ST078

MLH1 Promoter Methylation Analysis

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Background

Hereditary colon cancers of the nonpolyposis type (HNPCC) or Lynch Syndrome (LS) is an inherited cancer syndrome caused by a germline mutation in one of several genes involved in DNA mismatch repair (MMR), including *MLH1*, *MSH2*, *MSH6* and *PMS2*, or *EPCAM* gene. It is estimated to account for 1-3% of colorectal cancer (CRC). Although HNPCC is characterized by abnormal immunohistochemistry for MMR proteins and microsatellite instability (MSI-H), tumors exhibiting abnormal IHC for *MLH1* and MSI-H are most often sporadic (non-inherited) and due to abnormal methylation of the *MLH1* gene promoter or the *BRAF* V600E mutation, whereas tumors that show neither are most often caused by an inherited mutation. Testing for methylation of the *MLH1* promoter can distinguish sporadic from inherited cancers.

Methods

Colon and endometrium FFPE samples are prepared using the QIAamp DSP DNA FFPE kit (Qiagen). DNA then undergoes bisulfite conversion using the EZ DNA Methylation Kit (Zymo) followed by methylation-specific PCR of the MLH1 promoter. Assay's accuracy, repeatability, reproducibility, analytical sensitivity and stability were evaluated.

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Results

Of the specimens tested during validation, 33 colon and endometrium specimens were run and compared to Sanger sequencing. Results were 100% concordant. Repeatability (intra-assay precision) and reproducibility (inter-assay precision) were 100% using 13 specimens. This assay has a sensitivity to detect approximately 1-5% MLH1 promoter methylation in a background of unmethylated DNA. The DNA samples stored at 2-8°C were stable for at least 10 months. The bisulfite treated DNA samples were stable at -80°C for 10 days. The unstained slides stored at room temperature (15-30°C) were stable for at least 35 months.

Accuracy Validation Data

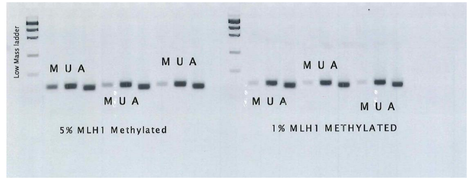
35 colon and endometrium FFPE specimens were run for both MLH1 methylation assay and Sanger sequencing. The results were 100%.

Precision

Repeatability (intra-assay precision) was 100% concordant for MLH1 using 7 MLH1 unmethylated specimens and 6 MLH1 methylated specimens.

Reproducibility (inter-assay precision) was 100% concordant for MLH1 using 7 MLH1 unmethylated specimens and 6 MLH1 methylated specimens.

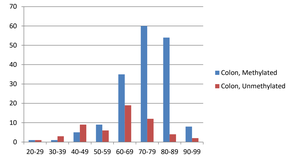
Detection Sensitivity



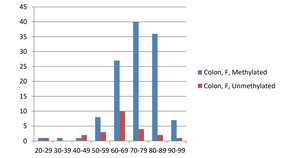
The MLH1 methylation assay reliably detected the MLH1 methylation status at 1-5%.

Clinical Testing Results

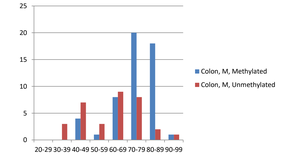
Colon, Methylated and Unmethylated Subject Age Distribution



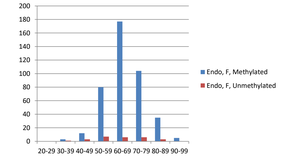
Colon, Female, Methylated and Unmethylated Subject Age Distribution



Colon, Male, Methylated and Unmethylated Subject Age Distribution



Endometrium, Methylated and Unmethylated Subject Age Distribution



The MLH1 promoter methylation assay has been offered as a clinical test based on the successful performance features. In a set of 442 endometrium specimens, 5.88% were unmethylated and 94.12% were methylated with 42.55% at age 60-69 and 86.78% at age 50-79. In a set of 229 colorectal specimens, 24.45% were unmethylated with 82.14% at age 40-79 and 75.55% were methylated with 86.12% at age 60-89. There was a significant shift of the MLH1 methylation age group vs unmethylation group for both female and male which could be due to other underlying germline mutations for colorectal cancer and aging effect. Similar findings were observed in other publications for the MSI unstable patient group. Besides one specimen with insufficient amount of DNA for testing, results were obtained for all other clinical specimens.

Conclusions

The MLH1 promoter methylation assay is a robust, reproducible and sensitive assay using FFPE specimens for distinguishing sporadic from inherited cancers.

References

Gausachs, M. et al. 2012. MLH1 promoter hypermethylation in the analytical algorithm of Lynch syndrome: a cost-effectiveness study. European Journal of Human Genetics. 20: 762-768.