Droplet digital PCR application for rapid aneuploidy and maternal cell contamination testing of invasive prenatal samples

Olga Mikhaylichenko[1], Endre Hegedus[2], Vanessa Felice[2], Chenyu Li[1], Nathan Hendel[1], Eric Hall[1], Nyari Dzvova[1], Monica Herrera[1], Richard Dannebaum[1], Severine Margeridon[1], Ronald Wapner[3], Vaidehi Jobanputra[2]

1- Molecular Diagnostics R&D – Clinical Diagnostics Group, Bio-Rad Laboratories Inc., Pleasanton, CA, USA
2- New York Genome Center, New York, USA; 3 - Columbia University Irving Medical Center, New York, USA

Background

Efficient aneuploidy screening and maternal cell contamination (MCC) control are essential in invasive sample analysis workflows, eliminating costs of whole-genome sequencing for contaminated or aneuploid samples.

This study introduces rapid aneuploidy screening using droplet digital PCR (ddPCR) on genomic DNA (gDNA) from fetal blood, amniocytes or chorionic villi, requiring as little as 5 ng of gDNA per sample. The method reduces analysis time to 9 hours for a batch of 46 samples, eliminating the need for imaging or capillary electrophoresis. MCC estimation is performed alongside aneuploidy analysis in one test to assess sample quality and aneuploidy status.

Invasive sample screening is part of prenatal care for high-risk pregnancies

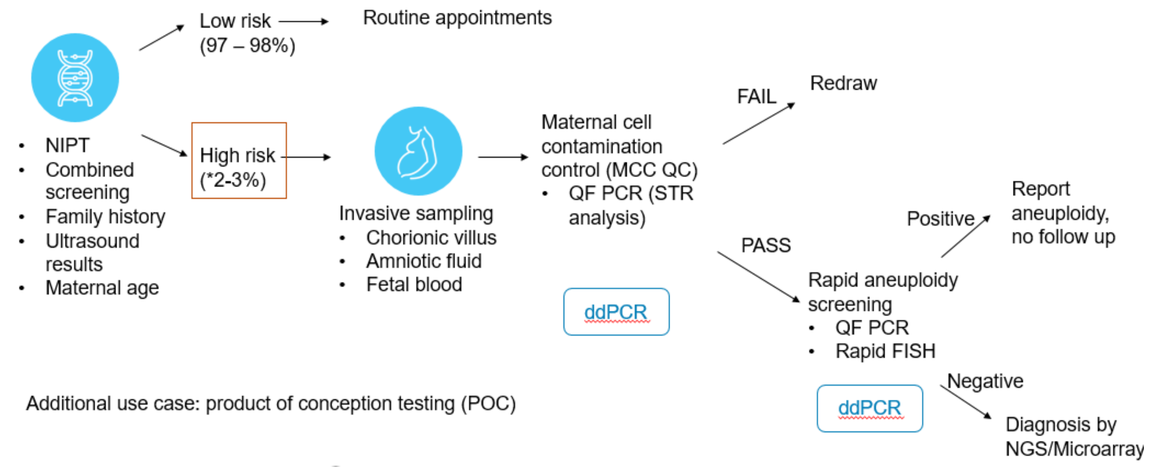


Fig. 1. Diagram representing diagnostic sampling workflow & timeline for patients in high-risk pregnancy category. We propose ddPCR as a method of choice for aneuploidy and maternal cell contamination screening.

ddPCR workflow: from sample to answer in 9 hours

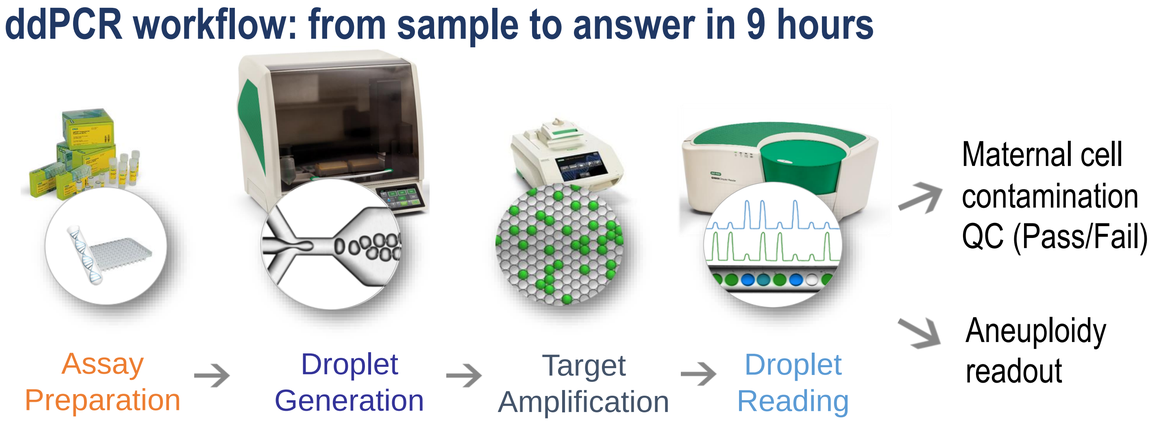


Fig. 2. ddPCR workflow from sample to readout includes the following steps: 1) assay preparation and ddPCR plate setup, 2) droplet generation (automatic or manual), 3) target amplification (PCR), 4) droplet reading

Multiplexed assays using universal probes, generated with a custom and automated primer design engine

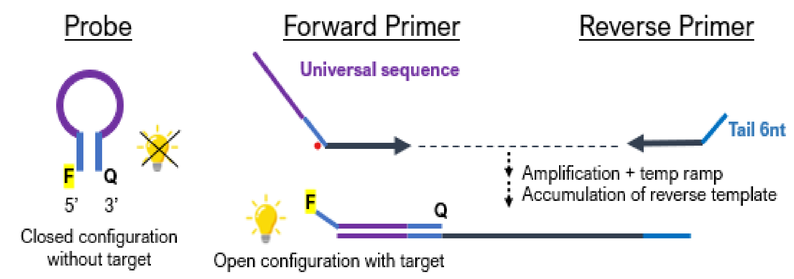


Fig. 3. Multiplex primer pairs targeting the same chromosome are combined in a single fluorescent channel using a unique universal probe.

Distinguishing fetal from maternal gDNA via ddPCR

- Methylation-sensitive restriction enzymes (MSREs) enable ddPCR to visualize differences in fetal and maternal DNA methylation
- MSRE digestion is performed in-droplet, with no disruption to the ddPCR workflow

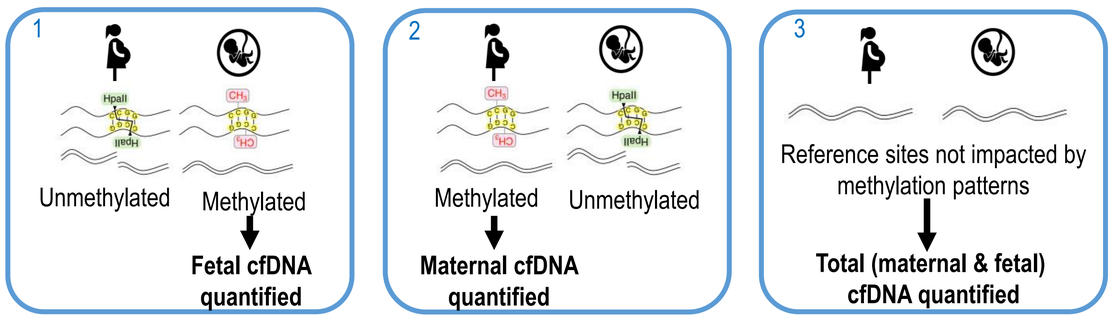


Fig. 4. Fetal and maternal cfDNA are quantified simultaneously in the same ddPCR reaction. 1) Hypermethylated fetal cfDNA is quantified after MSRE digestion of hypomethylated maternal cfDNA. 2) Maternal cfDNA is similarly quantified. 3) Total cfDNA is quantified from non-digested regions.

Multiplexed assays using universal probes, generated with a custom and automated primer design engine

Aneuploidy multiplex performance across 6 channels on QX600 instrument 2D amplitude plots

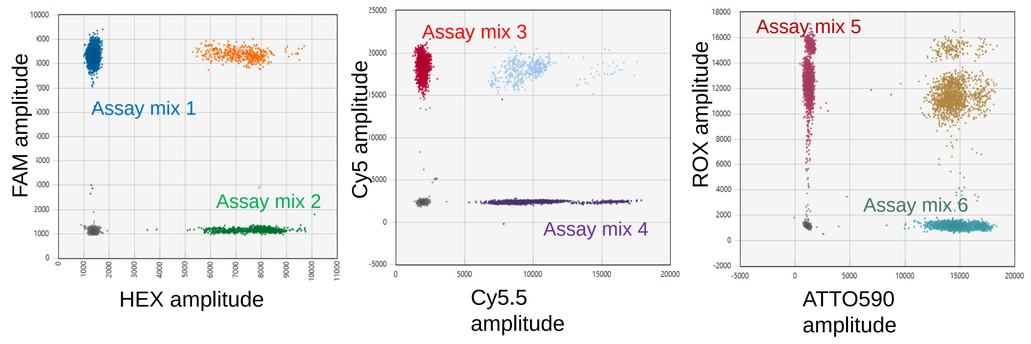


Fig. 5. 2D plots for Aneuploidy screening ddPCR assay. Each channel accumulates signal across multiplexed targets on the chromosome of choice through universal molecular beacon detection.

- A custom primer design engine (developed internally) performs ultra-fast generation of compatible primer pairs for high level multiplexing, minimizing off-target and heterodimer events.

Aneuploidy assay performance

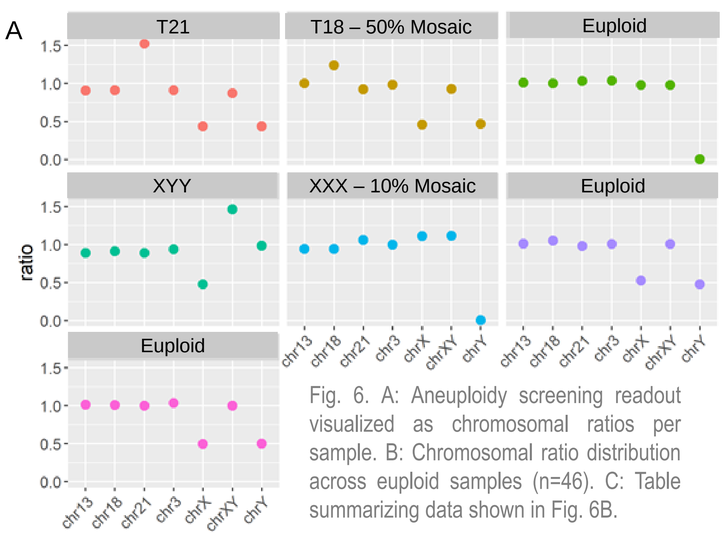
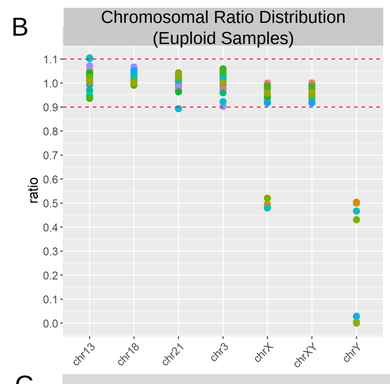


Fig. 6. A: Aneuploidy screening readout visualized as chromosomal ratios per sample. B: Chromosomal ratio distribution across euploid samples (n=46). C: Table summarizing data shown in Fig. 6B.



C

Target	Mean Ratio	St Dev Ratio
chr13	1.006	0.043
chr18	1.013	0.028
chr21	1.011	0.033
chr3	0.992	0.036
chrXY	0.979	0.030

Maternal cell contamination (MCC) assay performance

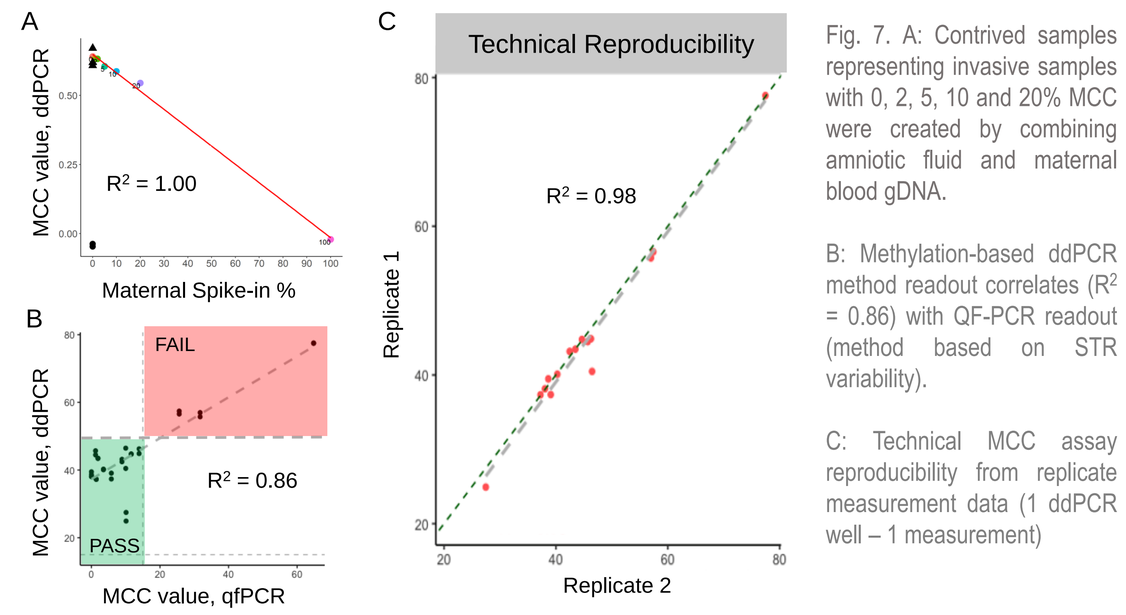


Fig. 7. A: Contrived samples representing invasive samples with 0, 2, 5, 10 and 20% MCC were created by combining amniotic fluid and maternal blood gDNA.

B: Methylation-based ddPCR method readout correlates (R^2 = 0.86) with QF-PCR readout (method based on STR variability).

C: Technical MCC assay reproducibility from replicate measurement data (1 ddPCR well – 1 measurement)

Conclusions

- ddPCR emerges as a fast, low-input, and cost-efficient alternative for rapid aneuploidy screening in invasive prenatal samples.
- Combining aneuploidy detection with MCC quality control in the same reaction plate using a methylation-based ddPCR approach reduces turnaround time and instrument costs, presenting a promising approach for invasive testing.

References

1. Okmen, F., Ekici, H., Hortu, I. *et al.* Comparison of indications and results of prenatal invasive diagnostic tests before and after the implementation of the use of cell-free fetal DNA: a tertiary referral center experience. *J Assist Reprod Genet* **37**, 2019–2024 (2020).
2. Ioannides, M. *et al.* Development of a new methylation-based fetal fraction estimation assay using multiplex ddPCR. *Mol Genetics Genom Medicine* **8**, e1094 (2020).