



R.C.P.U. NEWSLETTER

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Reproductive counseling in the modern era: The expanding role of cell free DNA (cfDNA) and non-invasive prenatal screening (NIPS)

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INTRODUCTION

Issues related to reproductive counseling have changed dramatically in the past decade for medical providers, and for parents who have, or are at risk to have, a child with a potentially heritable disorder. First, patients with conditions that may affect fertility have the option of meeting with a reproductive endocrinologist to provide information about their reproductive future. This can assist couples who are unsure of their ability to conceive to have clearer answers about the possibility of future children. Couples should also be aware of the options of pre-implantation genetic diagnosis (PGD). PGD is diagnostic testing performed on cells removed from an embryo or a polar body from an oocyte during the in-vitro fertilization process. To avoid a pregnancy with known heritable mutations or chromosomal abnormalities (eg, translocations) carried by one or both biological parents, only embryos that do not carry the genetic condition of concern are transferred into the uterus. Although this option is not financially feasible or emotionally acceptable to all patients, all families should be aware that this technology does exist.

Once pregnant, couples have many options for screening and diagnostic tests. These results can provide couples with reassurance about the pregnancy, aid them in decisions regarding continuation of a pregnancy or prepare them for the birth of a child with special needs. Amniocentesis (after 16 weeks) and chorionic villus sampling (after 10 weeks) are the gold standards of prenatal genetic diagnosis and are, in general, safe and well tolerated. These procedures, however, carry some risk of procedure-related miscarriage. This rate is in the range of 1 in 600-1000 for amniocentesis and 1 in 100-200 for CVS. Maternal blood analyte screening and ultrasound screening tests for chromosome abnormalities are often utilized to avoid this risk, however commonly utilized screens have suboptimal sensitivity (80-90%) and many false positives. In addition, this technology is designed to identify only a limited number of genetic conditions, and not the broad range of heritable genetic disorders. These tests do have some benefits, however, such as early diagnosis of serious birth defects with ultrasonography (first trimester screen) or adverse pregnancy outcome risk assessment (quad screen) although there are currently no guidelines to change management based on such an assessment. When cfDNA is used for aneuploidy screening, neural tube defect screen is still recommended (serum AFP or ultrasound).

The isolation of fetal DNA by non-invasive means can provide better screening for chromosome anomalies and potentially lead to a much broader range of genetic screening information. cfDNA is plentiful in the maternal blood stream and is unique to the current pregnancy because of their rapid clearance ($t_{1/2}=1h$). Intact fetal cells are also detectable in the maternal blood stream but are currently not a reliable source of fetal genetic material because they are relatively rare and may persist from prior pregnancies, making it impossible to determine if they are from the current fetus or a previous one. See test performance tables:

Performance Parameters of Noninvasive Screening Tests for Fetal Trisomy 21

Risk Factor/ Biomarker*	GA (wks)	Sensitivity (%)	Screen Positive (%)	Specificity	DR ^b (for a 5% FPR) (%)	FPR ^c (for an 85% DR) (%)
Age ≥ 35 y	—	30	5	1 in 100	30-50	48-55
First trimester risk assessment	11-14	82-87	5	1 in 25	79-90	5-9
Second trimester serum analyte (quad) screen	15-20	80	5	1 in 40	75-85 (only ~ 50% for twins)	5-10
Integrated screen	11-20	94-97	4-5	1 in 30	90-96	1-5

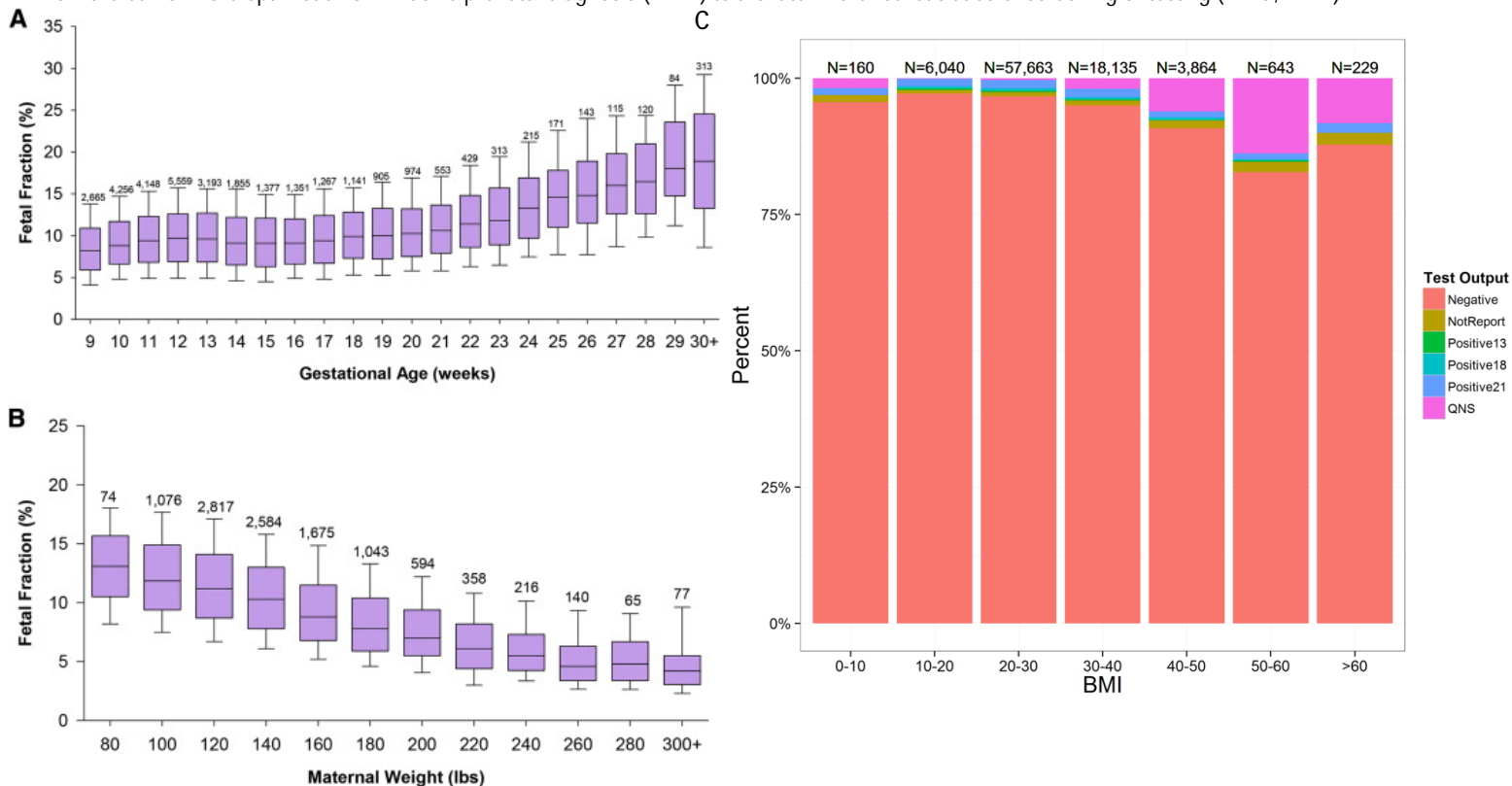
Comparison of Techniques for Prenatal Diagnosis Using Cell-Free DNA

Company	Technical Approach	Accuracy of Detection			Depends on Measurement of Specific Loci or Alleles?	Maternal/ Paternal Samples Used?
		Anomaly	Sensitivity (%)	Specificity (%)		
Sequenom Center for Molecular Medicine (San Diego, CA)	MPSS	T21	98.6	99.8	No	No
		T18	100	99.7		
		T13	91.7	99.0		
		45,X 47,XXY	—	—		
Verinata Health (Redwood City, CA)	MPSS	T21	100	100	No	No
		T18	97.2	99.8		
		T13	78.6	99.4		
		45,X 47,XXY	93.8	99.8		
Ariosa Diagnostics (San Jose, CA)	Targeted sequencing + DANSR + FORTE	T21	100	100	Yes (chromosome 21, 18 and 13)	No
		T18	98	100		
		T13	—	—		
		45,X 47,XXY	—	—		
Natera (San Carlos, CA)	Targeted sequencing + PS	T21	100	100	Yes (SNPs)	Yes
		T18	100	100		
		T13	100	100		
		45,X 47,XXY	100	100		

Cell free DNA

Although the majority of non-maternal cfDNA in the maternal blood stream arises from apoptosis of placental cells it is still referred to as fetal cfDNA since, the vast majority of the time, the placenta and fetus have identical constitution. Fetal cfDNA can be isolated from maternal blood as early as the 5th postmenstrual week, and almost always by the 9th postmenstrual week. Fetal cfDNA typically comprises 10-15% of the total cfDNA in the maternal circulation during the late first and early second trimesters of pregnancy. It is during this time that most prenatal testing is performed. In large studies, samples that contained too little fetal DNA to produce a valid result (QNS = quantity not sufficient, less than 4.0% cfDNA or less than 100 copies of cfDNA) occurred in 0.9% of samples. Two thirds of these results were followed up with repeat cfDNA analysis, and results of this repeat assay were reportable in over 70% of cases. This reduced the overall QNS rate to 0.54% in all patients. High BMI (body mass index) increases the risk of a QNS result with 18.3% of patients with BMI >60 kg/m² resulted as QNS. This is not different than the increased risk of difficulties interpreting other types of screening such as maternal analyte screening and ultrasound analysis in obese women.

To avoid false-negative tests caused by a failure to extract fetal cfDNA from the maternal circulation, a variety of methods are used to confirm the presence of fetal cfDNA. Most diagnostic and research laboratories amplify and measure the presence and amount of fetal cfDNA using real-time quantitative PCR. PCR works well when the pregnant woman does not have the fetal DNA sequence that is being amplified, such as the Y chromosome or a paternally-inherited autosomal allele. False negative or inadequate sample results can occur when the fetal cfDNA is at too low a concentration in the maternal plasma. This is more common in obese women because of their larger volume of distribution and increased amount of maternally derived cfDNA. False positive results can be due to factors such as maternal genetic abnormality, maternal cancer, vanishing twins or confined placental mosaicism. Acceptance of these sources of false positive results explain the evolution of the terminology for this technology, from the earlier more optimistic non-invasive prenatal diagnosis (NIPD) to the later more realistic titles of screening or testing (NIPS, NIPT).



A&B - the effect of gestational age and maternal weight on fetal fraction of cfDNA
 C - The effect of maternal BMI on rates of no-call results for low fetal fraction (QNS = quantity not sufficient)

Special considerations in cfDNA interpretation:

Demise of one twin — After the demise of a co-twin, the cfDNA contribution from the demised twin's placenta may persist after the demise occurs and compromise the remaining twin's results. In large reports, 0.4% of samples from presumed singletons are found to have >2 fetal haplotypes, indicative of either unreported twins, vanishing twin, or triploidy.

Donor oocyte — Pregnancies resulting from in vitro fertilization with a donor oocyte challenge the SNP technology used for some, or all, aspects of noninvasive prenatal testing because the inheritance pattern that makes the fetal cfDNA approximately 50% homologous to the maternal cfDNA does not exist. Although noninvasive prenatal testing is available for these patients from some companies, adjustments in the analyses must be made.

PRENATAL SCREENING APPLICATIONS of cfDNA

Screening for autosomal aneuploidies — Noninvasive prenatal screening for trisomies 13, 18 and 21 using cfDNA in the maternal blood was made available for clinical use in 2011. Because of the risk of a false-positive result as a result due to placental mosaicism, deceased co-twin, and other complications, this technology should still be seen as a screening rather than a diagnostic test, and positive result should be confirmed by amniocentesis for purposes such as termination decisions. In addition, the positive predictive value in a woman whose baseline risk of aneuploidy is 1% is only 50%. Clinical use has been mostly in patients considered at increased risk, but cfDNA is an excellent screening test in all women, and will likely replace previous screening techniques in the future although it is not currently recommended for use in low risk women by professional associations such as the American Congress of Obstetricians and Gynecologists (ACOG). One limiting factor in the application of NIPS in universal screening for aneuploidies is the cost of testing, but this is likely to come down with widespread uptake of the test.

Methods for detection vary by commercial provider but all perform comparably.

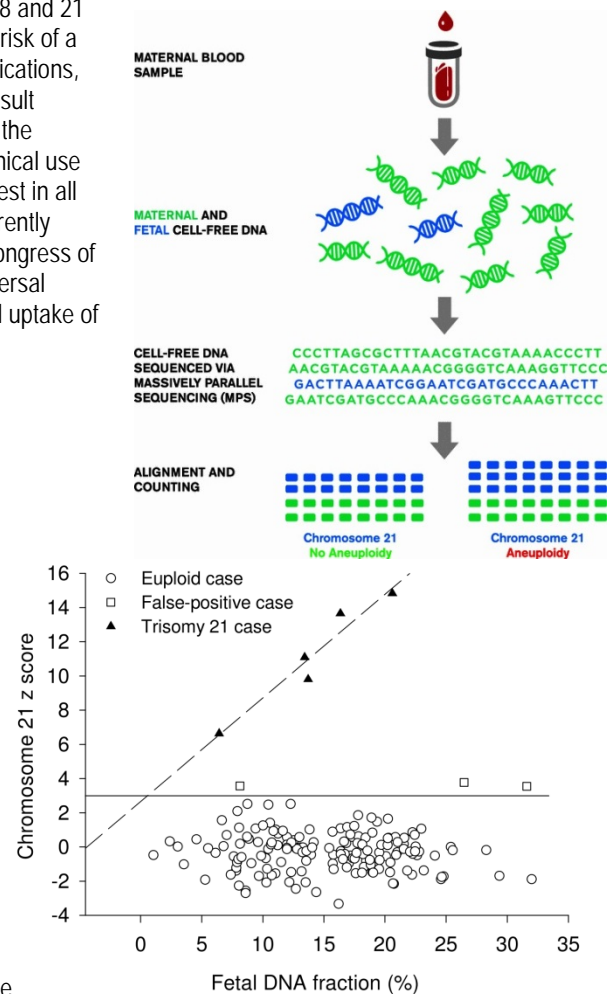
Methodologies include: chromosome-selective sequencing, digital analysis of selected regions (DANSR) followed by fetal fraction optimized risk of trisomy evaluation (FORTE) algorithm, polymerase chain reaction (PCR)-based amplification and analysis of single nucleotide polymorphisms (SNPs) specific to chromosomes of interest. Some reports have suggested a higher risk of inadequate fetal fraction of cfDNA in an aneuploid fetus and thus recommend invasive diagnostic testing rather than repeating cfDNA testing in 1-2 weeks. Other reports fail to show this association.

Multiple gestations — Testing is commercially available for trisomies 13, 18, and 21 in twin gestations, although there is less validation data available from twin gestations than from singletons. Because it is impossible to report which twin may be abnormal, results are reported for the entire pregnancy, and invasive testing is required to distinguish which twin, if either one, is affected.

Fetal sex determination — Determining fetal sex is perhaps the most straightforward use of cfDNA. Several common DNA sequences specific to the Y chromosome allow for the determination of fetal sex as early as the 7th postmenstrual week, with nearly 100% determination by the 10th postmenstrual week. Sex determination may be particularly useful as an initial step in the prenatal diagnosis of X-linked diseases, such as hemophilia or Duchene muscular dystrophy. In these cases, many carriers who know that their fetus is female do not pursue invasive fetal diagnosis since a female fetus will either be unaffected or an asymptomatic carrier of the mutation. On the other hand, if a male fetus is diagnosed, invasive testing for prenatal diagnosis may be desired if this information will affect pregnancy continuation or management. Direct-to-consumer marketing via the internet and print media for fetal sex testing services has made tests for fetal sex determination widely available, although the accuracy of tests from unregulated laboratories is questionable, and their availability raises important ethical concerns.

Sex-chromosome aneuploidy — Sex-chromosome aneuploidy is found in 1 in 400 live births, making it more common than autosomal aneuploidy. The commercial availability of testing for monosomy X, Klinefelter's syndrome (XXY), XYY syndrome, and other sex-chromosome aneuploidies is a new diagnostic study that has been added to prenatal screening by noninvasive prenatal testing. With the exception of monosomy X, which was sometimes diagnosed after finding a cystic hygroma or cardiac defect on prenatal ultrasound examination, most sex-chromosome aneuploidies do not have a phenotype that can be diagnosed by ultrasound or by serum screening. Furthermore, the use of noninvasive prenatal testing for these conditions may lead to the diagnosis of maternal sex-chromosome aneuploidy in women who are not aware that they have this condition. For example, up to 90% of women who carry an extra X chromosome may not be aware that they have triple X syndrome, and may be fertile. Thus, a determination of 47, XXX at the time of cfDNA testing may well reflect the mother's own karyotype as well as her offspring's.

Performance - In general, validation and experience reports have concluded similar test performance. However, a recent report of 109 cases of discordance between cell free DNA and cytogenetic results, reported a true-positive rate of 93% for trisomy 21 (38 of 41 confirmed) and 64% for trisomy 18 (16 of 25 confirmed). The true-positive rate was only 44% (7/16 cases) for trisomy 13 and 38% (6/16 cases) for sex chromosome aneuploidy. This may reflect the broad range of genotypic variability in certain disorders, such as monosomy X, about 60% of which arise from a single X chromosome while 16% is caused by isochromosomes, 5% by rings, 8% by Xp deletions, and even more rarely, mosaic or translocations.



Based On Clinical Validation				
Result	Sensitivity	Specificity	Expected False Positives	Expected False Negatives
T21	100–96.52	99.91–99.42	79 (4–497)	20 (0–341)
T18	100–87.99	100–99.61	0 (0–341)	0 (0–228)
T13	100–51.68	99.92–99.49	79 (4–320)	5 (0–483)
Based on Clinical Experience				
	Estimated	Estimated	Sensitivity	Specificity
	False Positives	False Negatives	(CI 95%)	(CI 95%)
All				
T21	4	6	99.6% (97.3–99.9)	99.9% (99.8–100.0)
T18	5	4	99% (93.3–100.0)	99.9% (99.3–99.9)
T13	13	2	98.9% (64.6–98.5)	99.9% (99.5–99.9)
Multiple Gestation				
T21	1	0	>99.9%	99.9%
T18	0	1	95.20%	>99.9%
T13	1	0	>99.9%	99.9%

Clinical validation expectation was based on the published validation study. Clinical experience estimates were based on clinical feedback of confirmed discordant results during the duration of the 100,000 testing.
doi:10.1371/journal.pone.0109173.t005

Rhesus typing — A noninvasive test for fetal Rh(D) cfDNA in the maternal circulation is commercially available in the United States, and is widely used in Europe to reduce the need for unnecessary fetal surveillance in isoimmunized Rh(D)-negative women carrying an Rh(D)-negative fetus. Another application is avoidance of unnecessary prenatal immunoglobulin injections for prevention of isoimmunization in Rh(D)-negative women carrying Rh(D)-negative fetuses. However, since the sensitivity and specificity of the test are not 100%, departures from traditional management strategies should be evaluated carefully.

Microdeletion syndromes — Some commercial testing companies offer testing for some of the more common microdeletion syndromes, although validating data are limited at this time. Also, a positive result could be maternal and must be followed with testing of the triad of mother, father and fetus/neonate for determination and counseling purposes.

Commercially available microdeletion assays at time of publication include:

MaterniT21: chromosome 22 and 16

Panorama: del 22q11.2 (VCFS); del 1p.36; del 15q (Angelman and PWS); 5p- (Cri-du-chat)

Single gene disorders — cfDNA detection of single gene disorders is in development, but is currently limited to detection of paternally-inherited mutations, which would not be present in the maternal genome (assuming the mother is unaffected). Paternally-inherited autosomal dominant diseases that have been detected using fetal cfDNA in maternal blood include Huntington disease, myotonic dystrophy, and Achondroplasia. However, techniques under study that measure the "relative mutation dosage" of a particular gene in all cfDNA in the maternal bloodstream may help overcome this limitation by assessing the fetal allelic contribution to the maternal cfDNA pool.

In the case of autosomal recessive conditions where the parents are carriers of different mutations, a "negative" test for the paternal mutation rules out an affected fetus. A "positive" test indicates that the fetus has inherited the paternal allele carrying the mutation; however, the test cannot distinguish the fetus that is an unaffected carrier of the paternal allele alone from the fetus that carries the paternal allele, as well as a maternal allele, and is therefore affected. It is unclear whether noninvasive tests for common single gene disorders such as cystic fibrosis or beta thalassemia will be commercially available in the next few years, although many observers think that their development is inevitable.

CONCLUSION

As technology races ahead and advances in reproductive science arise, we will constantly have to review what may be available and relevant to our patients. But most importantly, we must bring up the topics of reproductive potential and pre-implantation or early fetal diagnosis so that patients and families can undergo further evaluation if desired and make informed decisions.

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ABOUT THE RCPU:

The Raymond C. Philips Research and Education Unit began in 1978 when the legislature established section 393.20, F.S., of what is now known as the "prevention" legislation. It is named after Raymond C. Philips, who was the Superintendent of Gainesville's Tacachale (formerly Sunland) Center for 38 years, and was an acknowledged state and national leader in services for mentally retarded persons. The Unit is located on the Tacachale campus and is funded through a contract with the Department of Children and Families and the Department of Health.

The purpose of the R.C.P.U. is to treat, prevent, and/or ameliorate intellectual disabilities through medical evaluations, education and research. The unit provides direct evaluations and counseling to families and promotes service, education, and prevention projects.

Some of the conditions currently under study at the RCPU involve Angelman, Velo-Cardio-Facial, Prader-Willi, Fragile X, Williams and Smith-Lemli-Opitz syndromes.

The R.C. Philips Unit is a resource for all Floridians interested in the diagnosis, treatment and prevention of intellectual disabilities. Staff members are available for consultation and for educational programs for health professionals and for the community at large.

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