CPAL
Central **P**ennsylvania **A**lliance **L**aboratory

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BRAF V600 Mutation Test (Melanoma Only)

Contact:

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Effective Date:
November 8, 2011

Performed:
Typically set up Monday through Friday. The testing schedule may be adjusted week to week to maximize workflow efficiencies. Expected Turn-Around-Time (TAT) is 3-5 days from receipt of specimens at CPAL.

Mnemonic: BRAF MELANOMA

Method: cobas® 4800 BRAF V600 Mutation Test (FDA Approved)

Intended Use:
The **cobas®** 4800 BRAF V600 Mutation Test is an in vitro diagnostic device intended for the qualitative detection of BRAF V600E mutation in DNA extracted from formalin-fixed, paraffin-embedded human melanoma tissue. The **cobas®** 4800 BRAF V600 Mutation Test is a real-time PCR test on the **cobas®** 4800 system, and is intended to be used as an aid in selecting melanoma patients whose tumors carry the BRAF V600E mutation for treatment with vemurafenib.

Specimen (IMPORTANT):
One stained, cover slipped and marked slide and eight unstained (no cover slip) serial sections of paraffin embedded formalin fixed tissue on slides. The portion of tissue on slide to be sampled (tumor) for testing must be clearly indicated on the stained slide. An estimation of the neoplastic cell content of the selected "tumor" area **MUST** be

indicated. It is acceptable to indicate simply ≥50% neoplastic cell content or <50% neoplastic cell content. More specific estimates are acceptable.

Specimens in which no desired sampling area (tumor) and estimated neoplastic cell content is indicated will be returned to the client so that the proper region of interest and information can be indicated and resubmitted to CPAL.

Specimens should be transported and stored at room temperature.

Reference Ranges:

Not Detected (see also, **Summary** below)

Summary:

Activating mutations of the proto-oncogene BRAF occur in many human cancers, including malignant melanoma, colorectal cancer, ovarian cancer, and thyroid cancer.[1, 2] BRAF mutations have been identified in 40%-60% of malignant melanomas.[3] Mutations are also common in benign nevi,[4] suggesting that such mutations are a very early event. The discovery of such somatic mutations in the BRAF gene in melanoma and other human tumors has helped to elucidate the central role of the BRAF kinase in signaling pathways that control cellular proliferation, differentiation and cell death. In normal cells, BRAF is part of a highly regulated signaling pathway that mediates the effects of growth factor receptors (such as EGFR) through RAS, RAF, MEK and ERK. Oncogenic mutations in BRAF result in a gain of kinase function, rendering the RAF-MEK-ERK pathway constitutively active in the absence of the typical growth factors. The majority of BRAF mutations in melanoma and other human tumors occur in codon 600.[5] The predominant mutation at codon 600 is the V600E mutation (GTG>GAG). A number of dinucleotide mutations affecting codon 600 [V600K (GTG>AAG), V600R (GTG>AGG) V600E2 (GTG>GAA), and V600D (GTG>GAT)] have also been observed less commonly, primarily in melanoma and rarely in other tumors, such as colorectal cancer. The **cobas®** 4800 BRAF V600 Mutation Test is a real-time PCR assay designed to detect the presence of the V600E (T1799A) mutation. The **cobas®** 4800 BRAF V600 Test is used as a companion diagnostic test for vemurafenib, a compound which inhibits the mutant V600E version of BRAF. Clinical trials of vemurafenib in patients with advanced melanoma have shown that patients with a V600E-mutant tumor are likely to experience clinical benefit from the compound.[6, 7]

BRAF Reporting

The BRAF assay tests for the BRAF V600 mutation.
The results are reported as **NOT DETECTED** or **DETECTED**.
NOTE: This assay does not reliably detect non-V600E mutations.

Limitations of Procedure:

1. As with all laboratory analyses, BRAF V600 Mutation Test results should be interpreted in conjunction with clinical and other laboratory findings for the most accurate interpretation.

2. BRAF V600 analysis for indications other than melanoma can be performed by Sanger sequencing methods (Ordering mnemonic: **BRAF**).

3. Detection of a mutation is dependent on the number of mutant sequence copies present in the specimen and may be affected by specimen integrity, amount of isolated DNA, and the presence of interfering substances.

4. Though rare, mutations and variants within the regions of the BRAF gene covered by the primers or probes used in the **cobas®** 4800 BRAF V600 Mutation Test may result in failure to amplify the BRAF V600 allele or detect the presence of mutation in codon 600.

5. The presence of PCR inhibitors may cause false negative or invalid results.

6. The **cobas®** 4800 BRAF V600 Mutation Test shows limited cross-reactivity with non-V600E mutant specimens (V600K, V600D, and V600E2). Refer to the Non-clinical Performance Evaluation section for more details.

7. FFPE tissue specimens containing degraded DNA may affect the ability of the test to detect the V600E mutation.

References:

1. Davies H, Bignell GR, Cox C, et al. Mutations of the BRAF gene in human cancer. *Nature* 2002; **417**:949-54.

2. McCubrey JA, Steelman LS, Chappell WH, et al. Roles of the Raf/MEK/ERK pathway in cell growth, malignant transformation and drug resistance. Biochim Biophys Act 2007;**1773**:1263–1284.

3. Curtin JA, Fridlyand J, Kageshita T, et al. Distinct sets of genetic alterations in melanoma. N Engl J Med. 2005;**353**:2135-2147.

4. Pollock PM, Harper UL, Hansen, KS, et al. High frequency of BRAF mutations in nevi. *Nature Genetics* 2003; **33**:19-20.

5. COSMIC database (http://www.sanger.ac.uk/perl/genetics/CGP/cosmic), Release 52 (March 2011)

6. Flaherty KT, Puzanov I, Kim KB, et al. Inhibition of mutated, activated BRAF in metastatic melanoma. N Engl J Med. 2010 Aug 26; **363**(9):809-819

7. Chapman PB, Hauschild A, Robert C, et al. Improved survival with vemurafenib in melanoma with BRAF V600E mutation. N Engl J Med. 2011;**364**:2507-2516.