

# Molecular Genetic Testing For Langerhans Cell Histiocytosis *BRAF* (V600E) and *MAP2K1* Mutations

## Tests Available:

- *BRAF* (V600E) Genotype Analysis
- *MAP2K1* Full Gene Sequence Analysis

## Disorder: Langerhans cell histiocytosis (LCH)

is a rare disease characterized by clonal proliferation of Langerhans cells (Allen et al, 2010). LCH encompasses several disorders historically known as eosinophilic granuloma, Hand-Schuller-Christian disease, and Letterer-Siwe disease, among others. The granulomatous lesions characteristic of LCH are heterogeneous in cellular composition as well as physical distribution. The broad clinical spectrum ranges from localized disease with spontaneous remission to multisystem involvement and a 20% mortality rate (Brown et al, 2014). Common symptoms include lytic bone lesions, exophthalmos, polyuria, hepatosplenomegaly, lymphadenopathy, skin rash, and hematological compromise (Badalian-Very et al, 2011). LCH is generally considered a disease of childhood, although adults experience similar forms of disease.

**Genetics:** Although the pathophysiology of LCH remains unclear, activation of the MAPK/ERK signaling pathway appears to play a significant role (Nelson et al, 2014). A somatic activating mutation in the *BRAF* gene, V600E, is identified in approximately 60% of LCH granulomas (Badalian-Very et al, 2010). Somatic *MAP2K1* mutations have been identified in approximately 50% of *BRAF*-negative LCH samples (Brown et al, 2014). In addition, somatic mutations affecting other *BRAF* residues as well as the *ARAF* gene have recently been described (Satoh et al, 2012; Nelson et al, 2014).

**Indications:** *BRAF* V600E genotyping and *MAP2K1* sequencing are indicated in patients with suspected or confirmed LCH. Determination of the genetic mutation in LCH lesions has important implications for therapeutic treatment. *BRAF* inhibitors such as vemurafenib have been successfully used to treat LCH patients with the V600E mutation (Haroche et al, 2013). The presence or absence of one of these mutations in a tumor does not confirm or rule out a diagnosis of LCH; results from these tests should be correlated with clinical findings and histopathologic features.

## Sensitivity:

**Clinical Sensitivity:** The *BRAF* V600E mutation has been reported in 39-57% of Langerhans cell histiocytosis (LCH) lesions (Sahm et al, 2012; Badalian-Very, et al, 2010). Approximately 30% of LCH tumors have *MAP2K1* mutations (Brown et al, 2014). The combination of these two tests is expected to identify somatic mutations in 70-90% of LCH tumors.

**Analytical Sensitivity:** The TaqMan® assay used for detection of the *BRAF* V600E mutation identifies mutant DNA at a level of approximately 0.1% in a wild-type background. Sanger sequencing, used for *BRAF* V600E genotyping of FFPE tumors and *MAP2K1* sequencing, can detect somatic mutations present at a level of 20% or greater.

## Specimen Requirements:

Fresh frozen or paraffin embedded tissue (PET/FFPE) is acceptable for sequencing-based tests. Only fresh frozen tissue (no PET/FFPE samples), is acceptable for *BRAF* V600E by RT-PCR at this time. Tissue should be collected in sterile cell culture media and shipped immediately on dry ice to laboratory.

## Testing Methodology:

### **BRAF V600E:**

- The *BRAF* (V600E) mutation by Sanger sequencing uses a DNA-based PCR-sequencing assay to detect the V600E mutation.

### **MAP2K1:**

- *MAP2K1* sequencing uses PCR-based sequencing of the entire coding region and intron/exon boundaries of the *MAP2K1* gene.

## Turn-Around Times:

- **BRAF (V600E) Genotype:** 7 days
- **MAP2K1 Full Gene Sequence Analysis:** 4 weeks

## CPT Codes:

- **BRAF (V600E) Genotype: V600E:** 81210
- **MAP2K1 Full Gene Sequence Analysis:** 81406

**Cost:** Please call 1-866-450-4198 for current pricing, insurance preauthorization, or with any billing questions.

**Results:** Results will be reported to the referring physician or health care provider as specified on the test requisition.

## Shipping Instructions:

Please enclose **test requisition** with sample.

**All information must be completed before sample can be processed.**

Place samples in styrofoam mailer and ship at room temperature by overnight Federal Express to arrive Monday through Friday.

## Ship to:

Cytogenetics and Molecular Genetics Laboratories  
3333 Burnet Avenue NRB 1042  
Cincinnati, OH 45229  
513-636-4474

## References:

- Allen, C. E., Li, L., Peters, T. L., Leung, H.-C. E., Yu, A., Man, T.-K., ... McClain, K. L. (2010). Cell-specific gene expression in Langerhans cell histiocytosis lesions reveals a distinct profile compared with epidermal Langerhans cells. *Journal of Immunology (Baltimore, Md.: 1950)*, 184(8), 4557–4567. doi:10.4049/jimmunol.0902336
- Badalian-Very, G., Vergilio, J.-A., Degar, B. A., MacConaill, L. E., Brandner, B., Calicchio, M. L., ... Rollins, B. J. (2010). Recurrent *BRAF* mutations in Langerhans cell histiocytosis. *Blood*, 116(11), 1919–1923. doi:10.1182/blood-2010-04-279083
- Badalian-Very, G., Vergilio, J.-A., Degar, B. A., Rodriguez-Galindo, C., & Rollins, B. J. (2012). Recent advances in the understanding of Langerhans cell histiocytosis. *British Journal of Haematology*, 156(2), 163–172. doi:10.1111/j.1365-2141.2011.08915.x
- Brown, N. A., Furtado, L. V., Betz, B. L., Kiel, M. J., Weigelin, H. C., Lim, M. S., & Elenitoba-Johnson, K. S. J. (2014). High prevalence of somatic *MAP2K1* mutations in *BRAF* V600E-negative Langerhans cell histiocytosis. *Blood*, 124(10), 1655–1658. doi:10.1182/blood-2014-05-577361
- Haroche, J., Cohen-Aubart, F., Emile, J.-F., Arnaud, L., Maksud, P., Charlotte, F., ... Amoura, Z. (2013). Dramatic efficacy of vemurafenib in both multisystemic and refractory Erdheim-Chester disease and Langerhans cell histiocytosis harboring the *BRAF* V600E mutation. *Blood*, 121(9), 1495–1500. doi:10.1182/blood-2012-07-446286
- Nelson, D. S., Quispel, W., Badalian-Very, G., van Halteren, A. G. S., van den Bos, C., Bovée, J. V. M. G., ... Rollins, B. J. (2014). Somatic activating *ARAF* mutations in Langerhans cell histiocytosis. *Blood*, 123(20), 3152–3155. doi:10.1182/blood-2013-06-511139
- Sahm, F., Capper, D., Preusser, M., Meyer, J., Stenzinger, A., Lasitschka, F., ... von Deimling, A. (2012). *BRAF*V600E mutant protein is expressed in cells of variable maturation in Langerhans cell histiocytosis. *Blood*, 120(12), e28–34. doi:10.1182/blood-2012-06-429597
- Satoh, T., Smith, A., Sarde, A., Lu, H., Mian, S., Mian, S., ... Geissmann, F. (2012). *B-RAF* mutant alleles associated with Langerhans cell histiocytosis, a granulomatous pediatric disease. *PLoS One*, 7(4), e33891. doi:10.1371/journal.pone.0033891