DEFINITIVE NON-INVASIVE PRENATAL DIAGNOSIS USING MATERNAL BLOOD

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March of Dimes, White Plains, New York

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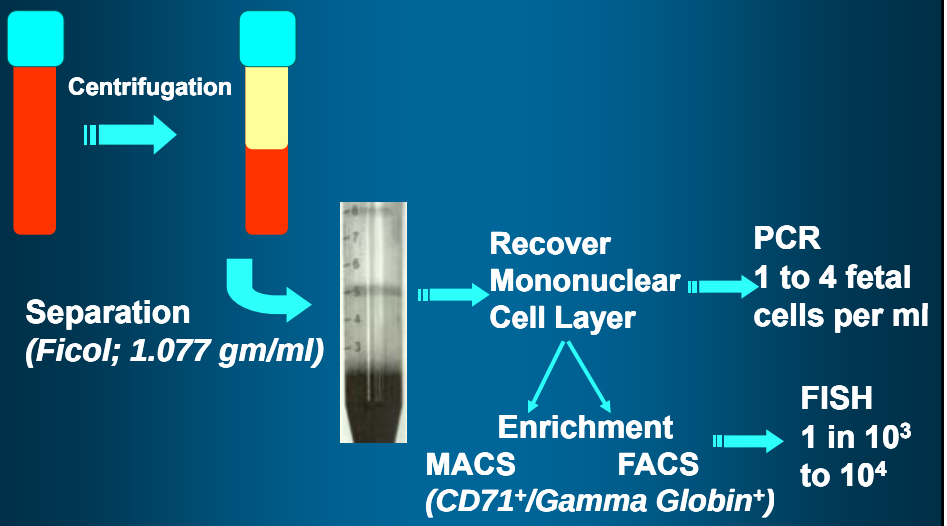
DISCLOSURE

- I have no financial conflicts relevant to information related in this talk. I have provided informal consultation for Ariosa, Natera, BioDx, and Rare Cells Diagnostics.

GENERAL STRATEGY (1990s, early 2000s) FOR RECOVERY OF INTACT FETAL CELLS



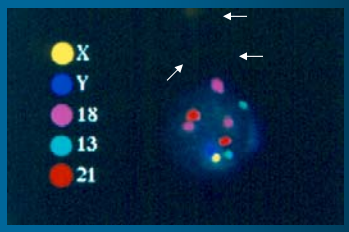
CIRCULATING CELLS AND DNA IN BLOOD: PREGNANCY

- First to detect fetal aneuploid cells in maternal blood:
  - Trisomy 18
    (Price, Elias, Wachtel, Simpson; 1991)
  - Trisomy 21
    (Elias, Price, Doktor, Simpson; 1992)
- 1994-2003 National Institutes of Health Fetal Cell Study Group (NIFTY)
  (Bianchi, Bischoff, Elias, Evans, Holzgreve, Jackson, Lewis, Simpson)

FIVE-COLOR FISH TO DETECT FETAL TRISOMIC CELLS IN ENRICHED POPULATION FROM MATERNAL BLOOD

Trisomy 21

Trisomy 18



Bischoff et al., Am J Obstet Gynecol 1998

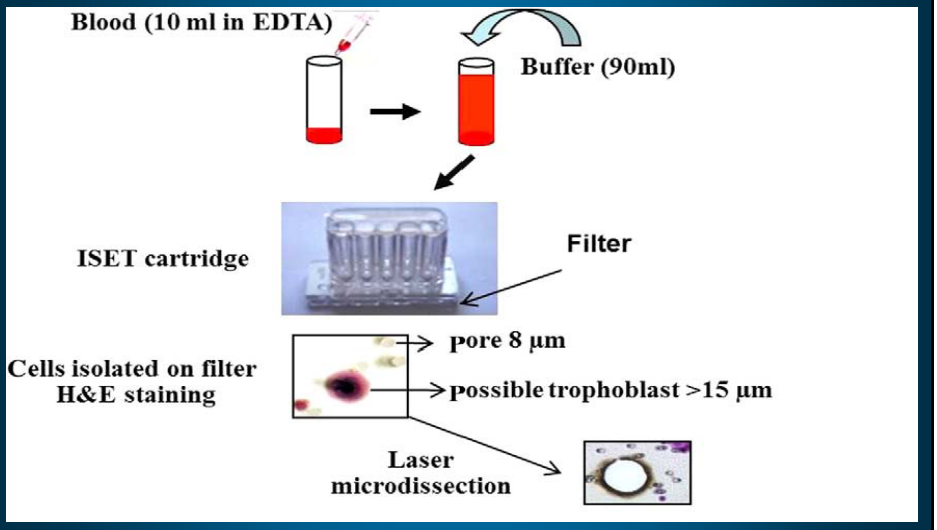
CONCLUSIONS (NIH): INTACT FETAL ERYTHROBLASTS

FISH to Detect Aneuploidies:

- 74% detection of fetal aneuploidy analyzing slides by fluorescent in situ hybridization (FISH); MACS preferable to FACS
- Enrichment and analysis inefficient and not consistently achieved. NICHD recommended biotech collaboration

Bianchi, Simpson, Jackson
Prenat. Diag., 2002

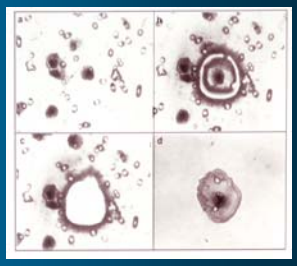
Isolation by Size Epithelial Cells/ Trophoblasts (ISET): 2012



Paterlini-Brechot, Rare Cells (2012)

ISET isolated cell

Single cell laser microdissection

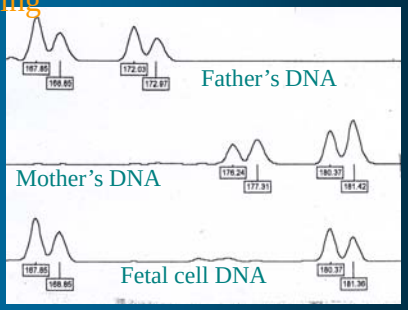


STR (Short Tandem Repeats)/ genotyping

(CA)1; (CA)3
CACACA
CA

(CA)5; (CA)7
CACACACACACACA
CACACACACA

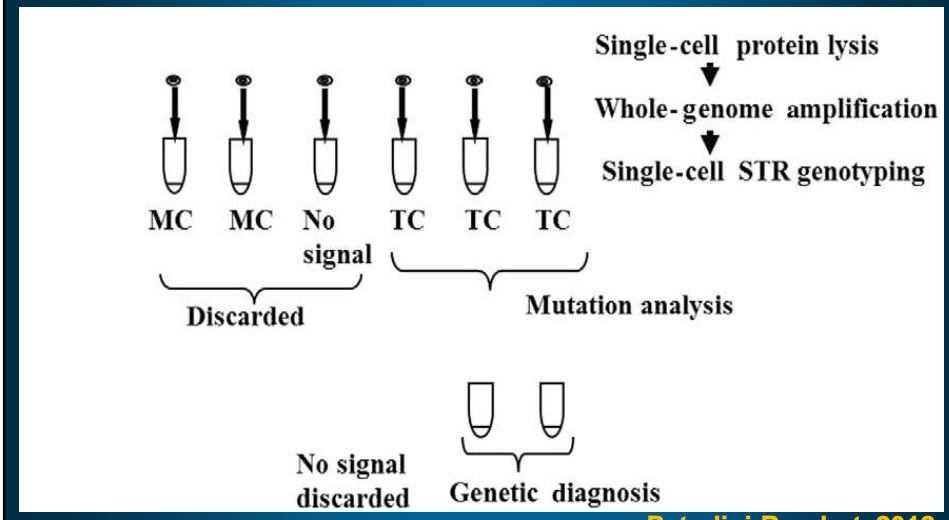
(CA)1; (CA)7
CACACACACACACA
CA



10 genomic analysis on the genome of a single cell

Vona et al, Am J. Pathol, 2002

Fetal Cell Isolation (Rare Cells)



Paterlini-Brechot, 2012

CLINICAL UTILITY OF TROPHOBLASTS (Paterlini-Bréchot)

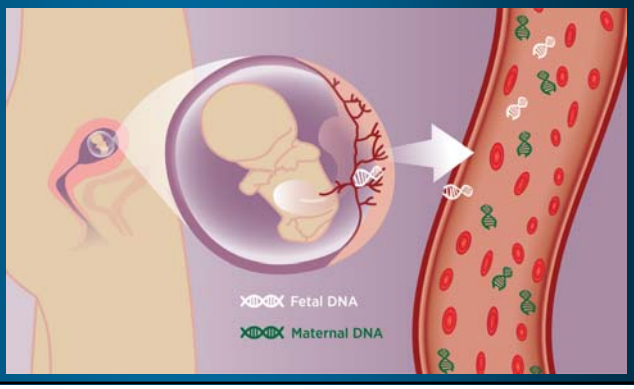
- Proof of principle reports (SMA, Lancet, 2003; Cystic fibrosis, Prenat. Diag., 2006)
- 63 consecutive correct cases (32 cystic fibrosis and 31 SMA) successfully diagnosed. (Reprod. Med. Online, 2012) All cases informative
- Trophoblasts recoverable from 5 weeks onward

CELL FREE FETAL DNA IN MATERNAL BLOOD

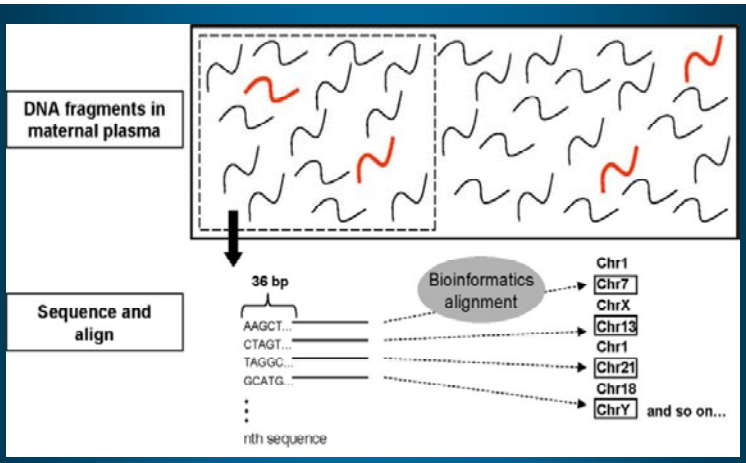
- Initial application by Lo (1990s) using plasma
- Current diagnostic approaches based on analyzing admixture of maternal and fetal cell free DNA (maternal blood)

Cell-Free DNA in Maternal Blood

- Cell-free DNA (cfDNA) are short DNA fragments
- In pregnancy, cfDNA from both the mother and fetus are in maternal blood
- Amount of fetal cfDNA present is a small fraction of the maternal cfDNA



Assessing Fetal 21 Transcripts by Parallel Genomic Sequencing (maternal and fetal transcripts)



Chiu, Lo, PNAS, 2008

Cell Free DNA for Diagnosis

- Qualitative Difference: Straight forward (e.g. Y-sequence)
- Quantitative Difference between mother and fetus: More difficult.

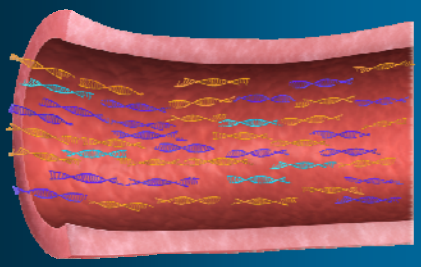
CELL FREE FETAL DNA TO DETECT PATERNAL ALLELE (thus *FETAL ALLELE*) NOT PRESENT IN MOTHER

1. Paternal mutations to detect mendelian mutation being transmitted to fetus (e.g., Marfan, Huntington). Presence of mutation in maternal blood must have originated from DNA of affected fetus.
2. Rh(D) to distinguish Rh negative (del/del) from Rh(D/del) fetus given RhD/del father. D in maternal blood can exist only if of fetal origin.

CELL FREE FETAL DNA FOR ANEUPLOIDY DETECTION

- Strategy: Increased trisomy 21 transcripts (maternal and fetal) in maternal blood of trisomic pregnancies compared to maternal blood of euploid (normal) pregnancies. Massive Parallel Genomic Sequencing (MPGS) [Massive Parallel Shotgun Sequencing – MPSS]
- Quantitative rather than qualitative difference must be shown for interrogated transcripts.

Cell-Free DNA in Maternal Blood



cfDNA in blood

Chr 21, 18, 13 cfDNA

Other Chr cfDNA

Unmapped cfDNA

Analysing Maternal Blood to Differentiate Euploid v Aneuploid Pregnancies

- **Massive Parallel Genomic Sequencing (MPGS) for all transcripts (maternal + fetal) [Sequenom; Verinata]**
- **Targeted: Chromosome-Specific DNA by hybridization of only selected chromosomes (e.g. 13,18,21)**
  - **Followed by either quantitative counting (Ariosa) or SNP analysis (Natera)**

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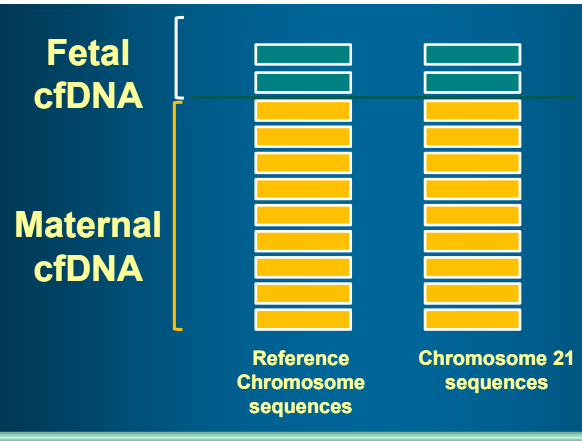
Fetal Trisomy Detection With cfDNA

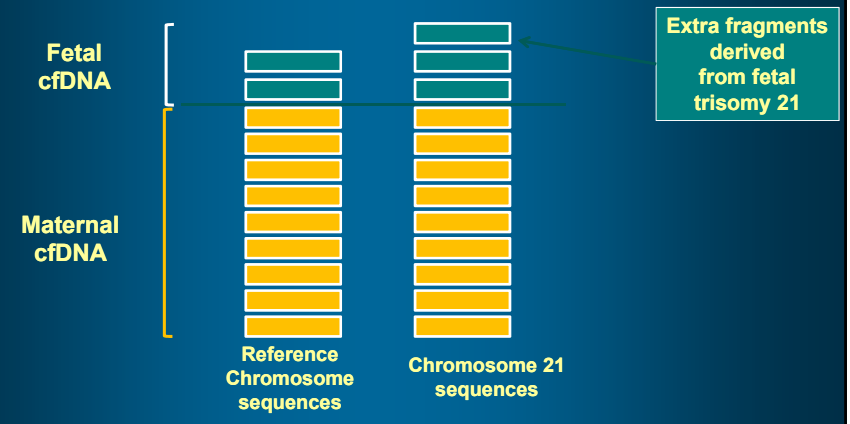


- Each bar represents thousands of cfDNA fragments
- Counting of chromosome cfDNA fragments done by DNA sequencing

Aneuploidy Detection (+21)

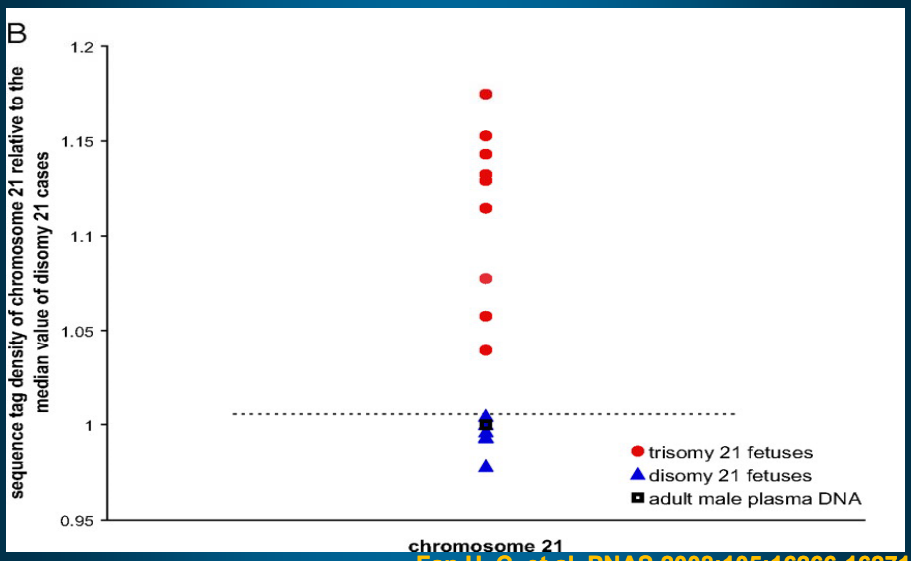
- Determine total chromosome 21 transcripts (maternal and fetal) in trisomic and non-trisomic pregnancy
- If 10% of cell free DNA in maternal blood is fetal, trisomic pregnancies should provide 5% greater chromosome 21 *fetal* transcripts than disomic pregnancies

Fetal Trisomy Detection With cfDNA



* The overabundance of chromosome 21 cfDNA fragments in trisomy 21, although small, can be measured with DNA sequencing

Massive Parallel Genomic Sequencing (MPGS) For Trisomy 21



Fan H. C. et.al. PNAS 2008;105:16266-16271

Sequenom Center for Molecular Medicine: Validation (2011)

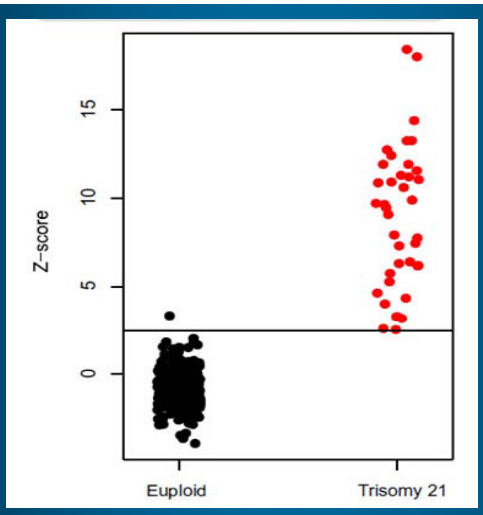
- Archived samples
- Blinded, nested case control study: match 1 trisomy 21 to 7 controls
- Illumina Hi Seq platform
- Z score > 3 = abnormal

Palomaki et al. Genetics in Medicine 13: 913, 2011

Trisomy 21 (Sequenom)

- 209/212 Trisomy 21 detected
  – (98.6%)
- False positive - 3/1471 (0.2%)
- Test failure - (0.8%)

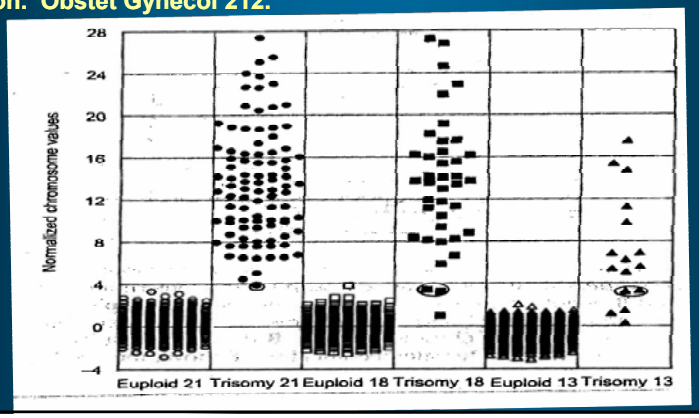
Cell Free Fetal DNA Trisomy 21 (Sequenom)



Ehrich et al., AJOG, 2011

MPGS FOR TRISOMIES (Verinata)

MPGS: (MELISSA:Verinata) Massively parallel sequencing normalized chromosome values compared with karyotype classifications for chromosomes 21, 18, and 13. Circles display classifications for chromosome 21, squares display classifications for chromosome 18, and triangles display classifications for chromosome 13. Unclassified samples with trisomy karyotypes have been circled. Bianchi. Genome-Wide Fetal Aneuploidy Detection. Obstet Gynecol 212.



Sex Chromosomal Abnormalities

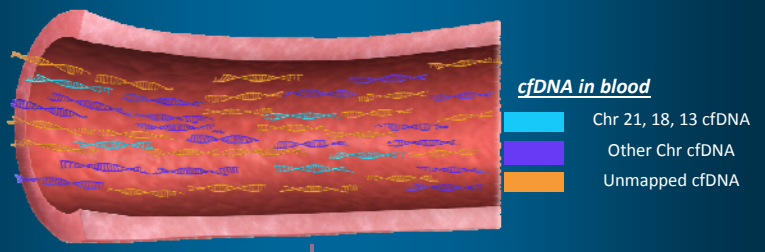
	Detection	
45, X	15/16	(4 no calls)
47, XXY	2/3	
47, XXX	3/4	
47, XYY	3/3	

Cell-Free Fetal DNA (BGI-Shenzhen)

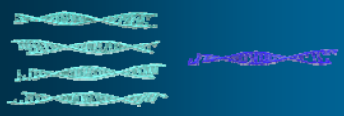
- N=11,105 (China)
- 42 centers high risk; 7 centers no prior risk assessment. No specific risk factors in 1387 (12.5%)
- 143/143 trisomy 21
- 47/47 trisomy 18
- False positives: 1 trisomy 21
  1 trisomy 18

Dan et al, 2012

Cell-Free DNA in Maternal Blood (Maternal + Fetal)

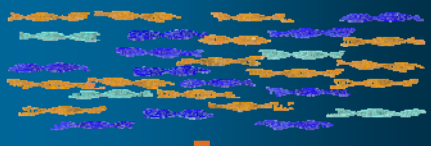


Directed



More efficient

MPSS (shotgun)



Random analysis of cfDNA

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- Massive Parallel Genomic Sequencing (MPGS) for all transcripts (maternal + fetal) [Sequenom; Verinata]
- Targeted: Chromosome-Specific DNA by hybridization of only selected chromosomes (e.g. 13,18,21)
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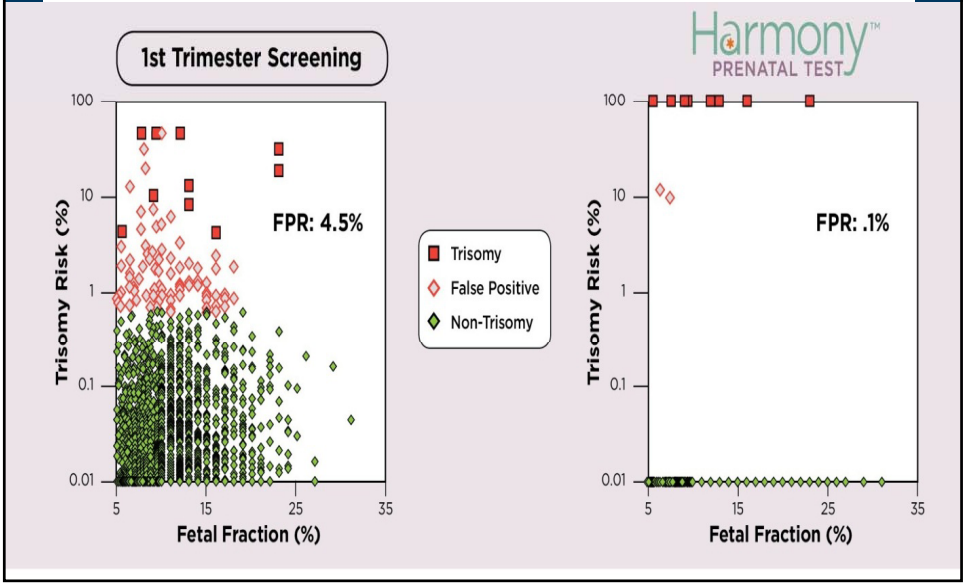
Ariosa Approach

- Targeted quantitative counting for chromosome specific transcripts
- Takes into account maternal age
- Provides risk based on >99% or <1% likelihood for trisomy
- Takes into account percent cffDNA

TARGETED Cell Free Fetal DNA Plus Likelihood Ratio



Targeted Fetal Cell DNA (Norton et al., 2012) (Ariosa)

- Maternal age 34.3y
  - Gestational age ~ 16 weeks
- 4.6% Non-informative:1.8% <4% fetal DNA; 2.8% assay failure.
- Detection Rate
  - 81/81 Trisomy 21
  - 37/38 Trisomy 18
- False Positive (0.1%):1/2228

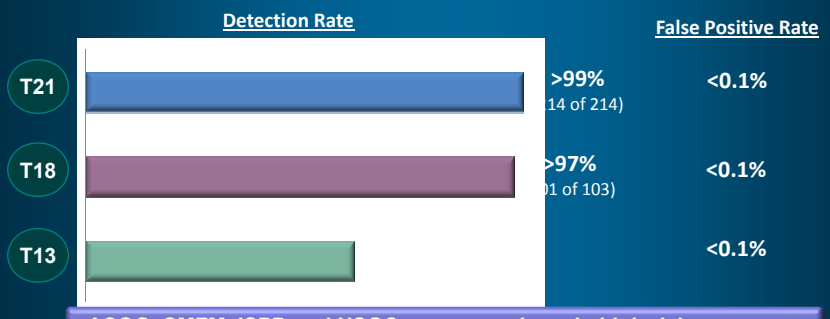
Targeted Fetal Cell DNA (Nicholaides et al. 2012) (Ariosa)

- Cohort study 2049 "routinely screened" first trimester cases (maternal age 23.4)
- 4.8% non-Informative (2.2%; <4% Fetal DNA; 2.6% assay failure including one Trisomy 18
- Detection: (100%)
  - 8/8 trisomy 21
  - 2/2 trisomy 18

False positive: (0.1%)
- 01/1939 trisomy 21
- 2/1939 trisomy 18

Clinical Performance (Ariosa)

Studied in over 6,000 patients, including >2,000 average-risk women



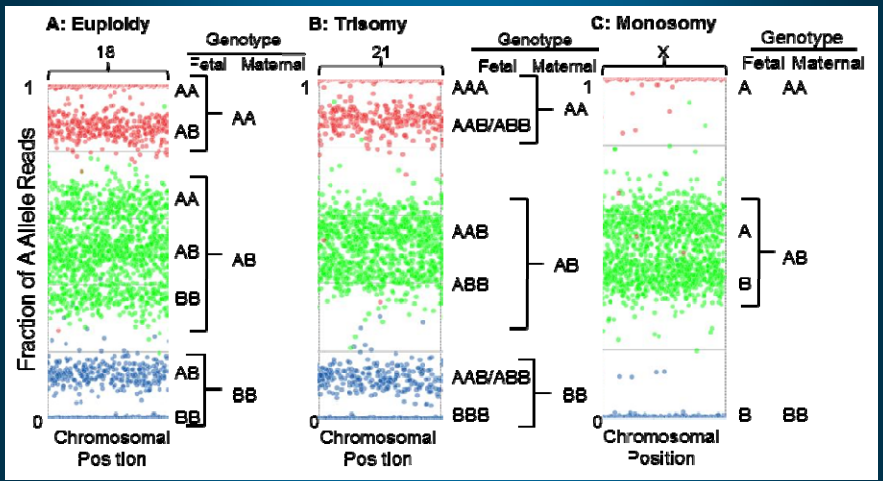
ACOG, SMFM, ISPD and NSGC recommend use in high-risk pregnancy

1. Sparks, A.B., Struble, C.A., Wang, E.T., Song, K., Oliphant, A., Non-invasive Prenatal Detection and Selective Analysis of Cell-free DNA Obtained from Maternal Blood: Evaluation for Trisomy 21 and Trisomy 18, Am J Obstet Gynecol. (2012), doi: 10.1016/j.ajog.2012.01.030.; 2. Ashoor, G., Syngelaki, A., Wagner, M., Birdir, C., Nicolaides, K.H., Chromosome-selective sequencing of maternal plasma cell-free DNA for first trimester detection of trisomy 21 and trisomy 18, *Am J Obstet Gynecol.* (2012), doi: 10.1016/j.ajog.2012.01.029.; 3. Sparks, A.B., Wang, E.T., Struble, C.A., Barrett, W., et al, Selective analysis of cell-free DNA in maternal blood for evaluation of fetal trisomy. Prenat Diagn (2012);32(1):3-9. doi: 10.1002/pd.2922. Epub 2012 Jan 6.; 4. Norton, M., Brar, H., Weiss, J., Karimi, A., et al. Non-Invasive Chromosomal Evaluation (NICE) Study: Results of a Multicenter, Prospective, Cohort Study for Detection of Fetal Trisomy 21 and Trisomy 18, *Am J Obstet Gynecol.* (2012), doi:10.1016/j.ajog.2012.05.021.; 5. Nicolaides KH, Syngelaki A, Ashoor G, et al. Noninvasive prenatal testing for fetal trisomies in a routinely screened first-trimester population. Am J Obstet Gynecol 2012;207:374.e1-6.; 6. Ashoor G, Syngelaki A, Nicolaides KH, et al. Trisomy 13 detection in the first trimester of pregnancy using a chromosome-selective cell-free DNA analysis method, *ULTRASOUND Obstet Gynecol.* (2012), DOI: 10.1002/uog.12299.

Targeted Fetal Cell-Free DNA (Natera Approach)

- Parental genotypes [Single nucleotide polymorphisms (SNPs] and used to determine potential trisomic, disomic, monosomic fetal genotypes
- Bioinformatics applied, to assess relative likelihood of fetal trisomy vs. fetal disomy

Natera Approach: Allele Fractions



Limitations using cell-free DNA approaches

- Lower fraction of cff DNA in obese patients
- Lower fraction cff DNA under 10 weeks
- Detecting single trisomic fetus in multiple gestation a concern, but recent work indicates high detection rates

Non-Informative Samples

- Initially 5% in reported series based on pre-set quality control standards.
- “No call results” may reflect poor DNA quality (sample degradation) or low fetal fraction. Will decrease with second sample.
- Obtaining new sample should result in higher cumulative rate of informative cases.

Detection Rates/False Negatives

- Detection rates in published reports >99% trisomy 21. ~ 98% for trisomy 18 and sex chromosomal abnormalities. Lower for trisomy 13.
- Detection rates higher than with maternal serum analyte/ultrasound screening (85-93+%).

False Positive Rates

- Much ≤ 1%
- Much lower than with maternal serum analyte/ ultrasound (5%)

Explanations

- "Vanishing" co-twin with placental tissue persisting
- Confirmed placental mosacism (CPM)
- Maternal low-grade trisomy 21 mosaicism in blood

ACOG Committee Opinion 545 (2012) Noninvasive Prenatal Testing for Fetal Aneuploidy

- "Tremendous potential as a screening tool"; "should be an informed patient choice"
- Should not be offered to low risk women "or in multiple gestations because it has not been sufficiently evaluated in these groups".
- Current indications include maternal age 35 years, fetal anomaly, prior trisomy, balanced Robertsonian translocation (13;21), positive serum analyte serum.

Obstet Gynecol 120:1532-1534, 2012

American College Medical Genetics Statement (ACMG)

- No statement on limiting to high risk women. Low risk women can be offered as is done for maternal serum analyte screening.
- Noted screening available for sex chromosomal abnormalities.

Genet. Med., 2013

Stated Limitations (ACMG)

- Cannot distinguish type of aneuploidy (e.g., translocation trisomy)
- Cannot identify *balanced* rearrangements or triploidy
- Does not screen for neural tube defects
- Does not obviate first trimester ultrasound, which is still useful for gestational age dating

NONINVASIVE PRENATAL GENETIC DIAGNOSIS: 2013

1. Multiple vendors offer cell free fetal DNA aneuploidy screening. Will not be labelled "test" but has low false positive rate. Detection rate is over 99% for trisomy 21, much higher than maternal serum analyte nuchal translucency screening (85 – 93%).
2. Likely to replace maternal serum analyte as primary aneuploidy screen.

NONINVASIVE PRENATAL GENETIC DIAGNOSIS: in 2013

3. Applicable from at least 10 weeks onward, with fraction fetal DNA minimally changing by gestational age.

4. Up to 5% non-informative cases, but with repeat samples lower per patient.

NONINVASIVE PRENATAL GENETIC DIAGNOSIS: STATUS in 2012

5. False positives much lower (<1%) than with maternal serum analytes but still require confirmation with invasive procedures before termination.

6. Intact fetal cell(s) — trophoblast — could provide information and diagnosis earlier in pregnancy (5 weeks).