

Molecular Genetic Testing For BRAF Mutations

Tests Available:

- BRAF V600E by real-time PCR
- BRAF V600E mutation only by Sanger sequencing
- BRAF full gene sequence analysis by Sanger sequencing

BRAF encodes a protein kinase that activates the MAP kinase/ ERK-signaling pathway. Activating mutations in *BRAF* play a role in oncogenesis in many different cell types. The most common *BRAF* mutation, V600E (c.1799 T>A), is particularly prevalent in malignant melanoma, papillary thyroid and colorectal cancers. In addition, patients with Langerhans cell histiocytosis commonly have this *BRAF* V600E mutation. Testing for this specific mutation is of critical therapeutic importance for these groups of patients.

Clinical Sensitivity:

Langerhans cell Histiocytosis: The *BRAF* V600E mutation has been reported in approximately 57% of Langerhans cell histiocytosis (LCH) lesions. The high prevalence of *BRAF* mutations in LCH indicates that LCH is a neoplastic disease that may respond to RAF pathway inhibitors. The presence or absence of the *BRAF* V600E mutation in a tumor alone does not confirm or rule out a diagnosis of LCH; the results should be correlated with clinical findings and histopathologic features. Somatic mutations in *MAP2K1* were recently described in approximately 50% of *BRAF*-negative LCH patients [Brown et al. 2014]. *MAP2K1* sequence analysis is indicated as a follow-up test in LCH patients with normal *BRAF* V600E test results, in order to direct appropriate therapy.

Malignant Melanoma: Activating mutations in *BRAF* are present in approximately 50-60% of malignant melanomas, the majority of which are V600E. Multiple studies have shown that patients with V600E positive tumors have overall improved response rates and progression-free survival with kinase inhibitor-directed

therapy. Testing whether *BRAF* mutation exists in melanoma is therefore of critical therapeutic importance. Full sequencing of *BRAF* may be indicated as a follow-up test in patients with malignant melanoma and normal *BRAF* V600E test results, in order to identify additional *BRAF* mutations that may respond to specific therapy. Somatic mutations in other genes including *CTNNB1*, *GNA11*, *KIT*, *MAP2K1* and *NRAS* [Yaman et al. 2014], as well as *TERT* promoter [Griewank et al. 2014] have also been reported in malignant melanoma, and additional genetic testing may be warranted in order to optimize treatment or modify prognosis.

Papillary Thyroid cancer: The *BRAF* V600E mutation is present in 18-87% of papillary thyroid cancers. It is associated with an aggressive tumor phenotype and predicts a poorer disease outcome [Xing et al. 2014]. Testing for this mutation may be useful for selecting initial therapy [Dadu et al 2014].

Metastatic colorectal cancer: The *BRAF* V600E mutation is found in approximately 14% of metastatic colorectal cancer (mCRC). At least one-half of *BRAF* mutations associated with mCRC are V600E. The presence of a *BRAF* mutation in mCRC has been reported to render the tumor resistant to anti-*EGFR* therapy. Full sequencing of *BRAF* may be indicated as a follow up test in patients with mCRC and normal *BRAF* V600E test results, in order to rule out other *BRAF* mutations that could result in drug resistance. Additional testing of other genes including *KRAS* may be warranted, in order to determine optimal therapy [Evaluation of Genomic Applications in Practice and Prevention (EGAPP) Working Group (2013)].

Other solid tumors: *BRAF* mutations have been found in many other cancer types, including lung cancer, sarcoma, glioma, ependymoma, non-Hodgkin lymphoma, acute lymphoblastic leukemia, liver cancer, stomach cancer, breast and ovarian cancer, and esophageal cancer, although at a low frequency. The clinical implication and utility of *BRAF* gene mutation testing in these types of cancers remain to be determined.

Available Tests and Analytical Sensitivities:

- The *BRAF* V600E by real-time PCR test uses a TaqMan® Mutation Detection Assay to detect the V600E mutation in exon 15 of *BRAF* in tumor (somatic) cells. The sensitivity of the TaqMan assay is ~0.2% mutant DNA in a wild-type background. Poor DNA quality, insufficient DNA quantity or the presence of PCR inhibitors can result in uninterpretable or (rarely) inaccurate results.
- The *BRAF* V600E mutation only by Sanger sequencing uses a DNA-based PCR-sequencing assay to detect the V600E in exon 15 of *BRAF*. The limit of detection for Sanger sequencing is > 20% mutant DNA in a wild-type background.
- The *BRAF* full gene sequence analysis by Sanger sequencing uses a DNA-based PCR-sequencing assay to detect point mutations in the coding sequence and intron/exon boundaries of the *BRAF* gene. The sensitivity of DNA sequencing is over 99% for the detection of nucleotide base changes, small deletions and insertions in the regions analyzed. Rare variants at primer binding sites may lead to erroneous results. The limit of detection for Sanger sequencing is >20%mutant DNA in a wild-type background.

Specimen Requirements:

Most sample types (eg. fresh/frozen, paraffin embedded tissue (PET/FFPE), blood, bone marrow, cell lines) are acceptable for *BRAF* V600E by Sanger sequencing or *BRAF* full gene sequence analysis. Only blood, bone marrow or cell line is acceptable for *BRAF* V600E by real-time PCR at this time.

Turn Around Times:

- *BRAF* V600E analysis by real-time PCR or Sanger sequencing: 7 days.
- *BRAF* full gene sequence analysis by Sanger: 21 days.

CPT Codes:

- *BRAF* V600E analysis by real-time PCR or Sanger sequencing: 81210
- *BRAF* full gene sequence analysis: 81406

Shipping Instructions:

Please enclose **test requisition** with sample. **All information must be completed before sample can be processed.**

Fresh frozen tissue should be collected in sterile cell culture media and shipped immediately on dry ice to laboratory. Place other sample types in styrofoam mailer and ship at room temperature by overnight Federal Express to arrive Monday through Saturday.

Ship to:

Laboratory of Genetics and Genomics
3333 Burnet Avenue NRB 1042
Cincinnati, Ohio 45229
513-636-4474

References:

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- Griewank, KG., R. Murali, et al. (2014) "TERT Promoter Mutation Status as an Independent Prognostic Factor in Cutaneous Melanoma." Journal of the National Cancer Institute 106(9):dju246.*
- Kirstein, M.M., A. Lange, et al. (2014) "Targeted Therapies in Metastatic Colorectal Cancer: A Systematic Review and Assessment of Currently Available Data. " Oncologist. 19(11): 1156-1168.*

Lade-Keller, KM RiiJmer, et al. (2013) "Evaluation of BRAF Mutation Testing Methodologies in Formalin-Fixed, Paraffin-Embedded Cutaneous Melanomas." *The Journal of Molecular Diagnostics* 15(1): 70-80.

Satoh, T., A. Smith, et al. (2012) "B-RAF Mutant Alleles Associated with Langerhans Cell Histiocytosis, a Granulomatous Pediatric Disease." (A. Y.W Chang, ed.) *PLoS ONE* 7(4): e33891.

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Xing, M, A. S. Alzahrani, et al. (2015) "Association Between BRAF V600E Mutation and Recurrence of Papillary Thyroid Cancer." *Journal of Clinical Oncology* 33(1):42-50.

Yaman, B., T. Akalin, et al. (2015) "Clinicopathological Characteristics and Mutation Profiling in Primary Cutaneous Melanoma." *The American journal of Dermatopathology* 37(5):389-97.