Colorectal Cancer – Predictive Testing for Anti-EGFR Therapy

### Indications for Ordering

Indicated for individuals with metastatic colorectal cancer (CRC) to guide treatment with anti-EGFR monoclonal antibodies (cetuximab and panitumumab)

### Test Description

**MassARRAY –** matrix-assisted laser desorption/ionization (MALDI) time-of-flight (TOF) mass spectrometry
- Simultaneous detection of mutations in **BRAF, KRAS, NRAS, PIK3CA**

**Pyrosequencing**
- Single gene assays for detection of mutations in **BRAF, KRAS, NRAS, PIK3CA**

**MassARRAY and pyrosequencing mutation detection**
- **BRAF** – codon 600
- **KRAS** – codons 12, 13, 61 (MassARRAY also detects codon 146)
- **NRAS** – codons 12, 13, 61
- **PIK3CA** – codons 542, 545, 1047

**Immunohistochemistry (IHC)**
- Detection of PTEN expression

**Next generation sequencing (NGS)**
- Extensive coverage of mutations in 48 genes, including **BRAF, KRAS, NRAS, PIK3CA, PTEN**
- Full gene and variant list at [www.aruplab.com/ngs-oncology-mutations](http://www.aruplab.com/ngs-oncology-mutations)

### Tests to Consider

**Primary test**

**Colon Cancer Gene Panel, Somatic 2011616**
- Use for individuals with metastatic CRC to guide treatment with anti-EGFR monoclonal antibodies (cetuximab and panitumumab)
- Detects mutations in **BRAF, KRAS, NRAS, extended KRAS, and PIK3CA**

**Related tests**

**Solid Tumor Mutation Panel by Next Generation Sequencing 2007991**
- Aids in therapeutic decisions for solid tumor cancers
- Simultaneously evaluates mutations in 48 genes, including **BRAF, KRAS, NRAS, PIK3CA**
- Predicts prognosis and therapeutic response in patients with solid tumor cancers

**KRAS Mutation Detection with Reflex to BRAF Codon 600 Mutation Detection 2001932**
- Determine eligibility for anti-EGFR (cetuximab and panitumumab) therapy in patients with metastatic CRC

**KRAS Mutation Detection 0040248**
- Predicts response to anti-EGFR and MAPK pathway therapies in a variety of malignancies (eg, CRC and lung cancer)

**BRAF Codon 600 Mutation Detection by Pyrosequencing 2002498**
- Use to detect activating **BRAF** mutations at codon 600
- Can indicate resistance to anti-EGFR therapy in CRC
- Also used within the Lynch syndrome reflex testing pathway (for CRC specimens only)

**BRAF V600E Mutation Detection in Circulating Cell-Free DNA by Digital Droplet PCR 2013921**
- Determines **BRAF V600E** mutation status in patients with solid tumors to select candidates for targeted therapy with kinase inhibitors (BRAF and/or MEK)
- Monitors response to therapy and disease progression in patients carrying **BRAF V600E** mutation

**PTEN by Immunohistochemistry 2004115**
- Detects loss of PTEN expression in tumor tissue
- Possibly associated with relative resistance to anti-EGFR therapy

**NRAS Mutation Detection by Pyrosequencing 2003123**
- Predicts response to anti-EGFR and MAPK pathway therapies in a variety of malignancies (eg, melanoma and CRC)
## Disease Overview

- CRC is 1 of the most commonly diagnosed malignancies worldwide
- EGFR represents an important therapeutic target in advanced CRC

- 2 anti-EGFR monoclonal antibodies (cetuximab and panitumumab) are available for treatment of advanced CRC
- KRAS, BRAF, and possibly PIK3CA, PTEN, and NRAS mutations are associated with resistance to anti-EGFR therapy

## Genetics and Test Interpretation

<table>
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<tr>
<th>Gene</th>
<th>Mutations</th>
<th>Sensitivity/Specificity</th>
<th>Results</th>
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</thead>
</table>
| KRAS | GTPase-encoding gene in the RAS/RAF/MAPK pathway | Majority of oncogenic mutations – codons 12 and 13 (>90%) | Clinical sensitivity – activating KRAS mutations found in ~40% of CRCs | Positive  
- Oncogenic KRAS mutation detected  
- Lack of response to therapy with antibodies targeted to EGFR is predicted | Limit of detection  
- MassARRAY and pyrosequencing – 10% mutant alleles  
- NGS – 5% mutant alleles  
- MassARRAY – oncogenic mutations outside of codons 12, 13, 61, 146 will not be detected  
- Pyrosequencing – oncogenic mutations outside of codons 12, 13, 61 will not be detected  
- A substantial portion of individuals with wild type KRAS still fail to respond to anti-EGFR agents, implicating downstream mutations |
| BRAF | Kinase-encoding gene in the RAS/RAF/MAPK pathway | Majority of activating mutations – codon 600 Mutually exclusive with KRAS mutations in individuals with CRC | Clinical sensitivity – activating BRAF mutation found in ~10% of CRCs | Positive  
- Oncogenic BRAF mutation detected  
- Available data suggest resistance to anti-EGFR therapy  
- Appears to be associated with a worse prognosis | Limit of detection  
- MassARRAY and pyrosequencing – 10% mutant alleles  
- NGS – 5% mutant alleles  
- MassARRAY and pyrosequencing – oncogenic mutations outside of codon 600 will not be detected |
| PIK3CA | Encodes a subunit of the PI3K protein | Exon 9 gain-of-function mutation (codons 542 or 545) requires interaction with RAS  
- May have no effect on cetuximab therapy  
- Exon 20 (codon 1047) mutation is independent of RAS binding  
- Appears to have a negative effect on response to cetuximab | Clinical sensitivity – oncogenic PIK3CA mutation found in 10-20% of CRCs, mostly exons 9 (60-65%) or 20 (20-25%) | Positive  
- Oncogenic PIK3CA mutation detected  
- Tumor may respond to therapies targeted at genes downstream of PI3K in the AKT/mTOR signaling cascade  
- Exon 20 (kinase domain) mutations may indicate resistance to anti-EGFR therapy in wild type KRAS tumors  
- Negative impact on the prognosis of advanced CRC | Limit of detection  
- MassARRAY and pyrosequencing – 10% mutant alleles  
- NGS – 5% mutant alleles  
- MassARRAY and pyrosequencing – oncogenic mutations outside of codons 542, 545, 1047 will not be detected |
| PTEN | Tumor suppressor gene in the PI3K/AKT pathway | Loss has been predicted to show resistance to anti-EGFR therapy | Clinical sensitivity – loss of expression found in up to 50% of CRCs | Abnormal  
- Possible resistance to anti-EGFR therapy  
- PTEN expression is intact | Normal  
- PTEN expression is intact |
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<tr>
<td>NRAS GTPase-encoding gene in the RAS/RAF/MAPK pathway</td>
<td>Majority of activating mutations – codon 61 Mutually exclusive with KRAS mutations in individuals with CRC Associated with relative resistance to anti-EGFR therapy</td>
<td>Clinical sensitivity – oncogenic NRAS mutation found in ~3% of CRCs Analytic sensitivity/specificity – 100%</td>
<td>Positive</td>
<td>• Oncogenic NRAS mutation detected • Predictive of relative resistance to anti-EGFR therapy</td>
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<td></td>
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<td>Negative</td>
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