

Title

Cell-free DNA testing for aneuploidy screening. Implications for unexpected results.

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Background/Synopsis -Cell-free DNA technology is commonly used as the initial screening method for detecting fetal aneuploidy during pregnancy. At least 13 U.S. labs offer cfDNA non-invasive prenatal screening for aneuploidy, utilizing methods such as SNP analysis, NGS, and microarrays. The choice of method depends on clinical needs, patient preferences, and insurance coverage. While technical advances are expected to improve sensitivity and accuracy, challenges like a lack of standardization, low fetal fraction, and biological variability can cause test failures. We will review the current platforms used for aneuploidy screening, along with common technical considerations and their implications in current practice. As clinical experience with these tests increases, we will develop a more detailed understanding of how to interpret phenotype-genotype discordance and unexpected results. The expected results are low risk or high risk, screen negative or screen positive. Up to 25% of the sample may not yield results. Test failures can be caused by technical reasons (tube broke, or the specimen was insufficient), failure of quality control (interfering substance like heparin, etc.), Low fetal fraction, Multiple aneuploidies (fibroids or maternal cancer) “ No Call,” is often seen in obese women drawn too early when the FF is low, but if you look at the no call group, the risk of an aneuploid fetus is up to 17%. There is no industry-wide standard on what or how to report these unexpected results.

Objective/Purpose -

We will summarize the various screening platforms, present the implications of unexpected test results for each of these currently used platforms.

Methods -

We performed a literature review of the 13 laboratories in the United States that receive clinical specimens for aneuploidy screening using either whole-genome sequencing or targeted sequencing. We evaluated the methodology each laboratory uses to determine the presence or absence of common aneuploidies using cell-free DNA analysis and their protocols for reporting normal and abnormal results.

Results -

Down syndrome (trisomy 21), Edwards syndrome (trisomy 18), and Patau syndrome (trisomy 13), along with sex chromosome aneuploidies, are the most commonly screened aneuploidies. Some laboratories also offer testing for rare autosomal trisomies, microdeletions or duplications, copy

number variations exceeding 7 megabases, and single-gene abnormalities. Each laboratory uses a proprietary bioinformatics protocol to analyze DNA fragment data, and all lab results can be influenced by factors such as low fetal fraction, background noise, interfering substances, and biological variability. Test failure may indicate an increased risk of fetal aneuploidy. Multiple autosomal monosomies, aneuploidy, or a "Globally chaotic pattern" with gains or losses across several chromosomes can increase the risk of maternal cancer.

Commercial aneuploidy screening options

LabCorp (Integrated Genetics) offers the MaterniT21 PLUS test, among others.

Natera: Provides the Panorama test.

Myriad Women's Health: Offers the Prequel test.

BillionToOne: Provides the UNITY Screen™.

Sequenom: Previously offered the MaterniT21 Plus test.

Ariosa Diagnostics: Offered the Harmony Prenatal Test.

Verinata Health (now Illumina): Offered the Verifi Prenatal Test.

Lab Genomics: Offers the Determine10 test.

ARUP Laboratories offers a prenatal cfDNA test using whole-genome sequencing.

Otogenetics offers EnVISION Non-Invasive Prenatal® Screening utilizing Illumina VeriSeq NIPT Solution with the VeriSeq Assay Software.

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Conclusion — Overall, targeted sequencing has a slightly higher risk of test failure compared to whole-genome sequencing. Each laboratory follows its own protocol for reporting unexpected results. Physicians need to understand these factors to provide essential genetic information and minimize risks to both parent and fetus. Physicians who order this type of antenatal testing must be familiar with their chosen laboratory's reporting practices and the implications of unexpected results.

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