ALLELIC LOSS (LOH) OF CHROMOSOME 1p: 19q BY MULTIPLEX POLYMERASE CHAIN REACTION ANALYSIS

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This test is indicated for the detection of LOH 1p:19q in all cases suspected of having oligodendroglioma and other malignant gliomas that may be found to be chemosensitive. It is an assessment of Allelic Loss or Loss of Heterozygosity (LOH) of chromosome 1p and 19q in suspected Oligodendroglioma and other malignant Glioma specimens.

Allelic loss of chromosome 1p is emerging as a marker of chemotherapeutic response and long term survival in patients with histologically defined anaplastic oligodendroglioma. Chromosome 1p loss may also identify other treatment-sensitive malignant gliomas, including rare glioblastomas. While 1p loss does not identify all chemosensitive tumors, nor do all patients whose tumors harbor 1p loss have long survival, those with combined chromosome 1p and 19q loss may have particularly favorable outcomes. Because LOH 1p:19q has clinical prognostic value and is strongly associated with classic histologic features of oligodendroglioma, testing for LOH may also be useful in the diagnosis of this clinical entity.

The routine analysis for LOH 1p:19q has been previously hampered by the common limitations of small sample size and the requirement of frozen tissue. Recent technical advances have given rise to the use of paraffin embedded fixed tissues for LOH analysis. Using PCR analysis of highly polymorphic microsatellite repeats and capillary electrophoresis detection, LOH 1p:19q analysis can now be performed in the clinical molecular diagnostic laboratory.

Figure 1 & 2. Example of patient normal tissue and tumor tissue showing allelic loss (Loss of Heterozygosity) on chromosome 1 (marker D1S199) and chromosome 19 (marker D19S206). Patient shows normal heterozygous microsatellite allelic pattern in normal (peripheral blood) and shows lost allele in tumor tissue.
To detect LOH 1p:19q in suspect tumor samples, three consecutive sections from the suspect tissue block are cut. The first section is stained and after pathologist evaluation, an area containing over 90% tumor is selected and marked on the slide. The tissue contained in the marked area is removed on the 2 subsequent unstained slides and DNA is extracted and purified. Peripheral blood DNA from the same patient is also prepared as a control for the baseline, normal allelic pattern in the patient. Multiplex PCR using fluorescent labeled primers to eight highly polymorphic microsatellite repeats flanking the areas of expected allelic loss on the chromosomes is performed.

Amplified products are analyzed using capillary electrophoresis on an ABI 3100-Avant Genetic Analyzer. This detection method for the amplicons produced in the multiplex reaction involves capillary electrophoresis and differential fluorescence detection of the amplified products. The primers used in the three reaction mixtures are labeled with different fluorescent dyes, each corresponding to a different microsatellite marker, and are detected simultaneously (4-color analysis). This detection system results in high sensitivity, single base resolution, differential product detection and relative quantitation. Fragment analysis of the amplified products, using GeneMapper software, and comparison of the normal versus the tumor genotype completes the analysis for possible allelic loss. The inter-assay and intra-assay reproducibility in size determination using capillary electrophoresis is approximately 1-2 base pairs. This reproducibility and sensitivity allows for accurate comparison of the patient’s normal allelic pattern with the pattern found in the submitted tumor.