University of Nebraska Medical Center
REGIONAL PATHOLOGY SERVICES

LAB ALERT

Date: October 21, 2015

Personalized Medicine now offered with NGS Panels for Melanoma, Gastrointestinal Tumors, CNS Tumors, and Systemic Mastocystosis

Dear Regional Pathology Clients,

We are pleased to announce the availability of Next Generation Sequencing (NGS) Panels available through our Molecular Diagnostics laboratory-***right here in Nebraska***. The panels available are:

- **Melanoma Mutation Panel-** ***New***
- **GIST Panel -** ***New***
- **IDH1/IDH2 Mutation Testing -** ***New***
- **KIT D816V Mutation Testing -** ***New***
- **50 Gene Cancer Panel**
- **5 Gene Colorectal Panel**

These tests were recently featured in an Omaha World Herald article
http://www.reglab.org/reglab/assets/File/Gene%20panel%20article.pdf

The following assays use multiplex DNA amplification for library preparation, emulsion PCR for clonal amplification and next generation sequencing to identify mutations. Two distinct bioinformatics software programs are employed to evaluate mutations. The limit of detection for these assays is approximately 5% mutant allele for single nucleotide variants and 10% for small insertions and deletions.

Melanoma Mutation Panel by Next Generation Sequencing

This test analyzes hotspot regions in the following genes BRAF, KIT, NRAS, GNA11, GNAQ, and HRAS. Approximately 50% of cutaneous melanomas demonstrate a mutation in BRAF codon 600 (V600E, V600K, V600R), more commonly in non-chronically sun damaged skin. Mucosal melanomas and acral melanomas more often have KIT mutations (approximately 10-20%). ***It is important to identify BRAF and KIT mutations because there are targeted therapies that are effective against BRAF and KIT-mutated melanomas.*** NRAS mutations are identified in 10-20% melanomas and GNAQ and GNA11 mutations have been identified in choroidal/uveal melanomas. HRAS mutations have been identified in Spitz nevi, but not in Spitzoid melanoma, assisting in differentiating between a benign and malignant condition. Please contact the laboratory for specific exon and codon coverage.

Test Name- Melanoma Mutation Panel (BRAF, KIT, NRAS, HRAS, GNAQ and GNA11)
Test code- MMP

Specimen requirements:

- FFPE tissue block, or 5 Unstained slides- 10µm thick with ≥20% tumor (with one adjacent H&E).

Ideal Volume: 5mmX5mm area if tissue is 100% tumor; 10mm X 10mm if tissue is ≥20% tumor

- Tissue, snap frozen, on dry ice (-80ºC)

Unacceptable specimens: Paraffin blocks with ≤10% tumor. Tissue fixed in heavy metal fixative or decalcified tissue.

Gastrointestinal Stromal Tumor (GIST) Panel by Next Generation Sequencing

This test analyzes hotspot regions in the following genes KIT, PDGFRA and BRAF. Gastrointestinal Stromal Tumor (GIST) is a soft tissue tumor arising most commonly in the stomach or small intestine. Approximately 85-90 % of GISTs demonstrate a primary mutation in one of three genes: KIT (85% of mutations), PDGFRA (5-10% of mutations) or BRAF (1% of mutations).
Identification of these mutations can determine how an individual may respond to various tyrosine kinase inhibitors, including imatinib. KIT exon 11 mutations appear to be most responsive to imatinib, however, other mutations in KIT exons and select PDGFRA mutations also demonstrate sensitivity. PDGFRA mutation p.Asp842Val has shown resistance to imatinib. BRAF p.V600E mutations are rarely found in GISTs, but are thought to be resistant to imatinib treatment. Please contact the laboratory for specific exon and codon coverage.

Test Name - GIST Mutation Panel (KIT, PDGFRA, BRAF) Test code- GIST

Specimen requirements:

- FFPE tissue block, or 5 Unstained slides- 10µm thick with ≥20% tumor (with one adjacent H&E).

Ideal Volume: 5mmX5mm area if tissue is 100% tumor; 10mm X 10mm if tissue is ≥20% tumor

- Tissue, snap frozen, on dry ice (-80ºC)

Unacceptable specimens: Paraffin blocks with ≤10% tumor. Tissue fixed in heavy metal fixative or decalcified tissue.

IDH1 and IDH2 mutation detection by Next Generation Sequencing

This test analyzes amino acids 101-134 of IDH1 (including hotspot codon 132) and 134-176 of IDH2 (including hotspot codon 172). IDH1 and IDH2 mutations are identified in various neoplasms. In CNS tumors, studies have indicated that their presence portends a better prognosis in gliomas. IDH mutations are more common in low-grade and secondary gliomas. IDH1/2 mutations can also be identified in 10% of Acute Myelogenous Leukemias (AML), more commonly in cytogenetically normal AMLs with intermediate prognosis. Studies have suggested that their presence in AML may correlate with a worse prognosis, especially in the presence of an NPM1-mutated/FLT3-wildtype background. IDH1/2 mutations (particularly IDH1) are common in enchondroma, periosteal chondroma, and conventional central chondrosarcoma, but are reportedly absent in osteochondroma/peripheral chondrosarcoma, among other cartilaginous tumor subtypes. Finally, IDH2 mutations are present in 30% of angioimmunoblastic T-cell lymphomas (AITL).

Test Name- IDH1 and IDH2 Mutation Detection panel/other (IDH12)
IDH1 and IDH2 Mutation Detection panel/blood (IDH12B)
Test code- IDH12 or IDH12B
Alternate Name- Isocitrate dehydrogenase 1 and 2 mutation detection

Specimen requirements:

- FFPE tissue block, or 5 Unstained slides- 10µm thick with ≥20% tumor (with one adjacent H&E).

Ideal Volume: 5mmX5mm area if tissue is 100% tumor; 10mm X 10mm if tissue is ≥20% tumor

- Tissue, snap frozen, on dry ice (-80ºC)
- Bone Marrow Aspirate, EDTA anticoagulated, minimum 1.0 mL
- Peripheral Blood, EDTA anticoagulated, minimum 2.0 mL

Unacceptable specimens: Paraffin blocks with ≤10% tumor. Tissue fixed in heavy metal fixative or decalcified tissue. Blood/Bone Marrow collected in heparin (green-top) tubes.

KIT D816V Mutation Detection

The KIT c.2447A>T; p.D816V mutation in exon 17 is present in approximately 95% or more of adult Systemic Mastocytosis (SM) and in approximately 33% of cutaneous mastocytosis in pediatric patients. ***It predicts resistance to imatinib therapy. This test is mainly used for diagnostic purposes in SM.***

Test Name: KIT Mutation Detection (D816V) -(KIT816)
KIT Mutation Detection/blood (D816V)-(KITPB)
Test code- KIT816 and KITPB
Alternate Name: D816V

Specimen requirements:

- FFPE tissue block, or 5 Unstained slides- 10µm thick with ≥20% tumor cells (with one adjacent H&E).

Ideal Volume: 5mmX5mm area if tissue is 100% tumor; 10mm X 10mm if tissue is ≥20% tumor

- Tissue, snap frozen, on dry ice (-80ºC)
- Bone Marrow Aspirate, EDTA anticoagulated, minimum 1.0 mL
- Peripheral Blood, EDTA anticoagulated, minimum 2.0 mL

Testing will be performed M-F; turnaround time is 7-14 business days, depending on the test. Inquires may be called to the laboratory at 559-7745, Jill Branson, Mgr, 559-7611, or Allison Cushman-Vokoun, Medical Director 559-3512.

For general inquiries or pricing you can contact one of our client coordinators, Dana El-Hajjar 402-559-9129 (delhajja@unmc.edu) or Brian Lenz, 402-559-7897 (blenz@unmc.edu)

24/7 Client Services 1-800-334-0459