Performance Evaluation of Methylation-Specific Real-Time PCR (MSP) Assay for the Detection of O (6)-methylguanine-DNA methyltransferase (*MGMT*) Promoter Methylation in Glioblastoma Multiforme (GBM)

Jessica Barry, Sameer S. Talwalkar (Division of Molecular Diagnostics, CPA Lab, Louisville, KY)

INTRODUCTION

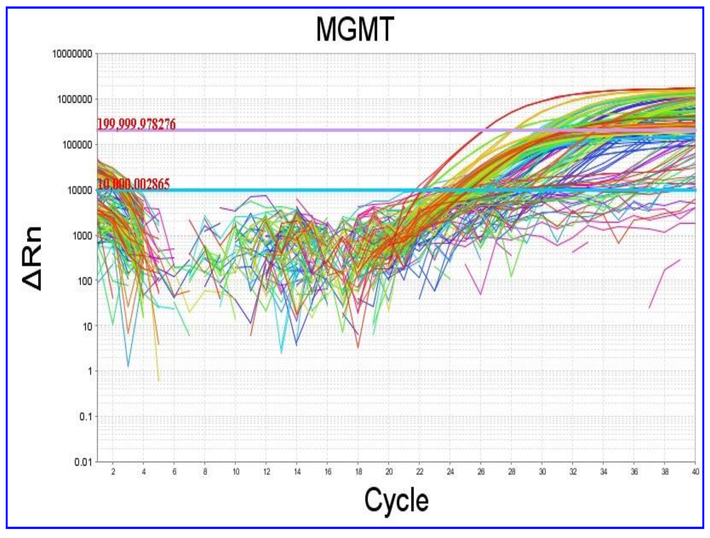
Methylation of *MGMT* promoter is associated with increased response to alkylating agents in patients with glioblastoma multiforme (GBM)

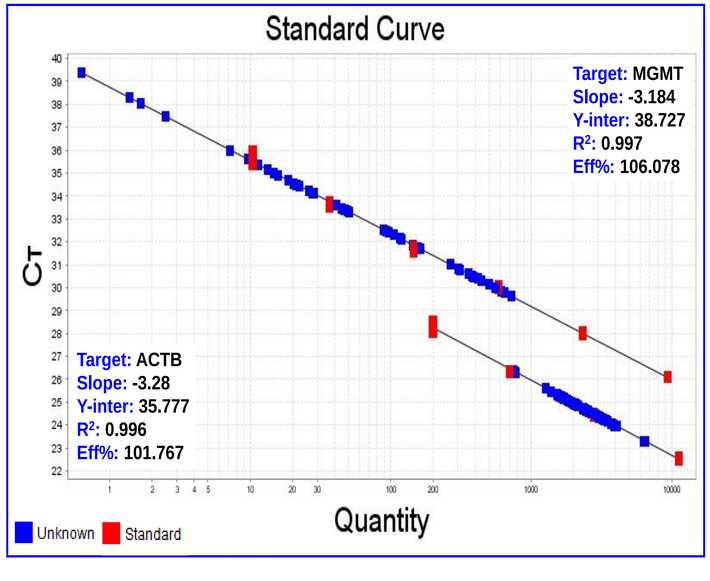
OBJECTIVE

Objective of this study was to test performance characteristics of the EntroGen *MGMT* assay in a mid-sized clinical reference laboratory

MATERIALS AND METHODS

- Genomic DNA extracted from macrodissected FFPE slides from patient samples (N=31), control materials (N=6), and the 2017 CAP GLI-B survey (N=3) were tested.
- All patient samples were diluted to 200-500ng and processed with the EZ DNA Methylation-Lightning™ Kit according to manufacturer's protocol with the modification of eluting into a 30uL volume.
- Control material with known methylation was tested to establish analytical measurement range (AMR).
- Assay includes 7 *MGMT* and *ACTIN-B* standards that cover the AMR as well as a no template control each tested in triplicate.
- Samples tested in triplicate with *ACTIN-B* (amplification control) and the average of three wells was used for result calculation.
- Samples used for validation were evaluated between performing technologists, shipments, multiple days, and correlation with a reference laboratory.





RESULTS

- Analytical measurement range (AMR) for methylated *MGMT* was determined to be between 10-9335 copies per 4uL (2.5-2333.75 copies/uL). Due to the broad AMR, a greater variability was observed at higher levels of methylation. Data was analyzed according to the parameters described in Table 1.
- 3 CAP *MGMT* proficiency testing (PT) survey samples (negative, low, and high positive) were tested for AMR verification. These demonstrated 100% correlation with CAP's intended responses for these specimens.
- Some discrepancies were observed between the reference lab methodology (Pyrosequencing) and the methylation-specific PCR (MSP), especially for samples which showed low level of methylation very near the lower limit of quantification. Using the resulting guideline (Table 1) helped eliminate this inconsistency for the MSP assay. MSP assay had 100% specificity as tested using 4 DNA samples extracted for other molecular assays (non-small cell lung carcinoma and melanoma) found to be negative for *MGMT* promoter methylation.

Table 1: *MGMT* Resulting Guidelines

Results valid if *ACTB* copy# is >200	
Unmethylated	*MGMT* copy# <10 **AND** %Methylation <5%
Methylated	*MGMT* copy# >10 **OR** %Methylation >5%

CONCLUSIONS

- ❖ The EntroGen MSP assay can be reliably used for the evaluation of *MGMT* promoter methylation on genomic DNA from FFPE-extracted tissue
- ❖ The assay is robust and has the capability to provide fast turn-around times
- ❖ This assay also provides quantitative assessment of methylation which can be used for assessment of treatment responsiveness and correlation with clinical outcome
- ❖ The resulting guideline can help in interpretation especially for samples with low level methylation
- ❖ Since going live, we have successfully completed two 2018 CAP PT surveys (GLI-A and GLI-B) with 100% correlation with intended responses (n=6).