurology service encompasses testing for disorders of the brain, spinal cord, peripheral nerve and muscle affecting infants, children and adolescents. These include arthrogryposis, holoprosencephaly, hydrocephalus, cerebral malformations, severe microcephaly and rare neuromuscular disorders.

## Referrals

For details of testing criteria for each Paediatric Neurology indication and the clinical specialties who would be expected to request the test (generally referrals will be accepted from Clinical Geneticists and Neurology services) please refer to the National Genomic Test Directory's Testing Criteria for Rare and Inherited Disease (https://www.england.nhs.uk/publication/national-genomic-test-directories/)

Referrals are accepted from English patients according to the agreed routing rules. Testing is also offered to the devolved nations and from paying overseas/private referral centres. A patient information leaflet for this test is available to aid in consenting; please contact the laboratory for a copy.

## Prenatal testing

Prenatal diagnosis may be offered as appropriate where pathogenic variants have been identified in accordance with expected inheritance pattern and appropriate parental testing and genetic counselling has been conducted.

## Service offered

Analysis of coding regions and intron/exon boundaries of the green genes listed in the virtual PanelApp panels (see below under the technical info for links to the gene lists). For moderate evidence amber genes, data may be collected and where clinically appropriate analysed, to gather further evidence for disease-association.

R83.3 Arthrogryposis

R381.2 Other rare neuromuscular disorders

R85.2 Holoprosencephaly

R86.3 Hydrocephalus

R87.3 Cerebral malformation

R88.3 Severe microcephaly

## Technical

Testing is performed by whole genome sequencing by Illumina and analysis using the NHSE decision support systems.

Details of the genes analysed for each test indication can be found on the NHS Genomic Medicine Service (GMS) Signed Off Panels Resource webpage.

## Target reporting time

Targeted routine testing is 42 days for whole genome sequencing data analysis once data is returned. Please contact the laboratory for urgent cases.

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# Ophthalmology

## Contact details

Rare & Inherited Disease Laboratory
North Thames Genomic Laboratory Hub
Levels 4-6, Barclay House
37 Queen Square
London, WC1N 3BH
T +44 (0) 20 7762 6888
E gos-tr.norththamesgenomics@nhs.net

## Samples required

- 5ml venous blood in plastic EDTA bottles (>1ml from neonates)
- Prenatal testing must be arranged in advance, through a Clinical Genetics department if possible.
- Amniotic fluid or CV samples should be sent to Cytogenetics for dissecting and culturing, with instructions to forward the sample to the Regional Molecular Genetics laboratory for analysis
- A completed DNA request card should accompany all samples

## Patient details

To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician

## Introduction

Ocular conditions are highly heterogeneous and show considerable phenotypic overlap. 1 in 2,500 children in the UK are diagnosed as blind or severely visually impaired by the time they reach one year of age. As many as half of these cases are likely to be inherited and remain undiagnosed due to the vast number of genes involved in these conditions. Many congenital eye disorders causing visual impairment or blindness at birth or progressive visual impairment also include syndromic conditions involving additional metabolic, developmental, physical or sensory abnormalities. Gene panels offer the enhanced probability of diagnosis as a very large number of genes can be interrogated. Ocular birth defects include all inheritance modalities. Autosomal dominant and recessive diseases as well as X-linked dominant and recessive diseases are seen. These conditions can also be caused by *de novo* variants.

## Referrals

Referrals are accepted from English patients according to the agreed routing rules. The North Thames Genomic Laboratory Hub also offers a national service for congenital fibrosis of the extraocular muscles as well as testing for Leber hereditary optic neuropathy in coordination with the Institute of Neurology. Referrals may be accepted from the devolved nations and from paying overseas/private referral centres.

Patients must meet the referral criteria for the specific test indication as outlined in the National Genomic Test Directory rare and inherited disease eligibility criteria document.
https://www.england.nhs.uk/publication/national-genomic-test-directories/

## Prenatal testing

Prenatal diagnosis is only offered to couples confirmed to be carriers of pathogenic variants and appropriately counselled by Clinical Genetics. Testing is undertaken by sequence analysis or an appropriate dosage assay for large deletions and duplications.

## Service offered

Analysis of coding regions and intron/exon boundaries of the genes listed in the approved green panels:

### R107 Bardet-Biedl syndrome (BBS)

Performed by next generation sequencing. Please refer to the national genomics test directory to check eligibility criteria before requesting this test.

### R31.1 Bilateral congenital or childhood onset cataracts

Performed by whole genome sequencing.

### R32 Retinal disorders

Performed by whole genome sequencing.

### R33 Possible X-linked retinitis pigmentosa

**RPGR* exon ORF15 analysis is performed as a first line of testing followed by whole genome sequencing if the result for the RPGR/ORF15 analysis is normal.

### R36 Structural eye disease

Performed by whole genome sequencing. Please refer to the national genomics test directory to check eligibility criteria before requesting this test.

### R38 Sporadic aniridia

# Ophthalmology

R38.1 analysis of *PAX6* and *WT1* is performed by MLPA and R38.2 is performed by next generation sequencing (small panel).

**R39 Albinism or congenital nystagmus**

Performed by next generation sequencing including analysis of the common hypomorphic variants in *TYR*: c.575C>A p.(Ser192Tyr) and c.1205G>A p.(Arg402Gln).

**R41 and R42 Optic neuropathy and Mitochondrial Leber hereditary optic neuropathy LHON**

For patients with a clinical suspicion of LHON, analysis includes targeted testing for three common mitochondrial variants and if negative result, the optic neuropathy panel will be activated which is performed by next generation sequencing.

**R46 Congenital fibrosis of the extraocular muscles (CFEOM)**

This test is offered nationally by the NHS North Thames Genomic Laboratory Hub and performed by next generation sequencing (small panel).

**R262 Corneal dystrophy**

Performed by next generation sequencing

**R43 Blepharophimosis ptosis and epicanthus inversus**

*R43.1 single gene testing of the *FOXL2* gene is performed by next generation sequencing. R43.2 includes *FOXL2* gene testing by MLPA and R.43.3 includes *FOXL2* gene testing by STR.

**R45 Stickler syndrome**

*R45.1 is performed by next generation sequencing (small panel) and R45.2 analyses the *COL2A1* / *COL11A1* genes by MLPA testing.

† details of the genes analysed for each test indication can be found on the NHS Genomic Medicine Service (GMS) Signed Off Panels Resource webpage: https://nhsgms-panelapp.genomicsengland.co.uk/entities

## Technical

In-house variant screening is carried out by Sanger sequencing or next generation sequencing with library preparation using a custom TWIST target enrichment followed by sequencing on the Illumina platforms. Read alignment and variant calling is performed by the Illumina Dragen platform followed by variant annotation and copy number analysis by an in-house pipeline.

Whole genome sequencing is performed by Illumina with analysis using the NHSE Decision support systems.

## Target reporting time

84 days for next generation sequencing screening of large gene panels (>10 genes) in an index case, 42 days for next generation sequencing screening of small gene panels (<10 genes) in an index case and for familial or targeted variant testing.

For whole genome sequencing the turnaround time for data analysis and reporting is 42 days once sequence data is returned from Illumina.

Please contact the laboratory for urgent cases.

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# Genetic epilepsy syndromes (R59.3)

## Contact details

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Levels 4-6, Barclay House
37 Queen Square
London, WC1N 3BH
T +44 (0) 20 7762 6888
E gos-tr.norththamesgenomics@nhs.net

## Samples required

- 5ml venous blood in plastic EDTA bottles (>1ml from neonates)
- Prenatal testing must be arranged in advance, through a Clinical Genetics department if possible.
- Amniotic fluid or CV samples should be sent to Cytogenetics for dissecting and culturing, with instructions to forward the sample to the Regional Molecular Genetics laboratory for analysis
- A completed DNA request card should accompany all samples

## Patient details

To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician

## Introduction

The developmental and epileptic encephalopathies (DEE) are a group of disorders characterized by early onset seizures and developmental delay. Many are associated with intractable seizures, severe developmental delay and require lifelong care. Early mortality is common amongst severely affected individuals due to seizures and/or respiratory tract infections.

An increasing number of individual genetic disorders are now recognised to cause DEE. In only a small subset of the disorders is the clinical phenotype sufficiently recognisable or distinctive to allow targeted testing of specific genes.

## Referrals

This is a core test. As a guide referrals will generally be accepted from Clinical Geneticists, Neurology and Metabolic Medicine services.

Patients must meet the referral criteria for the specific test indication as outlined in the National Genomic Test Directory rare and inherited disease eligibility criteria document. https://www.england.nhs.uk/publication/national-genomic-test-directories/

Whole genome sequencing test indications are only available to English NHS patients, private testing is not currently available. Referrals may be accepted from the devolved nations and from paying overseas/private referral centres offering an in-house version of the panel (please contact the laboratory for further details).

## Prenatal testing

Prenatal diagnosis may be offered as appropriate where pathogenic variants have been identified in accordance with expected inheritance pattern and appropriate parental testing and genetic counselling has been conducted.

## Service offered

Analysis of coding regions and intron/exon boundaries of the green genes listed in the virtual PanelApp panel (see below under the technical info for link to the gene list). For moderate evidence amber genes, data may be collected and where clinically appropriate analysed, in order to gather further evidence for disease-association.

## Technical

Testing is performed by whole genome sequencing by Ilumina and analysis using the NHSE Decision support systems.

Details of the genes analysed can be found on the NHS Genomic Medicine Service (GMS) Signed Off Panel Resource webpage.

## Target reporting time

42 days for whole genome sequencing data analysis and reporting once data is returned from Illumina. 42 days for targeted routine testing. 3 days for prenatal testing.

Please contact the laboratory for urgent cases.

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# Monogenic hearing loss R67

## Contact details

Rare & Inherited Disease Laboratory
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Levels 4-6, Barclay House
37 Queen Square
London, WC1N 3BH
T +44 (0) 20 7762 6888
E gos-tr.norththamesgenomics@nhs.net

## Samples required

- 5ml venous blood in plastic EDTA bottles (>1ml from neonates)
- Prenatal testing must be arranged in advance, through a Clinical Genetics department if possible.
- Amniotic fluid or CV samples should be sent to Cytogenetics for dissecting and culturing, with instructions to forward the sample to the Regional Molecular Genetics laboratory for analysis
- A completed DNA request card should accompany all samples

## Patient details

To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician

## Introduction

Pre-lingual non-syndromic hearing loss has an estimated prevalence of 2-3 cases per 1,000 individuals. Approximately half of sensorineural hearing loss (SNHL) in children is thought to be genetic comprising 70% non-syndromic and 30% syndromic. The presentation of patients with some of the syndromic forms of deafness can be highly variable and the hearing loss may appear to be isolated. The most common genetic cause of non-syndromic hearing loss is *GJB2* however there is high genetic and allelic heterogeneity with a large number of other causative genes.

## Referrals

Referrals for the R67 Monogenic hearing loss indication are accepted according to the National Genomic Test Directory's Testing Criteria for Rare and Inherited Disease.

Acceptable referring specialities include Audiovestibular Medicine, Clinical Genetics, Paediatrics, and Ear, Nose and Throat.

Testing is offered to eligible English patients according to the agreed routing rules and samples may be accepted from the devolved nations and from paying overseas/private referral centres.

A patient information leaflet for this test is also available to aid in consenting; please contact the laboratory for a copy.

## Prenatal testing

Prenatal testing is not routinely offered for pregnancies at risk of non-syndromic hearing loss. Prenatal testing for syndromic deafness may be offered if appropriate where pathogenic variants have been identified in accordance with expected inheritance pattern and appropriate parental testing and genetic counselling has been conducted.

## Service offered

Analysis of coding regions and intron/exon boundaries of the green high-evidence genes listed in the GMS signed off R67 Monogenic hearing loss panel.

The below analysis slices are available. All patients will have the connexin 26 (*GJB2*) slice and any clinically indicated syndromic slice analysed in the first instance. If no diagnosis is made during slice analysis then all the R67 green genes will be analysed.

1. Connexin 26
2. Pendred syndrome
3. Branchio-oto-renal syndrome
4. Waardenburg syndrome
5. Usher syndrome
6. Wolfram syndrome

## Technical

Variant screening is by next generation sequencing with library preparation using a custom TWIST targeted enrichment followed by Illumina NextSeq sequencing. Read alignment and variant calling on the Illumina Dragen platform followed by variant annotation and copy number analysis using an in-house pipeline.

## Target reporting time

12 weeks for routine analysis on the R67 panel for index cases. Targeted routine testing is 6 weeks. Please contact the laboratory for urgent cases.

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# Immunology Specialist Services

## Contact details

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## Samples required

- 5ml venous blood in plastic EDTA bottles (>1ml from neonates)
- Prenatal testing must be arranged in advance, through a Clinical Genetics department if possible.
- Amniotic fluid or CV samples should be sent to Cytogenetics for dissecting and culturing, with instructions to forward the sample to the Regional Molecular Genetics laboratory for analysis
- A completed DNA request card should accompany all samples

## Patient details

To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician

## Introduction

The laboratory offers a range of tests for the investigation and diagnosis of Primary Immunodeficiencies in accordance with the National Genomic Test Directory for England.

Single gene tests are available for a number of genes where there is a highly specific immunological, biochemical or clinical pattern associated with a particular gene.

A large panel of genes is available for the investigation of a broad range or Primary Immunodeficiencies including the eight International Union of Immunological Societies (IUIS) categories of primary immunodeficiency.

A medium panel of genes is available for the investigation of Autoinflammatory Disorders and is carried out at the highly specialised National Amyloidosis Centre.

## Referrals

Referrals for genetic and genomic tests should be directed to the local GLH to arrange testing. The North Thames GLH accepts samples according to the agreed routing rules.

Patients must meet the eligibility criteria for the specific test indication as outlined in the National Genomic Test Directory rare and inherited disease eligibility criteria document. https://www.england.nhs.uk/publication/national-genomic-test-directories/

Referrals may be accepted from the devolved nations and from paying overseas/private referral centres for any of the single gene tests or panel tests in the test directory offered by the North Thames GLH, please contact the laboratory for further details.

## Prenatal testing

Prenatal diagnosis is only offered to couples confirmed to be carriers of pathogenic variants and appropriately counselled by Clinical Genetics. Testing is undertaken by sequence analysis or an appropriate dosage assay for large deletions and duplications.

## Service offered

Analysis of the coding regions and intron/exon boundaries is carried out by next generation sequencing for the following single gene indications:

R155 Autoimmune Polyendocrine Syndrome (*AIRE*)

R16 Severe combined immunodeficiency with adenosine deaminase deficiency (*ADA*)

R235 Severe combined immunodeficiency with features of gamma chain deficiency (*IL2RG*)

R234 Severe combined immunodeficiency with PNP deficiency (*PNP*)

R17 Lymphoproliferative syndrome with absent SAP expression (*SH2D1A*)

R18 Haemophagocytic syndrome with absent XIAP expression (*XIAP*)

R232 Haemophagocytic syndrome with absent perforin expression (*PRF1*)

R19 Autoimmune lymphoproliferative syndrome with defective apoptosis (*FAS*)

R233 Agammaglobulinaemia with absent BTK expression (*BTK*)

R20 Wiskott-Aldrich syndrome (*WAS*)

Analysis of coding regions and intron/exon boundaries is carried out by whole genome sequencing for the green genes listed in the approved panel for the following indication:

R15 Primary Immunodeficiency or monogenic Inflammatory Bowel Disease

# Immunology Specialist Services

Droplet digital PCR testing is offered for the following indication:

R436 Hereditary alpha tryptasaemia (*TPSAB1* copy number analysis)

## Technical

In-house variant screening is carried out by next generation sequencing with library preparation using a custom TWIST target enrichment followed by sequencing on the Illumina platforms. Read alignment and variant calling is performed by the Illumina Dragen platform followed by variant annotation and copy number using an in-house pipeline.

## Target reporting time

84 days for next generation sequencing screening of large gene panels (>10 genes) in an index case, 42 days for next generation sequencing screening of small gene panels (<10 genes) in an index case and for familial or targeted variant testing. 42 days for whole genome sequencing data analysis and reporting once data is returned from Illumina.

Please contact the laboratory for urgent cases.

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# Inborn errors of metabolism

## Contact details

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## Samples required

- 5ml venous blood in plastic EDTA bottles (>1ml from neonates)
- Prenatal testing must be arranged in advance, through a Clinical Genetics department if possible.
- Amniotic fluid or CV samples should be sent to Cytogenetics for dissecting and culturing, with instructions to forward the sample to the Regional Molecular Genetics laboratory for analysis
- A completed DNA request card should accompany all samples

## Patient details

To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician

## Introduction

Inborn errors of metabolism (IEMs) are rare genetic or inherited disorders resulting from an enzyme defect in biochemical and metabolic pathways affecting proteins, fats, carbohydrates metabolism or impaired organelle function presenting as complicated medical conditions involving several human organ systems. Age of presentation can vary from infancy to adolescence and adulthood with the more severe forms appearing in early childhood accompanied by significant morbidity and mortality. Although rare individually, taken as a group, IEMs occur in approximately 1 in 2500 births. Most are inherited as autosomal recessive, but there are some X-linked and autosomal dominant disorders.

A small number of IEM disorders are tested for by newborn screening, including glutaric acidaemia I, MCAD deficiency, phenylketonuria, and isovaleric acidaemia.

As a number of the IEMs can be diagnosed biochemically prior to any genetic testing, there are individual single gene tests available for these diseases in the NHSE test directory (see below). If a specific gene test is not available, patients will undergo whole genome sequencing (WGS) using the large likely inborn error of metabolism panel. This gene panel offers the enhanced probability of diagnosis as a very large number of genes can be interrogated.

## Referrals

Referrals for all metabolic tests are accepted according to the agreed routing rules. Where appropriate, samples are sent to other laboratories for testing.

Patients must meet the referral criteria for the specific test indication as outlined in the National Genomic Test Directory rare and inherited disease eligibility criteria document. https://www.england.nhs.uk/publication/national-genomic-test-directories/

WGS test indications are only available to English NHS patients, and private testing is not currently available. Referrals may be accepted from the devolved nations and from paying overseas/private referral centres for any of the single gene tests or small panel tests in the test directory and for in-house slices of the R98.2 likely inborn error of metabolism panel (see below and please contact the laboratory for further details).

## Prenatal testing

Prenatal diagnosis is only offered to couples confirmed to be carriers of pathogenic variants and appropriately counselled by Clinical Genetics. Testing is undertaken by sequence analysis or an appropriate dosage assay for large deletions and duplications.

## Service offered

Analysis of coding regions and intron/exon boundaries of the genes listed in the approved green panels:

**R270 Smith-Lemli Opitz syndrome**

Performed by next generation sequencing and MLPA dosage where necessary.

**R231 Neuronal ceroid lipofuscinosis**

Performed by next generation sequencing. Patients suspected of having *CLN3*-related disease are tested for first with the common *CLN3* 1kb deletion assay. Patients must have a clinical phenotype in keeping with an NCL and/or histopathological evidence of an NCL storage disorder.

**R271 Neuronal ceroid lipofuscinosis type 2**

Performed by next generation sequencing following confirmed enzyme or histological diagnosis.

**R334 Cystinosis**

Patients are tested for the common 57kb deletion first followed by next generation sequencing if negative. A confirmed biochemical diagnosis is required prior to testing.

**R335 Fabry disease**

# Inborn errors of metabolism

Performed by next generation sequencing and MLPA dosage where necessary. Male patients must have a confirmed biochemical diagnosis. Female patients are tested if they have features suggestive of Fabry disease.

**R274 Glycogen storage disease**

Performed by next generation sequencing of a panel of 28 genes. Patients are required to meet the appropriate eligibility criteria: https://www.england.nhs.uk/publication/national-genomic-test-directories/

Targeted testing of the *GAA* gene is available for patients with a confirmed enzyme diagnosis of glycogen storage disease type II / Pompe disease.

**R276 Lysosomal storage disorder**

Performed by next generation sequencing following confirmed biochemical, enzyme, or histological diagnosis of a particular disorder.

**R277 Mucopolysaccharidosis type I**

Performed by next generation sequencing following confirmed enzyme diagnosis.

**R278 Mucopolysaccharidosis type II**

Performed by next generation sequencing following confirmed enzyme diagnosis. Testing for the common *IDS* inversion will also be carried out.

**R291 Mucopolysaccharidosis type IIIA**

Performed by next generation sequencing following confirmed enzyme diagnosis.

**R292 Mucopolysaccharidosis type IIIB**

Performed by next generation sequencing following confirmed enzyme diagnosis.

**R380 Niemann Pick disease type C**

Samples sent to appropriate laboratory following confirmed biochemical diagnosis.

**R324 Familial chylomicronaemia syndrome**

Samples sent to appropriate laboratory for patients with fasting triglycerides >20mmol/L and exclusion of secondary causes of hypertriglyceridaemia. A completed proforma is required (please contact the laboratory for a copy).

**R325 Lysosomal acid lipase deficiency**

Samples sent to appropriate laboratory following confirmed biochemical diagnosis.

**R323 Sitosterolaemia**

Samples sent to appropriate laboratory following confirmed biochemical diagnosis.

**R286 Tay-Sachs disease**

Samples sent to appropriate laboratory following confirmed biochemical diagnosis.

**R272 Gaucher disease**

Samples sent to appropriate laboratory following confirmed biochemical diagnosis.

**R273 Glycogen storage disease V**

Samples sent to appropriate laboratory following confirmed biochemical diagnosis.

**R288 GM1 gangliosidosis and mucopolysaccharidosis type IVB**

Samples sent to appropriate laboratory following confirmed biochemical diagnosis.

**R280 Krabbe disease – *GALC* deficiency**

Samples sent to appropriate laboratory following confirmed biochemical diagnosis.

**R281 Krabbe disease – saposin A deficiency**

Samples sent to appropriate laboratory following confirmed biochemical diagnosis.

**R287 Mucopolysaccharidosis type IVA**

Samples sent to appropriate laboratory following confirmed biochemical diagnosis.

**R289 Mucolipidosis II and III alpha/beta**

Samples sent to appropriate laboratory following confirmed biochemical diagnosis.

**R290 Mucopolysaccharidosis type VI**

Samples sent to appropriate laboratory following confirmed biochemical diagnosis.

# Inborn errors of metabolism

**R282 Niemann-Pick disease type A or B**

Samples sent to appropriate laboratory following confirmed biochemical diagnosis.

**R285 Sandhoff disease**

Samples sent to appropriate laboratory following confirmed biochemical diagnosis.

**Newborn Screening**

**R105 MCAD deficiency – *ACADM* common variant**

Targeted testing by Sanger sequencing.

**R403 MCAD deficiency – full *ACADM* sequencing**

Sanger sequencing of all coding regions and intron/exon boundaries of the *ACADM* gene.

**R283 Phenylketonuria**

Samples sent to appropriate laboratory for full *PAH* gene sequencing and MLPA dosage.

**R279 Isovaleric acidaemia**

Samples sent to appropriate laboratory for *IVD* common 'benign' variant targeted testing.

**R275 Glutaric acidaemia I**

Samples sent to appropriate laboratory for full *GCDH* gene sequencing.

**R98 Likely inborn error of metabolism**

Samples should be submitted for WGS testing by the local GLH for NHS patients from England. For patients from the devolved nations and from paying overseas/private referral centres, the following next generation sequencing subpanels are available:

ALD_slice_IEM_v2_3_R98 - adrenoleukodystrophy

CDG1A_slice_IEM_v2_3_R98 – congenital disorder of glycosylation type 1A

CDG1B_slice_IEM_v2_3_R98 - congenital disorder of glycosylation type 1B

CDG_slice_IEM_v2_3_R98 - congenital disorders of glycosylation

FATTYACIDOX_slice_IEM_v2_3_R98 – fatty acid oxidation disorders

FOLATE_slice_IEM_v2_3_R98 – folate metabolism and transport disorders

GALACTOSAEMIA_slice_IEM_v2_3_R98 – galactosaemia disorders

GLUTARICACID__slice_IEM_v2_3_R98 – glutaric acidaemia disorders

HOMOCYST_slice_IEM_v2_3_R98 – homocystinuria disorders

HYPERAMMONAEMIA_slice_IEM_v2_3_R98 – hyperammonaemia disorders

HYPERGLYCINAEMIA_slice_IEM_v2_3_R98 – hyperglycinaemia disorders

HYPEROXALURIA_slice_IEM_v2_3_R98 – primary hyperoxaluria disorders

KETOHYPOGLY_slice_IEM_v2_3_R98 – ketotic hypoglycaemia disorders

MMA_slice_IEM_v2_3_R98 – methylmalonic aciduria disorders

MPS_slice_IEM_v2_3_R98 – mucopolysaccharidosis disorders

MSUD_slice_IEM_v2_3_R98 – maple syrup urine disease

MTF_slice_IEM_v2_3_R98 – mitochondrial trifunctional protein deficiencies including LCHAD

NONKETOHYPER_slice_IEM_v2_3_R98 – non ketotic hyperglycinuria disorders

OTC_slice_IEM_v2_3_R98 – OTC deficiency

PEROXISOMAL_slice_IEM_v2_3_R98 – peroxisomal disorders

PEX_GENES_slice_IEM_v2_3_R98 – peroxisomal *PEX* genes only

PROPIONIC_slice_IEM_v2_3_R98 – propionic aciduria

# Inborn errors of metabolism

RCDP_slice_IEM_v2_3_R98 – rhizomelic chondrodysplasia punctata

REFSUM_slice_IEM_v2_3_R98 – Refsum disease

SCADS_slice_IEM_v2_3_R98 - short chain acyl-CoA dehydrogenase deficiency

TYROSINAEMIA_slice_IEM_v2_3_R98 – tyrosinaemia

UREACYCLE_slice_IEM_v2_3_R98 – urea cycle disorders

Information about the genes in these subpanels is available on request.

Details of the all of the genes analysed for each test indication can be found on the NHS Genomic Medicine Service (GMS) Signed Off Panels Resource webpage: https://nhsgms-panelapp.genomicsengland.co.uk/entities

## Technical

In-house variant screening is carried out by next generation sequencing with library preparation using a custom TWIST target enrichment followed by sequencing on the Illumina platforms. Read alignment and variant calling is performed by the Illumina Dragen platform followed by variant annotation and copy number analysis using an in-house pipeline.

Whole genome sequencing is performed by Illumina and analysis with NHSE decision support tools.

## Target reporting time

84 days for next generation sequencing screening of large gene panels (>20 genes) in an index case, 42 days for next generation sequencing screening of single gene and small gene panels (<20 genes) in an index case and for familial or targeted variant testing.

For whole genome sequencing the turnaround time for data analysis and reporting is 42 days once sequence data is returned from Illumina.

Please contact the laboratory for urgent cases.

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# Musculoskeletal

## Contact details

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E gos-tr.norththamesgenomics@nhs.net

## Samples required

- 5ml venous blood in plastic EDTA bottles (>1ml from neonates)
- Prenatal testing must be arranged in advance, through a Clinical Genetics department if possible.
- Amniotic fluid or CV samples should be sent to Cytogenetics for dissecting and culturing, with instructions to forward the sample to the Regional Molecular Genetics laboratory for analysis
- A completed DNA request card should accompany all samples

## Patient details

To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician

## Introduction

The Genomic Medicine Service (GMS) musculoskeletal rare disease speciality offers genetic testing for a wide range of disorders that affect the musculoskeletal system; specifically the bones, muscles, joints, cartilage, ligaments, tendons and bursae.

## Referrals

Referrals are accepted according to the agreed routing rules. Whole genome sequencing test indications (R100 and R104) are only available to English NHS patients, private testing is not currently available.

Patients must meet the referral criteria for the specific test indication as outlined in the National Genomic Test Directory rare and inherited disease eligibility criteria document.
https://www.england.nhs.uk/publication/national-genomic-test-directories/

## Prenatal testing

Invasive prenatal diagnosis may be offered as appropriate where pathogenic variants have been identified in accordance with expected inheritance pattern and where appropriate parental testing and counselling has been conducted. Invasive prenatal diagnosis may also be offered to confirm a suspected diagnosis of *FGFR3*-related skeletal dysplasia on antenatal ultrasound scan.

Non-invasive prenatal diagnosis is available for known familial variants or suspected diagnosis via ultrasound scan for Apert syndrome, Crouzon syndrome with acanthosis nigricans, *FGFR2*-relatated craniosynostosis syndromes and *FGFR3*-related skeletal dysplasia and is the preferred method of prenatal testing.

*Please note that NIPD analysis is not applicable in cases where the mother has the pathogenic variant.*

## Service offered

R52 Short Stature – SHOX deficiency *
*SHOX* single gene testing via Sanger sequencing and MLPA.

R24 Achondroplasia
Targeted *FGFR3* c.1138G>A p.(Gly380Arg) and c.1138G>C p.(Gly380Arg) testing via Sanger sequencing.

R382 Hypochondroplasia
*FGFR3* single gene testing via next generation sequencing.

R25 Thanatophoric dysplasia
*FGFR3* single gene testing via next generation sequencing.

R104 Skeletal dysplasia †
Gene panel testing via trio whole genome sequencing.
R99 Common craniosynostosis syndromes †
Gene panel testing via next generation sequencing, including dosage analysis.

R100 Rare syndromic craniosynostosis or isolated multisuture synostosis †
Gene panel testing via trio whole genome sequencing, R99 must be completed prior to activation of this test indication.

R416 Syndromic & non-syndromic craniosynostosis involving midline sutures only
*SMAD6* single gene testing via next generation sequencing.

# Musculoskeletal

R340 Amelogenesis imperfecta *†
Gene panel testing via next generation sequencing, North Thames referrals exported.

R23 Apert syndrome
Targeted *FGFR2* c.755C>G p.(Ser252Trp) & c.758C>G p.(Pro253Arg) testing via Sanger sequencing.

R101 Ehlers Danlos syndrome with a likely monogenic cause *†
Gene panel testing via next generation sequencing, North Thames referrals exported.

R102 Osteogenesis imperfecta *†
Gene panel testing via next generation sequencing, North Thames referrals exported.

R390 Multiple exostoses *
*EXT1* and *EXT2* gene testing, North Thames referrals exported.

* completed by an external laboratory, referrals within the North Thames region should be sent to this laboratory and will be exported as appropriate

† details of the genes analysed for each test indication can be found on the NHS Genomic Medicine Service (GMS) Signed Off Panels Resource webpage https://nhsgms-panelapp.genomicsengland.co.uk/entities

## Technical

In-house variant screening is carried out by Sanger sequencing (R24; R23) or next generation sequencing (R382; R25; R99; R416) with library preparation using a custom TWIST target enrichment followed by sequencing on the Illumina platforms. Read alignment and variant calling is performed by the Illumina Dragen platform followed by variant annotation and copy number analysis using an in-house pipeline.

## Target reporting time

84 days for next generation sequencing screening of large gene panels (>10 genes) in an index case, 42 days for next generation sequencing screening of small gene panels (<10 genes) in an index case and for familial or targeted variant testing. 42 days for whole genome sequencing data analysis and reporting once data is returned from Illumina.

Please contact the laboratory for urgent cases.

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# Renal

## Contact details

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E gos-tr.norththamesgenomics@nhs.net

## Samples required

- 5ml venous blood in plastic EDTA bottles (>1ml from neonates)
- Prenatal testing must be arranged in advance, through a Clinical Genetics department if possible.
- Amniotic fluid or CV samples should be sent to Cytogenetics for dissecting and culturing, with instructions to forward the sample to the Regional Molecular Genetics laboratory for analysis
- A completed DNA request card should accompany all samples

## Patient details

To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician

## Introduction

Renal conditions, those which affect the kidneys, are heterogeneous and may show considerable phenotypic overlap. More than 1.8 million people in England have diagnosed chronic kidney disease. Many common renal conditions have a genetic basis and accurate and timely diagnosis is helpful for patient management and family genetic counselling. Renal gene panels offer the enhanced probability of diagnosis as a very large number of genes can be interrogated. Renal conditions include all modes of inheritance. These conditions can also be caused by *de novo* variants.

## Referrals

For details of testing criteria for each renal indication and the clinical specialties who would be expected to request the test (generally referrals will be accepted from Clinical Geneticists and nephrologists; list not exhaustive), please refer the National Genomic Test Directory's Testing Criteria for Rare and Inherited Disease.

Samples are accepted for testing according to the agreed routing rules. The North Thames GLH is the single national provider for renal tubulopathies (R198) and *CFHR5* nephropathy (R196). Renal-related amyloidosis with no identifiable cause (R204) testing is performed by the National Amyloidosis Centre based at the Royal Free Hospital. In every case samples should be sent to the local GLH for DNA extraction and appropriate routing.

## Prenatal testing

Prenatal diagnosis is only offered to couples confirmed to be carriers of pathogenic variants and appropriately counselled by Clinical Genetics. Testing is undertaken by sequence analysis or an appropriate dosage assay for large deletions and duplications.

## Service offered

Analysis of coding regions and intron/exon boundaries of the green genes listed in the virtual PanelApp panels (see below for PanelApp links to gene lists):

**R193** Cystic renal disease*

**R194** Haematuria

**R196** *CFHR5* nephropathy (non-sequencing based duplication PCR assay)

**R198** Renal tubulopathies

**R256** Nephrocalcinosis or nephrolithiasis

**R257** Unexplained paediatric onset end-stage renal disease*

*Services provided centrally by WGS; referrals from outside of the North Thames region must be submitted for testing by the local GLH

## Technical

In-house variant screening is carried out by next generation sequencing with library preparation using a custom TWIST target enrichment followed by sequencing on the Illumina platforms. Read alignment and variant calling is performed by the Illumina Dragen platform followed by variant annotation and copy number analysis using an in-house pipeline.

## Target reporting time

84 days for next generation sequencing screening of large gene panels (>10 genes) in an index case, 42 days for next generation sequencing screening of small gene panels (<10 genes) in an index case and for familial or targeted variant testing. For whole genome sequencing the turnaround time for data analysis and reporting is 42 days once sequencing data is returned from Illumina. Please contact the laboratory for urgent cases.

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# Dermatology

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## Samples required

- 5ml venous blood in plastic EDTA bottles (>1ml from neonates)
- See Technical section for a particular note regarding skin samples
- Prenatal testing must be arranged in advance, through a Clinical Genetics department if possible.
- Amniotic fluid or CV samples should be sent to Cytogenetics for dissecting and culturing, with instructions to forward the sample to the Regional Molecular Genetics laboratory for analysis
- A completed DNA request card should accompany all samples

## Patient details

To facilitate accurate testing and reporting please provide patient demographic details (full name, date of birth, address and ethnic origin), details of any relevant family history and full contact details for the referring clinician

## Introduction

Inherited conditions affecting the skin, hair and nails consists of genetically heterogeneous disorders, which benefit from a virtual gene panel approach to maximise clinical sensitivity to potentially minimise time to diagnosis. Inheritance may be autosomal dominant, recessive, or X-linked, and mosaic dermatological conditions are increasingly recognised.

## Referrals

For details of testing criteria for each dermatology indication and the clinical specialties who would be expected to request the test (generally referrals will be accepted from Clinical Geneticists and Dermatologists), please refer to the National Genomic Test Directory's Testing Criteria for Rare and Inherited Disease.

Our in-house tests are to patients in the North Thames GLH catchment and to other English patients according to the agreed routing rules.. We are the single national provider for Mosaic skin disorders - deep sequencing (R327).

## Prenatal testing

Prenatal diagnosis may be offered as appropriate where pathogenic variants have been identified in accordance with expected inheritance pattern and appropriate parental testing and genetic counselling has been conducted.

## Service offered

Analysis of coding regions and intron/exon boundaries of the green genes listed in the virtual PanelApp panels (see below for PanelApp links to gene lists):

**R110** Segmental overgrowth disorders*

**R163** Ectodermal dysplasia

**R164** Epidermolysis bullosa and congenital skin fragility

**R165** Ichthyosis and erythrokeratoderma

**R166** Palmoplantar keratodermas

**R167** Autosomal recessive primary hypertrophic osteoarthropathy

**R227** Xeroderma pigmentosum, Trichothiodystrophy or Cockayne syndrome
This includes DNA repair defect testing.

**R230** Multiple monogenic benign skin tumours
This includes *FLCN* dosage analysis.

**R236** Pigmentary skin disorders
Please note *SPRED1* dosage would be included in the analysis of the pigmentary panel, but we are not able to provide a validated assay at this time for *SPRED1* dosage. Single gene testing is available if indicated.

**R237** Cutaneous photosensitivity with a likely genetic cause

**R239** Incontinentia pigmenti

**R255** Epidermodysplasia verruciformis

**R326** Vascular skin disorders

**R327** Mosaic skin disorders - deep sequencing*
Testing for McCune-Albright syndrome (MAS) is eligible under this clinical indication, to specifically interrogate the R201 and Q227 codons of the *GNAS* gene. MAS referrals can be accepted from Clinical

# Dermatology

Genetics, Dermatology, (Paediatric) Endocrinology specialties. If the patient is not presenting with a skin phenotype, they should have polyostotic fibrous dysplasia (+/- precocious puberty) to accept testing. Precocious puberty is insufficient on its own.

**R332** Rare genetic inflammatory skin disorders

**R424** Subcutaneous panniculitis T-cell lymphoma (SPTCL)

R164, R227 and R239 are completed by an external laboratory, referrals within the North Thames region will be exported as appropriate.

## Technical

Variant screening is carried out by next generation sequencing with library preparation using a custom TWIST target enrichment followed by sequencing on the Illumina platforms. Read alignment and variant calling is performed by the Illumina Dragen platform followed by variant annotation and copy number analysis using an in-house pipeline.

***Deep sequencing**

We have designed a custom panel covering the mosaic (R327) and segmental overgrowth (R110) indications, which is sequenced at high read depth (minimum coverage threshold of 700x) and with a specific bioinformatics pipeline for somatic variant detection. We have shown we are able to detect variants down to ~1% variant allele frequency (VAF). Confirmation of variants will be by Sanger sequencing (VAF>=10%) or droplet digital PCR (ddPCR) (VAF<10%).

We are much more likely to detect a causative variant on the mosaic (R327) and segmental overgrowth (R110) panels when the sample is from affected tissue, mainly skin biopsies to date. Direct DNA extraction is preferred. If there is no alternative, we can attempt to obtain a result from blood. Highly degraded DNA or FFPE derived DNA may not be appropriate for testing.

## Target reporting time

84 days for a full NGS screen in an index case (6 weeks if urgent). 42 days for familial testing (2 weeks if urgent).

Please contact the laboratory for urgent cases.

**PanelApp gene lists**

Details of the genes analysed for each test indication can be found on the NHS Genomic Medicine Service (GMS) Signed Off Panels Resource webpage.

The laboratory can be contacted for details of the sequence coverage for individual gene(s).