Non-Invasive Prenatal Testing (NIPT): Introduction and Technology Overview
Birth Defects: Rates and Causes

Adapted from Stevenson, RE and Hall, J. Human Malformations and Related Anomalies, 2nd ed. 2006
Prenatal Prevalence of Reported Chromosomal Abnormalities

Data adapted from Wellesley, D, et al., Rare chromosome abnormalities, prevalence and prenatal diagnosis rates from population-based congenital anomaly registers in Europe. *Eur J of Hum Gen* 11 January 2012.
Prenatal Screening and Diagnostic Testing

Prior to NIPT

1ST TRIMESTER

First day of LMP

13 wks

CVS

10-14 wks

12 wks

NT MEASUREMENT (LIMITED ANATOMY)

1ST trimester
Screen serum + U/S

2ND TRIMESTER

2nd trimester
Screen serum

13 wks

18 wks

amnio

16-22 wks

27 wks

3RD TRIMESTER

Term

40 wks

Term

12 wks

18 wks
### Conventional Prenatal Screening Options

**Detection Rates for Trisomy 21**

<table>
<thead>
<tr>
<th>Screening Option</th>
<th>Detection Rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st Trimester NT Ultrasound</td>
<td>64-70</td>
</tr>
<tr>
<td>1st Trimester Blood Screen</td>
<td>82-87</td>
</tr>
<tr>
<td>2nd Trimester Triple Screen</td>
<td>69</td>
</tr>
<tr>
<td>2nd Trimester Quadruple Screen</td>
<td>81</td>
</tr>
<tr>
<td>Integrated Screen</td>
<td>94-96</td>
</tr>
<tr>
<td>Serum Integrated</td>
<td>85-88</td>
</tr>
</tbody>
</table>

**False Positive Rate:** 5%

*ACOG Practice Bulletin No. 77, January 2007*

Actual detection rates and false positive rates will vary slightly based on the laboratory used.
Invasive Prenatal Testing

- Gold-standard diagnostic tests
  - Chorionic Villus Sampling (CVS) at 11-13 weeks
  - Amniocentesis at 15-20 weeks

- Present risk to patient and fetus
  - 0.4% risk of miscarriage with amniocentesis*
  - Risk of maternal bleeding, infection, leaking

Ultrasound Examination

- Useful because fetuses affected with aneuploidy often have anatomic changes or anomalies.

- A *genetic sonogram* uses ultrasound to assess the fetus for both structural anomalies and soft markers suggestive of aneuploidy.

- Invasive testing still is required to obtain a definitive diagnosis.

- First and Second trimester ultrasound
  - Can detect many other abnormalities that can be associated with other chromosomal/genetic syndromes
  - Can detect structural abnormalities not associated with genetic syndrome
What are the Goals of NIPT?

- Reduce exposure of risk to fetus
- Reduce false positives
- Testing that can easily be offered to pregnant women
- Enable a high detection rate
NIPT Technology Overview
Non-Invasive Prenatal Testing (NIPT)

A new category of prenatal testing

- Detect fetal aneuploidy using cell-free DNA from maternal blood
  - Analyzed by next-gen DNA sequencing

- Other used nomenclature:
  - NIPD: Noninvasive Prenatal Diagnosis
  - NIPS: Noninvasive Prenatal Screening
  - cfDNA: Cell-free DNA
  - cffDNA: Cell-free fetal DNA
  - DNA-based noninvasive prenatal screening
Cell-Free DNA (cfDNA)
A reliable analyte during pregnancy

- Released through apoptosis
  - Fetal cfDNA likely arises from cytotrophoblastic cells of placenta
- Released into bloodstream as small DNA fragments (150–200 bp)
- Maternal blood contains both fetal, maternal cfDNA
  - 2–20% of total cfDNA is fetal
- Fetal cfDNA reliably detected after 7+ weeks gestation
- Fetal cfDNA undetectable within hours postpartum

Massively Parallel Sequencing (MPS)

Fetal DNA fragments in maternal blood.

Cell free DNA fragments are then sequenced.

Compare the individual sequenced chromosomes against a reference for analysis.
Genome-Wide MPS
*Provides precise, across-the-genome coverage*

- Low assay failure rates
- Ability to add new content to test menu

**Benefits**
Targeted MPS

*Limited to few chromosomes, loci*

- High assay failure rates
- Limited ability to add new content without changing assay

Chromosome-Wide Coverage

NOT TO SCALE
Targeted MPS (SNP-Based Method)

Complex, failure-prone method

- High Assay Failure Rates
- Difficult to analyze egg-donation, surrogacy, consanguinity, maternal transplant, multiple gestation samples
Evidence for NIPT Performance with MPS

- Updated Meta-analysis: To review the clinical validation of cfDNA screening for fetal aneuploidies
- 37 publications on NIPT for detection of aneuploidies between 2011-2015

<table>
<thead>
<tr>
<th>Condition</th>
<th>DR (%)</th>
<th>95% CI</th>
<th>FPR (%)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trisomy 21</td>
<td>99.2</td>
<td>98.5-99.6</td>
<td>0.09</td>
<td>0.05-0.14</td>
</tr>
<tr>
<td>Trisomy 18</td>
<td>96.3</td>
<td>94.3-97.9</td>
<td>0.13</td>
<td>0.07-0.20</td>
</tr>
<tr>
<td>Trisomy 13</td>
<td>91.0</td>
<td>85.0-95.6</td>
<td>0.13</td>
<td>0.05-0.26</td>
</tr>
<tr>
<td>Monosomy X</td>
<td>90.3</td>
<td>85.7-94.2</td>
<td>0.23</td>
<td>0.14-0.34</td>
</tr>
<tr>
<td>Other sex aneuploidies</td>
<td>93.0</td>
<td>85.8-97.8</td>
<td>0.14</td>
<td>0.06-0.24</td>
</tr>
<tr>
<td>Twins T21</td>
<td>93.7</td>
<td>83.6-99.2</td>
<td>0.23</td>
<td>0.00-0.92</td>
</tr>
</tbody>
</table>

NIPT with Arrays?
An Unknown Limit of Detection

**Drawbacks**
- Array NIPT unproven
- High samples failure
  - Likely requires high FF call
- Late term
  - High FF means late term testing

Proof is in the Data

Published and presented samples

<table>
<thead>
<tr>
<th>Technology</th>
<th>Published and Presented Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>WGS</td>
<td>382,996</td>
</tr>
<tr>
<td>Targeted Sequencing</td>
<td>37,206</td>
</tr>
<tr>
<td>Array Technology</td>
<td>878</td>
</tr>
<tr>
<td>Targeted SNP Sequencing</td>
<td>32,916</td>
</tr>
</tbody>
</table>

1 A PubMed search for “cell-free, DNA, prenatal”, “noninvasive prenatal testing”, and “noninvasive prenatal screening” was performed on April 30, 2015. All validation and clinical studies using unique samples were included, where a current clinical NIPT provider performed sample analysis. Case studies and studies published in a language other than English were excluded. Data from a 2015 ESHG conference abstract was also included. A total of 45 published studies were surveyed. Data calculations on file. Illumina, Inc. 2015. NGS = next-generation sequencing; either whole-genome or targeted.
Illumina Technology Has Enabled NIPT

<table>
<thead>
<tr>
<th>Company</th>
<th>Approach</th>
<th>Sequencing Platform</th>
</tr>
</thead>
<tbody>
<tr>
<td>illumina®</td>
<td>Whole Genome</td>
<td>illumina®</td>
</tr>
<tr>
<td>SEQUENOM®</td>
<td>Whole Genome</td>
<td>illumina®</td>
</tr>
<tr>
<td>natera™</td>
<td>Targeted SNP Sequencing</td>
<td>illumina®</td>
</tr>
<tr>
<td>BerryGenomics</td>
<td>Whole Genome</td>
<td>illumina®</td>
</tr>
<tr>
<td>华大基因</td>
<td>Whole Genome</td>
<td>illumina®</td>
</tr>
</tbody>
</table>

*Illumina NGS platform clinically validated for NIPT on over 35,000 patients; over 1,000,000 clinical reports issued*
Clinical Implementation and Counseling Considerations
Professional Society Guidelines Endorsements
“Non-invasive prenatal testing based on massively parallel sequencing of circulating free fetal DNA (cfDNA) in maternal plasma has been shown to be highly effective for aneuploidy detection”

“[NIPT] would appear to be the most effective method for screening for fetal trisomy 21 and trisomy 18”

“The tests should not be considered to be fully diagnostic and therefore are not a replacement for amniocentesis and CVS”

“Laboratory providers should also be prepared to provide ongoing specifics on accuracy, test failure rates and turn-around time”

Also supporting NIPT for high risk pregnancies:
Some important points from position statement:

- cfDNA screening as a primary test offered to \textit{all} pregnant women
- cfDNA secondary to a high risk assessment based on serum and ultrasound screening
- cfDNA contingently offered to a \textit{broader group} of women ascertained as having high or intermediate risks by conventional screening
  - Contingent provision of cfDNA, could also include a protocol in which women with very high risks are offered invasive prenatal diagnosis while those with intermediate risk are offered cfDNA
Which Patients Should Be Offered NIPT?

Patients wanting early, accurate testing and are at high risk of aneuploidy due to:

- Maternal age-related risks
- Positive results on maternal-serum screening
- Abnormal ultrasound finding(s)
- History suggestive of increased risk for T21, T18,T13 or sex chromosome aneuploidy
- Parental translocation involving one of the tested chromosomes

Patients wanting early, accurate testing and are at average risk of aneuploidy
NIPT Failure Rates by Technology & Company


*Very limited data published using array technology, no clinical experience available
Why Do Test Failures Matter in NIPT?

- Actual sensitivity is less than claimed sensitivity
- Studies have shown a high rate of aneuploidy in test failures
- Redraw for NIPT is usually ineffective
  - High published redraw failure rates
  - Leads to increased turnaround time, office visits, patient/physician frustration

2. ACOG Committee Opinion Number 640, Sept 2015
Women whose results are not reported, indeterminate, or uninterpretable (a “no call” test result) from cell-free DNA screening should receive genetic counseling and be offered comprehensive ultrasound evaluation and diagnostic testing because of an increased risk of aneuploidy.
NIPT is now part of prenatal screening options.

Important to remember the benefits and limitations of the various prenatal tests
- NIPT does not test for all chromosome abnormalities, birth defects, genetic disorders or other pregnancy complications.
- No testing is 100%.

Labs have varying restrictions regarding gestational age, multiples, consanguinity and pregnancies conceived through the use of donor eggs/surrogacy.

Labs have varying test failure rates (some of which may include an increased risk of aneuploidy).

Possibility test results might not reflect the chromosomes of the fetus, but may reflect chromosomal changes to the placenta or of the mother.

Co-twin demise
Thank You
Appendix
Whole Genome Sequencing Has Benefits Over Targeted Sequencing & Arrays

**Benefits**
- Low assay failure rates (<1%)
- Ability to add new content to test menu

**Drawbacks**
- High assay failure rates (up to 12%)
- Limited ability to add new content without changing assay

**WGS provides precise counts, across the genome**

**Targeted sequencing is limited to few chromosomes, loci**
Fetal Fraction in NIPT

- Fetal Fraction = amount of fetal cfDNA in total cfDNA

- % fetal fraction (FF) affects ability of NIPT to detect fetal aneuploidy
  - Very low fetal fraction may lead to false negative results

- Several methods currently in use to estimate fetal fraction
  - Inaccurate at low fetal fraction (much variation in the measurement)

- Threshold for fetal fraction depends on coefficient of variation obtained for an individual chromosome
  - May be improved through algorithm improvements
Finding the Fetal Fraction

- Assay Quality
  - Lowers the limit of detection (LOD)
  - Based on sequencing methodology and analysis method

- Fetal Fraction
  - Lower fetal fraction demands a lower LOD
Why does anyone measure Fetal Fraction?

- NIPT assays with lower quality use Fetal Fraction to eliminate difficult samples
  - Eliminating samples with low fetal fraction increase sensitivity and specificity

- Some labs do not measure fetal fraction and do not eliminate samples from analysis

- Laboratory Clinical experiences
  - Assay failure rates
  - Negative Predictive Value (NPV)

- Lowest limit of detection
  - Combination of accurate sequencing and data analysis algorithms
Clinical Factors Affecting Fetal Fraction

- Significant correlation with aneuploidy
  - FF higher for trisomy 21
  - FF lower for trisomy 18, trisomy 13, monosomy X

- Correlation with gestational age
  - Slight increase from 10-21 weeks gestation
  - Significant increase after 21 weeks gestation

- Weak correlation with maternal BMI
  - Slight decrease in FF with maternal BMI
  - No specific threshold has been established where results cannot be obtained relative to maternal weight

- Not affected by maternal age, ethnicity, a priori trisomy risk

Fetal Fraction in NIPT

*Vast majority of samples have FF levels well above lower threshold*

![Graph showing relative frequency of fetal fraction](image)

Adapted from Rava et al, Circulating cell-free DNA fractions differ in autosomal aneuploidies and monosomy X, June 2013, submitted.

From Wang et al. Prenatal Diagnosis 2013 33, 1-5.
Comparison of NIPT Service Providers

**illuminava verifi® prenatal test leads in performance**

<table>
<thead>
<tr>
<th></th>
<th>Illumina verifi</th>
<th>MaterniT21 Sequenom</th>
<th>Harmony Ariosa</th>
<th>Panorama Natera</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Method</strong></td>
<td>Whole Genome</td>
<td>Whole Genome</td>
<td>Targeted/Array</td>
<td>Targeted</td>
</tr>
<tr>
<td><strong>Limit of Detection</strong></td>
<td>1.4–2.7¹</td>
<td>4%³</td>
<td>4%⁵ (unknown with microarray)</td>
<td>3.8–8.0⁸</td>
</tr>
<tr>
<td><strong>Specimen</strong></td>
<td>1 tube maternal blood</td>
<td>2 tubes maternal blood</td>
<td>2 tubes maternal blood</td>
<td>2 tubes maternal blood, paternal sample optional</td>
</tr>
<tr>
<td><strong>Failure Rate</strong></td>
<td>0.1%²</td>
<td>1.9%⁴</td>
<td>4.6–4.9%⁵,⁶ (unknown with microarray)</td>
<td>6.4–8.1%⁸,⁹</td>
</tr>
<tr>
<td><strong>Time to Report</strong></td>
<td>3–5 business days</td>
<td>5 business days</td>
<td>7–10 business days</td>
<td>9.2⁹ calendar days</td>
</tr>
<tr>
<td><strong>Egg Donors &amp; Twins</strong></td>
<td>Yes</td>
<td>Yes (13% failure rate⁷)</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td><strong>Microdeletions Offered</strong></td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>

## Comparison of NIPT Service Providers

**Illumina verifi Prenatal Test Leads in Performance**

<table>
<thead>
<tr>
<th></th>
<th><strong>Illumina verifi</strong></th>
<th><strong>NIFTY BGI</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Method</strong></td>
<td>Whole Genome</td>
<td>Whole Genome</td>
</tr>
<tr>
<td><strong>Failure Rate</strong></td>
<td>0.1%</td>
<td>~2% before redraw&lt;sup&gt;1,3&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Turn Around Time</strong></td>
<td>3–5 business days</td>
<td>10–15 business days&lt;sup&gt;2,3,4&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Sample (blood)</strong></td>
<td>1 tube maternal blood</td>
<td>2 tubes maternal</td>
</tr>
<tr>
<td><strong>Published Laboratory Clinical Experience</strong></td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Laboratory</strong></td>
<td>CLIA/CAP-certified laboratory</td>
<td>Non CLIA/CAP-certified laboratory</td>
</tr>
</tbody>
</table>

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3. [http://www.thisismy.co.uk/non-invasive-prenatal-testing-nipt/](http://www.thisismy.co.uk/non-invasive-prenatal-testing-nipt/)
NIPT Test Failure Rates

Failure rate in twins

1. With paternal sample
   - Natera\(^1,2\) 8.1%
   - Ariosa\(^3,4\) 4.6%
   - Sequentom\(^5,6\) 5.5%
   - verifi\(^7\) 1.9%
   - Requires retest of sample

2. Whole Genome Sequencing

3. Targeted Sequencing

Genetic Counseling NIPT Flipbook

The value of non-invasive prenatal testing (NIPT).

A supplement for a Genetic Counselor’s flipbook

- 10 pages with illustrations to help patients understand the NIPT testing process, conditions tested, result interpretation
- Used by healthcare providers prior to NIPT
- verifi prenatal test specific
- Available in 9 languages: English, Spanish, Portuguese, French, German, Italian, Korean, Japanese, Chinese
- Download at verifitest.com

Tools For Your Practice
Patient Education Video

- 12-minute video providing an overview of the benefits and limitations of various prenatal testing options
  - Prenatal screening (e.g. first trimester combined screen)
  - CVS/Amniocentesis
  - NIPT (verifi prenatal test specific)
    - Includes description of conditions tested

- Healthcare providers can direct their patients to watch this video in the clinic or at home prior to their OB appointment

- Available in 9 languages: English, Spanish, Portuguese, French, German, Italian, Korean, Japanese, Chinese

- Can be viewed at verifitest.com

Tools for Your Practice
- Downloadable to other Practices’ websites
NIPT Test Failures Not Only Due to Fetal Fraction Cutoffs

Comparison of test providers

![Graph showing test failure rates and LODs for different test providers]

Test Failure Rates Depend on Assay Reliability, Limit of Detection (LOD)

* Reasons include insufficient cfDNA, inability to measure fetal fraction, lab error, contamination, bad statistical fit, highly variable cfDNA counts, or other sequencing failure

<table>
<thead>
<tr>
<th>Screening Method</th>
<th>Detection Rate</th>
<th>Cases Detected</th>
<th>False Positive Rate</th>
<th>Failure Rate</th>
<th>Invasive Tests</th>
<th>Procedure Related Loss</th>
</tr>
</thead>
<tbody>
<tr>
<td>T21 (n=200)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maternal age</td>
<td>30%</td>
<td>60</td>
<td>5%</td>
<td></td>
<td>4,990</td>
<td>10</td>
</tr>
<tr>
<td>Integrated Screen</td>
<td>95%</td>
<td>190</td>
<td>5%</td>
<td></td>
<td>4,990</td>
<td>10</td>
</tr>
</tbody>
</table>

| Normal (n=99,800)      |                |                |                     |              |                |                        |
| Natera                 | >99.9%         | >199           | <0.1%               | 6.3%         | 6,287          | 12                     |
| Ariosa                 | >99.9%         | >199           | <0.1%               | 3%           | 3,094          | 6                      |
| Sequenom               | 98.6%          | 197            | <0.1%               | 1.9%         | 1,996          | 4                      |
| Verifi                 | >99.9%         | >199           | <0.1%               | 0.1%         | <100           | <1                     |

Theoretical population 100,000 Pregnancies, T21 Prevalence 1:500

Implications of Test Failure
NIPT as a Primary Screen

- Maternal age-related risks
- Abnormal ultrasound finding(s)
- Hx suggestive of increased risk for T21, T18, T13 or SCA
- Parental translocation involving one of the tested chromosomes

START HERE

NIPT

Aneuploidy detected or suspected?

YES

CVS/Amniocentesis (Invasive)

(Genetic counseling is recommended)

NO

Continue with pregnancy management according to your practice’s protocols

Continue with pregnancy management according to your practice’s protocols
NIPT as a Secondary Screen
(following a positive serum screen)

(START HERE)

Serum Screening
(per practice’s protocol)

Is the serum screen result positive?

YES → Counsel the patient about the verifi prenatal test and invasive test options

NO → Continue with pregnancy management according to your practice’s protocols

NIPT

Aneuploidy detected or suspected?

YES → CVS/Amniocentesis (Invasive)

(NO) (Genetic counseling is recommended)

NO → (START HERE)
<table>
<thead>
<tr>
<th>Test (Company)</th>
<th>Current Clinical NIPT Method</th>
<th>No. of Published NIPT Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bambni™ Assay (Berry Genomics)</td>
<td>Illumina NGS</td>
<td>2,351</td>
</tr>
<tr>
<td>MaterniT21 PLUS™ Test (Sequenom)</td>
<td>Illumina NGS</td>
<td>108,665</td>
</tr>
<tr>
<td>NIFTY™ Test (BGI)</td>
<td>Illumina NGS</td>
<td>160,667</td>
</tr>
<tr>
<td>Panorama™ Prenatal Screen (Natera)</td>
<td>Illumina NGS</td>
<td>32,916</td>
</tr>
<tr>
<td>PrenaTest (LifeCodexx AG/GATC Biotech AG)</td>
<td>Illumina NGS</td>
<td>504</td>
</tr>
<tr>
<td>verifi® Prenatal Test (Illumina)</td>
<td>Illumina NGS</td>
<td>113,367</td>
</tr>
<tr>
<td>Harmony™ Prenatal Test (Ariosa)</td>
<td>Illumina NGS</td>
<td>37,206</td>
</tr>
<tr>
<td>Harmony™ Prenatal Test (Ariosa)</td>
<td>Affymetrix Array</td>
<td>878</td>
</tr>
</tbody>
</table>

A survey of 45 published studies* revealed that 99.8% of reported NIPT samples run on Illumina NGS systems.

*A Pubmed search for “cell-free, DNA, prenatal”, “noninvasive prenatal testing”, and “noninvasive prenatal screening” was performed on April 30, 2015. All validation and clinical studies using unique samples were included, where sample analysis was performed by a current clinical NIPT provider. Case studies and studies published in a language other than English were excluded. Also included data from a 2015 ESHG conference abstract.*