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QUANT BCL2/IGH ® Cat.1.006
Real Time Quantitative PCR of t(14;18) (Bcl2/IgH) for diagnosis and monitoring of follicular Cell lymphoma

Follicular Cell Lymphoma (FCL) is the most common indolent non-Hodgkin's lymphomas (NHL), accounting for 25-35% of all adult NHL (1). In more than 80% of FCL cases, tumor cells carry the t(14;18) translocation which results in the juxtaposition of the BCL-2 gene, located on chromosome 18, to the joining region (JH) of immunoglobulin heavy chain genes, on chromosome 14 (Fig. 1) (2). In addition, the BCL2/IgH translocation is found in 30% of diffuse large B cell lymphomas (DLBCL). As a consequence of the t(14;18) rearrangement, the BCL2 gene undergoes the control of the em IgH enhancer, resulting in the constitutive hyper expression of the antiapoptotic BCL2 protein in lymphoma cells. BCL2/IgH rearrangements cluster to specific BCL2 breakpoint regions, with the majority of cases occurring in the major breakpoint region (MBR) (3), which comprises about 70% of all the breakpoints on chromosome 18. This region was identified in a 300 bp segment located upstream to the 3' untraslated region (3'UTR) of exon 3 of BCL2 gene (4). The breakpoint on chromosome 14 can be located at any consensus region of JH fragment of the IgH locus.

Methodology

The assay is based on REAL-Time PCR strategy able to quantify the number of cells carrying the BCL2/JH rearrangement (MBR) by the taqman technology. The exact amount of BCL2/IgH-containing gene copies, for each point of provided standard curve was obtained by a 10 fold serial dilution of a cloned primer-specific template, in turn exactly quantified by competitive PCR against an engineered "competitor" (a DNA fragment not present in the human genome to which BCL-2-specific primers were linked) (Fig. 2).

Principle of the method: a) genomic DNA extraction; b) Real-time amplification with a dual-labeled probe; c) revelation by a Real-time PCR sequence detection system compatible with taqman chemistry.

Applicability: on extracted DNA from peripheral blood, bone marrow and lymph node cells or other tissues.

Number of test: 132 PCR reactions (24 samples equivalent to 72 PCR reactions + 60 PCR reactions for standard curves and controls)

Stability: 18 months if correctly stored.

Method sensitivity: the limit sensitivity of the assay (tested on the DoHH2 cell line) is of 8.5 BCL2/IgH+ cells on 85000 analyzed cells (i.e. 500 ng of tested DNA) (Fig. 3), with an interassay variation ≤ 5% at the lowest dilution (Table 1).

Kit containing and storage

NAME	STORAGE
Standard dilution	
St-1: 9.0×10^{0} copies	**+4°**
St-2: 4.5×10^{1} copies	**+4°**
St-3: 9.0×10^{1} copies	**+4°**
St-4: 9.0×10^{2} copies	**+4°**
St-5: 9.0×10^{3} copies	**+4°**
St-6: 9.0×10^{4} copies	**+4°**
Real-time PCR	
10X BCL2/IgH (Primers/FAM-TAMRA probe)	**-20°C**
10X Albumin (Primers/FAM-TAMRA probe)	**-20°C**
Master mix 2X	**+4°C**
H2O RNase/DNase free	**-20°C**
Software for results calculation and interpretation	

Interpretation of results

The cycle number at which the reporter dye emission intensities rises above background noise is called the threshold cycle . The Ct is directly proportional to the copy number of the target template at beginning of the reaction (On a Taqman machine set a threshold at 0.1 and baseline between cycles 3 and 15). Using standard curves of known amounts of both the endogenous Albumin reference control and BCL2/IgH rearranged copies (DoHH2 DNA), allows the calculation of the ratio of specific BCL2/IgH gene copies to reference DNA (Albumin) in each sample. Given that 500 ng of DNA correspond to 85000 cells (5), the results are expressed as absolute number of BCL2/IgH+ cells over 85000 analyzed cells. A software for results interpretation and absolute quantification of BCL2/IgH+ cells in analyzed samples is included in the kit.

Appropriate applications of the test

The quantitative evaluation of BCL2/IgH+ cells in tissue samples (peripheral blood, bone marrow, lymph nodes, biopsies of other tissues) from patients with FCL and/or DLBCL, provides a useful tool for i) molecular diagnosis, ii) monitoring of minimal residual disease (MRD), iii) early evaluation of therapeutic efficacy of a given treatment, including chemotherapy, immunotherapy, chemo-immunotherapy, high-dose therapy and radio-immunotherapy, iv) monitoring of tumor cell contamination in bone marrow or peripheral stem cells collected for high dose therapy with autologous hemopoietic rescue (6). More recently, it has been shown by multivariate analysis that the absolute levels of BCL2/IgH+ cells in bone marrow of newly diagnosed patients with FCL, were the best predictor for the achievement of complete clinical and molecular response following CHOP plus Rituximab therapy (7). In this regard absolute quantization of BCL2/IgH+ cells by Real-time PCR at diagnosis provides an important new tool to predict treatment response and long-term clinical outcome in patients with FCL (7). Since the constitutive levels of BCL2 in tumor cells of DLBCL have shown to correlate with prognosis and resistance to chemotherapy (8,9),

quantitation of BCL2/IgH+ cells in lymph node samples may also be used as a prognostic predictor in t(14;18)$^{+}$ aggressive B cell NHL. Finally, quantitative evaluation of BCL2/IgH+ cells could provide a cut-off value able to discriminate the amounts of t(14;18) positive clones found in lymphoma patients versus those present in healthy subjects (5).

References

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