

Identification and quantitative measurement of BCR/ABL transcripts using real-time PCR

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Abstract:

The BCR/ABL translocation genotype (Philadelphia chromosome) is present in 95% of chronic myelogenous leukaemia (CML) patients ⁽¹⁾. The reverse transcriptase-polymerase chain reaction (RT-PCR) has become widely used for monitoring minimal residual disease (MRD) after allogenic stem cell transplantation.

Our laboratory diagnose ~ 225 samples a year. These are samples from ~ 110 follow-up patients and 45 newly diagnosed patients. During the last years, diagnostics have been performed using qualitative RT-PCR (qPCR). Recently, we established quantitative real-time PCR (RQ-PCR) approach for detection and quantification of the BCR/ABL fusion transcripts. This approach and the conventional qualitative RT-PCR were performed in parallel for comparison on 20 samples from different patients. There was a good compliance between the two methods.

Standardised protocol:

It is well established that quantification based on real-time RT-PCR for evaluation of MRD have a higher predictive value than qualitative methods.

The collaborative network "Europe against cancer" (EAC), has developed a standardised protocol for monitoring MRD. The intention is to have a quick and accurate method for quantification of fusion transcripts in blood samples. The automated real-time quantitative RT-PCR based method has been established as a standard method for monitoring MRD and is useful for measuring the risk of relapse in leukemia patients.

With this standardisation, disease development and affect of therapy can be surveyed by comparable assays performed at different times and places.

Method:

Isolation of leucocytes: RBC-lysis buffer (Ammonium bicarbonate and ammonium chloride based)

RNA extraction method: Trizol® Reagent (Invitrogen)

RT step: Superscript II (Invitrogen). The procedure is adapted from the BIOMED-1 protocol ⁽⁴⁾

RQ-PCR step: Platinum Quantitative Supermix-UDG (Invitrogen)

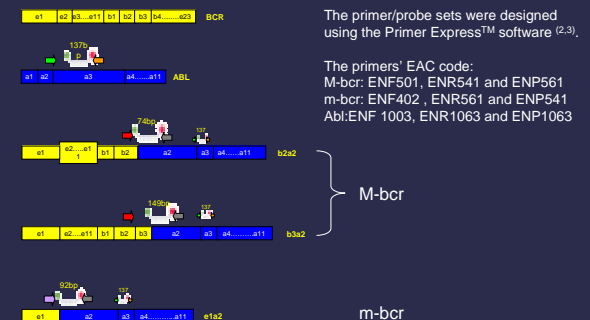
Taqman probes (Applied Biosystems)

Plasmid DNA calibrators containing the target gene sequence (Ipsogen, Marseilles, France)

Control gene: *ABL*

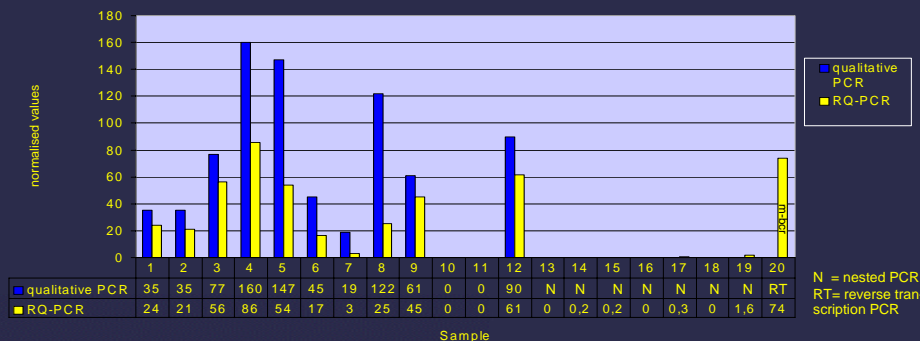
Performed on a ABI 7900 platform (Applied Biosystems)

Primers and probes:



Results:

Comparison of results



The table shows the normalised values obtained from the 20 samples. The products from the qualitative RT-PCR were analysed on an ABI 3100 genetic analyser. When possible to normalise values from qualitative PCR, the values were obtained by dividing the peak area value of fusion transcript by that of the Abl-transcripts. The normalised values from the RQ-PCR were obtained by using the equation:

$$\text{Qty fusion transcripts} / (\text{Qty abl transcripts} + \text{fusion transcripts}) \times 100$$

Conclusion:

There is a good compliance between the two methods

Real-time quantitative PCR is time and cost effective

qPCR is more accurate and results are easier to compare

Measures relapse risk in leukemia patients

Ref: ¹Melo JV, Blood. 1996 Oct 1;88(7):2375-84.
²Bellard E et al., Leukemia 2003 Dec; 17(12): 2474-86
³Gabert J et al Leukemia. 2003 Dec; 17(12): 2318-57
⁴van Dongen JJ et al., Leukemia 1999; 13: 1901-1928