KRAS Testing For Colorectal Tumors

Now Available at PhenoPath Laboratories

Testing for the presence of mutations in the KRAS gene, a finding present in 30-40% of colorectal adenocarcinomas, has become a critical and essential test to determine which patients will benefit from the addition of cetuximab (Erbitux™) therapy. KRAS is a G-protein that is a key component of the EGFR signal transduction pathway. Mutated KRAS protein is constitutively activated, and tumors carrying these activating mutations have been shown to be resistant to anti-EGFR therapy. In controlled clinical trials, patients with colorectal tumors having mutated KRAS were resistant to anti-EGFR antibody therapy and had similar progression-free survival (PFS) and overall survival (OS) compared to untreated control patients. In contrast, patients with unmutated KRAS did benefit from anti-EGFR therapy with improved PFS and OS compared to controls. Therefore, KRAS mutation status is a key predictor of patient responsiveness to anti-EGFR antibody therapy.

The most common activating mutations in KRAS have been identified in human cancers involving codons 12 and 13 in exon 2. These account for >98% of all known KRAS mutations. In the assay we have developed and validated at PhenoPath Laboratories, designed to perform optimally in deparaffinized, formalin-fixed tissue, these KRAS mutations are detected by the allele-specific PCR method (Amplification Refractory Mutation System [ARMS]). ARMS exploits the fact that oligonucleotide primers must be perfectly annealed at their 3’ ends for a DNA Polymerase to extend these primers during PCR. Oligonucleotide primers that match only a specific DNA point mutation in the KRAS gene and not the wild-type allele can therefore be designed. The high sensitivity of PhenoPath Laboratories’ ARMS assay for KRAS means that mutations can be detected even if mutated tumor accounts for ≤ 1% of the total DNA extracted from the tissue. You can be assured that slides from every case sent to PhenoPath Laboratories will be reviewed and tissue microdissected by one of our pathologists to ensure that the most appropriate portions of the tissue are analyzed, resulting in fewer quantity not sufficient (QNS) results.

**KRAS FAQs**

*What kind of specimen do I need to submit to PhenoPath Laboratories to permit KRAS mutational analysis?*

Paraffin blocks of formalin-fixed tissue, or cut sections from blocks, are most appropriate. Please contact PhenoPath Laboratories for recommendations regarding cut sections or the handling of scant or fresh specimens, including FNAs. Our staff will work with you to ensure proper handling of any type of specimen. We also have experience with DNA extractions performed on specimens fixed in non-formalin-based fixatives.

*What is the turnaround time for KRAS mutational analysis?*

Turnaround time is usually seven days or less from receipt of specimen.
How has PhenoPath Laboratories validated this assay?
The KRAS mutational assay has been rigorously validated by direct DNA sequence verification on control cell lines as well as on a large number of colorectal cancer specimens to ensure accurate results. Our assay can detect KRAS mutations when cells containing the mutation comprise ≤ 1% of the specimen, which is an industry standard for this assay. Additionally, the assay has been cross-validated with other methodologies.

How does PhenoPath Laboratories’ KRAS mutational assay differ from other laboratories’ assays?
We employ a robust and rapid proprietary DNA extraction method that maximizes DNA yields without sacrificing sensitivity and specificity, and allows for more rapid PCR assay turnaround time without sacrificing accuracy. Microdissection of all specimens is performed by a pathologist. Unlike other ARMS-based mutational assays, the design of our assay employs direct visualization of mutant and control KRAS gene PCR products, which decreases the likelihood of false positive results. The ARMS-based KRAS assay employed at PhenoPath Laboratories is more sensitive and specific than sequencing alone.

If you or your clinicians have any questions regarding PhenoPath Laboratories’ KRAS mutational assay, contact us at 206-374-9000, or by email at lab@phenopath.com.
Amplification of the HER2 gene and concomitant protein overexpression are both present in between 10 and 20% of primary breast cancers, and identification of this subset of breast cancers is key. In the October 2008 issue of *Modern Pathology*, the pathologists at PhenoPath Laboratories published a landmark study capping more than a decade of HER2 testing in breast cancer. Following publication of the ASCO-CAP Guidelines, questions were raised as to the ability of laboratories to attain the required 95% concordance between results of HER2 status, in positive and negative cases, as tested by immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH). This study demonstrates that the answer to that question is a resounding yes at PhenoPath Laboratories.

A total of 6,604 breast cancer specimens received over a four-year period were evaluated at PhenoPath Laboratories for HER2 status by both IHC and FISH using standard methodologies. Among the HER2 negative cases (as determined by IHC), the concordance with FISH was 99.2%; among the HER2 positive cases (as determined by IHC), the concordance rate was 94.7%. The tissues for this study were obtained prior to the ASCO-CAP Guidelines for HER2 testing, and thus the specimens, from hundreds of laboratories around the United States, had a wide range of fixation times. The study demonstrates that only when we modified the standard IHC scoring system with a “normalized” scoring system (in which the HER2 score was derived by subtracting the score on the non-neoplastic breast epithelium from that on the tumor cells), could we obtain such high levels of concordance between IHC and FISH for HER2 status. These results reflect the many years of experience of our pathologists and staff, our extensive and demanding quality assurance program for HER2 testing, and the overall dedication to quality testing at PhenoPath Laboratories.

Dr. Mahul Amin
At Our Spring Conference

Mahul Amin, M.D., of Cedars-Sinai Medical Center, Los Angeles, CA, will present "Role of Immunohistochemistry in Urologic Pathology – Selected Topics" at the PhenoPath Pathology/Immunohistochemistry Conference on Thursday, February 12, 2009. The format of the conference is a social hour commencing at 6:30 PM, followed by Dr. Amin's lecture at 7:30 PM. A light catered dinner will be served during the social hour.

Currently Professor and Chairman of the Department of Pathology and Laboratory Medicine, and the Fellowship Director in Urologic Pathology at Cedars-Sinai Medical Center, Dr. Amin is also a Professor of Pathology at the David Geffen School of Medicine at the University of California, Los Angeles (UCLA).

Recognized as one of the leading authorities in genitourinary pathology with extensive expertise in oncologic pathology of the genitourinary tract, Dr. Amin is also a national and international consultant in tumors of the genitourinary tract, including prostate, urinary bladder, kidney and testis. In addition to publishing over 219 peer-reviewed articles, Dr. Amin is also the co-author of the current classification systems for urothelial tumors and renal neoplasms, two Armed Forces Institute of Pathology fascicles, and a monograph on Gleason Grading of Prostate Cancer. He has authored two other books in genitourinary pathology including co-authoring a book on bladder biopsy interpretation. Dr. Amin is a highly sought-after speaker, having given over 200 national and international lectureships.