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

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Objective

Non-invasive prenatal tests for the detection of fetal aneuploidies are predominantly based on the analysis of cell-free DNA (cfDNA) from the plasma of pregnant women by next-generation sequencing (NGS). Compared to the 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

Multiplex digital droplet PCR (ddPCR) was used to detect 16 amplicons from chromosome 21 and 16 amplicons from chromosome 18 as the reference. Two fluorescently labeled 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 the plasma cfDNA of 26 pregnant women with euploid pregnancies and 16 plasma samples from pregnancies with trisomy 21 to determine the cutoff value for sample classification. In this pilot phase, we achieved 87.5% sensitivity with a positive predictive value (PPV) of 93.3% and a negative predictive value (NPV) of 92.6%. We validated the test on 30 plasma samples from pregnant patients with a risk for trisomy 21 ranging from 1: 4 to 1: 801. Our results were in complete agreement with the results of the invasive diagnostic procedure (sensitivity, specificity, PPV, and NPV of 100%).

Conclusion

We demonstrated the high potential of the developed methodology on a set of 30 patients in the blind part of the study. The low cost and speed of analysis predetermine this method for implementation into the clinical workflow as a screening alternative. However, a larger number of pregnant women must be examined to support our findings.