Somatic BRAF mutation analysis
Multiple hotspot mutations of the BRAF gene including V600E
melanoma, thyroid cancer, colorectal cancer, lung cancer

Synopsis
The BRAF gene encodes a serine/threonine kinase protein involved in the mitogen-activated protein kinase signaling pathway (MAPKs). Somatic mutations of BRAF have been estimated to occur in up to ~15% of human tumors\(^1,2\). They are most commonly found in melanomas (~50%) and papillary thyroid carcinomas (PTC, ~45%)\(^3\) but are also known to occur in other cancers including colorectal cancers (CRC, ~15%)\(^4,5\), and non-small cell lung cancer (NSCLC, ~4%)\(^6\). The most common BRAF mutation is the V600E change in exon 15 which has been shown to activate the kinase activity of BRAF by simulating phosphorylation\(^7\).

In malignant melanomas, BRAF V600E mutations have been strongly associated with sensitivity to BRAF inhibitor therapies\(^8-11\). Other studies have also associated BRAF mutation status with response to MEK inhibitors\(^12-14\).

Studies have demonstrated a strong association of BRAF V600E with aggressive forms of primary PTC\(^5\). Therefore, BRAF mutation status may provide additional information in prognosticating PTCs and how aggressively they should be treated.

In metastatic colorectal cancer (CRC), the V600E BRAF mutation has been associated with resistance to anti-EGFR therapies (panitumumab, cetuximab)\(^4\). This mutation has also been associated with CRCs having high microsatellite instability, but not in association with Lynch syndrome (Hereditary Nonpolyposis Colorectal Cancer, HNPCC)\(^3,7\), suggesting that V600E positive cases may not require testing of the mismatch repair genes associated with HNPCC (MLH1, MSH2, MSH6, and PMS2).

Indications for testing
BRAF mutation status may inform on prognosis and/or therapeutic selection for:

- melanoma
- CRC prior to initiating panitumumab or cetuximab therapy.
- MSI-high CRC, where there is uncertainty about the case being sporadic or hereditary (HNPCC)
- papillary thyroid cancer where additional prognostic information is desired

Methodology
Genomic DNA (gDNA) is extracted from micro-dissected cells from formalin-fixed, paraffin-embedded tissue. A targeted DNA library is generated using the Ion AmpliSeq\(^\text{TM}\) Cancer Hotspot Panel v2 Kit, and sequenced by semiconductor-based next-generation sequencing technology on an Ion Torrent PGM. This test targets 77 mutations within exons 11 and 15 (including V600E) of the BRAF gene.

Performance/Limitations
The gene is not sequenced in its entirety; only the regions including the targeted mutations are analyzed. The method will not detect gross genetic alterations including large deletions, duplications, and inversions. The minimum detectable mutant allele ratio is approximately 10%.
**Specimen Requirements**

**Formalin-fixed, paraffin-embedded (FFPE) tissue blocks or slides.**

The tissue sample should be large enough to provide at least 3000 tumor cells and at least 30% of tumor cells within the tissue. We prefer to receive FFPE tissue blocks (unused portion will be returned), but slides are also accepted. For slides:

- 10 slides, 10 micron serial sections, unstained, without coverslip.
- 1 representative H&E slide, 4 micron section, with a coverslip.
- If the sample is a needle biopsy or has very little tumor, please send 5 additional slides.
- Slides or blocks should be labeled with the accession number and patient name and accompanied by a copy of the corresponding pathology report.
- Place slides in appropriate container(s) to ensure against breakage.

**Test Request Form (TRF)**

A completed CMDL [TRF] must be submitted with each specimen. Complete testing and billing information must be provided before the specimen is processed.

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<th>Order Codes</th>
<th>CPT Codes</th>
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**References**