High Sensitive Molecular Detection of the BCR-ABL Major and Minor Fusion Transcripts in a Multiplex, Close-Tube Format by Isothermal Loop Mediated Amplification Reaction (RT-LAMP)

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INTRODUCTION
The molecular detection of the BCR-ABL fusion transcripts is necessary for the genetic confirmation of Chronic Myeloid Leukemia (CML) diagnosis and for the risk classification of Acute Lymphoblastic Leukemia (ALL) [1,2]. The molecular techniques currently used for this purpose are based on conventional RT-PCR [3], the main limitations are time-consuming and multi-step procedures that slow down the diagnostic labs routine and require highly specialized personnel. We developed an innovative non-PCR assay based on Loop mediated Isothermal Amplification [4] reaction for multiplex detection of BCR-ABL p190 and p210 fusion transcripts and of the endogenous GUS mRNA.

MATERIALS AND METHODS
The use of intercalating dye (Yo pro-1, Invitrogen) for a real-time reaction monitoring was done by incorporating a specific internal control (IC) (F3) for each reaction.

INDUCTION
The results showed that RT-LAMP was also validated on CML follow-up samples previously diagnosed and quantified by conventional RT-PCR in Ospedali Riuniti di Bergamo. The results are fully concordant and the high sensitivity of RT-LAMP allowed the detection of very low NCNL (Normalized Copy Number Levels).

RESULTS

Analytical Specificity
The analytical specificity was established on various fusion type cell lines: no false positive results have been obtained on 121 replicates (Table 1).

Role of Internal Control (IC):
- control the RT reaction procedure
- control the quality of RNA extracted
- control the correct reaction condition
- control of the presence of inhibitors that may interfere with RT.

Interpretation of results:
- 100% specificity
- 100% concordance on 126 clinical samples

RESULTS

Analytical Sensitivity
The analytical sensitivity was established on 500ng RNA extracted from p210 and p190 positive cell lines serially diluted into negative cell line RNA.

Table 4: RT-LAMP was also validated on CML follow-up samples previously diagnosed by conventional RT-PCR in Ospedali Riuniti di Bergamo. The results are fully concordant and the high sensitivity of RT-LAMP allowed the detection of very low NCNL (Normalized Copy Number Levels).

REFERENCES

CLINICAL VALIDATION: Comparison with PCR

Results on Clinical Sample degraded
6 clinical samples resulted invalid (no amplification of BCR-ABL, nor of GUS) by RT-LAMP. The quality of RNA was therefore tested by Agilent 2100 Bioanalyzer, resulting highly degraded.

Table 3: RT-LAMP was validated on clinical samples previously diagnosed by conventional RT-PCR in Ospedali Riuniti di Bergamo. The two methods showed 100% concordance on 114 clinical samples

Table 2: RT-LAMP was validated on clinical samples previously diagnosed by conventional RT-PCR in Ospedali Riuniti di Bergamo. The two methods showed 100% concordance on 114 clinical samples.