## Lynch Syndrome testing of Colorectal Cancer

*Colorectal cancer (CRC)* refers to cancer in the bowel and rectum. It is the 4th most common cancer in Wales, with 2,200 people being diagnosed every year.

Testing colorectal tumours using either microsatellite instability (MSI) or immunohistochemistry (IHC) testing for mismatch repair (MMR) proteins can identify people in which the cancer may have occurred because of Lynch syndrome.
Lynch syndrome, also known as hereditary non-polyposis colorectal cancer (HNPCC), is a type of inherited cancer syndrome associated with a genetic predisposition to different cancer types. This means people with Lynch syndrome have a higher risk of certain types of cancer.

After a diagnosis of Lynch syndrome, for some cancer sites, risk-reducing strategies can be offered to prevent or allow early diagnosis of associated cancers.

Testing all colorectal cancer patients in Wales may increase the detection of Lynch Syndrome. These families could then benefit from cascade genetic testing to identify further family members with Lynch syndrome. These individuals can be offered appropriate surveillance. A consequent improvement in patient outcomes is anticipated through earlier diagnosis and treatment.

Colorectal Cancer Multi-Gene Panel Molecular Analysis is also available in the laboratory for colorectal cancer patients; please see separate information sheet for sample requirements and testing information.

## Links for further information

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| Orphanet | www.orpha.net |
| EDDNAL | www.eddnal.com |
| OMIM | www.omim.org |
| Genetic Test Registry | www.ncbi.nlm.nih.gov/gtr |
| Cancer Research UK | www.cancerresearchuk.org |
| Bowel Cancer UK | www.bowelcanceruk.org.uk |

## All Wales Genomics Laboratory (AWGL)
Phone: 02920 742 641 Fax: 029 2074 4043
Email: lab.genetics@wales.nhs.uk Website:
http://www.wales.nhs.uk/AWMGS

## Microsatellite Instability Testing

All colorectal cancer (CRC) tumour samples with >30% tumour nuclei content are tested for microsatellite instability (MSI).

Microsatellite instability (MSI) of tumour tissue is assessed by analysis of five mononucleotide markers. A sample is reported as microsatellite unstable when >39% (at least 2 out of 5) microsatellite markers show instability.
Most Lynch syndrome colorectal tumours and some sporadic tumours exhibit microsatellite instability. If no instability is identified then no further analysis is necessary; the tumour is considered sporadic and the likelihood of association with Lynch syndrome is reduced.

### Immunohistochemistry (IHC) testing

All colorectal cancer (CRC) tumour samples with <30% tumour nuclei content have immunohistochemistry (IHC) testing for the mismatch repair (MMR) proteins performed in the Cellular Pathology Department. The test analyses the protein expression of MLH1, MSH2, PMS2 and MSH6.

Tumours from people with Lynch Syndrome are likely to demonstrate loss of mismatch repair protein expression. The pattern of loss observed can provide information about which gene is not functioning properly.

The IHC result can provide information about the likelihood of Lynch Syndrome and direct testing towards specific gene(s).

### BRAF V600E variant Testing

If instability (or loss of MLH1) is detected the tumour is tested for the presence of BRAF V600E.

BRAF V600E is detected by PCR amplification of codons 599, 600 and 601 of the BRAF gene followed by pyrosequencing analysis.

If the BRAF V600E variant is detected then the tumour is likely to be a sporadic (non-Lynch syndrome) tumour.

### MLH1 Promoter Methylation Testing

Tumours that show Microsatellite instability (MSI) and/or loss of MLH1 protein expression and no BRAF V600E variant can be tested for MLH1 methylation.

Bisulphite pyrosequencing of the MLH1 promoter region to detect methylated cytosines in tumour DNA detects the methylation status of the MLH1 promoter region.

Tumours without the BRAF V600E mutation but with MLH1 methylation are usually sporadic (not Lynch-associated). However, MLH1 methylation can be constitutional and even inherited therefore analysis of DNA from blood or normal tissue to investigate this possibility will be suggested.

## Testing Outcome

If the results indicate the tumour is sporadic (not Lynch- associated) no further Lynch testing is recommended. However, if these patients fulfil the criteria for referral to clinical genetics they should still be referred.
If the results indicate that Lynch Syndrome is likely then a referral to Clinical Genetics is required and gene screening may be offered.

## Information for requesters

All requests for testing should be made on an appropriate request form available on the AWGL website http://www.wales.nhs.uk/AWMGS. This request form should be sent with the FFPE sample (details below) and a copy of the pathology report to:

**All Wales Genomics Laboratory, Institute of Medical Genetics, University Hospital of Wales, Heath Park, Cardiff CF14 4XW**

Please contact the laboratory for pricing and further information details for:

- non-NHS patients
- testing of NHS patients outside of current government funding (e.g. WHSSC commissioned genetic testing in Wales or NHS England commissioning)
- research purposes

## Sample Requirements for Genetic testing/ IHC testing

- Unstained sections:
  - **If >30% tumour**: 8 x 4 micron sections (air-dried) mounted on slides
  - **If <30% tumour**: 4 x 4 micron sections (heated for 1 hour at 60°C or overnight at 37°C) mounted on charged slides
- 1 x 5 micron H&E stained slide with tumour area highlighted
- Pathology report

## Information

- Paraffin-embedded tumour tissue (FFPE) slides should be selected with the maximum quantity of viable tumour.
- Please label samples with three identifiers and date of collection.
- Where possible, the FFPE slides should be accompanied by the relevant histology report.
- All samples must be accompanied by a tumour request form: http://www.wales.nhs.uk/AWMGS

***NOTE:*** *Consent for genetic testing and DNA storage is assumed when a test request and samples are received.*

MSI results will be reported to the named healthcare professional/s on the request form within a target turnaround time of 14 calendar days. Follow-up BRAF/MLH1 methylation results are reported within a target turnaround time of 28 calendar days.