Microsatellite Instability Testing on Solid Tumors

Background

Deficiency in mismatch repair occurs in approximately 15-20% of sporadic colorectal and endometrial cancers, and to a lesser degree in a variety of other solid tumors. Mismatch repair deficiencies have been associated with disease prognosis and been shown to be linked with potential response to the checkpoint inhibitor Pembrolizumab (KEYTRUDA®) in various solid tumors. Defects in the mismatch repair process can be assessed by capillary electrophoresis and amplification patterns for normal and tumor tissue compared for the detection of specific microsatellite sequences in the genome. Tumor samples are indicated to be deficient in MMR when one or more of the MMR proteins are not expressed, or to have high levels of microsatellite instability (MSI-H). In this study, the clinical and analytical performance features of the Promega MSI assay are evaluated.

Methods

Genomic DNA was isolated from both tumor and normal specimens of the same individual. Multiplex polymerase chain reaction analysis was used to amplify the BAT-25, BAT-26, NR-21, NR-24 and MCDN-27 loci. The fluorescent amplification products were analyzed by capillary electrophoresis and amplification patterns for normal and tumor tissue compared for the detection of novel fragments in tumor DNA, indicative of microsatellite instability. The assay’s accuracy, repeatability, reproducibility, analytical sensitivity and stability were evaluated.

Results

Of the specimens tested during validation, 19 FFPE specimens with known MSI results were 100% concordant. Repeatability (intra-assay precision) and reproducibility (inter-assay precision) were 100%. This assay can detect 5-10% of mutant in a background of wild-type genomic DNA. The DNA stored at 2-8°C was stable for at least 4 weeks and the sectioned FFPE slides stored at room temperature were stable for at least 1 year.

Accuracy Validation Data

<table>
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<th>Marker Name</th>
<th>GeneBank</th>
<th>Size Range (bp)</th>
<th>K562 Alleles (bp)</th>
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<td>BAT-25</td>
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</table>

Detection Sensitivity

The MSI assay was designed to reliably detect the MSI-H high value at 20% mutant level in the background of wild type.

Clinical Testing Specimen Results

The Promega MSI assay has been offered as a clinical test based on its successful performance features. In a set of 1650 colorectal cancer clinical specimens 15% were MSI-H, 85% MSI stable; in 211 endometrial cancer specimens 31% were MSI-H, 69% MSI stable; and in 231 other solid tumor specimens 5% were MSI-H, 95% MSI stable. Results could not be obtained in 7.4% of the specimens. This was mainly due to a low amount of amplifiable DNA obtained from these samples.

Conclusions

The Promega MSI assay is a robust, reproducible and sensitive assay using FFPE specimen for mismatch repair deficiency assessment.

References