METHYLATION ASSAY

**MGMT Pyrosequencing Methylation Assay for Glioblastoma**

**Background**

Glioblastoma is the most common and most aggressive malignant primary brain tumor. While occurring in only two to three cases per 100,000 people in North America, glioblastoma represents 52% of all functional tissue brain tumor cases and 20% of all intracranial tumors. Prognosis for those diagnosed with glioblastoma is poor, with a median survival time of about 14 months.¹

Patients with glioblastoma can be treated with alkylating agents such as Temador® (temozolomide). Epigenetic silencing of the *MGMT* (O⁶-methylguanine-DNA methyltransferase) DNA-repair gene by promoter methylation compromises DNA repair and has been associated with longer survival in patients with glioblastoma who receive temozolomide.²,³

Temozolomide kills tumor cells by producing cross-links between DNA strands and inhibiting DNA replication. The most common alkylation site is the O⁶ position of guanine. O⁶-methylguanine DNA methyltransferase (*MGMT*) is a DNA repair protein that reverses such DNA alkylation and confers chemoresistance by repairing DNA damage. Temozolomide seems to work by sensitizing the tumor cells to radiation.⁴

Recent clinical studies confirm that the presence of *MGMT* promoter methylation in tumor samples corresponds to an increased likelihood that tumor cells would be responsive to temozolomide.³,⁴,⁵,⁶ If the promoter was methylated, temozolomide was more effective. It is estimated that approximately 40 to 50% of glioblastoma tumors exhibit *MGMT* gene methylation, which correlates significantly with reduced DNA damage repair induced by alkylating agents and significantly enhanced chemosensitivity.⁴

According to recent clinical trials, glioblastoma patients with *MGMT* methylation respond to temozolomide two to three times better than those lacking *MGMT* methylation. Prolonged overall and progression-free survival at 24 months was 80% for those with *MGMT* methylation vs. 20% for those lacking *MGMT* methylation.

**Clinical Indications**

For patients diagnosed with glioblastoma to determine if a methylated *MGMT* promoter is present, which is a favorable prognostic indicator for temozolomide treatment. Individuals without a methylated *MGMT* promoter do not have such a benefit. *MGMT* “silence” is the most significant guide for the treatment of glioblastoma. This assay is to validate the methylation status of the *MGMT* gene.

**Methodology**

Pyrosequencing technology, which is based on the principle of sequencing by synthesis, provides quantitative data in sequence context within minutes. Real-time sequence information is highly suitable for quantification of CpG methylation. We have validated pyrosequencing-based assay in detection of *MGMT* methylation in paraffin-embedded biopsy tissue specimens. With 10% average methylation as a cutoff, *MGMT* promoter methylation was detected in glioblastoma, but not detected in non-neoplastic brain tissue. The analytical sensitivity of the assay is 5% of target cells harboring *MGMT* methylation.

Diagnostic *MGMT* testing requires sufficient and optimally preserved tumor tissue. The biopsy should be at least 0.5 cm in size and necrosis should be less than 15%. Both frozen and paraffin-embedded tissue are suitable for the pyrosequencing-based *MGMT* methylation assay.
**Interpretation**

Positive for *MGMT* methylation: equal or greater than 10% of methylation in any CpG island or in average of all CpG islands analyzed.

Negative for *MGMT* methylation: less than 10% of any CpG island or in average of all CpG islands analyzed.

**References**


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**Test Overview**

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<th>Test Name</th>
<th><em>MGMT</em> Methylation Assay for Glioblastoma</th>
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<td>Ordering Mnemonic</td>
<td><em>MGMT</em> Methylation</td>
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<td>Methodology</td>
<td><em>MGMT</em> Methylation with Pyrosequencing</td>
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<tr>
<td>Specimen Requirements</td>
<td>The biopsy should be at least 0.5 cm in size and necrosis should be less than 15%. The best results with methylation-specific PCR are obtained with cryopreserved tumor specimens.</td>
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<td>Reference Range</td>
<td>Positive for <em>MGMT</em> methylation: ≥ of 10% of any CpG island or average of all 5 CpG islands. Negative for <em>MGMT</em> methylation: &gt;10% of methylation or average of all CpG islands analyzed.</td>
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<td>Billing Code</td>
<td>84235</td>
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<td>CPT Codes</td>
<td>81287 (x1); G0452 (x1)</td>
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