

Effects of different SybrGreen Master Mix manufacturing lots and suppliers on real-time PCR parameters

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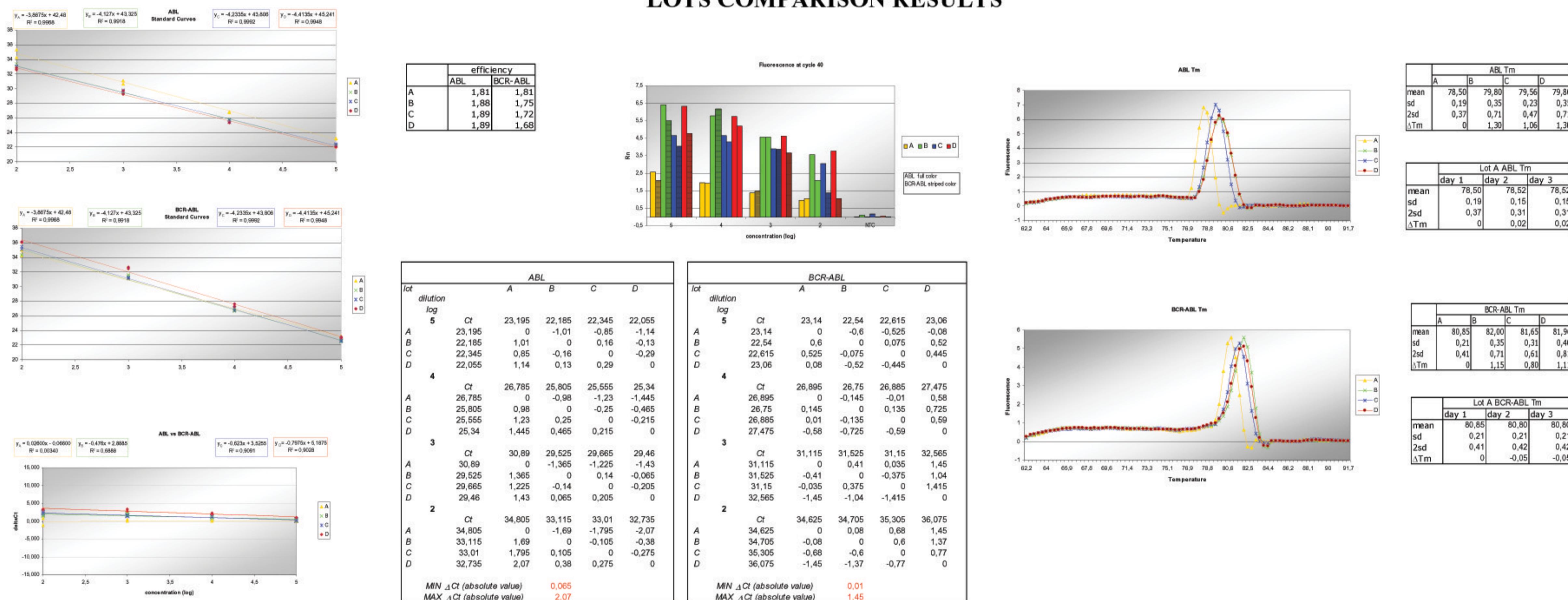
INTRODUCTION and AIM of the STUDY

Real-time polymerase chain reaction (PCR) methodology is now broadly used to quantitate target sequences for diagnostic purposes. Despite the growing availability of commercial *in vitro* diagnostic assay, "home-made" real-time PCR methods, using commercial reagent master-mix are still applied in many laboratories. Aim of the present study was to analyse the effect of different SybrGreen master-mix manufacturing lots and suppliers on real-time PCR parameters.

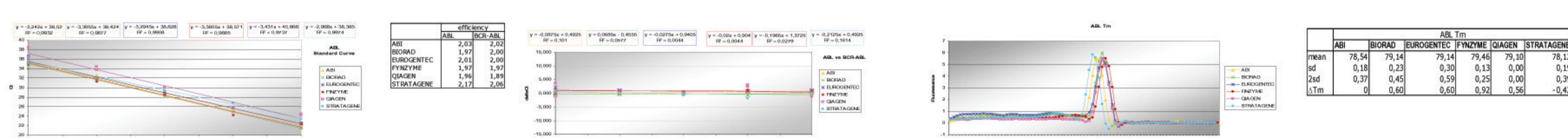
MATERIAL and METHODS

The analysis was conducted on a well-assessed "home-made" relative quantification BCR-ABL assay, using ABL as normalising gene and five log dilution of leukemic cell line K562 cDNA. To minimize variability due to reverse-transcription (RT), all the cDNA was obtained, from 1 µg total RNA, through the same RT reaction using random hexamers primers and SuperScript II (Invitrogen). We compared four different manufacturing lots of a same product (same p/o number), provided by a single supplier (Applied Biosystems) and six different suppliers of SybrGreen master-mix, with Rox (Applied Biosystems, Bio-Rad, Eurogentec, Finzyme, Qiagen and Stratagene). All the reaction (25 µl reaction volume, containing 1/10 RT volume) were conducted in duplicates on a GeneAmp5700 (Applied Biosystems), using the same production lot of primers and the same thermal conditions (40 cycles: 30" 95°C; 30" 60°C; 30" 72°C).

LOTS COMPARISON RESULTS



MASTER MIX COMPARISON RESULTS



CONCLUSIONS

As expected, variations in PCR parameters has been, in some cases, observed in master-mixes of different suppliers, due to differences in reagents composition. Differences in Tm values, Ct values, max deltaRn at the last PCR cycle and reaction efficiency have been also evidenced when comparing different lots of the same product; efficiency variations were not always comparable in the BCR-ABL and ABL assays. In conclusion, if master-mix composition is surely important during assay optimisation, particular care has to be taken introducing different lots of a same commercial master-mix, especially in diagnostic assay, and validation of the new preparation lot should be advisable.