Test Information Sheet

MLH1, MSH2 and MSH6

Genomic Diagnostics (GD)

Requirements for Testing:

- Peripheral blood (into EDTA tube) (2x9mL for full screen, 9mL for Predictive test), one buccal swab (supplied by GD) or high quality DNA (A260/280>1.7) (2ug for full screen and 0.5ug for Predictive test). [1]
- Test Request Form must be completed by referring clinician.

  Note: To optimize the accuracy of the personalised Cancer Risk and Management Tool for the patient, please complete entire form to avoid delays.
- Copy of patient's consent for the analysis.
- Completed payment form (for private funded tests only). Testing can only commence once a payment has been made

Description of Analysis

HNPCC Test:
This test includes the mutation analysis of the MLH1, MSH2, MSH6 genes.

Analysis consists of sequencing of all exons and adjacent intronic regions and large rearrangement (LR) testing of the MLH1, MSH2, MSH6 genes by multiplex ligation-dependent probe amplification (MLPA). Sequence analysis identifies mutations in all 19 exons and ~560 non-coding base pairs of MLH1, all 16 exons and ~480 non-coding base pairs of MSH2, all 10 exons, ~300 non-coding base pairs of MSH6.

The large rearrangement (LR) analysis is performed with Multiplex Ligation-dependent Probe Amplification (MLPA) Assay where the amplification products are separated by capillary electrophoresis. As a control, a number of probes for other human genes located on different chromosomes are included. Apparent deletions/duplication of a single/multiple exon(s) are confirmed by reanalysing the DNA from the original specimen in duplicate. To avoid the misinterpretation of the MLPA results due to close proximity of a mutation and/or polymorphisms to the probe ligation site, the apparent deletions (probe annealing site and nearby region (50bp)) detected by a single probe is always sequenced.

Single Gene Analysis: DNA sequence analysis and large genomic rearrangements analysis for a specified gene (MLH1 or MSH2 or MSH6).

Single Site Analysis (Predictive Testing): DNA sequence analysis (or MLPA analysis) for a specified mutation in MLH1 or MSH2 or MSH6 gene.

Description of Method:

DNA is extracted and purified from white blood cells or buccal cells isolated from each sample. Aliquots of patient DNA are each subjected to polymerase chain reaction (PCR) amplification. The amplified products are each directly sequenced in forward and reverse directions using fluorescent dye direct Sanger sequencing protocol. Chromatographic tracings of each amplicon are analysed by a proprietary computer-based review followed by visual inspection and confirmation.

Genetic variants are detected by comparison with a consensus wild-type sequence constructed for each gene. All potential mutations and genetic variants of unknown clinical significance are independently confirmed by repeated PCR amplification of the indicated gene region(s) and sequence determination as above.

For Single locus specific mutation analysis (Predictive Testing), the duplicate PCR reactions are performed and the PCR products are analysed by direct DNA sequencing. Controls for normal and mutated sequences (where available) are co-analysed for QA purposes.

Genomic rearrangements are detected by Multiplex Ligation-dependent Probe Amplification (MLPA) Assays. MLPA reactions result in a very reproducible gel pattern with fragments ranging from 130 to 490 bp. MLPA probes are able to discriminate between sequences that differ in only one nucleotide. Comparison of this gel pattern to that obtained with a control sample indicates which sequences show an aberrant copy number. Heterozygote deletions of probe recognition sequences should give a 35-50% reduced relative peak height of the amplification product of that probe. Alternatively, heterozygote duplication of probe recognition sequences should give a 35-50% enlarged relative peak area of the amplification product of that probe. The patient's DNA sample is tested in duplicate and compared with positive and negative controls for single/multiple exon deletions and duplications. To avoid the misinterpretation of the MLPA results due to close proximity of a mutation and/or polymorphisms to the probe ligation site, the apparent deletions (probe annealing site and nearby region (50bp)) detected by a single probe is always sequenced.

Performance Characteristics:

Analytic specificity: The incidence of a false report of a genetic variant or mutation resulting from technical error is considered negligible because of independent confirmation of all genetic variants (see above). The incidence of a false report of a genetic variant or mutation resulting from errors in specimen handling and tracking is estimated from validation studies to be less than one percent (<1%). Confirmation testing using a second blood sample for all mutations identified is recommended.

Analytic sensitivity: Failure to detect a genetic variant or mutation in the analysed DNA regions may result from errors in specimen handling and tracking, amplification and sequencing reactions, or computer-assisted analysis and data review. The rate of such errors is estimated from validation studies to be less than one percent (<1%).

Overall test accuracy: For a patient with at least a 10% probability of a positive test result based on a personal or family history of cancer.

Limitations of method: There may be limited portions of genes for which sequence determination can be performed only in the forward or reverse direction. Unequal allele amplification may result from rare polymorphisms under primer sites.

Interpretive Criteria:

"Positive for a deleterious mutation": Includes all mutations

[1] *For some test requests an alternative source of genetic material can be accepted for testing (please contact Genomic Diagnostics for details)*

(nonsense, insertions, deletions) that prematurely terminate ("truncate") the protein product of MLH1at least ten amino acids from the C-terminus, or the protein product of MSH2 at least 46 amino acids from the C-terminus (based on documentation of deleterious mutations in MLH1 and MSH2). In addition, specific missense mutations and non-coding intervening sequence (IVS) mutations are recognized as deleterious on the basis of data derived from linkage analysis of high risk families, functional assays, biochemical evidence and/or demonstration of abnormal mRNA transcript processing.

"Genetic variant of uncertain significance": Includes missense mutations and mutations that occur in analysed intronic regions whose clinical significance has not yet been determined, as well as chain-terminating mutations that truncate MLH1, MSH2 and MSH6 distal to amino acid positions 756, 934 and 1360, respectively. A genetic variant of uncertain significance in either MLH1 or MSH2 or MSH6 is considered to be less likely to be deleterious if it has been observed in one or more individuals with a known deleterious mutation in the same gene. The specific evidence supporting such an interpretation will be summarized for individual variants on each such report.

"No deleterious mutation detected": Includes non-truncating genetic variants observed at an allele frequency of approximately 1% of a suitable control population (providing that no data suggest clinical significance), as well as all genetic variants for which published data demonstrate absence of substantial clinical significance. Also includes mutations in the protein-coding region that neither alter the amino acid sequence nor are predicted to significantly affect exon splicing, and base pair alterations in non-coding portions of the gene that have been demonstrated to have no deleterious effect on the length or stability of the mRNA transcript. There may be uncommon genetic abnormalities in MLH1, MSH2, MSH6 genes that will not be detected by HNPCC test (see "Limitations of method", above). These analyses, however, is believed to rule out the majority of abnormalities in these genes, which are believed to be responsible for most hereditary nonpolyposis colorectal cancer (HNPCC). Data on polymorphic variants are available upon request.

"Specific variant/mutation not identified": Specific and designated deleterious mutations or variants of uncertain clinical significance are not present in the individual being tested. If one (or rarely two) specific deleterious mutations have been identified in a family member, a negative analysis for the specific mutation(s) indicates that the tested individual is at the general population risk of developing those cancers associated with HNPCC.

Change of interpretation and issuance of amended reports: If and whenever there is a change in the clinical interpretation of a specific reported variant, an amended test report will automatically be provided by Genomic Diagnostics.

Description of Nomenclature:

In the Human Genome Variation Society (HGVS) standard nomenclature (http://www.hgvs.org) the nucleotide numbering is in relation to the translation initiation codon, starting with number 1 at the A of the ATG translation initiation codon. The GenBank Accession numbers for MLH1, MSH2 and MSH6 genes are listed below:

	Genomic	Transcript	Protein
MLH1	NG_007109.1	NM_000249.3	NP_000240.1
MSH2	NG_007110.2	NM_000251.2	NP_000242.1
MSH6	NG_007111.1	NM_000179.1	NP_000170.1

Quality Assurance:

All tests are performed according to the AS/ISO15189 standard in a NATA and RCPA accredited laboratory. Accreditation No: 19619.