



TEST REPORT
<Date>

Asuragen Clinical Services
2150 Woodward St., Ste. 100
Austin, TX 78744
(877) 777-1874
CLIAServices@asuragen.com

Patient and Order Information

Patient Name:	DOB:	Sex: <M F>
Order ID:	Lab ID:	Type of Sample: FFPE
MRN:	Specimen ID:	
Attending Provider:	Date Collected:	Date Received:

KRAS and/or BRAF Mutation Testing Result

Mutation testing requested:

- 1. KRAS (Codon 12 and 13) 7 Mutations only
 If result negative, reflex to BRAF (Codon 600) Mutation Test
- 2. BRAF (Codon 600) Mutation only
- 3. KRAS (Codon 12 and 13) 12 Mutations and BRAF (Codon 600)
- 4. KRAS (Codon 12 and 13) 12 Mutations

Specimen ID	Mutation Test Result (Positive or Negative)	KRAS Mutation Detected	BRAF Mutation Detected*
XXXXXX	Positive*	G12V	none
YYYYYY	Negative**	none	NR

Results interpretation: **Positive*** – A positive result indicates detection of at least one mutated allele KRAS or BRAF;
Negative** – A negative result indicates that none of the KRAS and/or BRAF mutations tested for were detected. A negative test result does not indicate a wild-type BRAF or KRAS. This test detects mutations listed in the Assay Description and Methodology section of this report, and not other rare KRAS or BRAF mutations;
NR – test not requested.

Disclaimer:
***- in addition to V600E mutation (most common), this test may also detect rare, activating BRAF point mutations, such as V600E2, V600D, and V600K.

Comments

***CLINICAL SIGNIFICANCE:** Some mutations in the KRAS gene (about 40% of colorectal cancer patients) are associated with poor prognosis and lack of response to anti-EGFR therapy. In July 2009, the Food and Drug Administration (FDA) approved labeling changes to cetuximab and panitumumab stating that these agents are not recommended for the treatment of colorectal cancer (CRC) harboring KRAS mutations. Thus, determination of KRAS mutation status in these tumors is critical when evaluating a patient for anti-epidermal growth factor receptor therapy. The American Society of Clinical Oncology (ASCO) has further recommended that all patients with metastatic colorectal cancer for whom EGFR antagonists are being

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considered should be specifically tested for KRAS mutational status at codons 12 and 13. Data from the CRYSTAL trial suggests that BRAF mutations are also indicative of poor prognosis and the National Comprehensive Cancer Network (NCCN) Colon Cancer Guideline Update 2010 states that testing for mutations in BRAF (identified in 3% to 12% of CRC patients) should occur when KRAS testing indicates KRAS wild type.

REFERENCES:

1. Lievre A., et al. **Cancer Res** (2006), 66: 3992-3995; 2. Di Fiore F., et al. **Br J Cancer** (2007), 96:1166-1169; 3. Lièvre A., et al. **JCO** May (2008), 20: 2601-2602; 4. Lievre A., et al. **JCO** (2008), 26: 374-379; 5. Amado R.G., et al. **J Clin Oncol** (2008), 26:1626-1634; 6. De Roock W., et al. **Ann Oncol** (2008), 19:508-515; 7. Khambata-Ford S., et al. **J Clin Oncol** (2008), 25:3230-3237; 8. Freeman D.J., et al. **Clin Colorectal Cancer** (2008), 7:184-190; 9. Di Nicolantonio F. et al. **J Clin Oncol** (2008). 26(35):5705-12; 10. Loupakis F. et al. **Br J Cancer** (2009). 101(4):715-21. 11. Van Custem E. ASCO GI 2010. Abstract 281.

Assay Description and Methodology:

Formalin-fixed, paraffin-embedded tumor tissue sections are deparaffinized and DNA is extracted using the DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA). Mutated KRAS and BRAF oncogenes are detected using an in-house developed and validated test, which identifies 12 somatic mutations located in KRAS Codons 12 and 13 (see below) and one in BRAF codon 600 (V600E) using mutation-specific amplification followed by direct hybridization capture with an analytical sensitivity of at least 2% mutant DNA in a background of wild-type genomic DNA.

Gene	Mutations Detected
K-RAS 7 (Codon 12, 13)	Gly12Asp (GGT>GAT) Gly12Ala (GGT>GCT) Gly12Val (GGT>GTT) Gly12Ser (GGT>AGT) Gly12Arg (GGT>CGT) Gly12Cys(GGT>TGT) Gly13Asp (GGC>GAC)
K-RAS +5 (Codon 13)	Gly13Ser (GGC>AGC) Gly13Arg (GGC>CGC) Gly13Val (GGC>GTC) Gly13Cys (GGC>TGC) Gly13Ala (GGC>GCC)
BRAF (Codon 600)	Val600Glu (GTG>GAG)

Intended Use:

This laboratory developed test is intended to be used and be interpreted in conjunction with all other available clinical and laboratory information when evaluating anti-EGFR treatment options for colorectal cancer patients. The test is validated for use with CRC FFPE tissue specimens that contain at least 40% tumor area, or that can be enriched to that tumor content. in the course of a histological specimen review. The test has not been validated on other specimen types or other human malignancies.

CLIA Laboratory Director (Print & Sign)

Date

Disclaimer:

This laboratory developed test was developed and its performance characteristics determined by Asuragen's Clinical Laboratory. The Asuragen Clinical Laboratory is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA'88) as qualified to perform high complexity testing and is accredited by the College of American Pathologists (CAP). This test has not been cleared or approved by the US Food and Drug Administration. Although, such approval is not required for clinical implementation, Asuragen may choose to seek such approval.

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