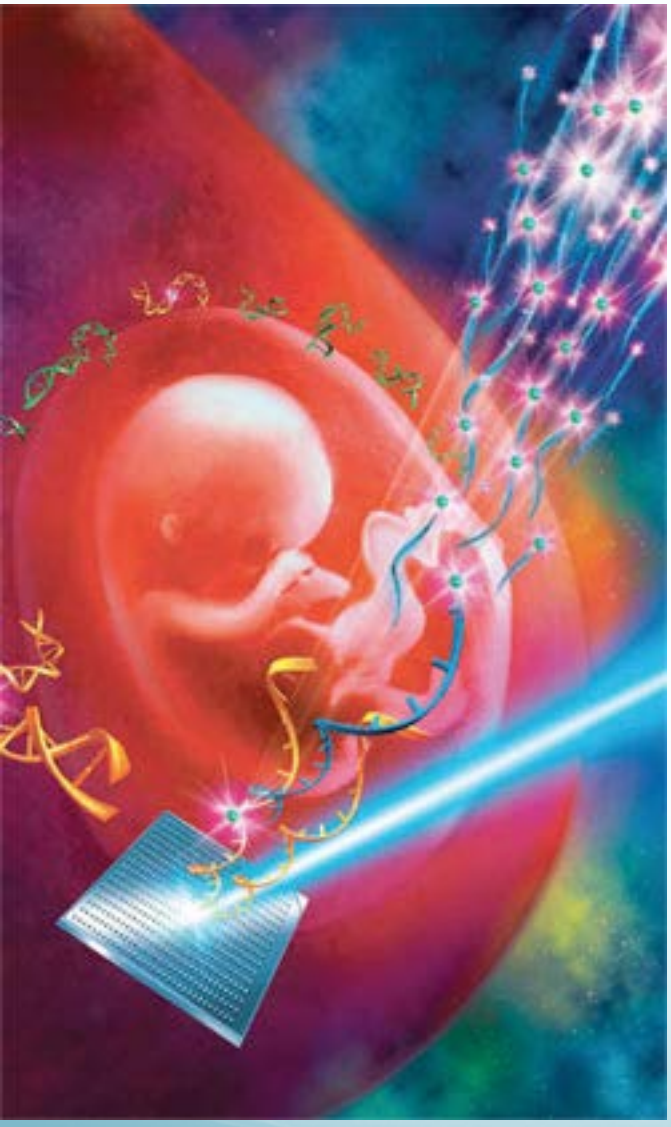


Non-invasive Prenatal Screening: The Clinical Perspective

Cecelia Bellcross, PhD, MS, CGC
Emory University School of Medicine
Department of Human Genetics
Nov 18th, 2015

Non-invasive Prenatal….

Diagnosis (NIPD)

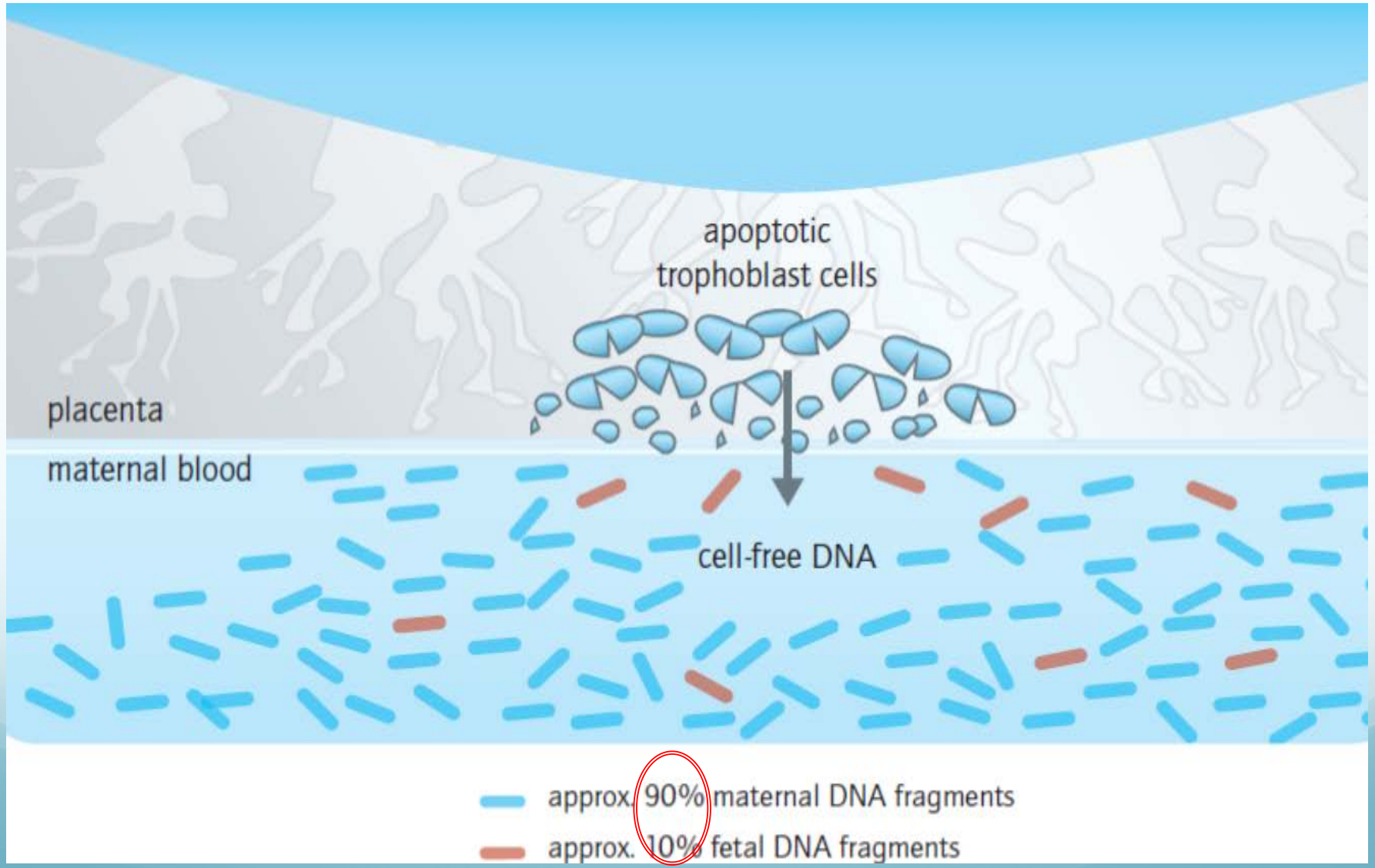
Testing (NIPT)

Screening (NIPS)

CffDNA Screening

What do we call it?

Cell-free Fetal (CFF) DNA



Comparison of Options

	CVS	Amnio	Sequential MSS	NIPT
Timing	11-13 weeks	≥ 16 weeks	10-22 weeks	≥ 10 weeks
Risk of miscarriage	<1%	~ 0.2%	None	None
Sensitivity	>99% all aneuploidies	>99% all aneuploidies	90% tri 21	>98% tri 21
False positive Rate	<2% all	<1% all	5% tri 21	<0.5% tri 21
Failure Rates	<1%	<1%	<1%	1-5%
Costs	~$2,000	~$1500	~$400	$800-$3,000

NIPT Challenges

- Fetal Fraction (FF)
  - 8% + needed for best performance
  - Affected by gestational age, maternal BMI, type of aneuploidy
- Triploidy
  - Lower fetal fraction
  - Missed by non-SNP methods
- Twins
  - Each fetus will have a different FF
  - Increased no call rate
  - If discordant for sex or aneuploidy
    - 10-15% FF < 4%
    - Increased false negative rate

NIPT False Positives

- Placental mosaicism
- Vanishing twin
- Maternal sex chromosome abnormality
- Neoplasia – apoptosis of cancer cells, aneuploidy common

Mosaicism

- **Confined placental mosaicism**
  - Follow up diagnostic testing recommended
  - Is Amniocentesis preferred over chorionic villus sampling?
- **Fetal mosaicism**
  - Identification of mosaicism will be less effective because the contribution from abnormal is partial (Canick 2013)
- **Maternal Mosaicism**
  - Sequencing of buffy coat may determine if maternal chromosome abnormality is confounding the results (1 in 3000)

Vanishing Twins (VT)

3% of pregnancies are twins

5-36% of twin gestations result in VT

ACOG Practice Bulletin 144, May 2014

"It is theoretically possible that apoptosis of cells from the fetoplacental remains of the non-viable fetus could interfere with the cfDNA result " (Benn, *2013)*

15% of **<u>discordant</u>** commercial results had VT

(Futch, 2013)

cffDNA seen at least 6-8 weeks post-demise

Mom Matters Too

Table 2. Contribution of an abnormal ChrX maternal karyotype in a prospective study of 187 discordant SCAs.

Clinical	NIPT findings	NIPT ChrX gain	NIPT ChrX loss	Total
NIPT follow-up	Abnormal NIPT for SCA, n	63	124	187
	Normal maternal karyotype, n	57	114	171
	Altered maternal karyotype, n	6	10	16
	Maternal mosaicism rate	9.52%	8.06%	8.56%

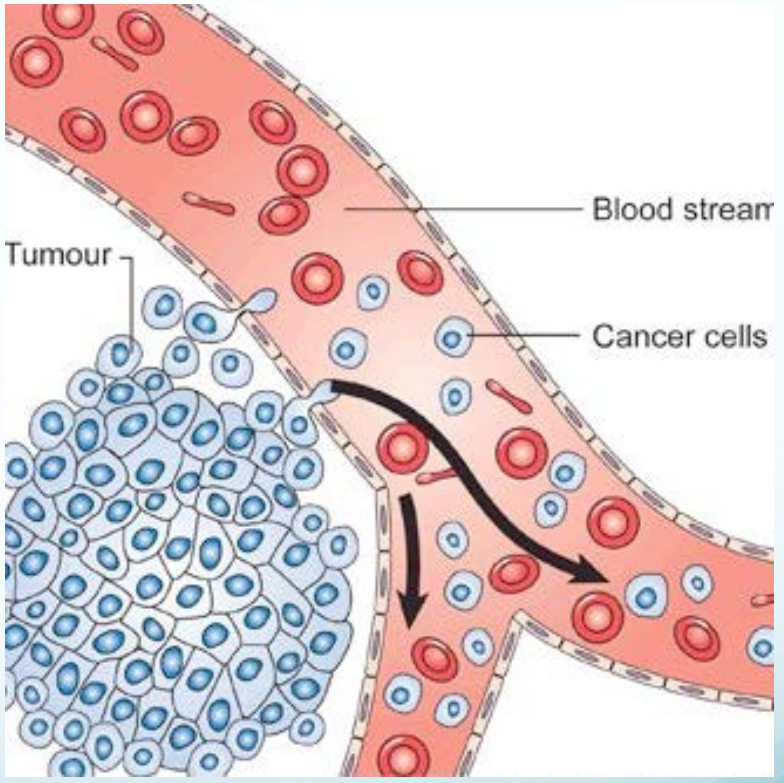
8.56% of called sex chromosomal aneuploidies were FP due to maternal mosaicism

"The relatively high frequency of maternal mosaicism warrants mandatory WBC testing in both shotgun sequencing– and single nucleotide polymorphism–based clinical NIPT after the finding of a potential fetal SCA."

Wang, Clin Chem, 2014

Maternal Malignancy

- 3757 NIPT positive for aneuploidy
- 10 cases of maternal cancer
- 39 cases multiple aneuploidy
  - 7 known maternal cancers (18%)
  - Monosomy/trisomy of 21, 13, 18, X
  - Clinical follow-up for maternal malignancy with double aneuploidies?



Bianchi, JAMA, 2015

NIPT in Low Risk Pregnancies

Table 2 Comparison of the detection rates, false-positive rates, and positive predictive values (PPV) for Down syndrome screening using conventional approaches (combined, quad, and sequential) and NIPT

Test	Detection rate (%)	False positive rate (%)	PPV high-risk population (1/100) (%)	PPV low-risk population (1/500) (%)
Combined (NT, PAPPA, hCG)	80	3	21	5
Quad (AFP, uE3, hCG, INH-A)	60	3	17	4
Sequential (combined & quad)	93	3	24	6
NIPT (composite of all methods)	99.3	0.1	91	67

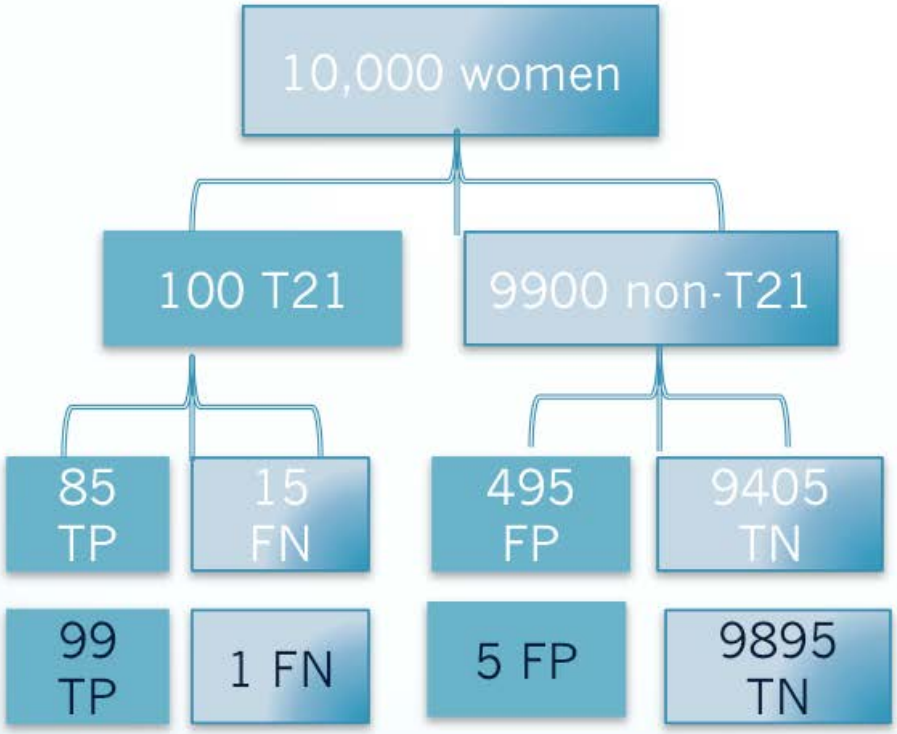
Positive Predictive Value (PPV)

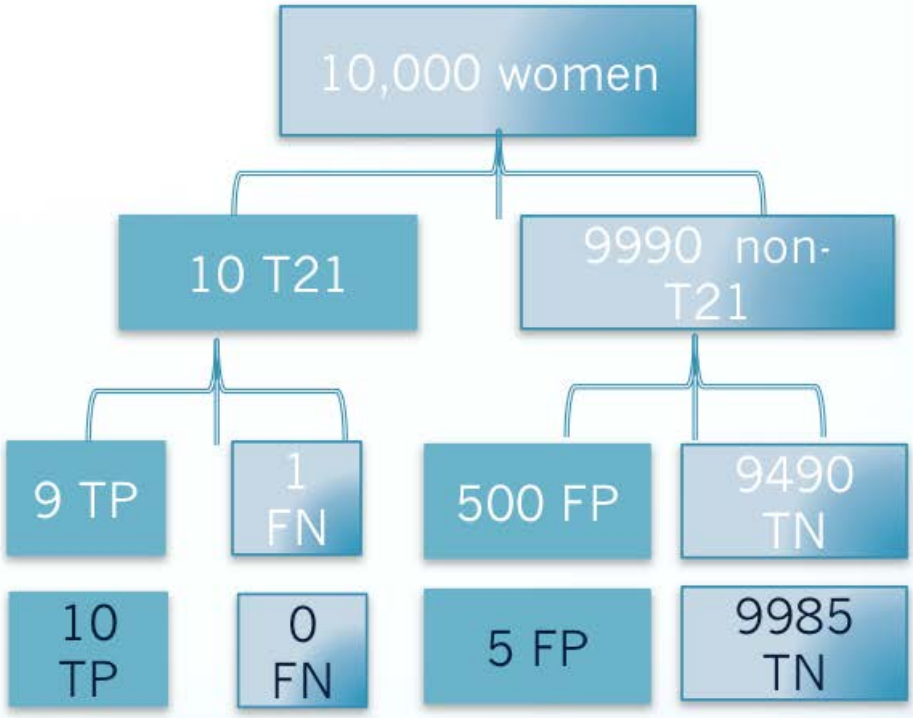
% of abnormal (positive) test results where fetus actually has the aneuploidy predicted

Dependent upon PREVELANCE of condition

Benn, J Fetal Med, 2014

85% Sens & 5% FPR = FTS
99% Sens & 0.05% FPR = NIPT





FTS: TP/(TP+FP) = 85/580 = **15% PPV**
NIPT = 99/104 = **95% PPV**

FTS = TP/(TP+FP)=9/509 = **2% PPV**
NIPT = 10/15 = **66% PPV**

High Risk 1/100

Low Risk 1/1000

Table 2. Test Performance for Trisomy 21 in the Primary Analysis Cohort, According to Maternal Age and Risk.*

Variable	Standard Screening	Cell-free DNA Testing		
	All Patients (N=15,841)	All Patients (N=15,841)	Maternal Age <35 Yr (N=11,994)	Low Risk (N=14,957)†
True positive — no.	30	38	19	8
True negative — no.	14,949	15,794	11,969	14,941
False positive — no.	854	9	6	8
False negative — no.	8	0	0	0
Sensitivity (95% CI) — %	78.9 (62.7–90.4)	100 (90.7–100)‡	100 (82.4–100)	100 (63.1–100)
Specificity (95% CI) — %	94.6 (94.2–94.9)	99.9 (99.9–100)§	99.9 (99.9–100)	99.9 (99.9–100)
Positive predictive value (95% CI) — %	3.4 (2.3–4.8)	80.9 (66.7–90.9)§	76.0 (54.9–90.6)	50.0 (24.7–75.3)
Negative predictive value (95% CI) — %	99.9 (99.9–100)	100 (99.9–100)¶	100 (99.9–100)	100 (99.9–100)

* P values are for the comparison between standard screening and cell-free DNA screening in the primary analysis cohort.
† Low risk was defined as a mid-trimester risk of trisomy 21 of less than 1 in 270 on standard screening.
‡ P=0.008
§ P<0.001
¶ P=0.005.

Norton ME, et al. NEJM, epub 4/1/15

Table 3. Test Performance for Trisomy 18 and Trisomy 13.*

Metric	Trisomy 18		Trisomy 13	
	Standard Screening (N=15,841)	Cell-free DNA Testing (N=15,841)	Standard Screening (N=11,185)	Cell-free DNA Testing (N=11,185)
True positive — no.	8	9	1	2
True negative — no.	15,782	15,830	11,155	11,181
False positive — no.	49	1	28	2
False negative — no.	2	1	1	0
Sensitivity (95% CI) — %	80.0 (44.4–97.5)	90.0 (55.5–99.7)	50.0 (1.2–98.7)	100 (15.8–100)
Specificity (95% CI) — %	99.7 (99.6–99.8)	100 (99.9–100)†	99.7 (99.6–99.8)	100 (99.9–100)†
Positive predictive value (95% CI) — %	14.0 (6.2–25.8)	90.0 (55.5–99.7)†	3.4 (0.1–17.8)	50.0 (6.8–93.2)
Negative predictive value (95% CI) — %	100 (99.9–100)	100 (99.9–100)	100 (99.9–100)	100 (99.9–100)

* Included in the trisomy 13 analysis are patients who were enrolled after September 2012.
† P<0.001 for the comparison with standard screening.

Norton ME, et al. NEJM, epub 4/1/15

TABLE 4

Clinical follow-up findings

N = 17,885[a]	Trisomy 21	Trisomy 18	Trisomy 13	Monosomy X	Total
High-risk calls	233[b]	55[b]	30	38	356
Confirmed outcomes					
True positive	140[c]	27	8	9	184
False positive	14[d]	2[e]	13[f,g]	9	38
Unconfirmed outcomes					
Suggestive[h]	8	9	0	2	19
Pregnancy loss[i]	18	6	3	9	36
Termination[j]	14	3	0	5	22
No follow-up[k]	39	8	6[l]	4	57

16.4%

- Confirmed Outcomes (62%)
  - **False positive = 17%** (includes 3 cases of CPM)
    - 79.2% FP with intermediate risk score (1/100 ≤ risk < 99/100)
    - 9.6% FP with maximum risk score (≥ 99/100)
  - PPV
    - Tri 21 – 90.9%, Tri 18 – 93.1%, Tri 13 – 38.1%, XO – 50%

Dar P, et al. Am J Obstet Gynecol 2014

Table 1 Concordant and discordant NIPT and cytogenetic results in a cohort of cases referred for cytogenetic studies ($N = 109$)

NIPT result	Specimen type	Number of cases	Concordant	Discordant	Specimen type of discordant cases	Cytogenetic results of discordant cases
Positive			True positive	False positive		
Trisomy 21	25 AF, 14 CVS, 1 FPB, 1 cord	41	38/41 (93%)	3/41 (7%)	2 AF, 1 FPB	Three normal
Trisomy 18	19 AF, 2 CVS, 2 FPB, 2 cord/POC	25	16/25 (64%)	9/25 (36%)	6 AF, 1 CVS, 1 FPB, 1 cord/POC	Eight normal, one balanced translocation
Trisomy 13	15 AF, 1 cord	16	7/16 (44%)	9/16 (56%)	8 AF, 1 cord	Nine normal
Sex chromosome aneuploidy	12 AF, 1 CVS, 2 FPB, 1 POC	16	6/16 (38%)	10/16 (62%)	7 AF, 1 CVS, 2 FPB	Nine normal, one with gain of 724 kb from 20p12.1
Trisomy 16	3 AF	3	1/3 (33%)	2/3 (67%)	2 AF	Two normal
Monosomy 21	2 AF	2	0	2/2 (100%)	2 AF	Two normal
Triploidy	AF	1	0	1/1 (100%)	1 AF	One normal
22q11.2 Microdeletion	AF	1	0	1/1 (100%)	1 AF	One normal
Negative			True negative	False negative		
	4 AF	4	0	4	4 AF	One trisomy 9, one trisomy 21, one marker chromosome, one 45,X/46,XY
Total	82 AF, 17 CVS, 5 FPB, 5 blood/POC	109	68/109 (62%)	41/109 (38%)	33 AF, 2 CVS, 4 FPB, 2 cord/POC	36 Normal, one trisomy 9, one trisomy 21, one autosomal balanced translocation, one marker chromosome, and one mosaic sex chromosome aneuploidy

AF, amniotic fluid; CVS, chorionic villus sampling; FPB, fetal peripheral blood; NIPT, noninvasive prenatal testing; POC, product of conception.

Wang et al. Genet Med 2005;17:234

Table 2 True-positive and false-positive rates in the NIPT-positive cases (*N* = 224)

NIPT result	Study by Choy et al.[2]	Study by Meck et al.[3]	Current study	Overall
Positive cases	80	46	98	224
True positive for trisomy 21	52/55	29/30	38/41	119/126 (94.4%)
False positive for trisomy 21	3/55	1/30	3/41	7/126 (5.6%)
True positive for trisomy 18	6/12	3/5	16/25	25/42 (59.5%)
False positive for trisomy 18	6/12	2/5	9/25	17/42 (40.5%)
True positive for trisomy 13	4/7	1/4	7/16	12/27 (44.4%)
False positive for trisomy 13	3/7	3/4	9/16	15/27 (55.6%)
True positive for SCA	4/6	1/7	6/16	11/29 (37.9%)
False positive for SCA	2/6	6/7	10/16	18/29 (62.1%)

NIPT, noninvasive prenatal testing; SCA, sex chromosome aneuploidy.

Wang et al. Genet Med 2005;17:234

Prenatal Diagnosis

Positive Predictive Value of Cell Free DNA Calculator

Baseline Risk
(•) Age-related risk ( ) A priori risk

Maternal Age (25)

20 22 24 26 28 30 32 34 36 38 40 42 44

Gestational Age in Weeks (10)

10 12 14 16 18 20

Test
(•) Harmony® ( ) Materniti 21® ( ) Panorama® ( ) Verifi®

	Trisomy 21	Trisomy 18	Trisomy 13
Age-related risk	1:712	1:1765	1:5621
Test Sensitivity	99	98	80
Test Specificity	99.97	99.93	99.9
PPV	82%	44%	12%

http://www.mombaby.org/nips_calculator.html

Expanded NIPT

- Non-viable trisomies (WHY????)
  - trisomy 16
  - trisomy 22

- Microdeletion syndromes (not associated with maternal age)
  - 1p36 del - PPV ~ 17%
  - 22q11.2 del - PPV ~ 5.3%
  - 5p minus - PPV ~ 5.3%
  - 15q11 del
    - Maternal, Angleman's – 3.8%
    - Paternal, Prader Willi – PPV ~ 4.6%

NIPT for Microdeletions: Issues

- Limited validation data
- SNPs used for ascertainment questioned
- Size of deletion matters
- Some conditions highly variable
- Parents may be affected (22qdel)
- Unanticipated results
  - Panorama - Opt out

Professional Recommendations

AMERICAN COLLEGE OF OBSTETRICIANS AND

ACMG

ISPD recognizes the challenge associated with explaining the expanding range of disorders that can be included in screening panels as well as the complexity of the various testing alternatives. To help meet this growing need, we support additional professional education for obstetricians and other healthcare personnel involved in screening, development of patient educational materials, and increased availability of genetic counseling.

ispd
International Society for Prenatal Diagnosis

2011, 2013, 2015

National Society of Genetic Counselors

Feb 2012

Society for Maternal-Fetal Medicine

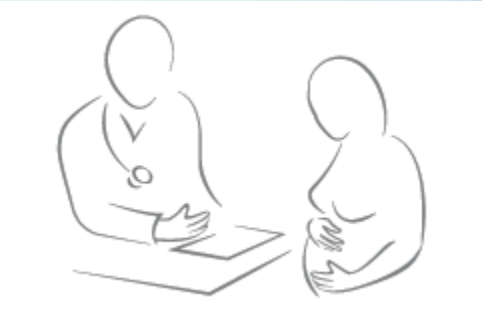
Summary recommendations

No.	Recommendations	GRADE
1	Optimal candidates for routine cfDNA aneuploidy screening are women with:	1B: Strong recommendation, moderate quality evidence
	Maternal age ≥35 years at delivery.	
	Fetal ultrasound finding that indicates an increased risk of aneuploidy, specifically for trisomies 13, 18, or 21.	
	History of previous pregnancy with a trisomy detectable by cfDNA screening (trisomies 13, 18, or 21).	
	Positive screening results for aneuploidy that include a first-trimester, sequential, integrated, or quadruple screen.	
	Parental balanced Robertsonian translocation with increased risk of fetal trisomy 13 or 21.	
2	Routine screening for microdeletions with cfDNA is not recommended.	1B: Strong recommendation, moderate quality evidence
3	For women who desire comprehensive testing for chromosomal disorders, diagnostic testing should be offered.	1B: Strong recommendation, moderate quality evidence
4	For women who undergo cfDNA aneuploidy screening, maternal serum alpha-fetoprotein, and/or second-trimester anatomy ultrasound scan should also be performed.	Best practice
5	Formal genetic counseling by maternal-fetal medicine subspecialist, geneticist, or genetic counselor after a positive cfDNA test is recommended	Best practice
6	Chorionic villous sampling or amniocentesis should be offered after a positive cfDNA screen to confirm the diagnosis.	Best practice
7	Traditional aneuploidy screening and cfDNA aneuploidy screening should not be performed at the same time.	Best practice
8	After a failed cfDNA test, genetic counseling should be performed that includes offering diagnostic testing (chorionic villous sampling or amniocentesis) and repeat cfDNA screening.	Best practice

Am J Obset Gynecol, 2015; 212:711-6

It’s Not Just About Aneuploidy

- NIPT does not provide comprehensive prenatal screening
  - Nuchal translucency
  - MSAFP
  - Second trimester ultrasound
- Even those with normal NIPT may want to consider invasive testing with ultrasound findings or family/medical history
- Other screening or testing may be better first approach depending on the indication
  - Karyotype
  - Microarray
    - (ACOG/SMFM Committee Opinion December 2013)



Informed Consent

- “However, published data and anecdotal experience suggest that many women do not fully understand implications of screening results & some were not fully aware that they were undergoing screening at all” *(Allyse, 2013)*

Pre-test Counseling

- Limitations
  - Not diagnostic!!!
  - Detects < 50% of genomic imbalances that could be serious
  - Limited and expensive for single gene disorders
  - Uninformative results
  - Does not address neural tube defects
  - More data needed on twins
  - No role in forecasting late pregnancy complications
  - ? Could reveal maternal malignancy ?

Pre-test Counseling – cont.

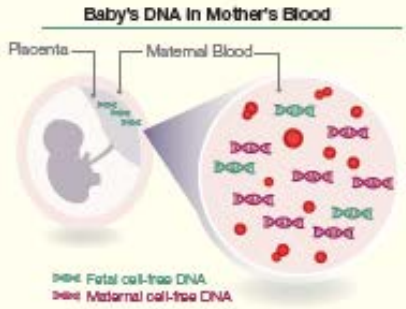
- Microdeletion syndromes
  - Opt out
  - Spectrum of conditions tested
  - Variability of conditions
  - Could reveal affected parent
- Benefits
  - *Performance appears better than any maternal serum screening test to date*
  - Risk assessment less dependent on gestation age

Congratulations on being pregnant!

What an exciting time. There are many things you are thinking about right now, one of which may be: "How healthy is my baby?" This is where Panorama™ can help you.

What is Panorama?

Panorama is a non-invasive prenatal screening test. During pregnancy, some of the DNA from the baby crosses into the mother's bloodstream. Panorama looks at this DNA to see if there is evidence of certain conditions that could affect the baby's health.



What does Panorama tell me?

Panorama gives you a personalized risk score and tells you if your baby is at high risk or low risk for certain genetic conditions, specifically:

- Down syndrome (T21)
- Edwards syndrome (T18)
- Patau syndrome (T13)
- Certain sex chromosome abnormalities:
  - Turner syndrome (monosomy X)
  - Klinefelter syndrome (XXY)
  - Jacob syndrome (XYY)
  - Triple X (XXX) or vanishing twin
- Triploidy
- Sex of the child (if requested)
- Microdeletions that are common and can be severe including 22q11.2 deletion syndrome (DiGeorge syndrome)*

How do I get a Panorama test?

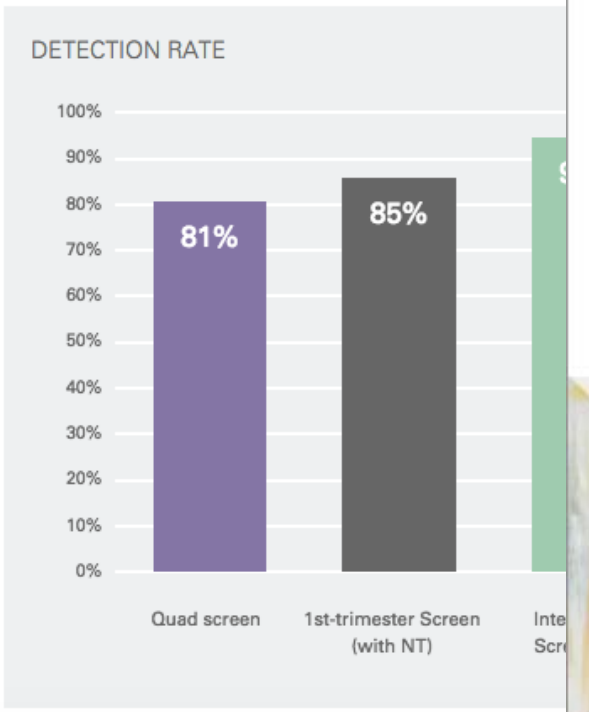
Your doctor orders the test, which is a simple blood draw from you. The baby's father can provide a cheek swab too, but it is not required, and will not affect the accuracy of the test. However, unlike other NIPTs that cannot use the father's cheek swab, this may increase the chance Panorama will be able to give you a result.

When can I get a Panorama test?

You can have this test as early as 9 weeks gestation. And your doctor gets your results back in 7-10 calendar days.

What other tests are available?

There are various other tests available. Traditional screening tests are not as accurate as Panorama, and diagnostic tests such as amniocentesis or chorionic villus sampling (CVS) have a slight risk of pregnancy complications including miscarriage.



harmony™
PRENATAL TEST

Clear ANSWERS to Questions that Matter

ty

surpassed accuracy, bringing clarity to genetic

ng methods using serum proteins and ultrasound
s, causing healthcare providers and their patients
onal invasive testing.

rmony correctly identified over 99% of cases of

ts can miss 15% or more of trisomy 21 cases

A more accurate tes

Harmony is more accurate than traditional Down
much less likely to give a false-positive result. Th
less chance your doctor would recommend follov
amniocentesis.

Harmony also tests for two other genetic conditi
syndrome) and trisomy 13 (Patau syndrome).

In addition, with Harmony you have the option to
chromosomes.

Non-invasive prenatal testing based on cell-free D
diagnostic. Once you have your Harmony test res
pregnancy care with your healthcare provider.

False Positive Rate*	Detection Rate**
Less than 1 in 1,600	More than 99 in 100
1 in 20	79 in 100

yndrome when it is NOT actually present
t for Down syndrome when it IS present

Post-test Counseling

- If a positive NIPT result:
  - Remember False Positives occur
  - What is the PPV for this patient?
  - Refer for **genetic counseling**
  - Always offer invasive testing for confirmation
    - Patients should *never* be offered the option of termination without confirmation
  - If parents decline invasive testing, postnatal confirmation should be completed
- If a Negative NIPT result:
  - Remember False Negatives occur – especially in higher risk pregnancies
  - Always *offer* invasive testing if parents want to “know for sure”

Thank you

cbellcr@emory.edu