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

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KRAS and BRAF Mutational Analysis

Contact:

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Effective Date:

June 1, 2010

Performed:

Typically set up Mondays and Wednesdays and resulted Tuesdays and Thursdays. The testing schedule will be adjusted week to week to maximize workflow efficiencies. Expected Turn-Around-Time (TAT) is 3-5 days from receipt of specimens at CPAL.

Mutational analysis can be ordered for KRAS (codons 12, 13 and 61) testing only (**KRAS - PDM 7000700**); BRAF (codon 600) testing only (**BRAF - PDM 7000750**); or as a reflex in which BRAF testing is performed in cases in which KRAS mutations are not detected (**KRAS RFX - PDM 7000725**).

Method:

PCR amplification and Di-deoxy chain terminating DNA sequencing chemistry (Sanger Method).

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. Blocks will not be accepted.

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

Tissue type should be indicated (colon, lung, etc).

Specimens should be transported and stored at room temperature.

NOTE: KRAS and BRAF mutational analysis can be performed from a single slide series specimen submission.

Reference Ranges:

Not Detected

Summary:

KRAS encodes for a GTP-binding protein that acts as a signal transducer, and is one of several downstream signaling pathways of the epidermal growth factor receptor (EGFR). This pathway is involved in regulating apoptosis, cell proliferation, cell differentiation, and angiogenesis. Oncogenic mutation of the KRAS gene leads to a constitutively activated protein which is commonly found in many carcinomas, including 30-50% of colorectal carcinomas, 15-30% of lung non-small-cell adenocarcinoma. Several studies have indicated that the presence of mutant KRAS in lung and colorectal tumors correlates with a poor prognosis and is associated with a lack of response to EGFR tyrosine kinase inhibitors.

BRAF is a component of the cytoplasmic RAS/RAF/MAPK signal transduction pathway, which mediates transcriptional protein regulation involving cellular proliferation. Signals from the extracellular EGFR receptor may activate several intracellular pathways. In the RAS pathway, BRAF is downstream from RAS, and mutations of BRAF are usually mutually exclusive of KRAS mutations. An oncogenic mutation (V600E) in exon 15 comprises 80-85% of BRAF mutations, and has been described in a variety of cancers, including melanoma (70%), thyroid (up to 30%), ovarian (up to 30%), and colorectal carcinomas (up to 15%). BRAF mutations are associated with sporadic microsatellite instability-high (MSI-H) colorectal carcinoma, but not with hereditary non-polyposis colorectal carcinoma. In colorectal carcinoma, the presence of a BRAF mutation predicts a poorer response to anti-EGFR monoclonal antibody therapeutics such as cetuximab and panitumumab.

The assays render genotype data for codons 12, 13 and 61 of the KRAS gene and codon 600 of the BRAF gene, which contain the majority of the clinically relevant mutations. Genomic DNA is extracted from formalin-fixed, paraffin embedded tissue. Polymerase Chain Reaction (PCR) is used to amplify the DNA of the target regions of KRAS codon 12/13 and codon 61 and/or codon 600 of the BRAF gene and Di-deoxy chain terminating (Sanger) methods are used to evaluate the DNA sequence in these regions.

The lower limit of sensitivity of this assay is 10%. There may be other mutations of the EGFR pathway that are contributory to poor prognosis and/or drug insensitivity that are not detected by this test.

Results should be interpreted in conjunction with clinical and other laboratory findings for the most accurate interpretation.

KRAS Reporting

KRAS results are reported as: Not Detected (**NOT DET**) or Detected (**DETECT**)

NOT DET means: KRAS normal (non-mutated): No KRAS Mutations detected (Wild Type) in the provided specimen of this individual.

DETECT means: KRAS abnormal (mutated): KRAS Mutation was identified in the provided specimen of this individual. In these cases the natures of the specific mutation will be identified (both nucleotide change and amino acid change).

BRAF Reporting

The BRAF assay tests for the BRAF V600E mutation.
The results are reported as Not Detected (**NOT DET**) or Detected (**DETECT**).
NOTE: Should an alternative BRAF mutation be identified in the region, the report will be appended to indicate the presence and nature of this mutation.

Limitations of Procedure:

These assays are sensitive and capable of detecting mutant alleles present at very low levels (5-15%) under ideal conditions. Effects of tissue processing, sampling, lymphocytic infiltration and normal variations in assay performance may affect this sensitivity. The results of this and all laboratory assays should be evaluated in conjunction with all clinical data. Mutations in the KRAS (EGFR) pathway are expected to occur early in carcinogenesis and therefore mutations should be well represented in carcinoma tissue sampled. Exceptions to this may occur.

Note:

This test was developed and its performance characteristics determined by The Central Pennsylvania Alliance Laboratory, LLC. It has not been cleared or approved by the U.S. Food and Drug Administration. The FDA has determined that such clearance or approval is not necessary. This test is used for clinical purposes. It should not be regarded as investigational or for research. This laboratory is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA) as qualified to perform high complexity clinical laboratory testing.