CGMP_50 Fact Sheet

CANCER GENE MUTATION PANEL_50 (CGMP_50)
BY TARGETED NEXT GENERATION SEQUENCING

TEST DETAILS

The CGMP_50 assay uses Targeted Detection of cancer-related mutations/variants using Next Generation Sequencing (NGS) technology in Colorectal, Lung, Brain, Ovarian, Thyroid cancers, Melanomas and other solid tumors. Genetic alterations in cancer are important diagnostic, prognostic, and predictive biologic markers. As more genetically targeted therapies become available, detection of targetable gene variants is increasingly important in order to drive therapy choice. The advent of targeted next-generation sequencing (NGS) technologies in clinical diagnostics promises to enhance the concurrent identification of several variants present in a panel of cancer-related genes. Targeted NGS technology can expand on genotyping of individual base pairs because it can detect single nucleotide variants and other mutations such as insertions and deletions across genes.

The targeted next generation sequencing test utilizes multiplex PCR with ion semiconductor sequencing (AmpliSeq, Ion Torrent/Life Technologies) to sequence a panel of key cancer gene variants including many clinically actionable mutations. This assay utilizes a minimal amount of DNA extracted from Formalin Fixed Paraffin Embedded (FFPE) tissue and cytology specimens to concurrently interrogate 2800 hotspots/variants in 50 cancer related genes in multiple specimens. The test can provide information for a more reliable prediction of personalized cancer therapies. The panel will also identify relevant genes that may have implications for enrollment of the patient in clinical trials. The genes interrogated in the panel are listed below:

<table>
<thead>
<tr>
<th>ABL1</th>
<th>AKT1</th>
<th>ALK</th>
<th>APC</th>
<th>ATM</th>
<th>BRAF</th>
<th>CDH1</th>
<th>CDKN2A</th>
<th>CSF1R</th>
<th>CTNNB1</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGFR</td>
<td>ERBB2</td>
<td>ERBB4</td>
<td>EZH2</td>
<td>FBXW7</td>
<td>FGFR1</td>
<td>FGFR2</td>
<td>FGFR3</td>
<td>FLT3</td>
<td>GNA11</td>
</tr>
<tr>
<td>GNAS</td>
<td>GNAQ</td>
<td>HNF1A</td>
<td>HRAS</td>
<td>IDH1</td>
<td>IDH2</td>
<td>JAK2</td>
<td>JAK3</td>
<td>KDR</td>
<td>KIT</td>
</tr>
<tr>
<td>KRAS</td>
<td>MET</td>
<td>MLH1</td>
<td>MPL</td>
<td>NPM1</td>
<td>NOTCH1</td>
<td>NRAS</td>
<td>PDGFRA</td>
<td>PIK3CA</td>
<td>PTEN</td>
</tr>
<tr>
<td>PTPN11</td>
<td>RB1</td>
<td>RET</td>
<td>SMAD4</td>
<td>SMARCB1</td>
<td>SMO</td>
<td>SRC</td>
<td>STK11</td>
<td>TP53</td>
<td>VHL</td>
</tr>
</tbody>
</table>

The analytical performance of this test is currently validated and approved by the New York State Department of Health (NYSDOH). The initial validation included variants in the BRAF, EGFR, KRAS and JAK2 genes. Ongoing validation is being performed for additional variants. All variants that are
documented in the Catalogue of Somatic Mutations in Cancer (COSMIC) database will be reported. Mutation details and corresponding COSMIC IDs for the genes in the panel can be obtained at http://cancer.sanger.ac.uk/cancergenome/projects/cosmic/. The mutation nomenclature used for reporting of variants is based on the convention recommended by the Human Genome Variation Society (http://www.hgvs.org/mutnomen/).

METHODOLOGY:
Tumor DNA is enriched by extraction from unstained macro-dissected, formalin-fixed, paraffin-embedded sections of the patient’s specimen with H&E guidance. Specimens with a minimum of 20% neoplastic content in the area selected for macro-dissection as indicated by a pathologist are preferred for analysis.

Genomic DNA is amplified, enriched and sequenced using next generation bidirectional sequencing technology on the Ion Torrent Personal Genome Machine (Life Technologies). The Ion Ampliseq Cancer Panel hotspot v2 targeted gene panel is designed to detect 2800 mutations/variants in 207 amplicons from 50 key cancer-related genes. Quality control measures are implemented at multiple levels of the sequencing process. Annotation of variants is performed using the Torrent Suite Software v 4.2 (Life Technologies) and/or NextGene Software (Soft Genetics).

This mutation panel is designed to detect targeted mutations only. The 50 genes are not sequenced in their entirety. Mutations outside the targeted regions will not be detected. The limit of detection is 5% at 400X coverage and 2.5% at 1000X coverage. This technology cannot reliably detect mutations at coverage below 200X. Confirmation of variants may be performed by Sanger Sequencing or real-time PCR if necessary.

SPECIMEN REQUIREMENTS AND SHIPPING INSTRUCTIONS:
A surgical pathology and/or cytology report and completed requisition form must accompany all specimens.

Specimen Type: Paraffin embedded tissue sections
• Tissue should be fixed in formalin and not exposed to decalcification solution. The paraffin block should contain at least a 3 mm area of tumor.
• The minimum amount of neoplastic cellularity acceptable in the tissue section is 20%.
• One H&E and 6 unstained sections are required for most of the tests. Ten unstained sections or more are required if the tissue is small. Please call the lab if you have questions.
• Slides should be properly labeled with a block label that matches the surgical pathology specimen number on the surgical pathology report.

Specimen Type: Fixed Fine Needle Aspiration (FNA) samples
• One H&E and 4-6 unstained sections from cell block are required.
• Fresh specimens should be collected into preservative solution
• Slides should be properly labeled with a number that matches the specimen number on the cytology report.

Specimen Type: Frozen tissue

• A minimum of 2 x 2 x 2 mm of frozen tissue containing at least 20% neoplastic cells is necessary

ORDERING THE TEST:

• Oncologists can request this test through Surgical Pathology at the time the specimen is sent for diagnosis. Surgical Pathology then orders the test through Molecular Pathology. **For now the order should specify testing for either, KRAS, BRAF or EGFR.** (A test order for all 50 genes will be available shortly). All tests where KRAS, BRAF or EGFR mutation analysis is requested will be sequenced on the CGMP_50 NGS assay.

• The NGS assay can accommodate most specimens sent for KRAS, BRAF and EGFR testing. However, in cases where the DNA concentration or quality is a limiting factor, which occurs in about 20% of the specimens, we may have to resort to the single gene assays that are currently utilized. In these cases a report for individual genes (KRAS, BRAF or/and EGFR) will be issued.

The results for the CGMP_50 NGS test will be reported in three different tiers as follows:

**Tier 1** – Clinical utility has been demonstrated - Actionable / Clinically Relevant variants (Having Therapeutic or Diagnostic or Prognostic implications)

**Tier 2** – Clinical utility is under evaluation – Variants associated with Clinical trials

**Tier 3** – VUS (Variants of Unknown Significance)

**Turn Around Time for the assay is 7-10 business days**

CONTACT INFORMATION:

For further information on the assay or for questions on the results of the CGMP_50 NGS test please contact the directors of the Molecular Pathology Laboratory:

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