MAYO CLINIC LABORATORIES

Test Definition: MTNON

Neuro-Oncology Genome-Wide Methylation Array Analysis, Tumor

Overview

Useful For

Assisting in tumor profiling by identifying methylation family and class that may support a diagnosis or help determine prognosis for patients with central nervous system tumors

This test is **not intended** for use in hematologic malignancies.

Genetics Test Information

This test uses a methylation array to evaluate genome-wide methylation of central nervous system (CNS) tumors. Results are analyzed using the NCI/Bethesda CNS tumor classifier v2.0 developed by the National Institutes of Health, an in-house nearest-neighbors assisted unsupervised analysis (NN Method), and the MGMT promoter methylation mgmtstp27 R package.

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 to ensure specimen adequacy.

Special Instructions

- Tissue Requirements for Solid Tumor Next-Generation Sequencing
- Primary Central Nervous System Tumor Genetic Testing Guide
- Reportable Methylation Families and Classes for Neuro-Oncology Genome-Wide Methylation Array Analysis

Method Name

Methylation Array

NY State Available

No

Specimen

Specimen Type

Varies

Ordering Guidance

This assay provides a genome-wide methylation profile and *MGMT* promoter methylation status of primary central

nervous system (CNS) tumors.

For next-generation sequencing to evaluate for microsatellite instability status, somatic mutations, and rearrangements involving targeted genes associated with CNS tumors, order NONCP / Neuro-Oncology Expanded Gene Panel with Rearrangement, Tumor.

For evaluation of copy number abnormalities and loss of heterozygosity, order CMAPT / Chromosomal Microarray, Tumor, Formalin-Fixed Paraffin-Embedded.

For test selection guidance for primary CNS tumors see Primary Central Nervous System Tumor Genetic Testing Guide.

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

Necessary Information

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. Pathologic diagnosis (final or preliminary)

Specimen Required

This assay requires at least 60% tumor nuclei.

-Preferred amount of tumor area with sufficient percent tumor nuclei: tissue 360 mm(2)
-Minimum amount of tumor area: tissue 144 mm(2)
-These amounts are cumulative over up to 20 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).
-If ordered in conjunction with other tests, please refer to specimen requirements for the specific tests.

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

Acceptable:
Specimen Type: Tissue slide
Slides: 1 Hematoxylin and eosin-stained and 20 unstained
Collection Instructions:
Submit the following slides:

1 Slide stained with hematoxylin and eosin
AND
20 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 20 slides from the same block.
Additional Information:
1. Unused unstained slides will not be returned.
2. Broken or cracked unstained slides will be rejected. Stained slides that were broken and repaired (ie, double mounted) at the client site will not be rejected, as, in most cases, they can still be reviewed under the microscope. However, due to the double mounting, digital scanning cannot be performed.

Forms
If not ordering electronically, complete, print, and send 1 of the following forms with the specimen:
-Oncology Test Request (T729)
-Children's Oncology Group Test Request (T829)

Specimen Minimum Volume
See Specimen Required

Reject Due To

Broken or cracked unstained slides	Reject
Broken or cracked stained slides that cannot be repaired	Reject

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Varies	Ambient (preferred)		
	Refrigerated		

Clinical & Interpretive

Clinical Information
Methylation array profiling has shown clinical utility in establishing and refining the classification of central nervous system (CNS) tumors and has been incorporated as either essential or desirable diagnostic criteria for over 50% of tumor types included in the 2021 5th World Health Organization (WHO) classification of CNS tumors. Two specific tumor types, diffuse glioneuronal tumor with oligodendroglioma-like features and nuclear clusters and high-grade astrocytoma with

piloid features, are definitively diagnosable only with the use of methylation profiling. The c-IMPACT-NOW working group (involved in planning of future WHO classifications) recently codified the utility of DNA methylation profiling as recommended for diagnostic practice, particularly for difficult-to-diagnose CNS tumors. *MGMT* promoter methylation status is also obtained using this test and is a well-established prognostic biomarker and a strong predictor of alkylating chemotherapy response for patients with glioblastoma, IDH-wildtype. *MGMT* promoter methylation testing using methylation array has been clinically validated in retrospective and prospective studies.

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 implications.

This assay has three reportable results:
1) Tumor classification at methylation family and methylation class levels generated by the NCI/Bethesda Classifier v.2.0, reported as shown in Table 1
2) Nearest neighbor Method (NN Method) class compared to the NCI/Bethesda Classifier v.2.0 methylation class results, reported as shown in Table 1
3) *MGMT* promoter methylation status, reported as methylated (score > or =0.358) or unmethylated (score <0.358)

Table 1. Classifier and Nearest Neighbor (NN) Method Interpretation

Classifier Family score	Classifier Class score	Classifier summary	NN Method Class concordant(a)	NN Method Class discordant(b) or not available(c)
> or =0.900	> or =0.900	Family MATCHED Class MATCHED	Supportive	Non-contributory
> or =0.900	0.840-0.899	Family MATCHED Class SUGGESTED	Supportive	Non-contributory
> or =0.900	<0.840	Family MATCHED Class NO MATCH	Non-contributory*	Non-contributory*
<0.900	Any	Family NO MATCH Class NO MATCH	Non-contributory*	Non-contributory*

(a) NN Method Class result is identical to Classifier Class
(b) NN Method Class result is different from Classifier Class
(c) NN Method Class result was not available as a consensus class among at least three of the five closest reference dataset samples (“neighbors”) was not reached
*For Classifier Class NO MATCH, there is no reportable Classifier Class to be compared with NN Method Class

Cautions

Abnormal methylation patterns sufficiently distinct from the reference dataset patterns can impact the ability of the classifier to provide high confidence results.

Discordant methylation profiling results may be obtained using different classifiers and different classifier versions.

The nearest-neighbors assisted unsupervised analysis (NN) method is complementary to the classifier and the results are not to be used as a stand-alone method.

Discordant *MGMT* methylation results may occur due to different testing methodologies and/or evaluation of different CpG sites.

Broken or cracked unstained slides are not compatible with the instrumentation used in our performing laboratories and will be rejected. Additional material may be requested.

Attempts will be made to repair broken or cracked stained slides; however, if they are unable to be repaired (ie, shattered in transit), they will be rejected and discarded due to safety concerns.

Supportive Data

Performance Characteristics
Validation studies demonstrated overall concordance of results between samples tested in-house and National Institutes of Health-tested samples was 88.7% (55/62 samples), with 91.9% (57/62) Family/Class classifier concordance, 98.4% (61/62) nearest-neighbors assisted unsupervised analysis concordance, and 98.4% (61/62) *MGMT* promoter methylation concordance.

Inter- and Intra-run reproducibility replicates were 100% concordant at limit of detection for all reportable results.

To ensure accuracy, this test will be performed on cases estimated by a pathologist to have at least 60% tumor cells and requires a minimum of 75ng of DNA.

Clinical Reference

1. WHO Classification of Tumours Editorial Board. Central nervous system tumours. 5th ed. World Health Organization; 2021. WHO Classification of Tumours. Vol 6
2. Aldape, K, Capper D, von Deimling A et al. cIMPACT-NOW update 9: Recommendations on utilization of genome-wide DNA methylation profiling for central nervous system tumor diagnostics. Neurooncol Adv. 2025:7(1): vdae228
3. Bady P, Delorenzi M, Hegi ME. Sensitivity analysis of the *MGMT*-STP27 model and impact of genetic and epigenetic context to predict the *MGMT* methylation status in gliomas and other tumors. J Mol Diagn. 2016;18(3):350-361
4. Bady P, Sciuscio D, Diserens AC, et al. *MGMT* methylation analysis of glioblastoma on the Infinium methylation BeadChip identifies two distinct CpG regions associated with gene silencing and outcome, yielding a prediction model for comparisons across datasets, tumor grades, and CIMP-status. Acta Neuropathol. 2012;124(4):547-560
5. Capper D, Jones DTW, Sill M, et al. DNA methylation-based classification of central nervous system tumours. Nature. 2018;555(7697):469-474
6. Capper D, Stichel D, Sahm F, et al. Practical implementation of DNA methylation and copy-number-based CNS tumor diagnostics: the Heidelberg experience. Acta Neuropathologica 2018; 136(2):181-210
7. Furtado L, Ikemura K, Benkli C, et al. General applicability of existing CAP accreditation requirements to clinical implementation of machine learning-based methods in molecular oncology testing. Arch Pathol Lab Med. 2025;149(4):319–327
8. Galbraith K, Vasudevaraja V, Serrano J, et al. Clinical utility of whole-genome DNA methylation profiling as a primary molecular diagnostic assay for central nervous system tumors-A prospective study and guidelines for clinical testing. Neurooncol Adv 2023;5(1):vdad076. (In eng). doi:10.1093/noajnl/vdad076
9. Kling T., Wenger A., Beck S., Caren H. Validation of the MethylationEPIC BeadChip for fresh-frozen and formalin-fixed paraffin embedded tumours. Clinical Epigenetics 2017;9:33
10. Wu Z, Abdullaev Z, Pratt D, et al. Impact of the methylation classifier and ancillary methods on CNS tumor

diagnostics. Neuro Oncol. 2022;24(4):571-581. doi:10.1093/neuonc/noab227

Performance

Method Description

This test uses the Illumina Infinium MethylationEPIC v2.0 kit to detect cytosine methylation at 935,000 CpG loci based on highly multiplexed genotyping of bisulfite-converted gDNA (Package insert: Infinium MethylationEPIC v2.0 BeadChip, Illumina Inc; 2022).

Sample data are analyzed using an in-house developed quality control module, the proprietary National Institutes of Health-developed NCI/Bethesda CNS (central nervous system) tumor classifier v2.0 and an in-house developed nearest-neighbors assisted unsupervised analysis (NN method).(Wu Z, Abdullaev Z, Pratt D, et al. Impact of the methylation classifier and ancillary methods on CNS tumor diagnostics. Neuro Oncol. 2022;249[4]:571-581. doi:10.1093/neuonc/noab227]) The NCI/Bethesda classifier generates tumor classification at family and class levels. It also provides a Uniform Manifold Approximation and Projection (UMAP) plot to visualize how the tested sample clusters relative to the reference dataset. Since UMAP is inherently subjective, we implemented the NN method to identify the five closest reference samples ("neighbors") to the test sample, enabling objective estimation of the test sample methylation class cluster. The NN method is complementary to the classifier and interpreted in the context of classifier results. For more information see Reportable Methylation Families and Classes for Neuro-oncology Genome-Wide Methylation Array Analysis.

The *MGMT* promoter methylation status is predicted using the mgmtstp27 R package.(Bady P, Sciuscio D, Diserens AC, et al. MGMT methylation analysis of glioblastoma on the Infinium methylation BeadChip identifies two distinct CpG regions associated with gene silencing and outcome, yielding a prediction model for comparisons across datasets, tumor grades, and CIMP-status. Acta Neuropathol. 2012;124[4]:547-560. doi:10.1007/s00401-012-1016-2)

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

PDF Report

No

Day(s) Performed

Varies

Report Available

10 to 16 days

Specimen Retention Time

Tissue blocks: Unused portions of blocks will be returned; Stained slides: Indefinitely.

Performing Laboratory Location

Mayo Clinic Laboratories - Rochester Main Campus

Test Definition: MTNON

Neuro-Oncology Genome-Wide Methylation Array Analysis, Tumor

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

81524

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
MTNON	Neuro-Onc Methylation Array, Tumor	In Process

Result ID	Test Result Name	Result LOINC® Value
623145	Result	82939-0
623146	Interpretation	69047-9
623147	Additional Information	48767-8
623553	Method	85069-3
623554	Disclaimer	62364-5
623148	Specimen	31208-2
623149	Source	31208-2
623555	Tissue ID	80398-1
623150	Released By	18771-6