Rapid non-invasive prenatal screening test for trisomy 21 based on digital droplet PCR

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Background: Non-invasive prenatal tests for the detection of fetal aneuploidies are based on the analysis of cell-free DNA (cfDNA) from plasma of pregnant women by next generation sequencing method. Compared to methods based only on the polymerase chain reaction (PCR), this is an expensive screening test. The development of alternative tests for routine genetic laboratories is therefore desirable.

Methods: We optimized the isolation of plasma cfDNA. Then we performed multiplex digital droplet PCR by detecting 16 amplicons from chromosome 21 and 16 amplicons from chromosome 18 as reference. Two fluorescently labelled lock nucleic acid probes were used for the detection of reaction products. The required accuracy was achieved by examining 12 chips from each patient using Stilla technology.

Results: We analyzed plasma cfDNA of 26 pregnant women with euploid pregnancies and 16 plasma samples from pregnancies with trisomy 21 to determine the cutoff level for sample classification. The test was validated on 30 plasma samples of pregnant patients with risk for trisomy 21 in the range from 1:4 to 1:801. Our results were in full agreement with the results of subsequent invasive diagnostic procedure. All test parameters (sensitivity, specificity, positive and negative predictive values) reached 100%.

Conclusions: High PPV, low cost and speed of analysis predetermine the method for implementation into the clinical workflow as a screening alternative offered to anxious patients having the risk for trisomy 21 before the confirmatory invasive procedure.

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