

Assay Summary

Somatic *MLH1* Promoter Methylation Analysis

Synopsis

MLH1 (MIM:120436) is a mismatch repair protein that, together with *PMS2* (MIM:600259), facilitates binding of other protein effectors of DNA repair.¹ Inactivation of both alleles of *MLH1* in tumor typically leads to microsatellite instability (MSI) in its target genes following DNA polymerase slippage errors,²⁻³ as well as to abnormal immunohistochemical staining (IHC) for *MLH1* and/or *PMS2*.

In general, MSI is observed in approximately 90% of cases of hereditary non-polyposis colorectal cancer (HNPCC) and in about 15% of sporadic colorectal cancer cases.⁴ MSI is usually a consequence of somatic *MLH1* promoter methylation.⁵ Therefore, as with *BRAF* V600E mutation, with which it is strongly correlated, *MLH1* promoter methylation testing helps to identify sporadic rather than hereditary microsatellite-unstable tumors,^{2,6} and, where indicated, guide further testing. A recent study of familial colorectal tumors with MSI and/or loss of mismatch repair protein expression demonstrated that *MLH1* promoter hypermethylation screening on HNPCC tumor biopsies outperforms *BRAF* mutation analysis in both analytical performance and cost-effectiveness.⁴

It is important to note that the analysis of the results of *MLH1* promoter methylation requires careful consideration of family and clinical histories. One reason is that up to 1% of HNPCC cases appear to be caused by constitutional *MLH1* epimutation.^{5,7} Secondly, *MLH1* promoter hypermethylation may represent a second hit, co-occurring with a germline mutation.^{4,8} Therefore, a diagnosis of HNPCC cannot be eliminated on the basis of a positive *MLH1* promoter hypermethylation result alone.*

* For more information on related tests offered at our laboratory please visit the following links:

- *BRAF* V600E mutation analysis: <https://www.cityofhope.org/clinical-molecular-diagnostic-laboratory/list-of-cmdl-tests/braf-ngs>
- HNPCC testing: <https://www.cityofhope.org/clinical-molecular-diagnostic-laboratory/list-of-cmdl-tests/hnpcc-dna-testing>

Indications for testing

- Symptoms suggestive of HNPCC history and absent *MLH1*/*PMS2* expression
- Sporadic colorectal cancer and absent *MLH1*/*PMS2* expression
- High MSI

Methodology

The MS-MLPA (Methylation sensitive multiple ligation-dependent probe amplification) enables detection of *MLH1* promoter and intron 1 methylation by providing 6 probes (kit ME011-B1; MRC-Holland, Amsterdam, The Netherlands) that harbor digestion sites for the methylation-sensitive *HhaI* endonuclease. The results are generated by comparing an undigested and an *HhaI*-digested sample from the same patient according to manufacturer's instructions. A probe signal is generated only in the presence of methylated target CpG islands in the patient's *MLH1*, as these are unavailable for digestion by *HhaI*. The MS-MLPA products are analyzed by DNA fragment analysis on an automated fluorescent sequencer (ABI 3730 DNA sequencers, Applied Biosystems).

Performance

MS-MLPA is a robust, clinically-tested method, with acceptable analytical performance on DNA obtained from formalin-fixed, paraffin-embedded tissue.⁴ Published reports suggest analytical sensitivity of MS-MLPA to be 10% and intra/inter-experiment variability within 1%.⁴ Absence of hypermethylation translates into a sensitivity of 57-100%^{4,6,9} and specificity of 66% for HNPCC.⁴ The specificity and sensitivity of the *MLH1* promoter hypermethylation testing for sporadic tumors is reported at 66% and 96%, respectively.⁴ Internal validation studies indicate that a methylation value cut-off of 30% in one target CpG island or 15% or higher in at least two *MLH1* promoter CpG islands provides acceptable sensitivity and specificity.

Limitations of MLPA

MS-MLPA probes selected CpG islands only. Moreover, a single HhaI site methylation status may not be representative of the status of the entire CpG island. Furthermore, some probe signals may be more sensitive to sample purity and experimental conditions. Probe signals may also be affected by mutations and/or polymorphisms situated in the vicinity of, or at the probe ligation site. Interpretation of test results should be conducted in the context of the patient's ethnicity, clinical and family histories, and other laboratory test results.

Specimen Requirements

We prefer to receive paraffin embedded tissue sample block. If blocks cannot be sent, please send six slides of tumor sample (5-micron serial sections, five unstained and one H/E stained). Ensure that the slides are clearly labeled with the patient name or identifier and date of birth and type of sample. Place slides in appropriate containers to ensure against breakage. Alternatively, the paraffin blocks may be submitted. Please include a copy of the pathology report.

Test Request Form (TRF)

- a) A completed CMDL [TRF](#) is required for each specimen. Please submit the completed TRF with the specimen. Complete testing and billing information must be provided before the specimen is processed.
- b) [HNPCC Patient Information Form](#): Include a completed HNPCC Patient Information, if applicable.

<i>Order Codes</i>	<i>CPT Codes</i>	<i>TAT</i>
MLH1-METH (MLH1 gene, Somatic Promoter Methylation analysis)	81288, 88381, G0452	2-3 wks

References

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4. Gausachs et al. (2012). Eur J Hum Genet. 2012 Jan 25. doi: 10.1038/ejhg.2011.277.
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6. Leite et al. (2010) Mol Biol Rep. 37(1):375-80.
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