Test Definition: MGMTD

MGMT Promoter Methylation Analysis, Tumor

Overview

Useful For

Prognostication of newly diagnosed patients with glioblastoma

Identification of newly diagnosed glioblastoma patients that may derive benefit from alkylating chemotherapy (ie, temozolomide)

Therapy selection for newly diagnosed glioblastoma in older patients (>60-65 years)

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.

Highlights

MGMT promoter methylation status has prognostic and predictive value for patients with glioblastoma.

Method Name

Droplet Digital Polymerase Chain Reaction (ddPCR)

NY State Available

Yes

Specimen

Specimen Type

Varies

Necessary Information

A pathology report (final or preliminary), containing the following information, is required and 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

Specimen Required

This assay requires at least 20% tumor nuclei.
-Preferred amount of tumor area with sufficient percent tumor nuclei: tissue 144 mm(2) tissue (4 x 6 mm x 6 mm areas)
-Minimum amount of tumor area: 36 mm(2) tissue (1 x 6 mm x 6 mm area)
-These amounts are cumulative over up to 10 unstained slides and must have adequate percent tumor nuclei.
-Tissue fixation: formalin-fixed paraffin-embedded (FFPE), non-decalcified

Submit only 1 of the following specimens:

Specimen Type: Tissue block
Collection Instructions: Submit a formalin-fixed non-decalcified, paraffin-embedded tissue block.

Specimen Type: Tissue slide
Slides: 1 Stained and 10 unstained
Collection Instructions: Submit 1 slide stained with hematoxylin and eosin and 10 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 10 slides from the same block.
Additional Information:
1. Hematoxylin and eosin-stained and 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 an Oncology Test Request (T729) with the specimen.

Specimen Minimum Volume

5 unstained slides at 5-microns thickness

Reject Due To

Specimens that have been decalcified (all methods)	Reject
Specimens that have not been formalin-fixed, paraffin-embedded	Reject
Extracted nucleic acid (DNA/RNA)	Reject
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

MGMT (O[6]-methylguanine-DNA methyltransferase) encodes a DNA repair enzyme. This enzyme rescues tumor cells from alkylating agent-induced damage and confers tumor resistance to chemotherapy with alkylating agents. Epigenetic silencing of *MGMT* by increased methylation of CpG sites within the promoter region results in decreased *MGMT* expression and reduces MGMT-mediated DNA repair of alkylating agent-induced DNA damage in tumor cells, increasing tumor sensitivity and response to alkylating chemotherapy.

MGMT promoter methylation status is a molecular biomarker for glioblastoma, which is the most frequent malignant primary central nervous system (CNS) adult-type tumor. In newly diagnosed patients with glioblastoma, *MGMT* promoter "methylated" status indicating increased levels of methylation is an independent favorable prognostic biomarker and a strong predictor of response to alkylating chemotherapy. Current standard of care treatment for glioblastoma patients consists of surgical resection followed by radiotherapy and alkylating chemotherapy with temozolomide. Older patients (>60-65 years) often have decreased tolerance for combined chemoradiation. For this group of patients, *MGMT* promoter methylation status guides treatment decision between monotherapy with the alkylating agent temozolomide versus radiotherapy alone.

Glioblastoma was originally defined solely based on histological features. Based on the 2021 World Health Organization (WHO) classification of CNS tumors, the original glioblastoma is now divided in "glioblastoma, IDH-wildtype, CNS WHO grade 4" (most cases) and "astrocytoma, IDH-mutant, CNS WHO grade 4".(1) In isocitrate dehydrogenase (IDH)-mutant diffuse gliomas, *MGMT* promoter "methylated" status is frequent and strongly associated with the IDH mutation-induced glioma CpG island methylator phenotype (G-CIMP), except for the uncommon infratentorial IDH-mutant astrocytomas. Therefore, the clinical significance of *MGMT* promoter methylation status in the context of IDH-mutant diffuse gliomas is less firmly established.

MGMT promoter methylation status may be evaluated by multiple methods, and the testing platform with most prospective clinical trial validation is methylation-specific polymerase chain reaction evaluating downstream CpG sites.

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

Test results should be interpreted in context of clinical findings, tumor sampling, and other laboratory data. If results obtained do not match other clinical or laboratory findings, contact the laboratory for possible interpretation. 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 types of fixatives are discouraged.

Improper treatment of tissues, such as decalcification, may cause polymerase chain reaction failure.

Rare polymorphisms exist that could lead to false-negative or false-positive results.

This test evaluates for the presence of increased levels of methylation of downstream CpG sites 75-80 and 84-87. Analytical validation studies showed that this assay requires at least 25 methylated copies for a positive result. Retrospective clinical validation study of approximately 200 patients with integrated diagnosis of glioblastoma, IDH-wildtype with grade 4 histological features who were treated with standard of care regiment including temozolomide established the cutoff of 6.50% fraction abundance to distinguish two groups of patients with statistically different overall survival rates. Using the combined cutoff of at least 25 methylated copies and 6.50% fraction abundance to define positive for increased promoter methylation ("methylated") status, patients with tumors positive for increased *MGMT* promoter methylation ("methylated") status have shown improved survival when compared to patients with tumors negative for increased *MGMT* promoter methylation ("unmethylated") status.

Negative results do not exclude the possibility that increased levels of methylation may be present but below the cut-offs for this assay due to low tumor purity. This assay requires at least 20% tumor.

Negative results do not exclude the presence of increased levels of methylation in other 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

Analytical validation studies demonstrated limit of quantitation for the assay to be 25 methylated copies. Fraction abundance (FA) threshold of 6.50% was above the estimated upper confidence interval limit of the 95th% quantile of the 95% confidence interval of values for non-neoplastic brain tissue. Clinical validation studies showed that this FA threshold favored identifying both low and high levels of methylation as positive for increased levels of methylation/"methylated" status, so that a negative result is more likely to indicate a truly negative result for increased levels of methylation/"unmethylated" status. Both values for methylated copies and FA will be used to determine if a sample is positive or negative for increased *MGMT* promoter methylation. A minimum of 360 unmethylated copies is required for a sample to be confidently resulted as negative.

Concordance between this test and the prior Mayo Clinic methylation-specific polymerase chain reaction and methylation array methods for detection of *MGMT* promoter methylation were 95% (194/205) and 97% (38/39), respectively.

The minimum viable tumor percent and DNA input required for testing are 20% and 25 ng, respectively.

Clinical Reference

1. WHO Classification of Tumours Editorial Board. Central Nervous System Tumours. 5th ed. IARC Press; 2021. WHO Classification of Tumours, Vol 6
2. Hegi ME, Diserens AC, Gorlia T, et al. *MGMT* gene silencing and benefit from temozolomide in glioblastoma. N Engl J Med. 2005;352(10):997-1003
3. Stupp R, Hegi ME, Mason WP, et al. Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomised phase III study: 5-year analysis of the EORTC-NCIC trial. Lancet Oncol. 2009;10(5):459-66
4. Wick W, Platten M, Meisner C, et al. Temozolomide chemotherapy alone versus radiotherapy alone for malignant astrocytoma in the elderly: The NOA-08 randomised, phase 3 trial. Lancet Oncol. 2012;13(7):707-715
5. Malmstrom A, Gronberg BH, Marosi C, et al. Temozolomide versus standard 6-week radiotherapy versus hypofractionated radiotherapy in patients older than 60 years with glioblastoma: the Nordic randomised, phase 3 trial. Lancet Oncol. 2012;13(9):916-926
6. Hegi ME, Genbrugge E, Gorlia T, et al. *MGMT* promoter methylation cutoff with safety margin for selecting glioblastoma patients into trials omitting temozolomide: A pooled analysis of four clinical trials. Clin Cancer Res. 2019;25(6):1809-1816
7. Mansouri A, Hachem LD, Mansouri S, et al. *MGMT* promoter methylation status testing to guide therapy for glioblastoma: refining the approach based on emerging evidence and current challenges. Neuro Oncol. 2019;21(2):167-178
8. Wen PY, Weller M, Lee EQ, et al. Glioblastoma in adults: a Society for Neuro-Oncology (SNO) and European Society of Neuro-Oncology (EANO) consensus review on current management and future directions. Neuro Oncol. 2020;22(8):1073-1113
9. Brat DJ, Aldape K, Bridge JA, et al. Molecular biomarker testing for the diagnosis of diffuse gliomas. Arch Pathol Lab Med. 2022;146(5):547-574
10. Hegi ME, Oppong FB, Perry JR, et al. No benefit from TMZ treatment in glioblastoma with truly unmethylated *MGMT* promoter: Reanalysis of the CE.6 and the pooled Nordic/NOA-08 trials in elderly glioblastoma patients. Neuro Oncol. 2024;26(10):1867-1875

Performance

Method Description

Droplet digital polymerase chain reaction using a TaqMan assay is performed on bisulfite converted DNA to evaluate for the presence of increased levels of methylation involving CpG sites 75-80 and 84-87 within the promoter region of the *MGMT* gene.(Unpublished Mayo method)

PDF Report

No

Day(s) Performed
Varies

Report Available
7 to 10 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; Digital images are obtained and stored for all slides used in testing.

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
81287
88381-Microdissection manual

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
MGMTD	MGMT Promoter Methylation Analysis	60252-4

Result ID	Test Result Name	Result LOINC® Value
621859	Result Summary	50397-9
621860	Result	60252-4
621861	Interpretation	69047-9
621862	Additional Information	48767-8
621863	Specimen	31208-2
621864	Source	31208-2
621865	Tissue ID	80398-1

621866	Method	85069-3
621867	Disclaimer	62364-5
621868	Released by	18771-6