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Analysis of Cell-Free Fetal DNA from Maternal Plasma and Serum Using a Conventional Multiplex PCR: Factors Influencing Success

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Abstract: Recent technology enables the use of cell-free fetal DNA in maternal plasma and serum for noninvasive prenatal genetic diagnosis. This study was designed to evaluate factors most likely to influence the success of a simple, cost efficient, reliable and replicable conventional PCR technique in the clinical routine of prenatal genetic diagnosis of selected cases. The results strongly suggest that DNA extraction and PCR cycle optimization are 2 major success-limiting steps and the maternal plasma is a better choice over serum for DNA extraction for such prenatal genetic diagnosis. In addition, the use of a ready-to-use PCR mixture containing heat-activated Taq polymerase significantly reduced the risk of nonspecific amplification and of primer dimerization formed at low temperatures during PCR setup and the initial PCR cycle eliminating false positive results and insufficient PCR amplification, respectively. Thus the ease, rapidity and effectiveness shown by the presented system requiring only optimization of routine PCR procedure and no additional sophisticated equipment could theoretically reduce the cost and number of invasive procedures required for prenatal diagnosis of X-linked recessive genetic disorders and of fetal RhD status.

Key Words: Noninvasive prenatal diagnosis, maternal plasma, maternal serum, fetal gender determination, conventional multiplex PCR

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