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Service information: Hereditary Non-Polyposis Colorectal Cancer (HNPCC)/ Lynch Syndrome

Gene/Locus: ***MLH1, MSH2, MSH6 & PMS2***
OMIM: ***120436 (*MLH1*), *609309 (*MSH2*), *600678 (*MSH6*), *600259 (PMS2)**

Referrals: Accepted from **Clinical Geneticists** only

Testing: Diagnostic testing offered in clinically affected patients, presymptomatic testing in individuals with a family history of HNPCC/Lynch syndrome and a familial pathogenic or likely pathogenic variant.

Target Reporting Times:

• Diagnostic screening	56 calendar days
• MLPA only diagnostic	28 calendar days
• Predictive testing for known pathogenic/likely pathogenic variants	14 calendar days
• Confirmatory testing for known pathogenic/likely pathogenic variants	28 calendar days

Test Details:

- **Diagnostic screening for HNPCC:** Next generation sequencing (NGS) of all coding sequence and intron/exon boundaries of *MLH1*, *MSH2, MSH6* and *PMS2*. Confirmations and repeats tested using Sanger sequencing.
- **Testing for known pathogenic and likely pathogenic variants** in individuals with a family history of Lynch Syndrome/HNPCC, by Sanger sequencing or MLPA, as appropriate.

Service Details:

Based on in-house test validation the analytical sensitivity of NGS testing is estimated to be greater than 98.6%. Diagnostic screening of the *MLH1*, *MSH2, MSH6* and *PMS2* genes for sequence variants and *MLH1*, *MSH2, MSH6, PMS2* and *EPCAM* copy number variants (CNVs) is predicted to detect pathogenic variants with high sensitivity in HNPCC/Lynch syndrome. However, if a pathogenic or likely pathogenic variant is not detected by the testing procedure, a diagnosis of HNPCC/Lynch syndrome cannot be excluded, as the patient may have a mutation in another gene or in a region of the gene(s) not covered by the above analysis. Please note that due to the presence of numerous pseudogene versions of the *PMS2* gene, the possibility that the *PMS2* result reflects that of a pseudogene cannot be excluded, particularly for exons 11 to 15 by sequencing and exons 12 to 15 by MLPA (van der Klift *et al* 2010, Hum. Mut., 31:578-587).

MLPA testing: Dosage analysis only by MLPA can be offered for the following genes: *MLH1/MSH2/EPCAM* (P003), *MSH2* (P072) and *PMS2* (P008)

Sample Requirements:

- **EDTA** blood sample (≥4 ml), labelled with patient's **full name**, **date of birth** and **NHS number**, or genomic DNA (≥5 μg at ≥50 ng/μl). Please send two separate blood samples for presymptomatic testing.
- Samples should be accompanied by a **fully** completed referral card which should include the patient's full name, date of birth and NHS number.
- Please also include details of the gene(s) to be tested, relevant clinical details and full details of the referring clinician and centre.
- Familial positive control samples are required for presymptomatic testing, if available.

Consent:

Please note that in submitting a sample, it is the responsibility of the clinician to ensure that consent has been taken i) for testing, ii) for storage, and iii) for the use of this sample and the information generated to be shared with the patient's relatives and their health professionals. Following testing, a sample of the patient's DNA may also be used anonymously to validate new tests and for internal quality control purposes.

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NGS Testing information.

NGS of all coding sequence and intron/exon boundaries (-30 to +15) of selected genes using a Sophia Genetics Custom HCS Panel and Illumina MiSeq platform. Data analysis using MiSeq Reporter software for FASTQ file generation followed by Sophia DDM for alignment of reads to genome build GRCh37, coverage analysis and variant calling.

The target region is covered at a minimum depth of 30x. Thresholds for variant calling: ≥15% total reads for indels and ≥20% total reads for single nucleotide variants (SNVs). Based on in-house test validation the analytical sensitivity of this testing is estimated to be greater than 98.6%. Variant confirmations and repeats are performed using Sanger sequencing. Copy number variation (CNV) confirmation by multiplex ligation-dependent probe amplification (MLPA).

If information on the coverage of individual genes is required, please contact the laboratory. Variants will be classified based on the ACMG variant interpretation guidelines (Richards et al (2015) Genet Med. 17:405-24; Ellard et al (2017) ACGS Best Practice Guidelines for Variant Classification 2017).Sequence variants defined by the laboratory as benign or likely benign variants are recorded but not reported. In some cases variants of uncertain clinical significance with no convincing evidence for pathogenicity may not be reported. NGS sequence data on other genes on the Sophia Genetics Custom HCS Panel that are not requested at the time of referral will be stored but not analysed.

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