MAYO CLINIC LABORATORIES

Test Definition: NONCP

Neuro-Oncology Expanded Gene Panel with Rearrangement, Tumor

Overview

Useful For

Identifying mutations and rearrangements that may support a diagnosis or help determine prognosis for patients with central nervous system tumors

Identifying specific mutations and rearrangements within genes known to be associated with response or resistance to specific cancer therapies

This test is **not intended** for use for hematological malignancies.

Reflex Tests

Test Id	Reporting Name	Available Separately	Always Performed
NONCM	Neuro-Onc Panel, Mutations Only	Yes	No
MCRSP	MayoComplete Targeted RNAseq Panel	Yes	No

Genetics Test Information

This test uses next-generation sequencing to evaluate for microsatellite instability (MSI) status, somatic mutations within 89 genes associated with tumors of the central nervous system, gene fusions within 1445 genes, known abnormal transcript variants in the *MET* and *EGFR* genes, and *BCOR* exon 15 internal tandem duplications. See Targeted DNA Gene Regions Interrogated by Neuro-Oncology Panel and Targeted Gene Fusions for the Neuro-Oncology Expanded Panel for details regarding the targeted gene regions identified by this test.

Of note, this test is performed to evaluate for somatic (ie, tumor-specific) mutations within the genes listed. Although germline (ie, inherited) alterations may be detected, this test cannot distinguish between germline alterations and somatic mutations with absolute certainty. Follow-up germline testing using non-neoplastic (normal) tissue can be performed for confirmation of suspected clinically relevant germline alterations. Germline testing should be performed along with genetic counseling.

Additional Tests

Test Id	Reporting Name	Available Separately	Always Performed
SLIRV	Slide Review in MG	No, (Bill Only)	Yes

Testing Algorithm

When this test is ordered, slide review will always be performed at an additional charge.

Standalone orderable tests will only be used if the specimen received is insufficient for both portions of testing.

Appropriate test code will be added per the direction of testing prioritization.

This test includes DNA mutation and RNA fusion analyses. A reflex test is added only when there is insufficient specimen for both test components. Indicate the preferred prioritization of testing on paperwork. If the specimen is insufficient for all portions of testing, the lab will use this prioritization to select the appropriate reflex test ID, reducing communication delays. If additional tests are ordered on same specimen, include them in the prioritization preferences.

Special Instructions

- RNA Targeted Gene Fusions and Abnormal Transcript Variants
- Tissue Requirements for Solid Tumor Next-Generation Sequencing
- Targeted Gene Fusions for the Neuro-Oncology Expanded Panel

Highlights

This next-generation sequencing tumor profiling assay interrogates targeted gene regions and rearrangements across genes associated with central nervous system tumors to assess for the presence of somatic mutations and rearrangements, such as mutations in *IDH1/2, TERT* promoter*, ATRX, TP53, H3-3A* (previously *H3F3A*)*, H3C2/H3C3 (previously HIST1H3B/C), BRAF, FGFR1, NF1, and SMARCB1,* and gene fusions including *KIAA1549::BRAF, ZFTA::RELA* (previously *C11orf95::RELA*), and *EGFR* transcript variants (eg, *EGFR* vIII).

Microsatellite instability (MSI) status is also determined (MSS, MSI-H) as part of this test and is often clinically actionable for determining the efficacy of immunotherapy in solid tumors.

Method Name

Sequence Capture and Targeted Next-Generation Sequencing (NGS)

NY State Available

Yes

Specimen

Specimen Type

Varies

Ordering Guidance

Multiple oncology (cancer) gene panels are available. For more information see Hematology, Oncology, and Hereditary Test Selection Guide.

Necessary Information

A pathology report (final or preliminary), at minimum containing the following information**, must accompany specimen for testing to be performed:**

1. Patient name
2. Block number-must be on all blocks, slides, and paperwork (can be handwritten on the paperwork)

3. Tissue collection date
4. Source of the tissue
5. Diagnosis, potential diagnosis, or differential diagnosis

Specimen Required

This assay requires at least 20% tumor nuclei.
-Preferred amount of tumor area with sufficient percent tumor nuclei: tissue 360 mm(2)
-Minimum amount of tumor area: tissue 72 mm(2)
-If ordered in conjunction with CMAPT / Chromosomal Microarray, Tumor, Formalin-Fixed Paraffin-Embedded, the preferred amount of tissue is 430 mm(2), the minimum amount is 180 mm(2).
-These amounts are cumulative over up to 15 unstained slides and must have adequate percent tumor nuclei.
-Tissue fixation: 10% neutral buffered formalin, not decalcified
-For specimen preparation guidance, see Tissue Requirements for Solid Tumor Next-Generation Sequencing. In this document, the sizes are given as 6 mm x 6 mm x 10 slides as preferred: approximate/equivalent to 360 mm(2) and the minimum as 4 mm x 4 mm x 10 slides: approximate/equivalent to 144 mm(2).

Preferred: Submit 2, if available, of the following specimens.
Acceptable: Submit **at least one** of the following specimens. Tissue blocks are preferred over tissue slides.

Specimen Type: Tissue block
Collection Instructions: Submit a formalin-fixed, paraffin-embedded tissue block with acceptable amount of tumor tissue.

Specimen Type: Tissue slide
Slides: 1 Hematoxylin and eosin-stained and 15 unstained
Collection Instructions:
Submit the followings slides:
1 Slide stained with hematoxylin and eosin
AND
15 Unstained, nonbaked slides with 5-micron thick sections of the tumor tissue.
Note: The total amount of required tumor nuclei can be obtained by scraping up to 15 slides from the same block.
Additional Information: Hematoxylin and eosin-stained and unstained slides will not be returned.

Forms

If not ordering electronically, complete, print, and send an Oncology Test Request (T729) with the specimen.

Specimen Minimum Volume

See Specimen Required

Reject Due To

All specimens will be evaluated at Mayo Clinic Laboratories for test suitability.

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container

Varies	Ambient (preferred)		
	Refrigerated		

Clinical & Interpretive

Clinical Information

Molecular biomarkers, including clinically relevant gene mutations (ie, sequence variants) and fusions, have been incorporated in the World Health Organization classification of central nervous system (CNS) tumors. This test evaluates targeted regions across genes associated with a variety of adult and pediatric-type CNS tumors for the presence of somatic mutations and rearrangements (fusions and abnormal transcript variants) including, but not limited to, mutations in *IDH1/2, TERT* promoter*, ATRX, TP53, H3-3A* (previously *H3F3A*)*, H3C2/H3C3* (previously *HIST1H3B/C*)*, BRAF, FGFR1*, *NF1* and *SMARCB1,* and *KIAA1549::BRAF* and *ZFTA::RELA* (previously *C11orf95::RELA*) fusions, and *EGFR* transcript variants (eg, *EGFR* vIII).

See Targeted DNA Gene Regions Interrogated by Neuro-Oncology Panel and Targeted Fusion Genes for Neuro-Onc Expanded Panel for details regarding the targeted gene regions identified by this test.

Reference Values

An interpretive report will be provided.

Interpretation

The interpretation of molecular biomarker analysis includes an overview of the results and the associated diagnostic, prognostic, and therapeutic implications.

Cautions

This test cannot differentiate between somatic and germline alterations. Additional testing may be necessary to clarify the significance of results if there is a potential hereditary risk.

Variants and fusions of uncertain significance may be identified.

A negative result does not rule out the presence of a variant that may be present below the limits of detection of this assay. The analytical sensitivity of this assay for sequence reportable alterations is 5% mutant allele frequency with a minimum coverage of 500X in a sample with 20% or more tumor content.

The sensitivity of this assay for gene fusions depends on several variables including decreased sensitivity with decreased tumor percentage, and decreased sensitivity with decreased level of expression of a variant. A negative result does not rule out the presence of a gene fusion, splice variant, or *BCOR* exon 15 internal tandem duplication that may be present but below the limits of detection of this assay. The analytical sensitivity of this assay for rearrangements is a minimum coverage of 5 unique variant molecules in a sample with at least 10% tumor content.

Point mutations and small deletion-insertion mutations (delins) will be detected in 89 genes. This test may detect single exon deletions but does not detect multi-exon deletions, duplications, or genomic copy number variants in any of the genes tested. Delins of 1000 base pairs or less are detectable with at least 50 supporting reads.

This test cannot reliably determine if a variant identified in *PMS2* exons 11-15 originated from *PMS2* or the highly homologous pseudogene *PMS2CL*. In the instance that a reportable variant is detected in *PMS2* exons 11-15, additional testing will be recommended in the patient report.

RNA is particularly labile and degrades quickly. Rapid preservation of the tumor sample after collection reduces the likelihood of degradation, but there are sometimes biological factors, such as tumor necrosis that interfere with obtaining a high-quality RNA specimen despite rapid preservation.

This assay can detect in-frame and out-of-frame fusions involving 1445 genes. Sensitivity for detecting out-of-frame fusions such as exon-intron, intron-intron or big insertions, may be lower due to bioinformatics detection limitations. This assay will only detect fusions involving at least 1 gene in the defined gene fusion target list of interest. This assay may not detect fusions involving deep intronic or intergenic regions and will not detect chromosomal rearrangements that do not create a fusion transcript (ie, enhancer repositioning). Variants not expressed, or expressed at very low level, are not detected by this assay.

Rare alterations (ie, polymorphisms) may be present that could lead to false-negative or false-positive results.

Test results should be interpreted in the context of clinical, tumor sampling, histopathological, and other laboratory data. If results obtained do not match other clinical or laboratory findings, contact the laboratory for discussion. Misinterpretation of results may occur if the information provided is inaccurate or incomplete.

Reliable results are dependent on adequate specimen collection and processing. This test has been validated on formalin-fixed, paraffin-embedded tissues; other fixatives are discouraged. Improper treatment of tissues, such as decalcification, may cause polymerase chain reaction failure.

Genes may be added or removed based on updated clinical relevance. Refer to the Targeted DNA Gene Regions Interrogated by Neuro-Oncology Panel and Targeted Fusion Genes for Neuro-Onc Expanded Panel for the most up to date list of genes included in this test.

Supportive Data

Performance Characteristics

DNA validation studies demonstrated concordance between this test and the reference method for detection of (single nucleotide variants [SNV] and deletions-insertions [delins]) is 99.7% (699/701) and 96.6% (226/234) of variants, respectively. Concordance for the detection of delins was 98.9% (186/188) in variants 1 to 10 base pairs (bp) in size, 95.8% (23/24) in variants 11 to 50 bp in size, and 88.9% (8/9) in variants 51 to 200 bp in size.

Microsatellite instability (MSI) evaluation is accurate at a tumor purity of at least 10% for colorectal tumors and 20% for other tumor types. During verification studies, 98% (200/204) concordance for MSI status was observed between this test and the reference method.

RNA validation studies demonstrated concordance between this test and the reference method for detection of gene fusions is 96.6% (256/265). No gene fusions were detected in 14 normal tissues, and no gene fusions were detected in the negative control sample (100% specificity). The sensitivity of this assay for detecting *CIC*::*DUX4* fusions is lower

(approximately 70%-80%), based on preclinical testing; tumors that are fusion negative but suspected to be *CIC*-rearranged may require orthogonal methods including immunohistochemistry, fluorescence in situ hybridization, real-time polymerase chain reaction, gene expression profiling, and/or methylation profiling.

To ensure this assay detects variants based on established sensitivity, this test will be performed on cases estimated by a pathologist to have at least 20% tumor cells.

Clinical Reference

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2. Zhang J, Wu G, Miller CP, et al. Whole-genome sequencing identifies genetic alterations in pediatric low-grade gliomas. Nat Genet. 2013;45(6):602-612
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8. Cancer Genome Atlas Research Network, Brat DJ, Verhaak RG, et al. Comprehensive, integrative genomic analysis of diffuse lower-grade gliomas. N Engl J Med. 2015;372(26):2481-2498
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14. Nabors LB, Portnow J, Ammirati M, et al. Central nervous system cancers, version 1.2015. J Natl Compr Canc Netw. 2015;13(10):1191-1202
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Performance

Method Description

Next-generation sequencing (NGS) is performed to determine microsatellite instability status and evaluate the presence of a somatic mutation in targeted regions of 89 genes. RNA-based NGS is performed to test for the presence of rearrangements involving 1445 genes, selected splice variants in *MET* and *EGFR* genes and *BCOR* exon 15 internal tandem duplications.

See Targeted DNA Gene Regions Interrogated by Neuro-Oncology Panel and Targeted Fusion Genes for Neuro-Onc Expanded Panel for details regarding the targeted gene regions identified by this test.(Unpublished Mayo method)

A pathology review and macro dissection to enrich for tumor cells is performed prior to slide scraping.

PDF Report
No

Day(s) Performed
Monday through Friday

Report Available
12 to 20 days

Specimen Retention Time
Tissue blocks: Unused portions of blocks will be returned; Tissue slides: Hematoxylin and eosin-stained and unstained slides will not be returned. Unused slides are stored for at least 5 years; Extracted DNA/RNA: 3 months

Performing Laboratory Location
Mayo Clinic Laboratories - Rochester Main Campus

Fees & Codes

Fees
- Authorized users can sign in to Test Prices for detailed fee information.
- Clients without access to Test Prices can contact Customer Service 24 hours a day, seven days a week.
- Prospective clients should contact their account representative. For assistance, contact Customer Service.

Test Classification
This test was developed and its performance characteristics determined by Mayo Clinic in a manner consistent with CLIA requirements. It has not been cleared or approved by the US Food and Drug Administration.

CPT Code Information

81455
81456

LOINC® Information

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Test Definition: NONCP

Neuro-Oncology Expanded Gene Panel with Rearrangement, Tumor

Test ID	Test Order Name	Order LOINC® Value
NONCP	Neuro-Onc Expanded Panel	73977-1

Result ID	Test Result Name	Result LOINC® Value
603048	Result Summary	50397-9
603049	Result	82939-0
603050	Interpretation	69047-9
603051	Additional Information	48767-8
603052	Specimen	31208-2
603053	Source	31208-2
603054	Tissue ID	80398-1
603055	Released By	18771-6