Multi-omic Medicine Service User Guide

Version number 3.0

Author Rebecca Ellmers / Alison Bybee

Authorised by Sammi Allouni

Issued on April 2025

Version Number	Change Details	Date
1.0	New Document	27/10/23
2.0	Added section 3.3.4 Sample Integrity – to include time restraints for samples as per change request	02/07/24
3.0	Added multiple sections for Liver Molecular Genetics, added author	April 2025

Contents

1. Introduction

1.1 Purpose and Scope

The purpose of this document is to act as a guide to the services provided by the Multi-omic Medicine Service (MMS) at King's College Hospital (KCH). The MMS consists of the Molecular Neuropathology (MNL) and Liver Molecular Genetics (LMG) departments. It further provides key contact points for service users. The information within this document can be used by any user that requires the investigations offered by the MMS.

1.2 Responsibilities

The department lead has overall responsibility for the accuracy of the information in this handbook. Executive responsibility for the upkeep of this document lies with the MNL Quality Manager, supported by the service manager.

1.3 Definitions

Term	Definition
FF	Fresh Frozen
FFPE	Formalin Fixed Paraffin Embedded
GLH	Genomics Laboratory Hub
GSTT	Guy's & St Thomas' Trust
LMG	Liver Molecular Genetics
KCH	King's College Hospital
KCL	King's College London University
MMS	Multi-omic Medicine Service
MNL	Molecular Neuropathology Laboratory
NGIS	National Genomics Informatics System
NGS	Next Generation Sequencing
NHSE	NHS England
SEGLH	South East Genomics Laboratory Hub
WGS	Whole Genome Sequencing

2. Health and Safety

Not applicable.

3. Procedure/Method

3.1 General information

The Molecular Neuropathology service is the designated laboratory within the South East Genomics Laboratory Hub (SEGLH) offering molecular analysis of neurological malignancies as part of the NHSE Genomics Medicine Service.

The Liver Molecular Genetics service conducts genetic tests and provides interpretation of results for inherited hepatic and gastrointestinal conditions. The laboratory is part of the South East Genomics Laboratory Hub (SEGLH) and delivers the NHSE

Gastrohepatology Genomics testing repertoire to more than half of the national population as part of the NHSE Genomics Medicine Service.

General information about the departments and services provided can be found on the respective websites:

Molecular Neuropathology Service | King's College Hospital NHS Foundation Trust (kch.nhs.uk)

Molecular Neuropathology Laboratory | Synnovis

Liver Molecular Genetics Laboratory | Synnovis

The Molecular Neuropathology and Liver Molecular Genetics services are under the control of Synnovis group. Synnovis Group is a limited liability partnership between Guy's and St Thomas' NHS Foundation Trust, King's College Hospital NHS Foundation Trust and Synlab UK & Ireland. Synnovis Analytics and Synnovis Services sit within the Synnovis Group: MNL and LMG sit within Synnovis Analytics. The corporate functions of strategy, management, finance, human resources and commercial are managed within the Synnovis Group. Synnovis Services manage the laboratory facilities, systems, equipment, consumables and maintenance. Synnovis Analytics manages the operations, diagnostics, research and development and clinical innovation.

3.2 Contact information

The Molecular Neuropathology service is located within King's College Hospital across two sites. The offices are located within the Academic Neuroscience Centre building (KCL). The Molecular Neuropathology Laboratory is located on the third floor of the Cheyne Wing (KCH). Address and key contacts are provided below:

Molecular Neuropathology

2nd Floor, Academic Neuroscience Centre

King's College Hospital

Denmark Hill

London

SE5 9RS

Laboratory Lead for Precision Medicine: Dr Barnaby Clark

Service Delivery Manager: Sammi Allouni

Operations Lead: Kelly Eggleton

Quality Manager: Elentina Gjoni

Office telephone: 020 3299 2375

Email: kch-tr.KCH.neurogenetics@nhs.net

The Molecular Neuropathology department is further integrated with the Department of Clinical Neuropathology which handles incoming samples for processing and distribution

to the Molecular Neuropathology Laboratory. Address and contact details provided below:

Department of Clinical Neuropathology

1st Floor, Academic Neuroscience Centre

King's College Hospital

Denmark Hill

London

SE5 9RS

Laboratory telephone: 020 3299 1957

The Liver Molecular Genetics service is located on the third floor of the Cheyne Wing (KCH). Address and key contacts are provided below:

Liver Molecular Genetics

The postal address is: -

Liver Molecular Genetics

Institute of Liver Studies

3rd Floor, Cheyne Wing

King's College Hospital, Denmark Hill, London SE5 9RS

Clinical Lead: Prof. Richard Thompson

Service Delivery Manager: Sammi Allouni

Operations Lead: Annabel Strickland

Quality Manager: Elentina Gjoni

Telephone: 020 3299 2253

Email: KCH-tr.KCHLMGadmin@nhs.net

3.3 Hours of work

Both the Molecular Neuropathology and Clinical Neuropathology departments are open from 8.30am to 5pm, Monday to Friday.

Liver Molecular Genetics is open from 0900 to 1730 Monday to Friday excluding Bank Holidays

There is **no** routine provision of on-call/out-of-hours service.

3.4 Sample information

3.4.1 Molecular Neuropathology sample requirements

Contact the Neuropathology Laboratory at King's College Hospital on **020 3299 1957** or email us at kch-tr.molecularneuropathology@nhs.net to let staff know when to expect delivery of samples. This telephone number can also be used for any difficulties or queries. The department is open from 8.30am to 5pm, Monday to Friday.

Either the FFPE block or ten unstained formalin fixed paraffin embedded (FFPE) tissue sections, cut at 10µm thick (from a representative block of tumour) are required for all molecular pathology tests. Alternatively, fresh tissue, preserved in RNALater can also be accepted instead of FFPE samples (please contact the laboratory for details).

Whole genome sequencing (WGS) can only be performed on fresh tissue and this is the preferred sample type of the molecular laboratory. For histopathology, we recommend FFPE tissue, therefore in some cases samples need to be divided and the molecular laboratory has no option but to work with FFPE tissue. Formalin fixation should be reduced to a minimum time interval at neutral pH to try and preserve the nucleic acid for molecular studies. Whole genome sequencing further requires a whole blood sample in EDTA (5ml BD Vacutainer) for confirmation of germline testing.

If you have insufficient material or if you require multiple tests on the same samples, please call the laboratory on **020 3299 2375** to discuss any additional requirements prior to sending.

Please complete the Molecular Neuropathology Request Form for all samples.

3.4.2 Liver Molecular Genetics sample requirements

For all tests:

Adults: 5 -10ml of peripheral blood in EDTA.

Paediatrics: 1-2ml of peripheral blood in EDTA

Our current request form is available to download from the South East GLH website at the following address:

https://southeastgenomics.nhs.uk/wp-content/uploads/2024/11/5.Gastrohepatology-Request-Form-Jan-2021.pdf

To request testing that is not part of the Gastrohepatology genomics repertoire and/or is not funded by NHSE please contact the laboratory.

3.4.3 Handling biological samples for the Multi-omic Medicine Service

- All patient samples must be treated with due care and respect.
- All blood and tissue samples from patients should be considered as a risk of infection and should be handled as such.
- Any spillages must be cleared up IMMEDIATELY and any possible contamination through cuts, finger pricks etc. reported as per local procedure.
- Biological spill kits and suitable disinfectant should be used to clear any biological fluid spillages. Chemical spill kits should be used to clear chemical spills.

- Staff employed by Synnovis group sign contracts with to ensure ethical conduct and confidentiality is maintained in handling human samples, tissues and remains. This policy also applies to bank and agency staff.

3.4.4 Sample collection and labelling for Molecular Neuropathology

When collecting samples from a patient, the clinician should positively identify the patient and check that any necessary preparation has been completed. All samples must be clearly labelled with the patient's identity. A minimum of two identifiers (name, hospital/NHS number or date of birth) are normally required to positively identify a sample. Samples that do not meet this standard may be accepted at the discretion of the service lead if the samples are regarded as "unrepeatable". The sample collector, date and time of collection should also be included with the sample request. All materials used in the sample collection process should be disposed of carefully in accordance with trust policy. Where several samples are collected from the same patient, including multiple pieces of tissue or slides, they should be appropriately labelled in order for correct identification.

3.4.1 Sample collection and labelling for Liver Molecular Genetics

A fully completed request form electronic or paper should accompany each sample. **Incomplete request forms may result in sample rejection.**

Routine samples originating from within King's College Hospital (KCH) should either be sent via main pathology reception or brought directly to the Liver Unit laboratories. KCH samples should be requested via EPIC. Tests can be found under Liver Laboratories orders or they can be searched for using the test name or by keywords. For external referrals if EPIC is unavailable request forms can be downloaded from the gastrohepatology page of GLH website or requested directly from the laboratory.

All samples from outside King's College NHS Trust must be accompanied by a written acceptance of the charges for testing and a clear indication of the person and/or department to whom the invoice should be sent.

Both electronic and paper requests **must** match the information on the specimen and should include the following information:

- Patient's full name and/or unique patient identifier (Hospital number and/or NHS number)
- Date of birth
- Sex
- Sender's lab reference number (requests external to KCH only)
- Sample type
- Date of specimen collection
- Test(s) required
- Pregnancy status (if relevant)
- Indication for testing and any clinical details that may influence the interpretation of results, e.g. medication, transfusion history, relevant laboratory results, family history and any previous genetic results.
- A secure address to which the results will be sent

3.4.2 Urgent requests for Liver Molecular Genetics tests

Urgent requests internal to KCH:

Specimens should be brought directly to the LMG Laboratory. Requirements for testing and results turnaround should be discussed with a senior member of LMG staff.

Urgent requests from external users:

Please contact the Laboratory directly to discuss.

3.4.3 Sample integrity for Molecular Neuropathology tests

Samples received have different time restraints for storage as follows:

- FFPE block and/or slides – no associated time restraints, can be stored indefinitely at room temperature.
- Fresh tissue preserved in RNALater – can be kept at room temperature for 2-3 days if required (i.e. whilst in transit) but should be stored in a fridge as soon as possible after collection/receipt in order to maintain sample integrity.
- Whole blood in EDTA – can be stored at room temperature for 2-3 days if required (i.e. whilst in transit) as long as the total time from collection to extraction does not exceed 72 hours. Ideally EDTA tubes should be stored at 4^{o}C, transported at 4^{o}C, and extracted within 72 hours of collection.

Refer to *KCH-MMS-GU-3 Sample Handling Guidance for Whole Genome Sequencing of Solid Tumour Samples* and *KCH-MMS-GU-4 Sample Handling Guidance for Whole Genome Sequencing for Germline Samples* for further details, or contact the Neuropathology laboratory using the phone number or email address stated above.

3.4.1 Sample integrity for Liver Molecular Genetics tests

The quality and quantity of extracted DNA will be affected if samples are collected incorrectly, stored at extreme temperatures, or delayed in transit. This may result in the failure of the test. EDTA samples which are grossly haemolysed or obviously clotted will not yield sufficient DNA for analysis. Samples with abnormal white blood cells or abnormal clotting may not be apparent until processing and are also unlikely to yield sufficient DNA for analysis.

Blood samples may contain donor DNA if obtained from patients who received heterologous blood transfusions or allogeneic blood or marrow transplantation. Results from samples obtained under these circumstances may not accurately reflect the recipient's genotype. For individuals who have received blood transfusions, the genotype is expected to revert to that of the recipient within 1 week (Adams et al (1992) Blood 80:551). For individuals who have received allogeneic blood or marrow transplantation, a pre-transplant DNA specimen is recommended for testing. For individuals who have received an organ transplantation and are being tested for a disorder with an aetiology in the donor organ, please contact the laboratory to discuss the suitability of DNA testing.

Specimens should be delivered to the laboratory as soon as possible after they are taken to ensure the quality of the specimen and the success of the results. Blood sample should be sent at room temperature (15°C - 25°C) and must arrive in the laboratory within 7 days from being taken. DO NOT FREEZE. DNA samples in a suitable buffer can be sent at room temperature (15°C - 25°C) and should arrive in the laboratory within 7 days of

being sent. Consider using local DNA extraction and sendaway services if sending samples from overseas.

EDTA samples which are grossly haemolysed or obviously clotted are not suitable for testing and should not be sent.

3.4.2 Transportation of biological samples for Molecular Neuropathology tests

All samples must be transported in the appropriate container (advice can be sought from the department).

Sample/specimens sent by post or courier must be correctly labelled and packaged (using UN 3373 compliant conditions).

Unstained slides must be packed into clean slide carriers taped shut to ensure they are not damaged during transit. A letter explaining the clinical details should accompany the sample. Post in a padded envelope to:

Department of Clinical Neuropathology
1st Floor, Academic Neuroscience Centre
King's College Hospital
Denmark Hill
London
SE5 9RS

3.4.3 Transportation of biological samples for Liver Molecular Genetics tests

For samples sent by post or by courier. All packaging should conform to PI650 standards. Otherwise, as for Molecular Neuropathology, except address to:

Liver Molecular Genetics

Institute of Liver Studies, 3rd Floor, Cheyne Wing

King's College Hospital

Denmark Hill

London

SE5 9RS

3.4.4 Sample retention in the Multi-omic Medicine Service

Samples received into Clinical Neuropathology (FFPE, FF, slides) are stored indefinitely and for a minimum of 30 years.

Samples are processed by the Molecular Neuropathology laboratory and DNA is extracted. DNA samples are stored indefinitely and for a minimum of 30 years.

DNA samples that have been extracted or received by the Liver Molecular Genetics laboratory are stored indefinitely and for a minimum of 30 years.

3.5 Requests

The department requires that all samples are accompanied with a formal request for analysis, whether this is in the form of a paper request, or one made electronically.

Solely verbal requests for analysis will not be accepted.

Please complete the Molecular Neuropathology Request Form or the Liver Molecular Genetics Request Form as appropriate.

The request form must include the following information:

- Name, date of birth and hospital number
- NHS number if applicable
- Relevant clinical details
- Type of specimen and provisional diagnosis
- Date of specimen collection
- Tests required
- Requester details including ID and location to which results are to be sent
- Identification of priority status if applicable

3.5.1 Rejection criteria

All samples must be clearly labelled with the patient's identity. A completed request form (electronic/paper) must accompany all samples.

Samples may be rejected if:

- They are the incorrect sample type for required test(s).
- They have leaked in transit.
- They are of insufficient volume/quantity.
- Blood is grossly haemolysed, or obviously clotted when in anticoagulant.
- The information on the request form and sample do not match or if there is insufficient information on either the sample or form.
- The specimen has not been processed/stored appropriately prior to referral or if there is a significant delay in specimen receipt.

PLEASE NOTE: specimens or request forms received without the minimum essential identification criteria may be rejected and/or may lead to a delay in reporting. Unlabelled specimens cannot be processed and may be discarded. Where contact details are provided, MNL and LMG will contact the referrer for additional details to prevent sample rejection if possible.

Requests that do not meet the above criteria may on occasion be accepted at the discretion of the Service Lead if the samples are regarded as "unrepeatable". Reports will indicate the nature of the problem and any possible consequence of this.

Failure to provide a reason for testing and further clinical details will limit the interpretation of the result. Providing a detailed family history and evidence of any previous genetic test results in the family is particularly important for guiding appropriate genetic analysis and interpreting the results, failure to provide these may result in delay, less interpretation on the report and/or less targeted testing.

3.5.2 Additional testing requests

Samples are stored indefinitely so there is no time limit for requesting additional tests on these samples.

If additional testing is required on a sample previously sent to MNL, please contact the department directly via email: kch-tr.molecularneuropathology@nhs.net. In exceptional circumstances, MNL could accept a verbal request for additional testing, please call the laboratory on **020 3299 2375** to discuss if required.

If additional testing is required on a sample previously sent to LMG, please contact the department directly via email: kch-tr.kchlmgadmin@nhs.net. Additional tests must be made in writing on a request form. In exceptional circumstances, LMG could accept a verbal request for additional testing, please call the laboratory on **020 3299 4625** to discuss if required.

3.6 Patient consent

All patients should give their consent to have samples collected for laboratory analysis. The patient should also be informed that it may be necessary to refer their samples on to another laboratory for analysis. If this proves necessary the department will only share the clinical information that is relevant to the sample request.

It is the referring clinician's responsibility to ensure that the patient/carer knows the purpose of the test and that a specimen may be stored for future diagnostic tests.

Consent for DNA testing and storage must be obtained from the patient by the referring clinician prior to referral of the sample. All genetic testing requires consent. This is not the responsibility of the laboratory staff.

The Molecular Neuropathology and Liver Molecular Genetics services adhere to KCH Trust policies; Synnovis policies and Caldicott principles to safeguard all patient information. Diagnostic material is stored according to The Royal College of Pathologists' guidelines.

Surplus diagnostic material from all referrals is retained for quality assurance purposes and may be used anonymously for the development of new tests unless consent for this is expressly denied on the request form.

3.7 Protection of Patient Information

All patient information is handled under the terms of the Data Protection Act 2018. All personal information received by Synnovis is dealt with according to the Synnovis Privacy, Data Protection & Cookie Policy which is available at Privacy and data protection policy | Synnovis.

3.8 Tests provided and turnaround times for Molecular Neuropathology

3.8.1 MGMT promotor methylation status

Testing for the methylation status of the O6-methylguanine DNA methyltransferase gene (*MGMT*) promoter methylation status performed using pyrosequencing technology and the therascreen *MGMT* pyro kit. This kit assesses four CpG sites, giving an average (%) methylation across the four.

Glioblastoma tumours with a methylated *MGMT* promoter are predictive of an improved response to alkylating chemotherapy (for example, temozolomide).

Turnaround time: 14 days from sample receipt

3.8.2 Methylation array

The molecular neuropathology department uses the Illumina 850k EPIC array to assess the DNA methylation status 850,000 individual CpG sites. The data is then processed via the 'Molecular Neuropathology Classifier' (MNP classifier), an algorithm developed the German Cancer Research Centre (Department of Neuropathology, University Hospital Heidelberg). This provides a tumour classification based on the methylation profile.

Turnaround time: 21 days from sample receipt

3.8.3 Multimodal NGS panel

The multimodal NGS panel assesses both DNA and RNA targets. Testing of the indicated genes is by the Qiagen QIAseq Multimodal Panel, using single primer extension with unique molecular indexes to assess a targeted DNA panel of 305 genes and a RNA panel of 76 genes associated with solid tumour fusions. Additional 'virtual' DNA panels may be applied as required, please contact the laboratory to discuss.

Samples are sequenced on an Illumina NextSeq2000, 2X150bp reads with a minimum coverage of 400x (minimum of 6 reads to make a mutation call). Variants at <2% are excluded from analysis and variants at <5% are not considered clinically significant and are therefore not reported. This test has been shown to have a minimum sensitivity of 98.3% for single nucleotide substitutions and small insertion/deletion variants for regions covered by >=400x with a 95% confidence interval.

Turnaround time: 21 days from sample receipt

3.8.4 NGS panels offered

Somatic Paediatric Neurological Tumours panel v5.0:

AKT1, ALK, ATRX, BCOR, BRAF, CDKN2A, CDKN2B, CTNNB1, DAXX, DDX3X, DICER1, DPYD, EZH2, FGFR1, FGFR4, IDH1, IDH2, KIT, MSH6, MYC, MYCN, NF1, NF2, NRAS, PDGFRA, PHOX2B, PIK3CA, PMS1, PMS2, PTCH1, PTCH2, PTEN, RAF1, RB1, SMARCA4, SMARCB1, SMARCE1, SMO, SUFU, TERT, TFE3, TP53, TSC1, TSC2, VHL, WT1, YAP1

Somatic Adult Neurological Tumours panel v5.0:

ATRX, BRAF, CDK4, CDK6, CDKN2A, CDKN2B, CTNNB1, DPYD, EGFR, FGFR3, H3C2, H3C3, H3F3A, H3F3B, IDH1, IDH2, KIAA1549, KRAS, MDM2, MDM4, MET, MYC, NF1, PDGFRA, PIK3CA, PIK3R1, PTEN, TERT, TP53, VHL

Germline Neurological Tumours panel v5.0:

AKT1, APC, ATM, BRCA1, BRCA2, DPYD, MLH1, MSH2, MSH6, NF1, NF2, PIK3CA, PMS1, PMS2, POLD1, POLE, PTCH1, PTCH2, PTEN, RAD51D, RB1, SMARCA4, SMARCB1, SUFU, TP53, TSC1, TSC2, VHL

RNA Neurological Tumours panel v5.0:

ABL1, AGK, AKAP9, ALK, ASPSCR1, BCOR, BCR, BRAF, BRD2, BRD3, BRD4, CBFB, CCDC6, CCNB3, CIC, DNAJB1, EGFR, EML4, ERBB3, ERG, ETV1, ETV6, EWSR1, FAM118B, FGFR1, FGFR2, FGFR3, FOXO1, FXR1, GNA11, HLA-A, HLA-B, HLA-C, KIAA1549, MACF1, MALAT1, MAML2, MET, MITF, MN1, MYB, MYBL1, MYC, MYH11, NFIB, NFIX, NPM1, NRG1, NTRK1, NTRK2, NTRK3, NUTM1, NUTM2B, NUTM2E, PML, PRCC, PRKACA, PVT1, QKI, RAF1, RARA, RELA, RET, ROS1, RUNX1, RUNX1T1, SRGAP3, TFE3, TFEB, TMPRSS2, TPM3, TTYH1, WWTR1, YAP1, YWHAE, ZFTA

Gene Target as per National Genomic Test Directory for Cancer v7.3 (updated 20 September 2023).

3.8.5 Whole Genome Sequencing

Whole Genome Sequencing (WGS) is an analytical technique that utilises Next Generation Sequencing (NGS) methodology, to analyse a patient's entire genetic make-up and thus offer vital information relating to diagnosis, prognosis and therapy response. Locally, WGS analysis is offered based upon the NHS National Genomic Testing Directory for Cancer (https://www.england.nhs.uk/publication/national-genomic-test-directories/). Patients fall within the Neurological and Paediatric tumour categories.

Analysis for patients is performed at the request of Consultant Neuropathologists, following correct genetic testing consent is obtained, which is recorded via a Record of Discussion (ROD) and Test Order Form (TOF). Each patient should have a solid tumour sample (biopsy) as well as a whole blood sample in EDTA (for germline testing).

The MNL will perform DNA extraction and quantification of the tumour sample. The extracted tumour DNA sample and the whole blood sample are then forward to Guy's & St Thomas' Trust (GSTT). GSTT perform DNA extraction and quantification on the blood sample; make sure that all information on the forms and samples are correct; request the testing within NGIS (National Genomics Informatics System); and then forward both tumour and blood DNA samples to the Birmingham Plating Laboratory. The Birmingham Plating Laboratory is then able to plate multiple samples (for higher throughput) and forward onto the Illumina Sequencing Laboratory in Cambridge. The service offered by the Illumina Sequencing Laboratory, is solely a pre-analytical service that is funded and overarched by Genomics England. Extracted DNA undergoes library preparation and NGS sequencing analysis upon the NovaSeq platform.

Following sequencing, WGS data is processed by Genomics England which requires multiple Bioinformatics pipelines within the "Cancer" analysis stream, in order to identify genetic aberrations in the patient genome.

Genomics England is not performing a clinical interpretation of the genome sequencing data. It is the responsibility of NHS GLH staff to perform a full clinical review, confirm the presence of selected variants where required, and report and authorise any results. WGS data are made available on the Interpretation Portal for interrogation by MNL scientists.

Turnaround time: 42 days from receipt of data

3.9 Tests provided and turnaround times for Liver Molecular Genetics

Please note that due to recent development work required to meet the requirements of the NHSE Genomics Test Directory R438 and R446 are currently out of UKAS accreditation scope (pending extension to scope applications).

3.9.1 Liver Molecular Genetics NGS panel (LLGP5)

Testing of indicated genes is performed using next generation sequencing for the genetic region known to contain the variants detected in other family members. Testing is by a custom probe based capture utilising the Agilent SureSelect XTHS platform followed by next generation sequencing. Samples are sequenced on an Illumina MiSeq, 2x220bp reads with a minimum coverage of 30x across >99% of the ROI unless otherwise stated. Additional virtual DNA panels may be applied as required, please contact the laboratory to discuss.

Read alignment and variant calling is performed by BWA-MEM 0.7.16a and GATK HaplotypeCaller 4.0.3.0. In house validation shows this analysis is expected to detect 99% of single nucleotide variants (SNVs) with a confidence interval of 95%, sensitivity for insertions and deletions (Indels) may be less. Except where indicated the coding exons and intron boundaries (+/-10bp) of the gene(s) have been analysed. Alignment of reads and calling of variants in genes with highly homologous pseudocopies can be challenging and such regions may display reduced sensitivity and/or specificity.

Sensitivity calculations are generated by in-house validation of the described tools and pipeline; the sensitivity figures given are representative of the validated sample set, and individual genes or regions may have a lower sensitivity due to local sequence context or genomic architecture. A familial positive control sample is not routinely utilised for targeted familial testing using next generation sequencing as there is not significant risk of alleleic dropout.

3.9.2 NGS panels offered

Genetic Cholestasis Panel v3.0:
*ABCB11, ABCB4, ABCC2, ADK, AKR1D1, ALDOB, AMACR, ATP7B, ATP8B1, BAAT, BCS1L, CFTR, CLDN1, COG7, CYP27A1, CYP7B1, DCDC2, DGUOK, FAH, GALE, GALM, GALT, *GBA, HADHA, HNF1B, HSD3B7, JAG1, KIF12, LIPA, MPI, MPV17, MVK, MYO5B, NBAS, **NOTCH2, NPC1, NPC2, NR1H4, PEX1, PEX12, PEX26, PEX6, PKHD1, POLG, RINT1, SERPINA1, SLC25A13, SMPD1, TALDO1, TJP2, TRMU, UGT1A1, UNC45A, USP53, VIPAS39, VPS33B, YARS, ZFYVE19*

Panel content as per ‘R171 Cholestasis’ GMS PanelApp panel version 3.0 which was signed off under NHS Genomic Medicine Service governance on 22 Mar 2023 (https://nhsgms-panelapp.genomicsengland.co.uk/panels/544/v3.0). Assay scope as per Clinical Indication ID: R171; Test IDs: R171.1 and R171.2 of the Rare and Inherited Disease National Genomics Test Directory v5.1. Unless otherwise stated analysis and reporting is carried out against the MANE Select transcript of the gene (or MANE Plus Clinical set where relevant). For details of the sequence coverage for individual gene(s) please contact the laboratory.

** Please note that sequencing of GBA is impacted by highly homologous pseudogenes. Assay sensitivity and/or specificity is likely to be lower for this region.*
*** Please note that exons 1-4 of NOTCH2 are refractory to sequencing by current methods and are not covered in this assay.*

Turnaround time: 42 days from sample receipt

Wilson disease single gene testing:
ATP7B

Assay content as per Clinical Indication ID: R172; Test ID: R172.1 of the Rare and Inherited Disease National Genomics Test Directory v5.1. Please note that unless otherwise stated analysis and reporting is carried out against the MANE Select transcript of the gene (or MANE Plus Clinical set where relevant). For details of the sequence coverage for individual gene(s) please contact the laboratory.

Turnaround time: 42 days from sample receipt

Pancreatitis Panel v3.0:
**PRSS1, SPINK1, CFTR, CELA3B*

Panel content as per 'R175 Pancreatitis' GMS PanelApp panel version 3.0 which was signed off under NHS Genomic Medicine Service governance on 30 Nov 2022 (https://nhsgms-panelapp.genomicsengland.co.uk/panels/386/v3.0). Assay scope as per Clinical Indication ID: R175; Test IDs: R175.1, R175.2, and R175.3 of the Rare and Inherited Disease National Genomics Test Directory v5.1. Unless otherwise stated analysis and reporting is carried out against the MANE Select transcript of the gene (or MANE Plus Clinical set where relevant). For details of the sequence coverage for individual gene(s) please contact the laboratory.

** Please note that PRSS1 is known to have multiple highly homologous pseudocopies that are likely to impact upon assay sensitivity and specificity in this region. All clinically significant SNVs detected within this gene are confirmed by LR-PCR.*

Turnaround time: 42 days from sample receipt

Polycystic Liver Disease Panel v1.26:
*ALG8, DNAJB11, GANAB, LRP5, *PKD1, PKD2, PKHD1, PRKCSH, SEC63*

Panel content as per 'R173 Polycystic Liver Disease' GMS PanelApp panel version 1.26 which was signed off under NHS Genomic Medicine Service governance on 22 Mar 2023 (https://nhsgms-panelapp.genomicsengland.co.uk/panels/653/v1.26). Assay scope as per Clinical Indication ID: R173; Test IDs: R173.1 and R173.2 of the Rare and Inherited Disease National Genomics Test Directory v5.1. Unless otherwise stated analysis and reporting is carried out against the MANE Select transcript of the gene (or MANE Plus Clinical set where relevant). For details of the sequence coverage for individual gene(s) please contact the laboratory.

** Please note that PKD1 is known to have multiple highly homologous pseudocopies that are likely to impact upon assay sensitivity and specificity in this region. All clinically significant SNVs detected within this gene are confirmed by LR-PCR.*

Turnaround time: 42 days from sample receipt

Intestinal Failure or Congenital Diarrhoea Panel v3.0:
ADAM17, ADAMTS3, AGR2, ANGPTL3, AP1S1, APOB, ARX, CCBE1, CD55, CLMP, CTLA4, DGAT1, EGFR, EPCAM, FAT4, FLNA, FOXP3, GUCY2C, ICOS, KMT2D, LCT, LRBA, MTTP, MYO5B, NEUROG3, PCSK1, PLVAP, RFX6, SAR1B, SI, SKIV2L, SLC10A2, SLC26A3, SLC39A4, SLC5A1, SLC9A3, SPINT2, STX3, STXBP2, TERT, TMPRSS15, TTC37, TTC7A, WNT2B, XIAP

Panel content as per 'R331 Intestinal Failure or Congeintal Diarrhoea' GMS PanelApp panel version 3.0 which was signed off under NHS Genomic Medicine Service governance on 22 Mar 2023 (https://nhsgms-panelapp.genomicsengland.co.uk/panels/514/v3.0). Unless otherwise stated analysis and reporting is carried out against the MANE Select transcript of the gene (or MANE Plus Clinical set where relevant). For details of the sequence coverage for individual gene(s) please contact the laboratory.

Turnaround time: 42 days from sample receipt

Hirschsprung disease single gene testing:
RET

Assay content as per Clinical Indication ID: R177; Test ID: R177.1 of the Rare and Inherited Disease National Genomics Test Directory v5.1. Please note that Unless otherwise stated analysis and reporting is carried out against the MANE Select transcript of the gene (or MANE Plus Clinical set where relevant). For details of the sequence coverage for individual gene(s) please contact the laboratory.

Turnaround time: 42 days from sample receipt

Paediatric Pseudo-obstruction Syndrome Panel v0.0:
*ACTA2, ACTG2, EDN3, EDNRB, ERBB3, FLNA, L1CAM, LIG3, LMOD1, MPV17, MYH11, MYLK, *PHOX2B, POLG, RET, SGO1, SOX10, TTC7A, TYMP, ZEB2*

Please note that this is an interim panel (hence versioned as v0.0) and does not completely conform to the current signed off GMS panel (see following information).

Panel content based on 'R438 Paediatric Pseudo-obstruction Syndrome' GMS PanelApp panel version 1.0 which was signed off under NHS Genomic Medicine Service governance on 22 Mar 2023 (https://nhsgms-panelapp.genomicsengland.co.uk/panels/1217/v1.0) but **does not currently include *PROK1*, *PROKR1*, and *PROKR2*.** Please note that unless otherwise stated analysis and reporting is carried out against the MANE Select transcript of the gene (or MANE Plus Clinical set where relevant). For details of the sequence coverage for individual gene(s) please contact the laboratory.

*Please note that *PHOX2B* contains a polyalanine repeat tract that is refractory to sequencing using current NGS methodologies. Additional Sanger sequencing is carried out in order to cover this region. Sensitivity and specificity metrics for this element of the assay are not currently available as validation is ongoing.

Turnaround time: 42 days from sample receipt

3.9.1 Targeted testing offered

Targeted Sanger sequencing of known familial variants:

Testing is performed using Sanger sequencing across the genetic region known to contain the variants detected in other family members. If the gene of interest is difficult to target using standard PCR techniques (e.g. there are multiple highly homologous pseudogenes) an additional Long-Range PCR step will be used in the protocol in order to ensure specificity of amplification. Only listed nucleotides are fully assessed in this analysis and we do not exclude the presence of other variants. In-house validation has shown sensitivity of the assay for germline variants (SNPs and small indels) to be >99%. Although primers have been designed to minimise to risk of allelic drop-out it remains possible that rare or novel polymorphisms within primer binding sites could interfere with this assay and confound the interpretation of results. Unless otherwise stated a familial positive control sample will have been utilised in testing to minimise the risk of false negative results. Results and interpretation are dependent on samples being correctly labelled and family relationships as indicated. Variant nomenclature is according to HGVS guidelines with numbering using the A of the ATG initiation codon as nucleotide 1.

Relevant test directory codes for this testing include:

R240 – Diagnostic testing for known variant(s) - Turnaround time: 42 days from sample receipt

R242 – Predictive testing for known familial variant(s) - Turnaround time: 14 days from sample receipt

R244 - Carrier testing for known familial variant(s) - Turnaround time: 42 days from sample receipt

R246 – Carrier testing at population risk for partners of known carriers of nationally agreed autosomal recessive disorders - Turnaround time: 42 days from sample receipt

Targeted genotyping for defined risk-associated variants:

Testing is carried out using TaqMan® genotyping assays and the Thermo Fisher Scientific StepOnePlus™ Real-Time PCR System. Extensive in-house validation has shown sensitivity for the variants of interest to be >99%. Although probes have been designed to minimise to risk of allelic drop-out it remains possible that rare or novel polymorphisms within probe binding sites could interfere with this assay and confound the interpretation of results. Any remaining DNA from this sample has been stored. Results are dependant on samples being correctly labelled and family relationships as indicated.

This is a targeted assay which interrogates the following *APOL1* variants only:

- 'G1': rs73885319: *APOL1* (NM_003661.4): c.1024A>G p.(Ser342Gly)
- 'G1': rs60910145: *APOL1* (NM_003661.4): c.1152T>G p.(Ile384Met)
- 'G2': rs71785313: *APOL1* (NM_003661.4): c.1164_1169del p.(Asn388_Tyr389del)
- 'M1': rs73885316: *APOL1* (NM_003661.4): c.792C>A p.(Asn264Lys)

No other variants within the APOL1 gene will be detected using this assay.

Relevant test directory codes for this testing include:

R446 – APOL1 kidney donor testing - Turnaround time: 42 days from sample receipt

3.10 Results - Molecular Neuropathology

3.10.1 Result enquiries

Result enquiries can be made directly to the department via email or telephone:

- kch-tr.molecularneuropathology@nhs.net
- 020 3299 2375

3.10.2 Result availability

Upon authorisation, Molecular Neuropathology reports are available on EPIC. In cases where requesters do not have access to EPIC, electronic copies of reports are emailed to secure nhs.net email addresses upon request.

3.11 Results – Liver Molecular Genetics

3.11.1 Result enquiries

Result enquiries can be made directly to the department via email or telephone:

- kch-tr.kchlmgadmin@nhs.net
- 020 3299 4625

3.11.2 Result availability

Upon authorisation, Liver Molecular Genetics reports are available on EPIC. In cases where requesters do not have access to EPIC, electronic copies of reports are emailed to secure nhs.net email addresses provided at time of referral.

3.12 Sample referral - Molecular Neuropathology

Samples are not generally referred to external laboratories from Molecular Neuropathology, with the exception of the WGS pipeline which is detailed in 3.8.5.

When contingency requires, samples will be referred for testing to external accredited referral laboratories. Where it is not possible to perform molecular testing in-house, samples are referred to:

Division of Neuropathology,
Queen Square House
UCL Institute of Neurology
The National Hospital For Neurology and Neurosurgery
Queen Square
London WC1N 3BG

UCLH.office.neuropathology@nhs.net
Telephone: 020 3448 4234

3.13 Sample referral – Liver Molecular Genetics

Samples are not generally referred to external laboratories from Liver Molecular Genetics.

When contingency requires, samples will be referred for testing to external accredited referral laboratories. Where it is not possible to perform molecular testing in-house, samples are referred to:

Sheffield Clinical Genetic Service

Outpatients Department 2,

Northern General Hospital,

Herries Rd, Sheffield S5 7AU

Phone: 0114 271 7025

Contact: Florentina SAVA florentina.sava@nhs.net

3.14 Clinical advice - Molecular Neuropathology

Clinical advice is available during service hours from:

- Clinical Scientists providing the service at a level commensurate with their seniority and expertise.
- Department lead in Molecular Neuropathology.

Please phone 020 3299 2375 and ask for Kelly Eggleton in the first instance.

Further clinical advice can be sought from the Consultant Neuropathologists, details below:

Professor Al-Sarraj telephone: 0203 299 1958

Dr Istvan Bodi telephone: 0203 299 1954

Dr Zita Reisz: 020 3299 1952

3.15 Clinical advice - Liver Molecular Genetics

Clinical advice is available during service hours from:

- Clinical Scientists providing the service at a level commensurate with their seniority and expertise.
- Lead-Clinician in Liver Molecular Genetics

Please phone 020 3299 2253 and ask for Sammi Allouni in the first instance
Further clinical advice can be sought from the Clinical Lead, details below:

Professor Richard Thompson telephone: 0203 299 4296

3.16 Agreements with laboratory users

The laboratory shall periodically review this document and the department website, in order to review the agreements for providing laboratory activities. This will ensure that:

- The laboratory continues to provide services which are both clinically appropriate and necessary;
    - As updates to molecular testing requirements are provided centrally by NHSE, e.g. to expand NGS panels, this shall be reflected in the testing provided as well as this document and the department website;
- All requirements are adequately specified in this document;
- The laboratory continues to have the capability and resources to meet the requirements specified herein;
    - The department commits to ensuring the ongoing availability and integrity of retained patient samples and records in the event of closure, acquisition or merger of the laboratory;
- The laboratory continues to advise users of the specific activities to be performed by referral laboratories and consultants;
- The laboratory continues to provide patients and users with publicly available information about the examination processes.

Users shall be informed of any changes to an agreement that can affect examination results through the review and update of this document as well as the department website. For any changes that directly impact patient results, laboratory users including patients shall be informed directly.

Queries from users and patients can be directed to the department via telephone (see 3.2 Contact Information section), including requests for relevant information.

Records of reviews, including any significant changes, shall be retained within the quality management system (Q-Pulse).

3.17 Complaints

Complaints may be made directly to the department, via PALS or via Synnovis Customer Support. Complaints are handled according to the Synnovis Complaints Policy and Procedure located at Customer Service | Synnovis.

The MMS has a complaints procedure in place, a copy of which is available on request: *QP-GEN-002USER User Satisfaction and Complaints*. When directly raising a complaint with the department, the initial point of contact should be the department's Quality Manager.

4. References and related information

Molecular Neuropathology Service | King's College Hospital NHS Foundation Trust (kch.nhs.uk)

Liver Molecular Genetics Laboratory | Synnovis

Molecular Neuropathology Request Form

Liver Molecular Genetics Request Form

Privacy and data protection policy | Synnovis

https://www.england.nhs.uk/publication/national-genomic-test-directories/

Customer Service | Synnovis

QP-GEN-002USER User Satisfaction and Complaints

KCH-MMS-GU-3 Sample Handling Guidance for Whole Genome Sequencing of Solid Tumour Samples

KCH-MMS-GU-4 Sample Handling Guidance for Whole Genome Sequencing for Germline Samples

5. Appendices

None