QUANT-BCL1/IgH MTC® Cat.1.007

Real-Time Quantitative PCR of t(11;14) (BCL-1/IgH) MTC for diagnosis and monito- ring of Mantle cell lymphoma

Introduction

Mantle cell lymphoma (MCL) is an aggressive form of non-Hodgkin (NHL) lymphoma, typically occurring in older males and usually associated with the BCL1/IgH gene rear- rangement. It accounts for 4-6% of all adult NHL (1) and is usually characterized by lym- phadenopathy, bone marrow (BM) and gastrointestinal tract involvement at diagnosis (2). MCL is characterized by t(11;14) translocation resulting in the juxtaposition of the BCL-1 (Cyclin D1) gene, located on chromosome 11q13, to the joining region (J) of immu- noglobulin heavy chain genes (IgH), on chromosome 14q32 (BCL-1/IgH rearrangement) (Figure 1) (3,4). Nearly half of these translocations cluster within a 500 base pair region, named major translocation cluster (MTC) region, located upstream to the 5' untraslated region (5'UTR) of the BCL-1 gene on chromosome 11q13 (Figure 1). The remainder of breakpoints on chromosome 11 are widely scattered over a region of approximately 120 kb, including a minor 500 bp translocation cluster region (mtc-1) located downstream the MTC. The breakpoints on chromosome 14 occur within the 5' region of one of the six J regions of the IgH gene (5). As a consequence of the t(11;14), the BCL-1 gene, not dis- rupted by the translocation, undergoes the control of the em IgH enhancer (Figure 1), re- sulting in the constitutive overexpression of the Cyclin D1 (BCL-1) protein in lymphoma cells (6). Only translocations involving the MTC region can be revealed by a routine PCR analysis. Thus 30% to 40% of MCL cases can be shown to carry the t(11;14) translocation by PCR methods. Very recently, the quantitative evaluation of cells carrying the t(11;14) translocation, following conventional and high dose therapy, was shown to represent a powerful predictor of long-term remission in MCL patients. Therefore the possibility of measuring molecular minimal residual disease (MRD) after appropriate therapy provides a powerful tool to define subgroups of MCL patients with a significantly different prognosis (7).

Appropriate applications of the test

Molecular diagnosis of MCL is usually made either through a qualitative PCR (able to detect 30-40% of cases) or, indirectly, through detection of Cyclin D1 mRNA overexpres- sion by REAL-time reverse transcriptase PCR. In this latter instance, Cyclin D1 overex- pression needs to be referred to a B cell marker (e.g. CD19 or CD20) (8,9) and cannot be exploited for direct MRD evaluation since it does not represent a direct measurement of neoplastic clone size. Alternative methods for direct MRD evaluation such as IgH clone- specific PCR (7) are cumbersome and time consuming. In contrast, the quantitative evalua- tion of BCL1/IgH+ cells in tissue DNA samples (peripheral blood, bone marrow, lymph nodes, biopsies of other tissues) from patients with MTC+ MCL, as performed with the present kit, provides a rapid, reliable and reproducible tool for i) molecular diagnosis of MTC+ MCL (30-40% of MCL cases), ii) accurate and direct monitoring of minimal resid- ual disease (MRD) of MTC+ cases, iii) early evaluation of therapeutic efficacy of a given treatment, including chemotherapy, immunotherapy, chemo- immunotherapy, high-dose therapy and radio-immunotherapy (7), iv) monitoring of tumor cell contamination in bone marrow or peripheral stem cells collected for high dose therapy with autologous hemopoi- etic rescue (10,11), v) early prognostic evaluation of MTC+ MCL patients (7).

Principle of assay: real-time PCR with SYBR Green® method
Applicability: On extracted and purified total DNA from peripheral blood or bone marrow lymph nodes or other tissue sample.
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 JVM-2 cell line) is of 9.0 BCL1/IgH+ cells on 90000 analyzed cells (i.e. 500 ng of tested DNA)

Kit containing and storage

Name	Storage
CD ROM 700 MB	
Software for calculation of results	
STANDARD DILUTIONS	
St1-pMTC 9x10^5 copies/2µl –Albumin 9x10^4 copies/2µl	-20°C
St2-pMTC 9x10^4 copies/2µl -Albumin 9x10^3 copies/2µl	-20°C
St3-pMTC 9x10^3 copies/2µl -Albumin 9x10^2 copies/2µl	-20°C
St4-pMTC 9x10^2 copies/2µl -Albumin 9x10^1 copies/2µl	-20°C
St5-pMTC 9x10^1 copies/2µl -Albumin 9x10^0 copies/2µl	-20°C
St6-pMTC 9x10^0 copies/2µl -	-20°C
REAL-TIME PCR	
10X Albumin Primers	-20°C
10X pMTC Primers	-20°C
SYBR Green® Master Mix 2X*	**+4°C**
H_2O RNase/DNase-free	-20°C

Analysis of results

Cycle threshold (Ct) is defined the cycle number at which the emission intensity of reporter dye is perceptible upon background noise. In a Real-time PCR, the Ct is directly related to the copies of target-template present at the beginning of reaction. In each sample the ratio between BCL-1/IgH and Albumin is calculated using specific standard curves. For a correct normaliza- tion, the efficiencies of Albumin and BCL-1/IgH must be very similar (In a PCR reaction, the maximum efficiency correspond to a standard curve theoretical slope of -3,32)..
Below are showed the relative plots of BCL-1/IgH amplification and Standard curves (Albumin and BCL-1/IgH) dilutions.

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References

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