**SOMATIC BRAF GENE MUTATION ANALYSIS BY**

**POLYMERASE CHAIN REACTION (PCR) AND SNPlex ASSAY**

**RESULT:**

**POSITIVE/NEGATIVE for BRAF V600E mutation**.

**INTERPRETATION:**

**BRAF V600E Mutation is/NOT detected in this specimen ( ).**

All controls show appropriate results.

BRAF encodes a protein belonging to the raf/mil family of serine/threonine protein kinases and plays a role in regulating the MAP kinase/ERKs signaling pathway, which affects cell division, differentiation, and secretion (1). The most common B-RAF mutation, a Thymidine to Adenosine transversion, converting Valine to Glutamate (V600E) in Exon 15, has been identified in malignant melanoma (27%-70%), papillary thyroid cancer (36%-53%), colorectal cancer (5%-22%) and serous ovarian and endometrium cancer (~30%). The association of the BRAF V600E mutation with prognosis in these tumors has been associated with a significantly poorer survival in microsatellite-stable colon cancers (2) and poor prognosis of papillary thyroid carcinomas when additional other gene alterations are present (3) . In addition, due to its absence in Lynch syndrome (LS), it has also been used as a guide regarding further work-up for LS (4, 5). If a BRAF V600E mutation is found (positive) in a microsatellite unstable tumor then the tumor is probably sporadic and further work-up for LS may not be warranted. If such mutation is not found (negative), then the tumor may be either sporadic or inherited, and further work-up for LS may be justified.

**METHODOLOGY:**

Genomic DNA was extracted from tumor tissue (Fresh, fresh frozen, or formalin fixed paraffin embedded tissue) and Exon 15 of the BRAF gene is amplified by Polymerase chain reaction (PCR), and subsequently a direct nucleotide sequencing by PCR-based cycle sequencing method was used to evaluate the point mutation causing BRAF V600E (see PCF Laboratories Interpretive Handbook for method details).

**LIMITATIONS:**

Mutations other than V600E mutation in BRAF gene will not be detected; furthermore, mutations within the primer regions could affect this assay. The presence of less than 5 percent to 10 percent of a mutant allele may not be detected. These tests were developed and their performance characteristics determined by the Pathology Core Facility of the Ohio State University Medical Center, 2001 Polaris Parkway, Columbus, OH. Performance characteristics refer to the analytical performance of the test. They have not been cleared by the US Food and Drug Administration. The FDA has determined that such clearance or approval is not necessary.

**REFERENCES:**

1. Robinson MJ and Cobb MH (1997) Mitogen-activated protein kinase pathways. **Curr Opin Cell Biol**, 9, 180–186.
2. Samowitz et al., 2005. Poor Survival Associated with the BRAF V600E Mutation in Microsatellite-Stable Colon Cancers. **Cancer Research**. 65:6063-6069.
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4. Domingo E, et al. 2004. BRAF screening as a low-cost effective strategy for simplifying HNPCC genetic testing. **J Med Genet**;41:664-8.

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