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 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 patients 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 Diagnostic and Clinical Support services at Maidstone Hospital.

The aim of the service is to produce a report in timely fashion, 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 together with support staff. Consultant Pathologists provide clinical support. The Lead Consultant Pathologist chairs the Molecular Pathology Governance group which provides clinical direction and leadership together with supporting the ongoing development of the department.

The 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 validation of all testing procedures prior to implementation. We participate in National External Quality 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

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Clinical Scientist (Lead for Molecular Pathology)

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Laboratory Manager

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Lead Consultant Pathologists:

Breast HER-2 testing:	Dr Sonia Saw
Gastric HER-2 testing:	Dr Gary Rushton
Lung Cancer Testing	Dr Dominic Chambers
Lower GI Testing:	Dr Dominic Chambers
Dermatology Testing:	Dr Ann Fleming

Scientific Team

Mr Ajay Ruparel
Mr Kwaku Ayensu
Mrs Ros Brewer
Mr Cimarun Gill

Clerical Team

Mrs Natalie Taylor
Mrs Mandy Bolton

Laboratory Support

Mrs Harminder Chagger
Miss Natasha Spiteri
Ms Stephanie Holmes

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

Laboratory Opening hours

The laboratory is open between 08:00 and 17:00 Monday to Friday. An on-call service is not provided, however if you require a result urgently, you should contact the Molecular team, 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. If this is necessary, you will be contacted and informed of the reasons for a delay.

Test	**Turnaround time** **(Calendar Days. Receipt in lab – authorisation)**	**Comments**
Breast HER-2 (IHC only)	7	
Breast HER-2 (incl FISH)	14	• Required in approx 22% of HER-2 cases. • Interim reports issued
Gastric HER-2 (IHC only)	7	
Gastric HER-2 (incl FISH)	14	• Required in approx 22% of HER-2 cases. • Interim reports issued
RAS	7 (BRAF/KRAS only i.e. 12/13 mutants) 21 (KRAS/BRAF/NRAS)	• BRAF provided on all cases • NRAS automatically provided if KRAS WT. • No interim reports issued. • NB KRAS 117/146 and NRAS provided off site but full interpretation provided at MTW so TATs for these cases will be within 21 days
BRAF (Melanoma)	7	
EGFR / T790M	7	
ALK IHC (NSCLC)	7	
PD-L1 (NSCLC)	7	• Clone SP263

PD-L1 (Triple negative breast Cancer)	7	• Clone SP142 • Currently outsourced.
PD-L1 (H&N) (pembrolizumab)	7-10	• Clone 22C3 • Currently outsourced
PD-L1 (Urology)	7-10	• Clone 22C3 (Pembrolizumab) • Clone SP142 (Atezolizumab) • Currently outsourced
PD-L1 (melanoma) (nivolumab)	7-10	• Clone 28/8 • Currently outsourced
ROS1 (NSCLC)	7	
ROS1 FISH (NSCLC)	7-10	• Currently outsourced
MMR	7	• BRAF / methylation testing if loss observed (7 additional days if required) • IHC Results on CPATH as part of histology report. • BRAF results on HAEM and e-mailed to requestor • Methylation testing outsourced and results e-mailed to requestors
Gene Expression Profiling		• Oncotype DX • Endopredict. • Prosigna • Outsourced

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 Laboratory Manager on request.

Some tests are currently within tariff and you will be charged for each test. These include HER-2 (all indications and tests performed), MMR assessment (including reflex tests) and EGFR testing.

NHSE currently fund ALK IHC, ALK FISH, RAS, BRAF, PD-L1, ROS1 and Breast gene expression profiling. Please discuss invoicing arrangements with the Molecular Management team.

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, 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.

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 the request form (see p18) and e-mailing to mtw-tr.molpathology@nhs.net

All requests require a completed request form, a copy of the histology report and FFPE block of representative tissue

Specimen Requirements

The request form 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 form:

- 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. Molecular Testing for Targeted Treatments in Lung Cancer

a. EGFR mutation Analysis

Epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) are currently used in patients with advanced metastatic non-small cell lung cancer (NSCLC). Somatic mutations in the EGFR gene are used to predict response to EGFR TKIs including Erlotinib (Tarceva®), Afatinib (Gilotrif®), and Gefitinib (Iressa®).

At present, mutation analysis of exons 18-21 of the EGFR gene are carried out as reflex tests on all biopsied or resected non-squamous NSCLCs (requested by Pathologists).

The test used is the Roche COBAS EGFR v2 kit. This method screens for 42 mutations in exons 18-21 (29 deletions in exon 19, T790M, L858R, G719X, S768I, L861Q, 5 Insertions in exon 20).

Reports are standalone reports available on the remote LIMS viewer or e-mailed to external users. The turnaround time for this test is 7 days.

b. ALK Testing

The Anaplastic Lymphoma Kinase (ALK) gene encodes a tyrosine kinase normally expressed in neuronal cells. The ALK gene can, by deletion or translocation, form a fusion protein, which is abnormally expressed in approximately 4-7% of NSCLC patients. The presence of this ALK gene fusion protein predicts response to ALK inhibitors.

Tests are carried out as reflex tests on all non-squamous NSCLCs (requested by Pathologists).

ALK testing is carried out using the Ventana D5F3 Rabbit monoclonal antibody. Reports are available on Telepath as a standalone report.

c. ROS1 Testing

The *ROS1* gene (6q22) encodes a transmembrane receptor protein with one of the largest extracellular domains amongst all human receptor tyrosine kinases. Genomic rearrangements involving *ROS1* occur in 1–2 % of NSCLCs and these tumours can now be treated with crizotinib (Xalkori®). See https://www.nice.org.uk/guidance/ta529.

Tests are requested as reflex tests on all non-squamous NSCLCs (requested by Pathologists). The test is performed using the Roche SP384 ROS1 monoclonal antibody (CE-IVD).

Reports are available on Telepath as a standalone report. The turnaround time for this test is 7 days.

Cases showing any level of staining are referred to Queen Elizabeth Hospital in Birmingham for FISH testing to make a definitive assessment of translocation status. A report will be available as a final report addendum to the ROS1 immunohistochemistry result within 2 weeks.

d. PD-L1 Testing

PD-L1 is a protein that binds to the PD-1 checkpoint on immune cells that can determine response to PD-1 checkpoint inhibitors such as Nivolumab (Opdivo®) and Pembrolizumab (Keytruda®). Several other PD-1/PD-L1 checkpoint inhibitors are also in late-stage clinical testing.

Currently PD-L1 tests are provided as a reflex test on all squamous and non-squamous NSCLCs.

The test provided is the Roche SP263 clone for which the interpretation must only be used for the following indications:

Indication for use	**Therapy**	**PD-L1 Expression-Therapeutic Line**
NSCLC	KEYTRUDA®	≥ 50% TC – First Line
		≥ 1% TC – Second Line
	OPDIVO®	≥ 1%, ≥ 5% and ≥ 10% TC – Second Line

Testing for melanoma, urothelial carcinoma, triple negative breast cancer, head & neck carcinomas and other indications will be outsourced. Please discuss with the management team and mark requests clearly, including the treatment being considered for the patient.

Sample Requirements:

Formalin fixed, paraffin embedded tumour blocks should accompany a copy of the Histopathology report along with a completed request form. 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 should 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 should 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.

2. Molecular Testing for Targeted Treatments in Colorectal Cancer

Anti-EGFR monoclonal antibody therapies such as Cetuximab and panitumumab target EGFR and are approved for use in patients with metastatic colorectal cancer. These are currently used in combination with chemotherapy in metastatic colorectal tumours that carry no mutations in the RAS family (KRAS and NRAS genes). Mutation testing is required to indicate suitability to receive anti-EGFR antibody therapy. The presence of a RAS mutation predicts the lack of response to these therapies.

a. RAS mutation analysis

All samples submitted for RAS analysis are assessed for the need to enrich the tumour by macro-dissection. This is an important prerequisite for molecular genetic testing in pathology as failure to enrich tumour DNA, which may harbour somatic mutations, can result in false negative results.

An H&E slide is requested with every submission which should be marked up with tumour circled together with an estimate of tumour burden within the marked area and identified by initials / date of the individual making the assessment (see example below). Please note we will retain this slide. A minimum of 10% tumour burden is required for the successful analysis of KRAS/NRAS genes

Our testing strategy is to test all patients with the Roche COBAS KRAS real time mutation assay for 19 different KRAS mutations covering the majority of clinically relevant ‘hot-spot’ mutations in codons 12, 13 and 61. Detection of a mutation will result in a final report being issued. Please note the mutation present will not be characterised. Those samples where no

mutation is detected will be outsourced for mutation detection in KRAS codons 117 & 146 as well as NRAS (codons 12/13, 59-61).

b. BRAF mutation analysis

BRAF is a proto-oncogene with a central role in signalling pathways that control cellular proliferation, differentiation and cell death/ apoptosis. In normal cells, BRAF is part of a highly regulated signalling pathway that mediates the effects of growth factor receptors. Oncogenic mutations in BRAF, V600E result in a gain of kinase function, activating signalling pathways in the absence of the typical growth factors.

Molecular Pathology performs the ROCHE cobas BRAF real-time PCR assay intended for the identification of mutations in codons V600E of the BRAF mutations in DNA derived from human colorectal tissues in all cases submitted for RAS analysis. A minimum of 50% tumour burden is required for the successful analysis of the BRAF gene.

RAS Reports are available following all tests performed as standalone reports on Telepath.

c. Mismatch Repair Testing

Microsatellite instability (MSI), a hypermutable phenotype caused by the loss of DNA, mismatch of repair activity is a mechanism of colorectal carcinogenesis. MSI is detected in about 15% of all colorectal cancers; 12% caused by sporadic, acquired hypermethylation and 3% associated with hereditary non-polyposis colorectal cancer (HNPCC), Lynch syndrome. Patients with HNPCC, Lynch syndrome, have an 80% percent chance of developing colorectal cancer. Tumours with MSI have slightly better prognosis. Discovery of MSI has increased awareness of the implications for specialised management of patients.

At Maidstone & Tunbridge Wells NHS Trust we perform MMR immunohistochemistry in order to assess the presence or loss of mis-match repair proteins MLH1, MSH2, PMS2 and MSH6 as a surrogate for MSI testing. No losses indicate preserved expression of the genes and sporadic nature of the disease.

If loss of expression is detected in MLH1 +/- PMS2, then a BRAF test is undertaken to elucidate whether or not a BRAF V600E mutation is present (which indicates a probable sporadic pathway). Those cases where no mutation is detected are followed up with MLH1 promotor methylation studies to further stratify those patient that need to be referred for specialist genetic testing and advice with regards to Lynch Syndrome. MLH1 promotor methylation testing is outsourced.

Currently some tests are provided as a reflex based upon the Bethesda criteria and others by clinical request on our request form or via e-mail to mtw-tr.molpathology@nhs.net .

Results of MMR immunohistochemistry can be found on the Histopathology report. If applicable, standalone reports will be made available for BRAF and MLH1 promotor methylation for interpretation and final report production by the Consultant Histopathologist.

3. Molecular Testing for Targeted Treatments in Malignant Melanoma

a. BRAF mutation analysis

Vemurafenib (Zelboraf®) is a BRAF enzyme inhibitor developed by Plexxikon and Genentech for the treatment of late-stage melanoma. Response to therapy is achieved in tumours that harbour BRAF codon 600 mutations.

Molecular Pathology performs the ROCHE cobas BRAF real-time PCR assay intended for the identification of mutations in codons V600E of the BRAF gene in DNA derived from human melanoma tissue. A minimum of 50% tumour burden is required for the successful analysis of the BRAF gene.

Some requests are reflexed based on diagnostic criteria and requests from clinicians are accepted via e-mail to mtw-tr.molpathology@nhs.net . BRAF Reports are available as standalone reports on Telepath.

4. Molecular Testing for Targeted Treatments in Breast and Gastric Cancers

The human epidermal growth factor HER-2/*neu*/c-*erb*B-2 (HER-2) is amplified in approximately 15% of invasive breast cancers. HER-2 status is of interest due to a number of targeted therapies in breast cancer. HER-2 positivity is a pre-requisite for the humanised monoclonal antibody Trastuzumab (Herceptin ™) and other HER-2 dependent therapies to be prescribed to patients. All invasive breast carcinomas are reflexed for a HER-2 at diagnosis.

Advanced gastric/GOJ cancers benefit from the administration of Herceptin in patients shown to over-express the protein due to gene amplification. Trastuzumab, in combination with cisplatin and capecitabine or 5-fluorouracil, is recommended as an option for the treatment of people with HER-2 positive metastatic adenocarcinoma of the stomach or gastro-oesophageal junction not previously having received treatment for their metastatic disease or have tumours expressing high levels of HER-2. HER-2 in gastric carcinomas are performed on clinical request only

a. Her-2 immunohistochemistry analysis

The antibody clone 4B5 from Roche is used on all cases and is scored using a semi-quantitative system. The majority will be reported as negative or positive on the immunohistochemistry results. Those falling into the equivocal category of 2+ are reflexed to be further assessed using Fluorescent in-situ hybridisation. An interim report is issued in these circumstances.

b. Fluorescent in-situ hybridisation (FISH) analysis

Fluorescent in-situ hybridisation (FISH) is carried out 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)

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 by UK National Coordinating Committee for Breast Pathology Group

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. Request Form

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
Speciality:		
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*