Bcl-2 / JH t(14:18) TRANSLOCATION DETECTION BY POLYMERASE CHAIN REACTION AND FLUORESCENT CAPILLARY ELECTROPHORESIS DETECTION

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This test is indicated for the accurate differentiation of malignant vs. benign lymphoproliferative disorders and for establishing B tumor cell lineage. It is an assessment of the presence of the Bcl-2/JH t(14:18) translocation. This translocation is found in 70-90% of Non-Hodgkin Follicular Lymphomas, 50% of Undifferentiated B-cell Lymphomas and 20-30% of Diffuse Large B-cell Lymphomas. In tissue containing the translocation, the 5' Bcl-2 proto-oncogene is translocated on chromosome 14 near the JH and C junction genes of IgH. The breakpoint in Bcl-2 is either in the 3' untranslated region of exon 3 (major breakpoint region or MBR) in 70% of cases, or more distally 3' of exon 3 (minor cluster region or MCR) in the remaining 30%. This illegitimate recombination forms no fusion protein, but does create a promoter exchange whereby the immunoglobulin gene enhancer stimulates the expression of Bcl-2. Since Bcl-2 is an apoptosis inhibitor, cell death is delayed, and there is cell accumulation more than real transformation. The translocation interferes with normal apoptosis and results in the prevention of programmed cell death through deregulated expression of bcl-2.

The Bcl-2/JH assay is useful for establishing diagnosis, predicting prognosis and monitoring therapeutic response in the above named patient population. The presence of the bcl-2 translocation is also an indicator of poor prognosis in diffuse large B cell lymphomas and may be helpful in the clinical management of these patients. The addition of this assay to the pathology service presents a useful adjunct to our molecular testing repertoire for lymphoma. The Bcl-2/JH assay can be used to determine the presence of a monoclonal B cell population in a submitted sample, indicative of a neoplastic lymphoproliferative disorder. The Polymerase Chain Reaction (PCR) is utilized to detect the clonal population and can be used on fresh or paraffin embedded material.

To detect Bcl-2/JH translocation, primers are constructed to amplify the DNA flanking the regions where the translocation is known to occur. The primers target the joining region of the immunoglobulin heavy chain gene (chromosome 14) and distinct regions of the Bcl-2 gene (chromosome 18). In the natural condition, no PCR product would be generated when these primers are used to amplify human DNA since the targeted regions lie on different chromosomes. In contrast, when DNA containing a Bcl-2/JH translocation t(14:18) is amplified, a PCR product of a size within an expected bp range is formed. The assay utilizes 3 master mixes to detect the Bcl-2/JH translocation. One pair targets the major breakpoint region or MBR. The other 2 pairs are nested primers targeting the minor cluster region or MCR.

A powerful detection method for the amplicons produced in the multiplex reaction involves capillary electrophoresis and differential fluorescence detection of the amplified products. Some of the primers used in the master mixes are labeled with different fluorescent dyes, each corresponding to a different target region (MBR or MCR). Reaction products from the different master mixes can be pooled, fractionated using capillary electrophoresis and detected simultaneously. This detection system results in unsurpassed sensitivity, single base resolution, differential product detection and relative quantitation. Further, differential detection allows accurate, reproducible and objective interpretation of primer-specific products and automatic archiving of data. The limit of detection of this assay has been determined to be approximately 1 clonal cell in approximately 10,000 normal cells. The inter-assay and intra-assay reproducibility in size determination using capillary electrophoresis is approximately 1-2 base pairs. This reproducibility and sensitivity allows monitoring and tracking of individual tumors.
Figure 1 & 2. Example of Archived CAP Survey sample DNAs demonstrating Bcl-2/JH t(14:18) translocation in the MBR (Figure 1-black peak) and MCR (Figure 2-green peak) regions.