Molecular Neuropathology User Guide

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1. Introduction

1.1 Purpose and Scope

The purpose of this document is to act as a guide to services provided by the Molecular Neuropathology (MNL) department at King's College Hospital. It further provides key contact points for service users. The information within this document can be used by any user that requires neuropathological investigation.

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
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. General information about the department and services provided can be found on the Molecular Neuropathology Service website:

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

The Molecular Neuropathology service is 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 sits 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

3.2.1 Hours of work

Both the Molecular Neuropathology and Clinical Neuropathology departments are open from 8.30am to 5pm, Monday to Friday. There is **no** routine provision of on-call/out-of-hours service.

3.3 Sample information

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.

3.3.1 Sample requirements

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.

3.3.2 Handling biological samples

- 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.

3.3.3 Sample collection and labelling

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.

3.3.4 Transportation of biological samples

- 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

3.3.5 Sample retention

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.

3.4 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 for all samples. 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

3.4.1 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 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.

3.5 Additional testing requests

Samples are stored indefinitely (see 3.3.5), 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.

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 service adheres 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

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 Results

3.9.1 Result enquiries

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

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

3.9.2 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.

3.10 Sample referral

Samples are not generally referred to external laboratories from Molecular Neuropathology, with the exception of the WGS pipeline which is detailed in 3.7.5.

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

3.11 Clinical advice

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 Bodi telephone: 0203 299 1954

Dr Zita Reisz: 020 3299 1952

3.12 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).

3.13 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 Molecular Neuropathology department 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)

Molecular Neuropathology 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

5. Appendices

None