

Abstract

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Improved real-time multiplex polymerase chain reaction detection of methylenetetrahydrofolate reductase (MTHFR) 677C>T and 1298A>C polymorphisms using nearest neighbor model-based probe design.

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BACKGROUND: The disorders of folate metabolism caused by methylenetetrahydrofolate reductase (MTHFR) gene polymorphisms may lead to several disease states including coronary heart disease, venous thrombosis, and several types of cancer.

OBJECTIVE: We have developed a real-time multiplex single-tube polymerase chain reaction procedure on the LightCycler for the detection of the two most commonly occurring variants, 677C>T and 1298A>C, in the MTHFR gene.

METHODS: An improved probe design, based on the nearest neighbor model for nucleic acid-probe duplex stability, resulted in a better separation (DeltaTm approximately 10 degrees C) of melting peaks of the wild-type and mutant alleles than that by the existing method (DeltaTm approximately 3 degrees C) for specimens heterozygous for the 1298A>C polymorphism.

RESULTS: Of the 333 blood specimens analyzed by this procedure, we did not find any samples that gave ambiguous results. The specimens with homozygous mutation for one polymorphism were of the wild type for the other variant. The assay was validated by the comparison of the genotyping results of 50 blood specimens from the LightCycler polymerase chain reaction with the conventional restriction fragment length polymorphism procedures. There was 100% concordance of the test results obtained by the two techniques.

CONCLUSION: This assay is reliable, economical, and can be performed by less trained technologists compared with the procedure performed by the conventional restriction fragment length polymorphism technique.

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