UPDATE ON ANTENATAL TESTING

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CENTER FOR GENETICS AND MFM
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Disclosure

- I have nothing to disclose
- I have no affiliation with any company mentioned during this presentation
Objectives

- Describe the different techniques to evaluate fetal DNA
- Distinguish between the different screening and diagnostic options
- Identify appropriate candidates for non invasive prenatal testing (NIPT)
All pregnant women should be offered screening.

Invasive diagnostic testing for aneuploidy should be available to all women, regardless of maternal age.

Pretest counseling should include a discussion of the risks and benefits of invasive testing compared with screening tests.
Basic Molecular and Cytogenetics

- Karyotype
  - Chromosomal constitution of an individual
# Most common chromosomal abnormalities

<table>
<thead>
<tr>
<th>Chromosomal abnormality</th>
<th>Incidence Rates</th>
</tr>
</thead>
<tbody>
<tr>
<td>XXY, XXX, Turner Syndrome</td>
<td>1/500</td>
</tr>
<tr>
<td>Down Syndrome</td>
<td>1/700</td>
</tr>
<tr>
<td>Cystic Fibrosis</td>
<td>1/2,500</td>
</tr>
<tr>
<td>Trisomy 18</td>
<td>1/5,000</td>
</tr>
<tr>
<td>Fragile X</td>
<td>1/8,000</td>
</tr>
<tr>
<td>SMA</td>
<td>1/10,000</td>
</tr>
<tr>
<td>Trisomy 13</td>
<td>1/16,000</td>
</tr>
</tbody>
</table>
Age-related risk at live birth

<table>
<thead>
<tr>
<th>Maternal age at delivery</th>
<th>Risk of Down syndrome</th>
<th>Risk of any chromosomal abnormality</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>1/1,200</td>
<td>1/476</td>
</tr>
<tr>
<td>30</td>
<td>1/1,000</td>
<td>1/385</td>
</tr>
<tr>
<td>35</td>
<td>1/400</td>
<td>1/192</td>
</tr>
<tr>
<td>40</td>
<td>1/100</td>
<td>1/66</td>
</tr>
<tr>
<td>45</td>
<td>1/35</td>
<td>1/21</td>
</tr>
</tbody>
</table>
So, what is a screening test?

- Goal is to define risk for a disease in an asymptomatic population
- Ideal characteristics
  - Identify common and important fetal disorders
  - Cost-effective and easy to perform
  - High detection rate
  - Reliable and reproducible
  - Test for disorders that have a diagnostic test available
  - Allow time to act on results if desired
History of antenatal testing

- 1966- amniocentesis
- 1970’s- age alone
- 1980’s
  - AFP
  - Triple screen- 72% detection 5% False positive
  - Quad screen- 79% detection 5% False positive
  - Penta screen- 83% detection 5% False positive
- 1980’s- CVS
- 1990’s –
  - First Trimester screen- 80-85% 5% False positive
  - Sequential screen 90-94% detection 3-5% FP
- 2010’s- Non-invasive prenatal diagnosis(fetal DNA)
Current Methods of Aneuploidy Detection

- **Confirmation of Diagnosis**
  - Invasive detection methods
    - Chorionic villous sampling – first trimester
    - Amniocentesis – second trimester
    - Potential complications: procedure-related loss 1/200

New kid on the block….

- Non-invasive prenatal testing (NIPT)
Non-invasive prenatal diagnosis

- Intact fetal cells
  - 1 in a million of total cell population
  - Can persist for years
- Cell-free DNA
- Cell-free mRNA
Circulating Cell Free Fetal DNA

- 1997 article by Lo in Lancet
    - Based on previous findings of tumor DNA present in patients with cancer
    - 30 pregnant women with male fetuses
    - Serum and plasma analyzed
    - 70 – 80% detection rate

Circulating Cell Free (ccf) Fetal (ccff) DNA

- Source of ccff is thought to be from placental cells through breakdown of fetal cells in circulation.
- Circulating fetal DNA thought to comprise 3 – 6% of all DNA in circulating maternal plasma. Now it’s known to range from 3 – 40%, with an average of about 10%.
- Detection starting at as early as 5 weeks. Consistently seen at 10 weeks.
- Little risk of interference of ccff from previous pregnancies.
- Half-life of ccff is 15 minutes and is undetectable within 2 hours postpartum.

Ehrich et al. Noninvasive detection of fetal T21 by sequencing of DNA in maternal blood. AJOG 2011
Clinical uses of cffDNA

- **RhD Genotyping**
  - Fetal DNA collected from maternal serum
  - Used for women who with partners who are heterozygous for RhD allele
  - Assays used in RhD genotyping are reliable, reproducible, and routinely used in Europe

- **Sex-determination**
  - For inherited disorders
    - CAH, hemophilia, duchenne’s

Noninvasive Aneuploidy Detection

- Aneuploidy detection is much more challenging
  - Single nucleotide polymorphisms
  - DNA methylation
  - Fetal mRNA
Cell-free fetal DNA

- **2007 Digital PCR**

- **2008 detection of Trisomy 21**
Massively Parallel Shotgun Sequencing (MPSS)

- Start with DNA fragments in maternal plasma
- Sequence and align the fragments (figure out if fragment is fetal or maternal, and on which chromosome it belonged)
- Sequences that belong to potentially more than one chromosome are thrown out of the count
- Determine the % contribution of unique sequences mapped to each chromosome (unique count for chrN/total unique count)
- Compare the % chrN to a predetermined z-score to detect a potential aneuploid
- Z-score of aneuploid chromosomes is expected to be higher (z-score >3.0)

Principles of Fetal Trisomy 21 Testing From a Maternal Blood Sample Using DNA Sequencing

- ~10% of the DNA fragments in a pregnant woman’s blood are from the fetus (orange)
- ~90% are from the mother (blue)

Schematic of DNA Fragments Isolated From Maternal Plasma Containing Maternal DNA and Euploid Fetal DNA

Schematic of DNA Fragments Isolated From Maternal Plasma Containing Maternal DNA, Fetal DNA and Extra Fragments of Chromosome 21 Contributed by a Fetal Trisomy 21

Euploid Fetus

Fetus with Trisomy 21
Principles of Fetal Trisomy 21 Testing From a Maternal Blood Sample Using DNA Sequencing

Sequencing tells you which chromosome the ccf fragment comes from.
Principles of Fetal Trisomy 21 Testing From a Maternal Blood Sample Using DNA Sequencing

The total number of ccf-fetal fragments vs. ccf-maternal fragments of any one chromosome is proportional to the size of the chromosome, and is consistent from sample to sample, and patient to patient.

Sequencing tells you which chromosome the combined maternal and fetal fragments come from.

Chromosome 1

Chromosome 21
Principles of Fetal Trisomy 21 Detection Using DNA Sequencing

**DNA MPS** does not differentiate which fragments come from the mother and which from the fetus.

The quantitative over-representation of Trisomy 21 fragments in an affected pregnancy is significant and can be measured with high precision.

- Unaffected Fetus
- Fetus with Trisomy 21

* MPS - Massively Parallel Sequencing
Schematic illustration of the procedural framework for using massively parallel genomic sequencing for the noninvasive prenatal detection of fetal chromosomal aneuploidy.

Fetal or maternal? Which chromosome Does it belong to?

% Chromosome N

Z-score

Chiu R W K et al. PNAS 2008;105:20458-20463
Fetal aneuploidy is detectable by the overrepresentation of the affected chromosome in maternal blood.

Fan H C et al. PNAS 2008;105:16266-16271
DANSR vs Massively Parallel Shotgun Sequencing Assay Comparison

**DANSR (Directed analysis)**

- Same cfDNA fragments from select chromosomes analyzed every time
- Only relevant cfDNA fragments go onto DNA sequencing
- 96 patient samples analyzed simultaneously

**MPSS (Random analysis)**

- Random cfDNA fragments from all chromosomes analyzed every time
- All cfDNA fragments go onto DNA sequencing regardless of relevance
- 4-8 patient samples analyzed simultaneously

**cfDNA in blood**

- Chr 18 and 21 cfDNA
- Other Chr cfDNA
- Unmapped cfDNA
Relative Chromosome Dosage (RCD)

- Measures the total amount of a specific locus on a potentially aneuploid chromosome (ex: Chr21)
- Measures both maternal + fetal total
- Total dosage is compared to reference
Current NIPTs

- **Sequenom MaterniT21 plus** – launched 10/2011
- **Verinata Verifi** - launched 3/2012
- **Ariosa Harmony** – launched 6/2012
- **Natera**- no published clinical data
## Technology

<table>
<thead>
<tr>
<th>Sequenom</th>
<th>Verinata</th>
<th>Ariosa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Massively parallel shotgun sequencing (MPSS)</td>
<td>Massively parallel shotgun sequencing (MPSS)</td>
<td>Digital analysis of selected regions (DANSR)</td>
</tr>
</tbody>
</table>
Risk Calculation

- **Z-score (Sequenom)**
  - Total maternal and fetal/total reference
  - Mean chromosomal amount + 3 SD
  - >3 is positive, <3 is negative

- **Normalized chromosomal value (Verinata)**
  - Total maternal and fetal/custom reference
  - >4SD is positive, 2.5-4 is no-call, <2.5 is negative

- **FORTE (Fetal-fraction optimized risk of Trisomy Evaluation) (Ariosa)**
  - \( LR = \frac{\text{cfDNA of specific chr}}{\text{total cffDNA}} \)
  - Provides individualized risk score
  - Maternal age + GA x LR of result
  - Accounts for fetal fraction
## Study Data

<table>
<thead>
<tr>
<th></th>
<th>Sequenom</th>
<th>Verinata</th>
<th>Ariosa</th>
</tr>
</thead>
<tbody>
<tr>
<td>total n=</td>
<td>2,437</td>
<td>651</td>
<td>4097</td>
</tr>
<tr>
<td># of published</td>
<td>4</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>studies</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T21</td>
<td>249</td>
<td>102</td>
<td>205</td>
</tr>
<tr>
<td>T18</td>
<td>62</td>
<td>44</td>
<td>113</td>
</tr>
<tr>
<td>T13</td>
<td>12</td>
<td>15</td>
<td>-</td>
</tr>
<tr>
<td>XO</td>
<td>?</td>
<td>19 - 20</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Sequenom</td>
<td>Verinata</td>
<td>Ariosa</td>
</tr>
<tr>
<td>------------------</td>
<td>--------------</td>
<td>----------</td>
<td>---------</td>
</tr>
<tr>
<td><strong>T21 detection</strong></td>
<td>249/251 (99.2%)</td>
<td>102/102 (100%)</td>
<td>205/205 (100%)</td>
</tr>
<tr>
<td><strong>FPR</strong></td>
<td>4/1880 (0.2%)</td>
<td>0/438</td>
<td>3/3547 (0.08%)</td>
</tr>
<tr>
<td><strong>T18 detection</strong></td>
<td>59/59 (&gt;99.9%)</td>
<td>43/44 (97.7%)</td>
<td>111/113 (98.2%)</td>
</tr>
<tr>
<td><strong>FPR</strong></td>
<td>5/1688 (0.3%)</td>
<td>0/499</td>
<td>2/3547 (0.05%)</td>
</tr>
<tr>
<td><strong>T13 detection</strong></td>
<td>11/12 (91.7%)</td>
<td>11/15 (73.3%)</td>
<td>-</td>
</tr>
<tr>
<td><strong>FPR</strong></td>
<td>16/1688 (0.9%)</td>
<td>0/531</td>
<td>-</td>
</tr>
<tr>
<td><strong>XO detection</strong></td>
<td>NA</td>
<td>15/16 (93.8%)</td>
<td>NA</td>
</tr>
<tr>
<td><strong>FPR</strong></td>
<td>NA</td>
<td>0/464</td>
<td>NA</td>
</tr>
</tbody>
</table>
MaterniT21 plus (Sequenom)
How Reliable is it in detecting T21?

- Multicenter (27 international sites) trial funded by Sequenom
- Blinded nested case control of 1694 women (212 DS/1484 controls) undergoing invasive testing
- DS detection rate was 98.9% (209/212)
- Detection rate improved to 210/212 after accounting for GC content in DNA
- FP rate of 0.2% with testing failure in 0.8%

Palomacki et al. Genetics in Medicine; 13(11); 2011.
MaterniT21plus (Sequenom) How reliable is it for T18 or T13?

- Nested case control of 62 women with T18 and 12 with T13 matched with euploid controls

  - T18 (62 samples)
    - 3 with insufficient fetal DNA
    - 59 sampled had z-scores > 3.88 c/w T18 (DR 100%)
    - FP rate of 0.3% (5/1688 euploid samples)

  - T13 (12 samples)
    - 11/12 with z-scores > 7.17 (DR 91.7%)
    - 1/12 with normal z-score (FN 8%).
    - 16/1688 with abnormal z-score (FP rate 0.9%)

Palomacki et al. DNA sequencing of maternal plasma reliably identifies T13 and T18. Genetics in Medicine 2011.
MaterniT21 plus (Sequenom)

- Results: “Positive” vs. “Negative” based on z-score.
- 1% chance for no result
- Eventual/potential ability to give results on other chromosomes including X and Y
- Will accept samples on twins and IVF/egg donor
- Long term potential for genome wide studies
MaterniT21 (Sequenom)
Verifi (Verinata)
MELISSA study

- Prospective, blinded
- 532 samples
  - 89/89 T21 100%
  - 35/36 T18 97.2%
  - 11/14 T13 78.6%
  - 232/233 XX 99.6%
  - 184/184 XY 100%
  - 15/16 XO 93.8%
  - Also reported on complex variations 3/3 translocation 21, 1/1 mosaic T18, mosaic 2/7 monosomy X

**Verifi (Verinata)**

- Results: “Aneuploidy detected/Aneuploidy not detected”
- Least amount of published data
- “no call” results for patients between 2.5-4 SD-2.6%
- Failure rate – 2.6% (2% could be re-run)
- Eventual/potential ability to give results on other chromosomes including X and Y
- Will accept IVF/egg donor samples
- Monosomy X available when cystic hygroma is diagnosed
- Long term potential for genome wide studies
ANEUPLOIDY DETECTED
See Below

REPORT DATE AND TIME
02/06/2012 12:51 PM

Prenatal Aneuploidy Test Report

PROVIDER INFORMATION
Jane Doctor, MD
800 Saginaw Drive
Redwood City, CA 94063
Phone: 650-123-4567
Fax: 1-650-362-9313

PATIENT INFORMATION
Name: Jane Patient
DOB: 1/1/1976
Medical Record/Patient ID: MRN1234
Gestational Age at Draw in weeks: 10
 Ordering Physician: Jane Doctor, MD

CLIENT SAMPLE ID:

<table>
<thead>
<tr>
<th>CHROMOSOME TESTS</th>
<th>RESULTS</th>
<th>INTERPRETATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromosome 21</td>
<td>Aneuploidy detected</td>
<td>Results consistent with aneuploidy for chromosome 21</td>
</tr>
<tr>
<td>Chromosome 18</td>
<td>No aneuploidy detected</td>
<td>Results consistent with diploid Chromosome 18</td>
</tr>
<tr>
<td>Chromosome 13</td>
<td>Unclassifiable</td>
<td>Analysis could not be accurately assigned a ploidy status</td>
</tr>
</tbody>
</table>

SAMPLE ID
Order Id: 202000012
Sample Id: 20000105
Date of Collection: 02/02/2012
Draw Time: 9:25 AM
Receipt Date: 02/03/2012

TEST CLAIMS

<table>
<thead>
<tr>
<th>CHROMOSOME</th>
<th>SENSITIVITY (%)</th>
<th>95% Confidence Interval</th>
<th>SPECIFICITY (%)</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromosome 21</td>
<td>100.0</td>
<td>[99.9-100.0 (N=89)]</td>
<td>99.1-100.0 (N=404)</td>
<td></td>
</tr>
<tr>
<td>Chromosome 18</td>
<td>97.2</td>
<td>[95.5-99.9 (N=36)]</td>
<td>99.2-100.0 (N=460)</td>
<td></td>
</tr>
<tr>
<td>Chromosome 13</td>
<td>78.6</td>
<td>[76.6-80.9 (N=14)]</td>
<td>99.2-100.0 (N=688)</td>
<td></td>
</tr>
</tbody>
</table>

Prenatal aCGH is a genomic, whole-genome, molecular diagnostic test for the detection of aneuploidy and copy number variants (CNVs). The test is performed on maternal plasma DNA in the second trimester of pregnancy. The test identifies CNVs and aneuploidies in all chromosomes. 

For more information, please contact Verinata Client Services: Phone 1-866-946-MKIDS (6543), Fax 866-946-3021
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CAP 761931 CLIA: 0202413601 California Lic. #: CL0089417H http://www.verinata.com
Laboratory Director: John C. Spinks, MD, PhD, Laboratory Director
Bernard S. Chang, MD, Associate Laboratory Director, Kurt McWhorter, MD, Associate Laboratory Director

Verinata Part No. LB-0007, Rev. A
Harmony (Ariosa)

- Prospective, blinded
  - NICE trial
    - 81/81 T21 1/2888 FP 0.03%
    - 37/38 T18 2/2888 FP 0.07%
  - 4.5% failure rate
  - 39% of abnormal karyotypes were other than T21/T18

Harmony (Ariosa)

- Up to 4.6% chance for no result
- Can’t do on twins or IVF with egg donor at this time
- Technology may be limiting in the future
- Offering to the general population
- Results predict PPV and NPV allowing providers to counsel patients appropriately
- Largest published data set
- Inclusion of clinical information in risk assessment
- Targeted sequencing means decreased cost
- Future studies
  - T13 validation
  - NITE- 500 subjects. European eval of T21 and T18
  - NEXT- 25,000 subjects comparing Harmony to current 1\textsuperscript{st} trimester screening for T21
Harmony (Ariosa)

### Test Results

<table>
<thead>
<tr>
<th>CHROMOSOME</th>
<th>RESULT</th>
<th>RISK SCORE</th>
<th>RECOMMENDATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trisomy 21 (T21)</td>
<td>HIGH RISK</td>
<td>Greater than 99/100 (99%)</td>
<td>Genetic counseling and additional testing</td>
</tr>
<tr>
<td>Trisomy 18 (T18)</td>
<td>Low Risk</td>
<td>Less than 1/10,000 (0.01%)</td>
<td>Review results with patient</td>
</tr>
</tbody>
</table>

- **Risk Levels:**
  - Low Risk: 1/10,000 (0.01%), 1/1,000 (0.1%)
  - High Risk: 1/100 (1%), 10/100 (10%), 50/100 (50%), 90/100 (90%), 99/100 (99%)
Natera

- Technology based on Parental Support™
  - Targeted sequencing approach measuring SNP’s, then incorporates high fidelity parental allelic information and crossover frequency data to model a set of hypothesis (monosomy, disomy and trisomy). Gives maximum likelihood estimation.
- Abstract data
  - 166 samples
    - >99% detection for euploid, T21, T18, T13, X and Y
    - 10% failure rate requiring redraw then <1% failure
- PreNatus trial- enrolling 1,000 pts.
- No NIPT published
- Has used technology for Paternity and preimplantation genetics for some time
Still a screening test

- Positive results need to be taken in the context of disease prevalence
- The less prevalent a disease, the more likely a positive test is a false positive

**Test accuracy:**
- 100% detection
- 0.2% false positive

**T21 prevalence:**
- 1 in 1,000

Positive test result is truly right only 1/3 of the time at extreme test performance
# Probability of Trisomy 21

<table>
<thead>
<tr>
<th>Maternal age(years)</th>
<th>Pretest Prob at 12 weeks</th>
<th>Positive test result</th>
<th>Negative test result</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>1 in 1068</td>
<td>1 in 23</td>
<td>1 in infinity</td>
</tr>
<tr>
<td>25</td>
<td>1 in 946</td>
<td>1 in 21</td>
<td>1 in infinity</td>
</tr>
<tr>
<td>30</td>
<td>1 in 626</td>
<td>1 in 14</td>
<td>1 in infinity</td>
</tr>
<tr>
<td>32</td>
<td>1 in 461</td>
<td>1 in 11</td>
<td>1 in infinity</td>
</tr>
<tr>
<td>34</td>
<td>1 in 196</td>
<td>1 in 8</td>
<td>1 in infinity</td>
</tr>
<tr>
<td>36</td>
<td>1 in 196</td>
<td>1 in 5</td>
<td>1 in infinity</td>
</tr>
<tr>
<td>38</td>
<td>1 in 117</td>
<td>1 in 3</td>
<td>1 in infinity</td>
</tr>
<tr>
<td>40</td>
<td>1 in 68</td>
<td>1 in 2</td>
<td>1 in infinity</td>
</tr>
</tbody>
</table>

Impact of using MPSS as a secondary test among high risk patients

Currently:

2.8 million screening tests

T21: 4362/5156 → 85%
T18: 722/888 → 87%
Euploid: 148,079/2.8 mil → 5.3%

114,307 invasive tests

548 procedure related losses

Palomacki et al. DNA sequencing of maternal plasma reliably identifies T13 and T18. Genetics in Medicine 2011.
NIPT as a secondary test

MPSS will be offered to those with a screen POSITIVE test

- T21: 4340/4384 → 98.9%
- T18: 763/772
- 1,386 with failed testing
- 0.2% of 148,000 women with FP results = 296

6,792 women who undergo invasive testing

23 – 34 procedure related losses

Palomacki et al. DNA sequencing of maternal plasma reliably identifies T13 and T18. Genetics in Medicine 2011.
## Cost and TAT

<table>
<thead>
<tr>
<th></th>
<th>Sequenom</th>
<th>Verinata</th>
<th>Ariosa</th>
</tr>
</thead>
<tbody>
<tr>
<td>List price</td>
<td>$2400</td>
<td>$1,200</td>
<td>$795</td>
</tr>
<tr>
<td>Cash pay</td>
<td>$475 up front</td>
<td>$495 pt billed</td>
<td>$795 pt billed</td>
</tr>
<tr>
<td>PPO</td>
<td>$235 pt billed</td>
<td>$200 pt billed</td>
<td>$0 or $795 pt billed depending on insurance</td>
</tr>
<tr>
<td>OHP</td>
<td>$475 or sliding scale</td>
<td>$0</td>
<td>$0</td>
</tr>
<tr>
<td>DSHS</td>
<td>$0</td>
<td>$0</td>
<td>$0</td>
</tr>
</tbody>
</table>
Medical and Financial Costs with NIPT

100,000
Screen positive women

3,000
Down syndrome

2,958
NIPT +

42
NIPT -

97,000
Euploid

198
NIPT +

96,026
NIPT -

776
Failure

Palomaki et al. Genetics in Medicine 2011
## Medical and Financial Costs with NIPT

<table>
<thead>
<tr>
<th>DS+ / MPSS+ (TP)</th>
<th>DS+/MPSS positive (FP)</th>
<th>MPSS positive (TP)</th>
<th>MPSS negative (TN)</th>
<th>MPSS Failure</th>
<th>Cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diagnostic test costs with full uptake</td>
<td>2958</td>
<td>0</td>
<td>198</td>
<td>96,026</td>
<td>776</td>
</tr>
<tr>
<td>NIPT reduced diagnostic test costs</td>
<td>2958</td>
<td>0</td>
<td>198</td>
<td>0</td>
<td>776</td>
</tr>
<tr>
<td>Potential savings</td>
<td>0</td>
<td>42</td>
<td>0</td>
<td>96,026</td>
<td>0</td>
</tr>
<tr>
<td>Cases not detected</td>
<td>0</td>
<td>42</td>
<td>0</td>
<td>96,026</td>
<td>0</td>
</tr>
</tbody>
</table>
Women need detailed pretest counseling
Not fully diagnostic and is an advanced screening test
Confirmation of results through invasive testing is required
Cautioned that before routine population screening for fetal Down syndrome is introduced, additional trials are needed, particularly in low-risk populations.
Genetic counseling

- General discussion of NIPT (not lab specific)
- Specific chromosomes evaluated
- Detection rate, false positive and false negative rate
- Not a diagnostic test
- Failed specimen possibilities
- Advocate against the use of NIPT in twin pregnancies until more data is published
- Will facilitate testing if requested after informed consent
# Test Restrictions

<table>
<thead>
<tr>
<th></th>
<th>Sequenom</th>
<th>Verinata</th>
<th>Ariosa</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gestational age</strong></td>
<td>10+ wks</td>
<td>10+ wks</td>
<td>10+ wks</td>
</tr>
<tr>
<td><strong>Multiple gestation?</strong></td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td><strong>Assisted Reproduction?</strong></td>
<td>Yes</td>
<td>Yes</td>
<td>No egg donor pregnancies</td>
</tr>
<tr>
<td><strong>Intended for-</strong></td>
<td>AMA, abnormal serum screen, abnormal US, fam hx</td>
<td>AMA, abnormal serum screen, abnormal US, fam hx</td>
<td>General population – data not validated</td>
</tr>
</tbody>
</table>
Points to consider

- Case by case challenges
- Discomfort/legalities of not offering test
- How to implement for the general population
  - Await low-risk studies
  - Build infrastructure
  - Decrease cost
- Rapidly moving target
- Social and ethical concerns (ie universal disclosure of gender)
Future

- Monitor results of test introduced into practice
- Study performance in low-risk population
- Further testing in multiple gestation
- Further testing in mosaic conditions
- Detection of subchromosomal abnormality, single gene disorders
- Complete fetal genome sequencing
  - Implications for in utero therapy
  - Transcriptomic evaluation of gene activity
- Prediction of pregnancy-associated complications
Thank you for your attention

Questions?
Points to consider

- Only validated for high-risk population - ? Ariosa
- Lack of large scale clinical trials/data – data starting to come in but not much
- **DNA is placental in origin**
- **Maternal DNA not separated from fetal DNA**
- Mosaicism
- Multiples
- Egg donors
- Unable to distinguish trisomy vs translocation
- Diagnostic testing recommended for confirmation
- Increased detection rate and increased power to exclude
- Decrease in losses due to fewer procedures
- Positive result = very high risk for aneuploidy
- Cost - $795 - $1900
- Routine screening and diagnostic testing still indicated for other issues
Nuchal translucency ultrasound

- Correct gestation age
- Early diagnosis of fetal malformation
- Diagnosis of multifetal gestation and chorionicity
- Can see cystic hygroma: 50% aneuploidy
  - Most are T21 or Monosomy X, but can be others
- Screening for low risk populations
## FORTE vs. Z-score Comparison

<table>
<thead>
<tr>
<th>FORTE</th>
<th>Z-Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Provides individualized risk score</td>
<td>- Groups all trisomies into one category</td>
</tr>
<tr>
<td>- Accounts for fetal fraction</td>
<td>- Does not factor in fetal fraction</td>
</tr>
<tr>
<td>- Can incorporate other clinical risk factors</td>
<td></td>
</tr>
</tbody>
</table>

**FORTE** (Fetal-fraction Optimized Risk of Trisomy Evaluation) – refers to the algorithm that incorporates DANSR assay results (chromosome counts, fetal fraction), and other clinical information to provide a individualized risk score.
## Normalized chromosomal value vs. Z-score method

<table>
<thead>
<tr>
<th>NCV</th>
<th>Z-score</th>
</tr>
</thead>
<tbody>
<tr>
<td># counts Chr 21/Counts on Custom reference Chr</td>
<td># counts Chr 21/# counts all Chr</td>
</tr>
<tr>
<td>High precision, removes variation</td>
<td>GC correction</td>
</tr>
<tr>
<td>Maximizes dynamic range</td>
<td>Z-score result</td>
</tr>
<tr>
<td></td>
<td>Sample to sample variability</td>
</tr>
<tr>
<td></td>
<td>Reduces dynamic range</td>
</tr>
</tbody>
</table>