

Assay Summary

Somatic BRAF Genotyping BRAF Gene, V600E Mutation Analysis

Synopsis

The BRAF gene encodes a serine/threonine kinase protein involved in the mitogen-activated protein kinase signaling pathway (MAPKs). BRAF somatic mutations are found in approximately 15% of colorectal cancers (CRC)^{1,2} and approximately 45% of papillary thyroid carcinomas (PTC)³. The most common alteration of the BRAF gene is a thymidine to adenine change at nucleotide 1799, resulting in an amino acid change from valine to glutamic acid at codon 600 (V600E). This alteration has been shown to activate the kinase activity of the BRAF protein by simulating phosphorylation⁴.

The V600E BRAF mutation has been associated with resistance to Panitumumab or Cetuximab in metastatic colorectal cancer. One publication reported that none of their BRAF positive cases responded to therapy ($p=0.029$), and that these same individuals had a shorter progression-free survival ($P=0.022$) and overall survival ($p<0.0001$) compared to wild-type². This mutation has also been associated with CRCs having high microsatellite instability, but not linked with Hereditary Nonpolyposis Colorectal Cancer (HNPCC)^{1,4}, suggesting that V600E positive cases may not require testing of the mismatch repair genes associated with HNPCC (MLH1, MSH2, MSH6, and PMS2).

The vast majority of thyroid carcinomas are the papillary type. Disease recurrence after surgical treatment is common. The extent of initial surgical resection is determined by balancing the risks of recurrence with the risks of adverse outcomes, though reliable pre-operative indicators for recurrence have not been readily available. Recent studies have demonstrated a strong association with BRAF V600E and aggressive forms of primary PTC. One study found that BRAF V600E, detected by fine needle aspiration biopsy, strongly predicted extrathyroid extension ($p=0.039$), thyroid capsular invasion ($p=0.045$), and lymph node metastasis ($p=0.002$)⁵. Therefore, BRAF mutation status may provide additional information in prognosticating PTC's and how aggressively they should be treated.

Indications for testing

Individuals with a diagnosis of CRC prior to initiating Panitumumab or Cetuximab therapy. Individuals with a MSI-high CRC, where there is uncertainty about the case being sporadic or HNPCC. Newly diagnosed thyroid cancers where additional prognostic information is desired.

Methodology

BRAF gene, V600E: A real-time PCR assay for the c.1799 T>A (V600E) mutation in the BRAF gene has been optimized, and validated by the Molecular Diagnostic Laboratory (MDL). Testing is performed on micro-dissected cells from formalin fixed, paraffin embedded tissue/cell block.

Performance

The sensitivity of this assay can detect down to 1 in 100 mutation-bearing cells in a micro-dissected area. This method will not detect mutations other than the one listed above.

Specimen Requirements

BRAF gene, V600E: We prefer to receive paraffin embedded tissue samples, and also accept cell blocks obtained from fine needle aspirations. Cell blocks with limited cellularity may not be sufficient for testing. 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 completed MDL [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.

<i>Order Codes</i>	<i>CPT Codes</i>	<i>TAT</i>
BRAF-V600E (BRAF Gene, V600E Mutation Analysis)	83890, 88323, 88381, 83892, 83898, 83909, 83912	1 wk

References

1. Kadiyska, T. K. et al., (2007) *Can Det and Prev*; 31:254-256
2. Nicolantonio, F. D. et al., (2009) *J Clin Onc*; 26:5705-5712
3. Chiosea, S. et al., (2009) *Endo Path*; 20:122-126
4. French, A. J. et al., (2008) *Clin Can Res*; 14:3408-3415
5. Xing, M. Et al., (2009) *J Clin Onc*; 27:2977-2982

NOTE: This test is performed pursuant to a license agreement with Roche Molecular Systems, Inc.