

The ROYAL MARSDEN



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Clinical Genomics Molecular Diagnostics Service Guide

The Centre for Molecular Pathology
The Royal Marsden NHS Foundation Trust
15 Cotswold Road, Sutton, Surrey
SM2 5NG

Enabling personalised cancer medicine



Molecular Diagnostics Laboratory

This document describes the service provided by the Molecular Diagnostics Laboratory. The laboratory is accredited by UKAS standard ISO15189 (Lab no: 9839).

a) Staff and contact numbers:

For enquiries please contact the laboratory on the telephone number below:

General office enquiries: 020 8915 6565

Results enquiries: 020 8915 6536

Dr Lisa Thompson, Head of Clinical Genomics (Diagnostics); 020 8915 6543

Dr Michael Hubank, Head of Clinical Genomics (Translational Research); 0208 915 6544

Ms Julie Howard-Reeves, Deputy Head Clinical Genomics, Head Cytogenetics; 020 8722 4232

Dr Sandra Hanks, Laboratory Manager; 020 8915 6561

Ms Dorte Wren, Consultant Clinical Scientist, Haemato-oncology; 020 8915 6587

Dr Suzanne MacMahon, Senior Clinical Scientist, Solid tumours; 020 8915 6521

Emma Poyastro-Pearson, Diagnostics Sequencing Scientist; 020 8915 6535

Ms Dee Collins/ Ms Marion Sullivan; Team Administrator; 020 8915 6565

Fax: 020 8915 6566

We can also be contacted by email:

rmh-tr.moleculardiagnosics@nhs.net

b) Location:

Clinical Genomics Department
Molecular Diagnostics
The Centre for Molecular Pathology
The Royal Marsden Hospital NHS Foundation Trust
15 Cotswold Road, Sutton, Surrey, SM2 5NG

c) Service provided:

The laboratory provides rapid and accurate molecular profiling of solid tumour and haematological malignancies. Working together with immunophenotyping, cytogenetics and histology services, Molecular Diagnostics forms part of the integrated Haematological Malignancies Diagnostic Service, which operates in compliance with NICE Improving Outcomes Guidance. Working with our Translational Research team we are able to rapidly translate novel techniques into the diagnostic setting, contributing to the development of new cancer diagnosis tools for precision medicine.

d) Hours of service:

9.00am to 5.00pm, Monday to Friday.

e) Out of hours service:

Currently no out of hours service is provided. Samples received after 5.00pm will be processed the following working day.

f) Instructions for completion of the request form:

Please fill **ALL** the fields provided with the relevant demographic/clinical information. For patient's details, stickers with the patients information can be used. Similarly, stickers can be used for the Consultant and referring hospital information.

If some information is unknown or not applicable please fill the field in with "UNKNOWN" and "N/A", respectively, rather than leaving it blank. Samples with attached request forms that do not contain all the relevant information can be rejected by the lab or put "on hold" until a new and completely filled request form has been received.

Please tick which investigations are to be performed and in the case of FFPE samples the box corresponding to the pathological assessment. If in doubt please contact us on the numbers given above.

g) Sample acceptance / rejection

Liquid samples e.g. peripheral blood, bone marrow, must be labelled with the patient first name and surname, D.O.B., hospital number and the date and time the sample was taken. In the case of FFPE sections and blocks, the histopathology number must be clearly displayed. The details on the sample must correspond to those on the request form. Unlabelled samples will **NOT** be accepted.

h) Transportation of samples:

Internal samples - will be sent -together with the request form- to Clinical Pathology (RMH, Sutton, 1st floor), as per Trust policy, as soon as possible after taking the sample. In the case of samples sent from the Chelsea site, they will be transported in the pathology containers on the RMH inter-site coach, to be delivered to Clinical Pathology. Samples will be then collected from Clinical Pathology by Laboratory staff and taken to The Centre for Molecular Pathology. See HSE guidance on <http://www.hse.gov.uk/biosafety/biologagents.pdf>

External samples - please package samples according to the HSE guidance and address specimens to:

Clinical Genomics Department
Molecular Diagnostics Laboratory
The Centre for Molecular Pathology
The Royal Marsden Hospital NHS Foundation Trust
15 Cotswold Road
Sutton
Surrey, SM2 5NG

i) Availability of Clinical advice and interpretation:

Please contact us on the numbers provided above if you need help with the interpretation and/or clinical significance of the results issued by our laboratory. For clinical advice, however, please contact any of the Consultants in the appropriate clinical unit at the RMH.

j) Specimens required, laboratory repertoire, turnaround times and performance issues:

We can process Formalin-fixed paraffin embedded (FFPE) sections and blocks, fresh frozen tissue and peripheral blood (circulating tumour DNA; ctDNA) for solid tumours or peripheral blood/bone marrow in EDTA for haematological malignancies.

We prefer samples collected in EDTA since heparin can inhibit the PCR reaction. However, we can process heparin samples if needed. In the case of peripheral blood samples requiring circulating tumour DNA (ctDNA) extraction (please see investigations performed), please contact the laboratory for cell free DNA collection tubes.

Samples should be sent on the day of collection and at ambient temperature. DNA studies (i.e. mutation detection, clonality assessment, IGHV mutation status, chimersim analysis) can also be performed on blood or bone marrow films (either stained or not). Clotted samples are unsuitable for molecular analysis, in these situations please obtain a new sample if possible.

Peripheral blood (PB) in EDTA - please send 3-10 mL of PB except in the case of MRD and Chimerism studies where 10-20 mL of blood is recommended.

Peripheral blood in cell free DNA collection tubes for ctDNA mutation detection - please ensure the stabilisation tube is filled to the line (8.5 mL PB) and inverted 8-10 times following venepuncture. Failure to do so may compromise the ctDNA obtained from the sample. Please dispatch within 24 hours of sample collection at ambient temperature.

Bone Marrow (BM) in EDTA – please send at least 1 mL of BM unless this is not feasible (e.g. paediatric samples).

Body fluids can also be processed if the cell count is at least 10^{5-6} .

Solid tissue samples can be processed either from Formalin-fixed paraffin embedded (FFPE) sections/blocks or from fresh (in isotonic media) or frozen tissues.

FFPE blocks (recommended) – please send a representative tissue block with a return address.

FFPE sections - please send one H&E section and three 10 µm mounted sections (unstained) for mutation analysis. If ALK, PD-L1 and ROS1 IHC testing is required, please add an additional H&E section and two 4-5 µm mounted sections (unstained and unbaked) per test. IHC testing is provided by the RMH Histopathology department and separate reports will be issued for the tests performed.

For more information on how to process these samples and for request forms please contact us by phone or email.

Investigations performed:

We provide both qualitative and quantitative PCR analyses, depending on the type of test required. Quantitative PCR by Real-Time PCR (RT-PCR) is normally performed for gene expression profiling and Minimal Residual Disease (MRD) monitoring, whilst semi-quantitative PCR is performed for chimerism or certain mutation analysis.

The percentage conformance turnaround time is provided for each test type and is dependent on the sample quality, percentage tumour infiltration and additional testing requirements e.g. confirmation testing by alternative methods. In cases where more than one test is requested an interim report will be generated.

1. Mutation detection

We perform mutation detection using a bespoke pan-cancer targeted Next Generation Sequencing DNA panels encompassing frequently mutated genes. A laboratory defined virtual panel specific to the tumour type (see table below) will be applied and reported in line with the NHSE National Genomic Test Directory for Cancer. The panel can detect single nucleotide variants and small insertion/deletions. Supplementary methods e.g. fragment analysis, direct Sanger sequencing, CE-SSCA and ARMS/ASO-PCR will be employed for complex mutations, confirmation of low level variants, large insertion/deletions or in cases of low quantity/quality DNA obtained from the sample.

Tumour assessments are undertaken on solid tumour investigations, to determine the cellularity and percentage of neoplastic cells present in the sample prior to DNA extraction.

| Clinical Indication | Target (genes)* |
|---------------------|------------------------------------------|
| Colorectal | <i>KRAS, NRAS, BRAF, PIK3CA, TP53</i> |
| Lung | <i>EGFR, KRAS, BRAF, PIK3CA</i> |
| Melanoma | <i>BRAF, NRAS, KIT</i> |
| GIST | <i>KIT, PDGFRFA, BRAF</i> |
| Glioma | <i>EGFR, IDH1, IDH2, TP53, BRAF</i> |
| Ovarian | <i>BRCA1, BRCA2 (somatic)</i> |
| Thyroid | <i>BRAF, KRAS, NRAS, HRAS, TP53, RET</i> |
| Head & Neck | <i>CDKN2A, EGFR, TP53</i> |
| Extended NGS panel | Please contact the laboratory |

* only specified gene regions are sequenced

In cases where the quantity of DNA obtained for the sample does not meet the quality criteria for NGS testing (<0.3ng/ul), an alternative testing platform will be used, which may provide a limited test scope.

Turn Around Times Percentage conformance

>85% of results expected within 14 calendar days

ctDNA EGFR mutation detection in lung cancer can be performed in patients at diagnosis, presenting with advanced non-small cell lung cancer, where a tissue sample is unavailable or at radiological progression, while on first line EGFR TKI treatment to detect resistant EGFR mutations. Testing is performed using real time PCR.

Turn Around Times Percentage conformance

>85% of results expected within 14 calendar days

Tumour BRCA1/2 mutation detection in ovarian cancer is supported by Astra Zeneca as a service to medicine.

Turn Around Times Percentage conformance (tBRCA 1/2 NGS panel)

>85% of results expected within 21 calendar days

TP53 mutation detection in CML is supported by Janssen-Cilag Ltd.

Turn Around Times Percentage conformance

>85% of results expected within 14 calendar days

In the case of urgent samples, please contact the laboratory, to expedite priority testing.

For further information and request forms for the services provided above, please contact the laboratory on the above numbers.

2. **Clonality assessment:** clonality tests for B-cell receptor (*IGH* and *IGK* genes) and T-cell receptor (*TCRB* and *TCRG*) are performed by fluorescent PCR amplification using the BIOMED2 protocols, followed by GeneScan analysis. For **B-cell clonality**, we analyse at least 5 of the following targets: FR1, FR2, FR3 and DJ for the *IGH* genes, and VJK and KDE for the *IGK* genes. **T-cell clonality** analyses are performed using 5 different targets: 2 PCRs for the *TCRB* VDJ rearrangements, 1 for *TCRB* DJ rearrangements and 2 PCRs for *TCRG* VJ rearrangements. Analysis of *TCRD* rearrangements is only performed under specific request or special circumstances. By combining the different targets the sensitivity of the analysis is >95%.

Turn Around Times Percentage conformance

>85% of results expected within 14 calendar days

3. **Mutational status of the Ig Variable regions:** the mutational status of *IGVH* in CLL is routinely analysed for prognostic purposes if requested.

Turn Around Times Percentage conformance

>85% of results expected within 21 calendar days

4. The analysis of **fusion genes in acute leukaemias** is performed quantitatively by Real-Time PCR on RNA samples from AML, ALL and CML patients. We currently have one panel of fusion genes for AML (*PML-RARA*, *RUNX1-RUNX1T1*, *CBFB-MYH11* and *BCR-ABL1*) and another one for ALL (*BCR-ABL1*, *ETV6-RUNX1*, *TCF3-PBX1*, *KMT2-AFF1* and *STIL-TALI*—only for T-cell leukaemias). *BCR-ABL1* is also analysed in suspected CML. We perform MRD analysis of acute leukaemias (or CML) only if we know that one of the markers was present at diagnosis.

Turn Around Times Percentage conformance

>85% of results expected within 14 calendar days

In the case of urgent haemato-oncology RT-PCR >90% of results expected within 5 calendar days

5. **Chimerism analysis:** For this analysis we require a donor sample and a sample from the patient prior to transplantation. We perform lineage-specific Chimerism analysis (CD3 v CD15) ONLY in peripheral blood samples unless otherwise stated.

Turn Around Times Percentage conformance

>85% of results expected within 14 calendar days

6. **Soft-tissue Sarcomas:** we currently offer a routine service for the analysis of the following STS fusion transcripts: *SS18-SSX1/SSX2*, *PAX3/PAX7-FOXO1A*, *EWSR1-FLI1*, *EWSR1-ERG*, *EWSR1-WT1*, *EWSR1-NR4A3*, *TAF15-NR4A3*, *EWSR1-ATF1*, *ASPSCR1-TFE3*, *FUS-*

DDIT3, FUS-CREB3L2, JAZF1-SUZ12, EWSR1-CREB1, ETV6-NTRK3 and YWHAE-NUTM2A/B. Confirmation testing by FISH can be arranged through our Cytogenetics laboratory. For further details, please contact the laboratory on 020 722 4232.

Turn Around Times Percentage conformance

>85% of results expected within 14 calendar days

- 7. Immunohistochemistry (IHC):** we currently offer a routine service for the ALK and PD-L1 expression studies in NSCLC via our Histopathology department. ROS1 IHC testing is provided by an approved reference centre. For further details, please contact the Histopathology laboratory on 020 8915 6549.

Performance issues:

Fresh liquid samples **MUST** be kept at ambient temperature and reach our lab within 24-48 hours from venepuncture to allow for successful DNA/ctDNA/RNA extraction (except frozen samples, FFPE tissue samples or PB/BM films, where there is no limit). Occasionally, samples older than 48 hours will be analysed (i.e. samples collected over the weekend/bank holiday periods), although in these cases the chances of a successful report are significantly diminished.

Other factors affecting the reliability of results include:

- Clotted samples
- Inadequate samples
- Leaking samples
- Inappropriate sample
- Incorrect collection tube used i.e. anticoagulant
- Inadequate sample labelling
- Inadequate request data quality

k) Time limit for requesting additional examinations:

All DNA and RNA samples are stored indefinitely for all examinations. FFPE blocks will be returned to the referrer once testing is complete.

Please note that we only store DNA or RNA, depending on the investigation originally requested, and therefore we will not be able to perform a DNA-based investigation on a sample where we only stored RNA and vice versa.

l) Organisational and departmental approach to protection of personal information

The Trust and all laboratories operate in conformance with the Data Protection Act 1998 and Computer Misuse Act 1990 applied by the Confidentiality and Data Protection Act Policy. All staff must undertake annual mandatory training in Information Governance.

m) Complaints procedure

The Trust has a formal Concerns and Complaints Policy and Procedure: all concerns and complaints are taken seriously and fully investigated with responses provided to the persons raising these. Pathology also has a policy and procedure for dealing with complaints addressed directly to the laboratories.