

Abstract

Digital PCR (Polymerase Chain Reaction) is a method that enables absolute quantification of DNA sequences and therefore finds application in many diagnostic disciplines. We focused on the diagnosis of trisomy 21, which manifests itself as Down syndrome. Information about the fetal genome can be obtained from free fetal DNA that is released into the mother's bloodstream from the placenta during pregnancy. In the first trimester, fetal DNA constitutes approximately 5-10% of free DNA and is therefore a suitable target for non-invasive prenatal testing (NIPT). However, we need a technique that is able to distinguish this relatively small fraction of free fetal DNA and differentiate it from free maternal DNA. Precisely digital PCR provides various ways to achieve this goal thanks to its wide multiplexing possibilities. We focused on optimizing the multiplex reaction, which in one fluorescence channel distinguishes the number of copies of the reference chromosome 18 and the observed chromosome 21. The resulting determination of this ratio provides us with information regarding the balance between the chromosomes and can thus confirm or refute the presence of a trisomic fetal fraction. We tested the usability of the proposed method for clinical application by examining DNA samples isolated from 25 plasma samples from pregnant women with healthy fetuses and from 15 plasma samples from pregnant women with fetuses affected by Down syndrome. ROC analysis of the obtained data led to the determination of a cut-off value of the measured ratio of 1.144 to achieve a sensitivity of 73.3% and a specificity of 80%. We identified methodological approaches with the potential to further improve the parameters of the developed test.

Keywords: ddPCR, NIPT, duplexing, Down syndrome, optimization, prenatal diagnostics, aneuploidy