Clinical Validation of the Agena Bioscience MGMT Promoter Quantitative Methylation Profiling Assay for use in Glioma Patient Management in a Healthcare System

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Abstract ST023

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Introduction

The methylguanine-DNA methyltransferase (*MGMT*) gene is epigenetically silenced by promoter hypermethylation in gliomas. Clinical trials have shown that hypermethylation of the *MGMT* promoter serves as a strong prognostic factor for increased responsiveness to Temozolomide. We describe here the validation of a quantitative *MGMT* methylation assay to replace our existing qualitative assay.

Design

The Agena *MGMT* Methylation Profiling Panel detects methylation through a multistep process that includes bisulfite conversion, PCR amplification, and transcription into a single-stranded RNA which is then cleaved at uracil sites. Cleavage products are dispensed onto a support matrix (SpectroCHIP® Array) and loaded into the MassARRAY MALDI-TOF for data acquisition. Data acquired by the MassARRAY Analyzer is processed using MassARRAY EpiTyper software. The assessed region within the MGMT promoter consists of 12 CpG sites within 5 cleavage fragments. Sample methylation level is the average methylation of all CpG sites.

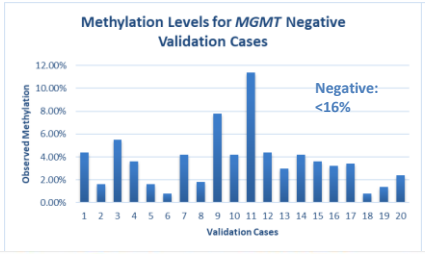
Coverage:
The CpG island coverage is chr10:129,467,212-129,467,309 with twelve CpG units organized into five CpG clusters as follows: CpG1.2, CpG3.4.5, CpG6.7, CpG8.9.10, and CpG11.12

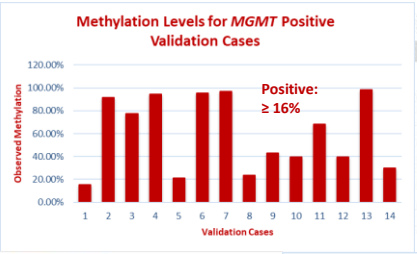
Reference

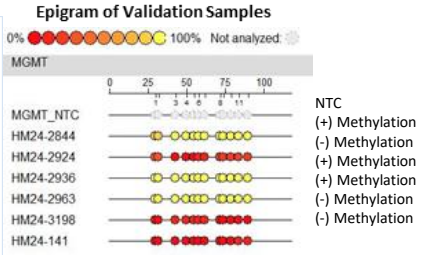
1. Agena Bioscience. (n.d.). EpiTYPER V1.4 Release Notes.
2. Agena Bioscience, Inc. (2018). EpiTYPER User Guide.
3. CpG and Epigram Images copied with permission from Agena.

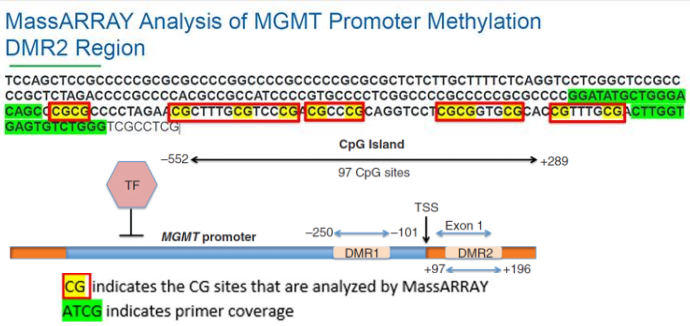
Results

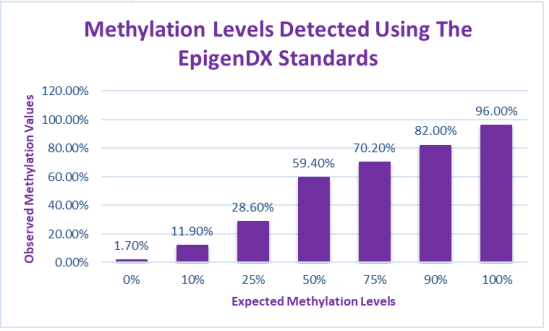
Thirty-four previously evaluated clinical specimens for *MGMT* promoter methylation were used, in addition to *MGMT* methylation standards obtained from EpigenDx. The standards were at: 0%, 10%, 25%, 50%, 75%, 90%, and 100% methylation. Samples and standards were tested over several runs and all yielded the expected results. Twenty of the 34 clinical samples were known negatives and were negative with the Agena methylation assay demonstrating 100% specificity. All negative specimens yielded methylation levels ranging from 0.8%-11.4% in keeping with a cutoff of ≥16% methylation for a positive call. Fourteen previously positive clinical specimens and 5 standards were also positive ≥16% methylation. Reproducibility and precision of the assay was established according to guidelines. Limit of detection was determined to fall at ≥5% tumor cellularity.











Conclusions

The EpiTyper *MGMT* assay was validated for clinical use and was shown to robustly and reliably detect methylation levels in 12 CpG units within the *MGMT* promoter. Result concordance was demonstrated for 34 previously characterized clinical specimens and commercial standards. Methylation cutoff is set at 16% in keeping with the literature and previously characterized qualitative positives. A sensitivity and specificity of 100% was shown. Limit of detection was determined at 5% tumor content.