BRAF V600E Mutation and *MLH1* Promoter Methylation Detection in Colon Cancer

FOR DIFFERENTIATING SPORADIC FROM INHERITED FORMS OF MICROSATELLITE UNSTABLE COLORECTAL CANCERS

Test Highlights

- Detects the *BRAF* V600E mutation and *MLH1* promotor methylation in colorectal tumors.
- Indicates whether further work-up for hereditary non-polyposis colorectal cancer (HNPCC) is recommended.

Disease Overview

- In the United States, colorectal cancer is the third most common form of cancer; individuals have a 6 percent lifetime risk of developing this disease.
- Most colorectal cancer is sporadic and is not hereditary.
- Microsatellite instability (MSI) testing by PCR and immunohistochemistry is performed on colorectal tumors to determine the likelihood of the individual having HNPCC.
- Approximately 15 percent of colon tumors have MSI but only about 10 percent of those are actually caused by germline HNPCC mutations.
- *BRAF* is a kinase-encoding gene in the RAS/RAF/MAPK pathway. The presence of a *BRAF* V600E mutation in a microsatellite unstable tumor indicates that the tumor is probably sporadic and not associated with hereditary non-polyposis colorectal cancer (HNPCC). The lack of this mutation indicates that a tumor may either be sporadic or HNPCC-associated.

Indications for Ordering

Colorectal tumors with microsatellite unstablility by PCR and/or lack of *MLH1* immunostaining.

Interpretation

- If either the *BRAF* V600E mutation or *MLH1* promoter methylation is found in a microsatellite unstable tumor, then the tumor is probably sporadic and further work-up for HNPCC may not be warranted.
- If no *BRAF* mutation or *MLH1* methylation is found, then the tumor may be either sporadic or inherited, and further work-up for HNPCC is recommended.
- Since there rare reports of either *BRAF* mutation or *MLH1* methylation in HNPCC-associated tumors, all results should be interpreted within the clinical context.

Limitations

- Mutations other than the *BRAF* V600E mutation will not be detected.
- Rare diagnostic errors may occur due to primer- or probe-site mutations.
- The presence of less than 10–20 percent of a mutant allele may not be detected.
- Methylation at locations other than those covered by the primers and probes will not be detected.
- Methylation levels less than 10 percent are not reported.
- Results of these tests should always be interpreted within the clinical context and other relevant data, and should not be used alone for a diagnosis of malignancy.
- These tests are not intended to detect minimal residual disease.

Methodology

- Slides are prepared from sections of paraffin-embedded tissue blocks.
- DNA is isolated from tumor tissue that is microdissected from prepared slides.
- Exon 15 of the *BRAF* gene is amplified using polymerase chain reaction (PCR), followed by probe melting curve analysis to determine mutation status.
- If the sample is negative for the *BRAF* V600E mutation, it is reflexed to *MLH1* methylation testing. The DNA is treated with sodium bisulfite, followed by amplification of a segment of the *MLH1* promoter region using methylation-specific real-time PCR. The *MLH1* methylation level is calculated by comparison to the amplification of a reference gene.

Related Tests

- HNPCC/Lynch Syndrome, Microsatellite Instability by PCR (0051740)
- Microsatellite Instability by Immunohistochemical Stain (0049302)

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References

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2. Samowitz WS, et al. Evaluation of a large, population-based sample supports a CpG island methylator phenotype in colon cancer. *Gastroent* 2005;129:837–45.
3. Weisenberger DJ, et al. CpG island methylator phenotype underlies sporadic microsatellite instability and is tightly associated with BRAF mutation in colorectal cancer. *Nat Genet* 2006;38:787–93.

Test Information

0051750	***BRAF* V600E mutation with reflex to *MLH1* promoter methylation, paraffin**
0049001	***BRAF* V600E Detection of Colon Cancer (Unstable Tumors) by PCR**
2002327	**Microsatellite Instability by IHC with Reflex to *BRAF* V600E Mutation & *MLH1* Promoter Methylation**

For specific collection, transport, and testing information, refer to the ARUP Web site at www.aruplab.com.

For information on test selection, ordering, and interpretation, refer to ARUP Consult® at www.arupconsult.com.