Molecular Pathology Department
Maidstone and Tunbridge Wells NHS Trust

Information for Users

Molecular Pathology
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Introduction

Maidstone and Tunbridge Wells NHS Trust (MTW) is a large acute hospital Trust in the South East of England. The Trust prides itself in putting the patient first with respect to innovation, service delivery and excellence.

Molecular tests offer novel and state-of-the-art laboratory methods primarily used to personalise patient's treatment based on the molecular phenotype of a tumour (somatic mutation profile, gene and protein expression).

The Molecular Pathology Department occupies a purpose-built suite of laboratories (opened in 2011) which is situated within the Cellular & Molecular Pathology department within the Division of Core Clinical Services at Maidstone Hospital.

The aim of the service is to provide (either by testing in-house or by working with our local Genomics Laboratory Hub), a comprehensive advanced predictive testing service to the patient in a clinically relevant timeframe, incorporating appropriate interpretation of results primarily to predict response to therapies, but also to aid prognosis and diagnosis where appropriate.

The service is led by a Clinical Scientist and the team comprises of a number of dedicated specialist Biomedical Scientists (BMS's) together with support staff. Consultant Pathologists provide clinical support. There is a Lead Consultant Pathologist who provides clinical direction and leadership together with supporting the ongoing development of the department.

The in-house testing repertoire of the service is under constant review and a full development programme is ongoing to allow for repatriation of clinically appropriate outsourced tests.

The department has undertaken full verification and/or validation of all testing procedures prior to implementation. We participate in National External Quality Assurance Schemes for all tests in our repertoire. We are currently accredited to ISO15189:2012 by UKAS and our schedule of accreditation can be found on the UKAS website .

Molecular Pathology Team

Clinical Lead for Cellular Pathology

Dr Dominic Chambers MBBS BSc (Hons) FRCPath. Consultant Histopathologist

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Clinical Scientist

Mrs Gillian Donald MSc CSci FIBMS

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Lead Biomedical Scientist

Mr Peter Deal BSc(Hons) CSci FIBMS

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Lead Consultant Pathologists:

Breast HER-2 testing:	Dr Sonia Saw
Gastric HER-2 testing:	Dr Monika Verma
Lung Cancer Testing	Dr Dominic Chambers
Lower GI Testing:	Dr Monika Verma
Dermatology Testing:	Dr Ann Fleming
Head & Neck Testing:	Dr Ann Fleming

Scientific Team

Mr Ajay Ruparel
Mr Kwaku Ayensu
Mrs Ros Brewer
Mr Abdul Wahhab-Wahid

Clerical Team

Mrs Natalie Taylor
Mrs Mandy Bolton
Miss Natasha Spiteri
Mrs Kay Jones
Mrs Beelynda Polanco-Lerma

Laboratory Support

Mrs Harminder Chagger
Ms Stephanie Holmes
Mrs Lyndsey Bilverstone
Miss Akua Oppey

e-mail: mtw-tr.molpathology@nhs.net **Tel: 01622 225643**

Laboratory Opening hours

The laboratory is open between 08:00 and 18:00 Monday to Friday. An on-call service is not provided, however if you require a result urgently, you should contact the Clinical Scientist or lead BMS, who will discuss your needs with you and do everything possible in order to provide this. Urgent results can be given on request by the Clinical Scientist/Consultant Pathologist.

Reports, Results and Turnaround Times

Authorised standalone Molecular Pathology reports are available on Telepath and remote access is available via Allscripts. We distribute reports by e-mail to nhs.net generic e-mail addresses. Hard copies of reports can be sent on request.

Cases requiring a second opinion will be referred to colleagues in accredited laboratories (see appendix 1). If this is necessary, you will be contacted and informed of the reasons for a delay.

Test	**Reflex / on request**	**Turnaround times (target = 90%)** (Calendar Days. Receipt in lab – authorisation)	**Comments**
<u>Breast Cancer Panel</u> 1. HER-2 (IHC only) 2. HER-2 IHC & FISH 3. PIK3CA 4. PD-L1 (atezolizumab) 5. PD-L1 (pembrolizumab) 6. Gene Expression profiling a. Prosigna (node negative patients) b. Oncotype DX (N1-3 patients)	1. Reflex 2. Reflex 3. on request 4. on request (TNBC) 5. on request (TNBC) 6. on request	1. 7 days 2. 10 days 3. 28 days 4. 10 days 5. 10 days 6. 21 days	1. In house 2. In house 3. GLH 4. External lab 5. External lab 6. External lab
<u>Lower GI Panel.</u> 1. KRAS/BRAF/NRAS*/** 2. MMR** including BRAF**/MLH1PM* where appropriate 3. HER-2 incl FISH if indicated ** 4. PD-L1** CPS / TC	1. On request 2. Reflex 3. On request 4. On request	** 7 days (in house testing) * 28 days (GLH 21 days plus 7 days preparation time)	*NGS testing provided at GLH ** In-house testing - Salvage pathway (urgent / too small / test not provided at GLH)

<u>Upper GI Panel.</u> 1. KRAS/BRAF/NRAS*/** 2. MMR** including BRAF**/MLH1PM* where appropriate 3. HER-2 incl FISH if indicated ** 4. PD-L1** CPS / TC 5. Cholangiocarcinoma panel*	1. On request 2. Reflex 3. On request 4. On request 5. On request	** 7 days (in house testing) * 28 days (GLH 21 days plus 7 days preparation time)	*NGS testing provided at GLH ** In-house testing - Salvage pathway (urgent / too small / test not provided at GLH)
<u>Skin Panel</u> BRAF/NRAS (Melanoma)*/** PD-L1 (nivolumab)	On request	** 7 days (in house testing) * 28 days (GLH 21 days plus 7 days preparation time)	*NGS testing provided at GLH ** In-house testing - Salvage pathway (urgent / too small / test not provided at GLH)
<u>Anaplastic Thyroid carcinoma</u> BRAF*/**	On request	** 24 hours (in house - urgent) * 28 days (GLH 21 days plus 7 days preparation time)	*NGS testing provided at GLH ** In-house testing - Salvage pathway (urgent / too small / test not provided at GLH)
<u>Lung Panel</u> 1. EGFR*/** 2. KRAS/BRAF*/** 3. Gene Fusion panel (RET/METex14/ALK/ROS1)*/** 4. ALK IHC** 5. ROS1 IHC** 6. PD-L1 IHC** 7. ROS1 FISH* 8. ALK FISH* 9. NTRK*	Reflex (all NSCLC) Others – on request	** 7 days (in house testing) * 28 days (GLH 21 days plus 7 days preparation time)	*NGS testing provided at GLH ** In-house testing - Salvage pathway (urgent / too small / test not provided at GLH)

Urology 1. MMR** including BRAF**/MLH1PM* where appropriate 2. tBRCA1/2* 3. PD-L1*** pembrolizumab 4. PD-L1*** atezolizumab 5. PD-L1*** aveulmab 6. PD-L1*** nivolumab	On request	** 7 days (in house testing) * 28 days (GLH 21 days plus 7 days preparation time) *** 10 days external lab	*NGS testing provided at GLH ** In-house testing - Salvage pathway (urgent / too small / test not provided at GLH) *** external lab
Gynaecology Panel 1. MMR** including BRAF**/MLH1PM* where appropriate 2. POLE 3. BRCA/HRD	1. Reflex 2. On request 3. On request	** 7 days (in house testing) * 28 days (GLH 21 days plus 7 days preparation time)	*NGS testing provided at GLH ** In-house testing - Salvage pathway (urgent / too small / test not provided at GLH)

Clinical Advice

Clinical advice can be provided from the Clinical Scientist or relevant pathologists involved in the service. Please contact a member of the Molecular team who will put you in contact with the relevant individual.

Costs

Price of testing is available from the Lead BMS on request.

Some tests are currently within tariff or funded by the GLH/NHSE. Please discuss with the team for individual needs. Invoices are prepared on a monthly basis to those departments not eligible for free testing and private providers.

Prices are reviewed in line with inflation prior to the start of the new financial year and users are informed of impending increase during March by email.

Consent, Storage and Authorisation

Please note, in accordance with the requirements of the Human Tissue Act (HTA), it is the responsibility of the referring clinician to ensure that appropriate informed consent has been obtained before any testing is undertaken. The laboratory must be informed of any restrictions to this consent. Unless stated, the laboratory will process all samples with the understanding all

appropriate consent has been obtained from the patient for the tests requested, and for storage of the derived DNA for future use.

Samples from patients who do not consent to storage and future use of their DNA must be accompanied by a "closed consent" form indicating the limits of the consent granted. If in doubt, contact a member of the Molecular Pathology team to discuss.

Transport of Samples

It is the responsibility of those taking and dispatching specimens to the laboratory to ensure that these samples are sent in accordance with any national guidelines and/or local policies for the packaging, labelling and transport of biological material.

Please email the team in advance to let them know what you are sending so that we can ensure its safe arrival corresponds to your list. We will inform you of arrival in the department

Problems and Queries

For any further information, issues, complaints please contact a member of the Molecular Pathology team who will refer you appropriately.

Requests

Requests can be made using e-mail or completing the request form (see appendix B) and e-mailing to mtw-tr.molpathology@nhs.net

All submissions for testing require a written request, a copy of the histology report and a Formalin Fixed Paraffin Embedded (FFPE) block of representative tissue.

Specimen Requirements

The request and FFPE should be sent to the laboratory following local protocols for transport of pathology samples. The following information must be legible on the request:

- Patient hospital number
- NHS number

- Surname
- Forename
- Date of Birth
- Gender
- Details of GP
- NHS/PP status

If the sample is from a private patient, we will invoice you as the requestor.

FFPE blocks must be labelled with the unique identifier. Accompanying slides must be labelled with the unique identifier and patient Surname and Forename (or initial). Samples without minimum data, or when data on the sample and accompanying documents do not match, will not be accepted and will be returned to the sender. Failure to comply with this policy will result in the sample being rejected or the result delayed.

Requesters should ensure invasive carcinoma is in the block sent for testing and that the sample represents the tumour.

Please note, we are unable to accept unstained slides

Quality Assurance and National Guidelines for Testing

All assays are subject to rigorous Internal Quality Control (IQC) measures and the laboratory participates in External Quality Assurance (EQA) schemes for which results are available on request:

- UK NEQAS Molecular Genetics for Colorectal cancer, lung cancer and Melanoma testing
- UK NEQAS ICC & ISH for Breast and Gastric Cancer, PD-L1, MMR, ROS1 and ALK testing

Service users can be assured with the large number of samples being tested, extensive experience with all methodology, complete training of all staff a high quality and accurate service is provided. Centralised testing also gives value for money.

Uncertainty in assessment and reporting has been considered for all tests provided and multiple measures are in place to mitigate these risks where they exist. Details are available from the laboratory on request.

Factors known to affect Molecular Pathology testing

Testing can be performed on metastatic deposits, core biopsies, cell blocks of cytology specimens, EBUS samples and samples of the resected tumour. There is some evidence that elements of processing the FFPE block may interfere with molecular analysis. Specialised Molecular Pathology analyses can be problematic in obtaining optimal results, particularly with factors outlined below. Invalid tests will be repeated once (at no additional cost). The service user will be contacted and informed of the reasons for a delay.

- Decalcification in an acid containing solution, fast acid decalcification (including surface decalcification) or fixation in Bouin's solution is known to degrade the DNA within a sample making subsequent molecular analysis difficult. Please provide decalcification details if performed (chemical used and duration).

- Alcohol fixation is a contraindication with many immunohistochemical assays. If the sample has been exposed to an alcoholic fixative (e.g. clots / EBUS etc.), this should be made clear on the request form. Failure to do so may result in false negative results.

- Tumour tissues, particularly small biopsies, may yield too little DNA for molecular analysis. If this is the case the sample will be reported as insufficient.

- DNA from non-neoplastic cells within the sample may dilute the analyte of interest beyond the level of detection for the assay. Tumour burden and macrodissection if considered necessary, is undertaken in order to minimise this occurrence and will be present on the report.

- The quality of tissue fixation- including ischaemic time, length of fixation, the size of the sample, penetration of formalin and processing protocols can affect the quality of DNA isolated from FFPE tissues; and therefore the results of molecular testing. Large, poorly fixed samples often produce inconsistent and technically difficult results.

- Marking ink and Eosin can interfere with fluorescent signals during FISH interpretation.

1. Genomic Testing for Solid Cancers

Next Generation Sequencing

The National Test directory (NHS England » National genomic test directory) details genomic testing targets for adult solid cancers which can be requested via the department.

On your request, you must be specific regarding which tests you require by ensuring you specify the test code.

The laboratory will then identify the most suitable sample for testing, assess and prepare the sample and dispatch to the relevant GLH for testing. Please note, the current Turnaound time (TaT's) is 7 working days for selection and preparation and dispatch of material, and 21 days for testing once received at the GLH.

A report will be added as a supplementary report to the histology report.

In the event of a sample being clinically very urgent, please telephone and discuss the case with senior managers who will be able to advise of alternative processes if they exist.

A small number of targeted hot-spot PCR assays can be undertaken in-house using the Idylla system and CE-IVD assays in such circumstances, or when sample volumes are too small for NGS testing. The assays available currently are EGFR, KRAS, BRAF, NRAS, Genefusion (includes RET, MET 14 skipping mutation, ROS1, ALK). Turnaround times for in-house assays are 7 working days, but most results will be available within 2-3 days.

Whole Genome Sequencing (WGS)

This is not a currently funded service and is in development. The only cases being considered for WGS are Carcinoma of Unknown Primary with unknown treatment options. Please contact the senior Managers if you are considering WGS as the requirements are very specific and logistics complex. Turnaround times for WGS are currently 3-4 months.

2. Specialist Predictive Immunohistochemistry Testing

We provide the following tests either in-house or by outsourcing to specialist accredited reference laboratories (appendix A). These tests predict suitability to a wide range of treatments and when requesting, please ensure the treatment being considered is specified to ensure the appropriate test is performed. Please ensure material is representative of the tumour and that no unusual processes have been undertaken. Any pre-analytical processes outside of the

routine paraffin preparation (examples include, but are not limited to, prolonged fixation; Neutral buffered Formalin (NBF) not used, extended cold ischaemic time, decalcification etc) must be declared at the time of submission in order to prevent false negative results.

1. ALK Testing
2. ALK FISH
3. ROS1 testing
4. ROS1 FISH
5. PD-L1 testing
6. HER-2 testing (gastric and breast)
7. HER-2 FISH (gastric and breast). Please note HER-2 FISH is a reflex test from all HER-2 IHC cases that are 2+ or equivocal. For further details, see below

Sample Requirements:

FFPE tumour blocks must accompany a copy of the Histopathology report along with a completed request. Samples can be sent according to local protocols. Please note, we cannot accept unstained slides

FFPE blocks must be labelled with your unique identifier. Accompanying slides (if present) must be labelled with the unique identifier as well as patient family name and given name (or initial). Samples without minimum data, or when data on the sample and accompanying documents do not match, will not be accepted and will be returned to the sender.

Requesters must ensure appropriate carcinoma is in the block sent for testing and that the sample represents the tumour. If more than one tumour lesion is present representative blocks of each tumour must be sent.

If requesting multiple tests on small samples, it is helpful to indicate which tests should be given priority. If no indication is given, PD-L1 testing will take precedence.

3. HER-2 Fluorescent in-situ hybridisation (FISH) analysis

Fluorescent in-situ hybridisation (FISH) is carried out in house on equivocal 4B5 cases as a reflex test on both breast and gastric cases. The HER-2 gene is examined using fluorescent labels to ascertain evidence of gene amplification. Scoring is carried out according to UK guidelines (Rakha EA et al, *J Clin Pathol* 2014;**0**:1-7. Wong NACS et al, *J Clin Pathol* 2018;**71**:388-394. Rakha EA et al, *J Clin Pathol* 2022;**0**:1-11.).

Cases showing HER-2 / CEP17 ratio of <1.79 and average copy numbers of <4.0 per cell are considered NEGATIVE (NOT AMPLIFIED).

Cases showing HER-2: CEP17 ratio of 1.8 – 1.99 or <2.0 with average copy numbers of 4.0 – 5.99 per cell are considered EQUIVOCAL/ BORDERLINE NON-AMPLIFIED. A repeat test is requested, either on the excision when available, another block of the same sample, or another sample. Following the repeat test, readings in these ranges are reported as Not amplified or Amplified.

Cases showing HER-2: CEP17 ratio >2.0 or with average copy numbers of >6.0 are reported as POSITIVE (AMPLIFIED).

Breast FISH cases showing HER2:CEP17 ratio >=2.0, HER2 copy number <4 are reported following current guidance issued in 2022 (see above reference).

Occasionally, the clinical picture may dictate an alternative outcome to that described above and all such cases will be fully explained within the comments of the report. Further clinical advice and guidance can be sought from the Clinical Scientist.

Appendix A. List of Referral Laboratories

Department	Address	ISO Accreditation number and status
Histopathology	Addenbrooke's Hospital, Hills Road, Cambridge, CB2 0QQ	ISO 8038 Accredited Issue Date: 23/12/2019
Molecular Pathology	Birmingham Women's Hospital Mindelsohn Way Edgbaston Birmingham, BT15 2TG	ISO 8176 Accredited Issue Date: 11/03/2019
Cellular Pathology	HSL-Advanced Diagnostics Ground Floor, 60 Whitfield Street London, W1T 4EU	ISO 9007 Accredited Issue Date: 29/09/2021
Histopathology	King's College Hospital, Denmark Hill, London, SE5 9RS	ISO 9705 Accredited Issue Date: 20/01/2022
Molecular Pathology	Myriad Genetics Royal Marsden Hospital, 15 Cotswold Rd, Surrey, SM2 5PT	ISO 9839 Accredited Issue Date 05/08/2021
Molecular Pathology	All Wales Genomics Laboratory Institute of Medical Genetics University Hospital of Wales Heath Park Cardiff, CF14 4XW	ISO 8988 Issue Date 04/08/2021
Molecular Pathology	Poundbury Cancer Institute Newborough House, 3 Queen Mother Square, Poundbury Dorchester, Dorset, UK, DT1 3BJ	ISO 9387 Issue Date 27/10/2021
Molecular Pathology	Source Bioscience 1 Orchard Place Business Park Nottingham, NG8 6PX	ISO 9571 Issue Date 27/06/2022
Molecular Pathology	Bristol Genetics Laboratory Pathology Sciences Building Southmead Hospital Westbury on Trym, BS10 5NB	ISO 9307 Issue Date 16/12/2021
Molecular Pathology	South East Genomic Laboratory Hub Cancer Genetics, Genetics Laboratories, 5th Floor Tower Wing, Guy's Hospital, London, SE1 9RT	ISO 8688 Issue Date 17/12/2021
Molecular Pathology	Exact Health Sciences USA Sent using the provided FedEx US Air waybill	(USA Based) CLIA accreditation number: 05D1018272 **Dr. William P. Joseph, M.D.**

Appendix B. Request Form

Pathology Molecular Pathology RWF-CP-MOL-F74 Revision 7.2

Kent Molecular Pathology External Request Form

Please ensure all details are provided when completing this form. Processing may be delayed if information is incomplete

Patient Details:			***Patients Address:***
Family Name:	Date of Birth: (DD/MM/YY)	Gender:	
	Hospital Number:		
Given name(s):	NHS Number:		Postcode:
	NHS Patient Private Patient (delete as appropriate)		GP:

Tissue-based testing:				***Your Case Number:***
	RAS Analysis on Colorectal Samples		**MMR** Testing	
	EGFR Mutation		**HER-2** Status BREAST / GASTRIC circle as appropriate	
	ALK Rearrangement (by IHC)		**BRAF** V600E for melanoma / MMR	***Please attach a copy of the report***
	SP263 IHC (**PD-L1 for NSCLC**)		**SP142** IHC (**PD-L1 for TNBC**)	Material sent:* see below for RAS/BRAF (e.g. Paraffin block) NB unstained slides not accepted
	ROS1 IHC (NSCLC)		**Other**: (please specify)	

Clinical Details/ Comments:

RAS/BRAF Testing: Please tick to confirm marked* H&E sent (non-returnable) ☐

* Please send an H&E with the block/report with tumour clearly marked. Tumour volume within marked area should be estimated and written on the slide which will be retained within the department and not returned. Please note a minimum tumour volume of 10% is necessary for KRAS testing and 50% for BRAF. Please note we will test high grade dysplasia in the absence of invasive malignancy.

Requester details:		***Please send or e-mail request to:***
Name:		Molecular Pathology Department Cellular Pathology Maidstone and Tunbridge Well NHS Trust Hermitage Lane, Maidstone, Kent ME16 9QQ
Specialty:		
Report Address:		
Signature:	Date requested:	
Send a copy of the report to: (electronic = nhs.net only)		***For all enquires please contact:***
		01622 225643 or mtw-tr.molpathology@nhs.net

For Office Use Only:	Date Received:	Material received:	*Molecular Pathology Number*

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