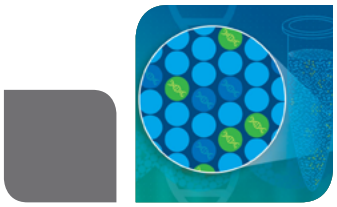
Reproductive and Women's Health using Droplet Digital PCR



Recommended Reading

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Non-Invasive Prenatal Testing

Detection of Aneuploidy with Digital Polymerase Chain Reaction
Fan, H and Quake, S. 2007. Anal. Chem. 79, 19, 7576–7579
This seminal work indicates that digital PCR could be used for a reliable discrimination between normal and aneuploid samples even when the aneuploid material represented 10% of the total material being examined. Implies that it may be possible to use this technology to detect fetal aneuploidies directly from cell-free DNA in maternal plasma.

Digital PCR for the molecular detection of fetal chromosomal aneuploidy
Lo, YM., et al. 2007. PNAS 104 (32) 13116-13121 D
Demonstrated two digital PCR strategies for the noninvasive detection of fetal aneuploidy in microwell plates. By the use of digital PCR analysis of maternal plasma samples, they were able to distinguish four aneuploid fetuses from nine normal healthy ones.

Microfluidic digital PCR enables rapid prenatal diagnosis of fetal aneuploidy
Fan, H., et al. 2009. Amer. J. of Obs and Gyn.200(5), P543.E1-543.E7 Digital PCR allows detection of fetal chromosomal aneuploidy utilizing uncultured amniocytes and chrorionic villus tissue in less than 6 hours.

A multiplex droplet digital PCR assay for non-invasive prenatal testing of fetal aneuploidies
Tan, C. et al. 2019 Analyst. Issue 7.
In this study a multiplex ddPCR assay used universal locked nucleic acid (LNA) probes to reliably identify fetal aneuploidies. They found that the accessibility and cost-effectiveness of multiplex ddPCR-based NIPT made it a competitive prenatal testing method in clinical use.

Non-invasive prenatal testing of fetal aneuploidies using a new method based on digital droplet PCR and cell free fetal DNA
Haidong, W., et al. 2020. medRxivdoi: https://doi.org/10.1101/2020.12.19.2024855353
This work shows the development of a ddPCR based assay called iSAFE NIPT using cell free fetal DNA (cffDNA) for detection of fetal trisomies 13, 18 and 21 in a single reaction. Based on clinical validation, the iSAFE NIPT has high diagnostic sensitivity and specificity. It can be decentralized in routine clinical laboratories, is fast, easy to use and economical comparing to current NIPT.

Development of a new methylation - based fetal fraction estimation assay using multiplex ddPCR
Ioannides, M., et al. 2020. medRxiv https://doi.org/10.1002/mgg3.109453
In this paper a novel set of fetal-specific differentially methylated regions (DMRs) and methylation sensitive restriction digestion (MSRD) were used to develope a multiplex ddPCR assay for accurate detection of fetal fraction in maternal plasma.

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Non-Invasive Prenatal Diagnosis – Single Gene Disorders

Noninvasive Prenatal Diagnosis of Single-Gene Disorders by Use of Droplet Digital PCR

Camunas-Sole, J. et al. 2018. Clinical Chemistry, Volume 64, Issue 2, 1, Pages 336–345

In this study a protocol was developed for noninvasive prenatal diagnosis of inherited single-gene disorders using ddPCR from cfDNA in maternal plasma. These included cases at risk of hemophilia, ornithine transcarbamylase deficiency, cystic fibrosis, β-thalassemia, mevalonate kinase deficiency, acetylcholine receptor deficiency, and DFNB1 nonsyndromic hearing loss. This method detects single-nucleotide mutations as early as the first trimester of pregnancy.

A Non-Invasive Droplet Digital PCR (ddPCR) Assay to Detect Paternal CFTR Mutations in the Cell-Free Fetal DNA (cffDNA) of Three Pregnancies at Risk of Cystic Fibrosis via Compound Heterozygosity

Debrand, E., et al. 2015 PLOS ONE

This work describes a ddPCR assay designed to inform the testing options for couples whose offspring are at risk of suffering from cystic fibrosis via compound heterozygosity. The assay correctly and unambiguously recognized the ΔF508-MUT CFTR allele in the cffDNA of all three proband fetuses and none of the six unaffected control fetuses. ddPCR was found to be a cost-effective and technically undemanding platform for designing bespoke NIPD assays.

Fetal Genotyping in Maternal Blood by Digital PCR: Towards NIPD of Monogenic Disorders Independently of Parental Origin

Perlado, S., et al. 2016. PLOS ONE

This work shows a validation study of ddPCR for analysis of both paternally and maternally inherited fetal alleles. A SNP validation strategy was used to mimic the inheritance pattern of monogenic disorders (autosomal dominant and recessive diseases and X-linked disorders). Demonstrates the potential of ddPCR for NIPD fetal genotyping independently of the inheritance pattern and the parental origin of the alleles.

Detection of cell-free foetal DNA fraction in female-foetus bearing pregnancies using X-chromosomal insertion/deletion polymorphisms examined by digital droplet PCR

Zednikova, I., et al. 2020. Sci Rep 10, 20036

This study presents a methodology allowing the detection of paternal X-chromosomal alleles on maternal background and the confirmation of female sex of the foetus by positive amplification signals. ddPCR was used to examine X-chromosomal INDEL (insertion/deletion) polymorphisms: rs2307932, rs16397, rs16637, rs3048996, rs16680 in buccal swabs. This was successfully applied in prenatal diagnostics in a family with Wiscott–Aldrich syndrome and in pregnancies tested for the risk of RhD incompatibility.

Digital PCR Analysis of Maternal Plasma for Noninvasive Detection of Sickle Cell Anemia

Barret, A., et al. 2012. Clinical Chemistry 58:6 1026–1032.

The objective of this study was to determine the feasibility of using digital PCR for NIPD in pregnancies at risk of sickle cell anemia. Digital PCR can be used to determine the genotype of fetuses at risk for sickle cell anemia. Optimization of the fractional fetal DNA concentration is essential.

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Endometriosis

Cancer-Associated Mutations in Endometriosis without Cancer
Anglesio, M., et al. 2017. N Engl J Med; 376:1835-1848.
In this paper epithelial and stromal components of lesions from 12 patients were analyzed by means of a ddPCR assay for recurrent activating KRAS mutations. Although endometriosis is considered to be a benign disorder from both a clinical and a histopathological perspective, well-known cancer-associated somatic mutations were found in the glandular epithelium of some deep infiltrating endometriosis lesions.

Frequent PIK3CA mutations in eutopic endometrium of patients with ovarian clear cell carcinoma
Murakami, K., et al. 2021. Mod Pathol. https://doi.org/10.1038/s41379-021-00861-3
This work investigated PIK3CA mutations (PIK3CAm) for three hotspots (E542K, E545K, H1047R) in eutopic endometrium in patients with ovarian cancer and endometriosis from formalin-fixed paraffin-embedded specimens by laser-capture microdissection and droplet digital PCR.

Iatrogenic endometriosis harbors somatic cancer-driver mutations
Lac, V., et al. 2018. Human Reproduction, Volume 34, Issue 1
In this study, ddPCR assays were created to rule out false positive and false negative next-generation sequencing-based errors related to KRAS. Patient samples were analyzed for all common KRAS activating G12 mutations (G12A, G12C, G12D, G12V, G12R, G12S, a subset of samples also included G13D).

Is endometriosis metastasizing? Shared somatic alterations suggest common origins across endometriotic lesions
Praetorius, T., et al. 2021. medRxiv 2021.04.12.21255355; doi: https://doi.org/10.1101/2021.04.12.21255355
Here selected alterations were tested by ddPCR in all available lesions from a given patient if they were observed either in any one lesion from a given patient (subjected to the FIND IT panel assay).

Carrier and Newborn Screening

Multiplex Droplet Digital PCR Method Applicable to Newborn Screening, Carrier Status, and Assessment of Spinal Muscular Atrophy
Vidal-Folch, N. et al. Clinical Chemistry 64(12): 1753-1761 (2018)
This paper describes the development of a multiplex, ddPCR assay for the detection of both SMN1 and SMN2 copy number states in dried blood spots and other tissues for use in newborn screening and carrier status assessment.

A Droplet Digital PCR Method for Severe Combined Immunodeficiency Newborn Screening Vidal-Folch, N. et al. Journal of Molecular Diagnostics 19(5): 755-765 (2017)
The method described is able to measure low TREC levels (LOD 4 copies/ul) and detect infants with SCID, leaky SCID and other TCLs with a comparable cost to current qPCR methods and can be utilized for high-throughput screening.

Presymptomatic Diagnosis of Spinal Muscular Atrophy Through Newborn Screening
Chien, Y-H., et al. The Journal of Pediatrics 190:124-9 (2017)
This work describes the utility of ddPCR as a second-tier newborn screening test for SMN1 and SMN2.

19-2170

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