

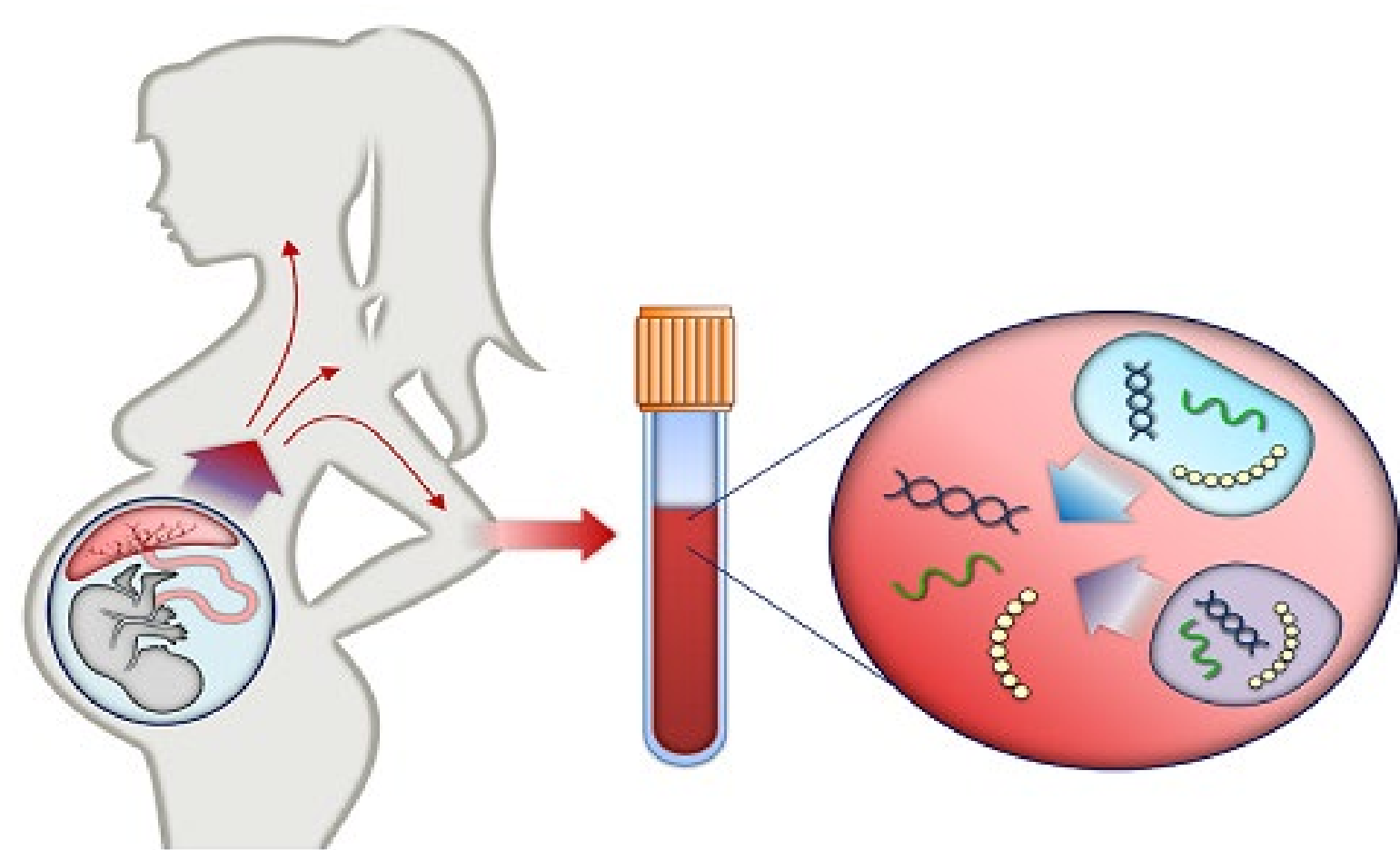


Cell-free DNA Testing for Aneuploidy Screening: Implications for Unexpected Results

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RATIONALE

- Cell-free DNA (cfDNA) is a noninvasive screening test used for detecting fetal aneuploidy as early as 8-9 weeks gestation
- At least 13 U.S. labs offer cfDNA and utilize methods such as targeted sequencing (TS), whole genome sequencing (WGS), and single nucleotide polymorphism
- The choice of method depends on cost, clinical needs, and patient preference
- The test is designed through sequencing and bioinformatics to determine either high risk or low risk for aneuploidy
- There is no industry wide standard or reporting protocol for uninformative results
- Unexpected results can be due to test failure, multiple gestations, low fetal fraction, assay or DNA library complications, maternal copy number variation, interfering substances (e.g., heparin) or maternal conditions (e.g., fibroids, lupus).



OBJECTIVE

- We aim to summarize the various screening platforms and present the implications of uninformative test results for each of these currently used platforms.

METHODS

- We performed a review of the U.S. laboratories that receive clinical specimens for commercial aneuploidy screening using either WGS or TS.
- The methodology used by each laboratory was evaluated for the presence or absence of common autosomal trisomy (CAT) using cfDNA, as well as their protocol for reporting results.

RESULTS

Testing methods

- Massively Parallel Sequencing (MPS)
 - Randomly sequences fragments from all chromosomes in the genome
- Single Nucleotide Polymorphism (SNP)
 - Compares the SNP profile of fetal DNA to the mother's
- Quantitative Counting Template (QCT)
 - Use of synthetic DNA fragments that mimic the biochemical properties of the gene of interest and identify it with a sequence tag

Laboratory Company	cfDNA Test Offered	WGS or TS	Method	Tests for:			Uninformative results rate
				CAT	RAT*	MMS**	
Ariosa Diagnostics	Harmony Prenatal Test	TS	SNP	✓		✓	0.5-2.9%
ARUP Laboratories	Non-Invasive Prenatal Anueploidy Screen by Cell-Free DNA Sequencing	WGS	MPS	✓			1-5%
BillionToOne	UNITY Screen	TS	QCT	✓			0.1%
Illumina/Verinata Health	Verifi Prenatal Test	WGS	MPS	✓		✓	0.1%
LabCorp (Intergraded Genetics)	MaterniT21 PLUS test	TS	MPS	✓		✓	0.9%
Lab Genomics	Determine 10 Test	WGS	MPS	✓		✓	0.1%
Myriad Women's Health	Prequel test	WGS	SNP	✓	✓	✓	0.1%
Natera	Panorama	TS	SNP	✓		✓	1.4%
Otogenetics	EnVISION Non-Invasive Prenatal Screening	WGS	MPS	✓		✓	0.08%

Chart 1: Laboratory companies and their commercial aneuploidy screening options with screening method, tested conditions, and rate of uninformative results. *RAT- rare autosomal trisomy, **MMS- microdeletion syndromes

Tests can be “uninformative” due to:

- Low fetal fraction
- Incorrect gestational age
- Background noise
- Interfering substances
- Biological variability
- Assay quality control failures
- Maternal cancer
- Maternal disease
- Maternal CNV
- Maternal fibroids
- Combined placental mosaicism

DISCUSSION

- Laboratories compete for market share by
 - Expanding detection of rare trisomies and microdeletions
 - Enhancing test performance and reducing the rate of uninformative tests.
- Specific patterns of cfDNA results may indicate maternal origin rather than placental origin including cancer, fibroids, and maternal CNV.
- The findings of multiple aneuploidies on NIPT has been linked to cancer in both pregnant and non-pregnant women.

CONCLUSIONS

- Each laboratory follows its own protocol for reporting unexpected results
- Most provide a re-draw test that then produces a conclusive result
- The rate of receiving an informative result after a re-draw tests is around 60-75% depending on the laboratory
- Physicians need to understand the rationale for the tests they order and the factors that can interfere with an informative result 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.
- When unexpected NIPT results occur, providers must have established patient counseling strategies or appropriate referral networks with domain experts.

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