NON-INVASIVE ANEUPLOIDY TESTING

Kathy Morris, MSSW, LCGC
UNM Dept OB/GYN
Objectives

• Participants will be able to
  
  • Identify the four current indications for non-invasive prenatal testing (NIPT)
  
  • Name two advantages of NIPT compared to standard screening (e.g. quad marker screen)
  
  • Name two limitations of NIPT compared to diagnostic testing (e.g. amniocentesis)
  
  • Know how to facilitate NIPT for their patients
• Maria is a 37 year old G3P3002 who presents for prenatal care at 11 weeks’ gestation. She is healthy, her prior obstetric history is unremarkable, and she has no family history of birth defects or genetic disorders. You plan to discuss aneuploidy testing options due to her age.
Maria

- Which tests is it appropriate to offer Maria?

  - A. Chorionic villus sampling
  - B. Sequential screen
  - C. Amniocentesis
  - D. Non-invasive prenatal aneuploidy testing
  - E. All of the above
You tell Maria that NIPT is better (higher detection rate, lower false positive rate for aneuploidy) than which of these tests?

A. Contingency screen
B. CVS
C. 20 week ultrasound
D. Amniocentesis
E. A and C
You tell Maria that NIPT is best at detecting:

- A. Down syndrome
- B. Trisomy 18
- C. XXY
- E. Trisomy 13
- F. Depends on which laboratory does the testing.
Maria

- Maria thinks she wants NIPT. When can you do it?

- A. After 10 menstrual weeks
- B. Between 10 and 20 weeks
- C. Between 15 and 21 weeks.
- D. Only after 16 weeks.
Maria

- Where would you send Maria to get her blood drawn?

- A. Your local Tricore laboratory
- B. SED labs
- C. Your local genetic counselor
- D. Sequenom
- E. You could draw the blood in your office.
- F. C and E
Evolution of Prenatal Diagnostic Testing For Chromosome Abnormalities

Amnio routine for karyotyping since 1970’s
Results took 4-5 weeks

PUBS used for rapid karyotyping

1980’s Chorionic villus sampling

Current: Improved turnaround for amnio and CVS
FISH: 1-3 days
Final: 7-10 days
Invasive procedures carry a small risk
# Invasive Diagnostic Options

<table>
<thead>
<tr>
<th>Trimester - Test</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1\textsuperscript{st} – CVS</td>
<td>99.25%\textsuperscript{1}</td>
<td>98.65%\textsuperscript{1}</td>
</tr>
<tr>
<td>2\textsuperscript{nd} - Amniocentesis</td>
<td>99.4%\textsuperscript{2}</td>
<td>99.5%\textsuperscript{2}</td>
</tr>
</tbody>
</table>


Complete karyotype, all types of chromosome abnormalities detectable
Evolution of **Prenatal Screening** for Chromosome Abnormalities

- Began as screening for NTDs, and realized that MSAFP could be used to screen for Down syndrome
- Most babies with Down syndrome are born to young parents
- Large scale screening of “low risk” population began in 1980’s
- MSAFP → multiple marker screen (triple, quad, penta)
- Expanded to include risk estimates for trisomy 18
- Some screening programs include detection of Smith-Lemli-Opitz syndrome
- Expanded to include first trimester US and serum analytes
- Can also incorporate 2^{nd} trimester US data for risk estimate
- Focus of screening has been improved detection rate for Down syndrome
Evolution of **Prenatal Screening** for Chromosome Abnormalities

- **Advantages of standard screening protocols:**
  - Non-invasive
  - Widely available
  - Detect many affected pregnancies that would not have been identified
  - Moderately expensive, depending on which test is done
  - Long track record, often covered by insurance
Evolution of **Prenatal Screening** for Chromosome Abnormalities

- **Disadvantages of standard screening protocols:**
  - 5% initial positive rate overall, higher in AMA patients
  - May lead to “unnecessary” invasive procedures
  - Poor positive predictive value
  - Anxiety engendered by positive results
  - Not cheap
  - “Negative” results may be misinterpreted as ruling condition out
Screening and Testing Options for Maria in 2011

1st Trimester:
- Screen serum + U/S

2nd Trimester:
- Screen serum
- CVS: 10-13 wks
- Amnio: 15-16 wks and beyond
- NT measurement: 12 wks
- Full anatomy: 18 wks

3rd Trimester:
- 27 wks
- 40 wks Term

First day of LMP: 40 wks Term
Evolution of **Prenatal Screening** for Chromosome Abnormalities

- **November, 2011:**
  - Non-invasive testing for Down syndrome becomes clinically available

- **2011-now**
  - Trisomy 13 and 18 tests added
  - Sex chromosome aneuploidy tests added
  - One lab offers testing for trisomy 16 and 22
  - Multiple gestation testing available
  - Microdeletion testing (e.g. DiGeorge) available
  - Multiple labs, very competitive
  - Different labs give different information
What are the Goals of NIPT?

- Reduce exposure of fetus to risk
- Reduce false positives
- Enable a high detection rate
- Testing that can easily be offered to all pregnant women*

*When data supports testing in all patients, instead of only high risk patients.
What is NIPT?

- Technology
  - Makes use of fetal DNA in maternal blood
Two Sources of Fetal DNA in Maternal Blood

- **Fetal cells**
  - 1 in a billion of total cell population
  - Requires fetal cell isolation via mechanical and/or biochemical means
  - Can stay in maternal circulation for years, into subsequent pregnancy

- **Cell-free DNA (cfDNA)**
  - 2–20% of total cfDNA is fetal
  - Requires DNA isolation and counting
What is cell free DNA?

• Released during apoptosis
  • Small (~150-200 base pairs) fragments
  • Fetal DNA enters into maternal circulation

• Maternal blood contains both maternal and fetal cell-free DNA
  • Most fetal DNA is placental in origin
Fetal Cell-Free DNA

- Reliably detectable as early as 7 weeks (though clinical testing is not available until 9 or 10 weeks)

- Half life of about 16 minutes
  - Virtually undetectable about 2 hours post-partum

What do you do with it?
Four US Laboratories using Three Technologies

- **Verinata Health (Verifi) and Sequenom (MaterniT21 Plus)**
  - Massively Parallel Sequencing across the genome
  - Trisomy 21, 13, 18, and sex aneuploidy optional
  - Twin gestation testing available
  - Microdeletion testing (22q and others from Sequenom)

- **Integrated/Ariosa (Harmony)**
  - Targeted Analysis
  - Trisomy 21, 13, 18, and sex aneuploidy optional
  - Twin gestation test available, but without sex chromosome aneuploidy

- **Natera (Panorama)**
  - Next Generation Aneuploidy Testing using SNPs
  - DS, Trisomy 13, 18, and Monosomy X, Triploidy
Massively Parallel Sequencing (MPS)

Verifi, Materni-T 21 Plus

cfDNA → DNA Sequencing → Alignment

CGATTAACT...

> 5,000,000 “counts” per blood sample
Detection of Fetal Aneuploidy

MPS Enables Precise Molecular Counting

Fetal cfDNA (20%) VS Maternal cfDNA

Chromosomes: 1 2 3 ... 21

10% more Chr21 cfDNA in T21

Trisomy 21
Dual Threshold Classification

Indicates Borderline Results

verifi® prenatal test
Dual Threshold

Sentbi

ANAEUPLAIDY
SUSPECTED

DETECTED

NOT DETECTED

VS.

Single Threshold Method

Materni-T 21 Plus

Trisomy

Diploid

POSITIVE

NEGATIVE

Verifi
DANSR™* (Harmony test) vs. MPSS

DANSR™ (Directed)

Directed analysis

More efficient

MPSS (Shotgun)

Random analysis of cfDNA

*DANSR™ = Digital Analysis of Selected Regions
FORTE™ Aneuploidy Algorithm

**Inputs**

- Chr 13, 18 and Chr 21 cfDNA counts
- Fetal fraction
- Clinical information (e.g. maternal and gestational age)

**FORTE Algorithm**

\[
P(x_j | T)P(T) \quad \frac{P(x_j | D)P(D)}{}
\]

**Outputs**

- Trisomy 21 risk value
- Trisomy 18 risk value
- Trisomy 13 risk value

*FORTE - Fetal-fraction Optimized Risk of Trisomy Evaluation*
NATUS Technology

Panorama Test

1. Mother’s blood sample is separated into layers via centrifuge. The three layers consist of the plasma, the buffy coat, and the red blood cells, top to bottom respectively.

2. The sample is amplified then analyzed using a sequencing machine. This measures genetic variations of single-nucleotide polymorphisms (SNPs) between the maternal and fetal genomes.

3. Data from the sequencing is then analyzed using a proprietary algorithm called NATUS. This distinguishes the fetal DNA signal from the maternal DNA signal.

4. Peace of Mind. A report is generated for chromosomes 13, 18, 21, X, and Y, and includes risk scores that incorporate chromosome-specific calculated accuracies.

A SNP (Single-Nucleotide Polymorphism) is a DNA sequence variation occurring when a single nucleotide — A, T, C, or G — in the genome is changed, which is part of the natural genetic variation within a population.
Take-home messages

• Different labs use different technologies

• Different tests provide different information

• Each has advantages and disadvantages

• Information you get, and how it is presented, is slightly different from lab to lab
How good is NIPT at detecting aneuploidy?
# Large Clinical Studies on cfDNA

<table>
<thead>
<tr>
<th></th>
<th>Palomaki et al.(^1)</th>
<th>Bianchi et al.(^2)</th>
<th>Norton et al.(^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Eligibility</strong></td>
<td>High Risk undergoing invasive prenatal procedure (9-22 wks)</td>
<td>High Risk undergoing invasive prenatal procedure (8-22 wks), including ART</td>
<td>Undergoing invasive prenatal procedure (10-22 wks)</td>
</tr>
<tr>
<td><strong>Total Cohort</strong></td>
<td>4,664</td>
<td>2,882</td>
<td>4,003</td>
</tr>
<tr>
<td><strong>Selected Cohort</strong></td>
<td>1,988</td>
<td>534</td>
<td>3,228</td>
</tr>
<tr>
<td><strong>T21 Analysis</strong></td>
<td>212 (non-mosaic)</td>
<td>90 + 3 mosaic</td>
<td>84</td>
</tr>
<tr>
<td><strong>T18 Analysis</strong></td>
<td>62 (non-mosaic)</td>
<td>38 + 1 mosaic</td>
<td>42</td>
</tr>
<tr>
<td><strong>T13 Analysis</strong></td>
<td>12 (non-mosaic)</td>
<td>16</td>
<td>N/A</td>
</tr>
<tr>
<td><strong>Other Abnormal</strong></td>
<td>none</td>
<td>73 abnormal karyotypes, analyzed across all chromosomes</td>
<td>81 abnormal karyotypes collected</td>
</tr>
<tr>
<td><strong>Controls</strong></td>
<td>Euploid</td>
<td>Euploid + all abnormal karyotypes</td>
<td>Euploid</td>
</tr>
</tbody>
</table>

## MELISSA Study – Verinata Health

<table>
<thead>
<tr>
<th>Classified</th>
<th>Sensitivity (%)</th>
<th>95% CI</th>
<th>Specificity (%)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromosome 21</td>
<td>100.0 (89/89)</td>
<td>95.9 - 100.0</td>
<td>100.0 (404/404)</td>
<td>99.1 - 100.0</td>
</tr>
<tr>
<td>(n=493)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chromosome 18</td>
<td>97.2 (35/36)</td>
<td>85.5 - 99.9</td>
<td>100 (460/460)</td>
<td>99.2 - 100.0</td>
</tr>
<tr>
<td>(n=496)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chromosome 13</td>
<td>78.6 (11/14)</td>
<td>49.2 - 95.3</td>
<td>100.0 (485/485)</td>
<td>99.2 - 100.0</td>
</tr>
<tr>
<td>(n=499)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*<1% unclassifiable per chromosome*

*2.8% per sample*

*0% False Positives*
Monosomy X Analysis

Analyzed MELISSA Samples with Cystic Hygroma

<table>
<thead>
<tr>
<th>Classified</th>
<th>Sensitivity (%)</th>
<th>95% CI</th>
<th>Specificity (%)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monosomy X (n=74)</td>
<td>95·0 (19/20)</td>
<td>75·1 - 99·9</td>
<td>100·0 (54/54)</td>
<td>93·4 - 100·0</td>
</tr>
</tbody>
</table>

0% False Positives

Publication in process

1 Verinata Health, Inc. Data on File.
### Panorama Test: Data from Natera Website

<table>
<thead>
<tr>
<th>Abnormality</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Trisomy 21</strong></td>
<td>83/83</td>
<td>1108/1108 &gt;99%, CI 99.7-100%</td>
</tr>
<tr>
<td></td>
<td>&gt; 99%</td>
<td></td>
</tr>
<tr>
<td><strong>Trisomy 18</strong></td>
<td>27/27</td>
<td>1164/1165 &gt;99%, CI 99.5-99.9%</td>
</tr>
<tr>
<td></td>
<td>&gt; 99%</td>
<td></td>
</tr>
<tr>
<td><strong>Trisomy 13</strong></td>
<td>13/13</td>
<td>1179/1180 &gt;99%, CI 99.7-100%</td>
</tr>
<tr>
<td></td>
<td>&gt; 99%</td>
<td></td>
</tr>
<tr>
<td><strong>Monosomy X</strong></td>
<td>11/12</td>
<td>1179/1180 &gt;99%, CI 99.5-99.9%</td>
</tr>
<tr>
<td></td>
<td>92%</td>
<td></td>
</tr>
<tr>
<td><strong>Triploidy</strong></td>
<td>8/8</td>
<td>272/272 &gt;99%, CI 98.7-100%</td>
</tr>
<tr>
<td></td>
<td>&gt; 99%</td>
<td></td>
</tr>
</tbody>
</table>
Limitations of NIPT...

- **MOSAICISM**- (Some cells euploid, some aneuploid)
  - Limited data
  - Very small numbers
  - Suggest test will detect at least higher levels of mosaicism

- **TWIN GESTATION**
  - Limited data
Twin Gestations

• One publication in peer review journal from Hong Kong, 2013, n=12

• Verinata website  115 twin pregnancies
  • 3/3 Down syndrome, one twin affected, correctly identified
  • 1/1 trisomy 18, both twins affected (monochorionic)
  • 91/91 at least one male correctly identified
  • No false positives

• “Fetal fraction” important, lower “per twin” than with singleton pregnancies  (Srinivasan, et al, abstract from SMFM, 2013)
Limitations of NIPT

- Limited number of chromosome abnormalities detected

Most tests will NOT identify aneuploidy of chromosomes apart from 21, 18, 13 and sex chromosomes

Materni-T 21 Plus, trisomy 16, 22

MaterniT 21 Plus: some chromosomal deletions

A few papers re: method of detection of deletions/duplications

Panorama test said to detect triploidy
Limitations of NIPT

• Will not (yet) identify most translocations, balanced or unbalanced, will not identify most deletions, duplications
NIPT Test Reports

• Vary by laboratory

• Some provide “yes or no” answer
  • Positive/negative
  • No aneuploidy detected/Results consistent with aneuploidy
  • Occasional unclassifiable result

• Others provide risk numbers
  • High risk of aneuploidy, > 99% risk
  • Low risk of aneuploidy, < 1 in 10,000 risk
  • Most risk numbers are at these extremes
**Prenatal Aneuploidy Test Report**

**REPORT DATE AND TIME:**
02/25/2013 12:31 PM

**PROVIDER INFORMATION**
Lisa Moore
UNM Maternal Fetal Medicine
2211 Lomas Blvd North East
Albuquerque, NM 87131

Phone (505) 273-2521
Fax (505) 272-1311

**PATIENT INFORMATION**

Name: 
DOB: 
Medical Record/Patient ID: 
Gestational Age at Draw in weeks: 21
Ordering Physician: Lisa Moore

**Client Sample ID:** 5128754/7296508

**PRENATAL ANEUPLOIDY TEST RESULTS**

<table>
<thead>
<tr>
<th>Chromosome</th>
<th>No aneuploidy detected</th>
</tr>
</thead>
<tbody>
<tr>
<td>21</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Sex Chromosomes</td>
<td>No aneuploidy detected</td>
</tr>
</tbody>
</table>

**INTERPRETATION**

Results consistent with diploid chromosomes 21, 18, 13 with two sex chromosomes (XX)

**SAMPLE ID:** 30021591

**Test Method:**
Non-invasive DNA analysis, DNA sequencing, and analysis of sequencing results to determine fetal aneuploidy.

**Limitations of Test:**
These results do not eliminate the possibility that a pregnancy may be affected with other chromosomal anomalies, such as other complications. The test is designed to detect any chromosomal aneuploidy, and has been validated for chromosomes 21, 18, 13, X, and Y. This was only performed for two sex chromosomes and genetic analysis for any additional aneuploidy. A negative result does not preclude the possibility of any other aneuploidy.

**Disclaimer:**
This information is not intended to guide patient care and is not the responsibility of the healthcare provider. It should not be used for the purpose of clinical care or genetic counseling. This information is subject to change and should be used in conjunction with the provider's written instructions.

**Verinata Health, Inc.** 1936 South Sixth Street, Suite 100. Redwood City, CA 94063 Phone 1-855-Biovance (236-8236) CA: 01363 CLIA: 9007800D California License 12-2015-001002 State of California, Department of Public Health

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Negative result
**ANAELOPLOIDY DETECTED**

- **Chromosome 21**: Aneuploidy detected. Results consistent with trisomy for chromosome 21
- **Chromosome 18**: Aneuploidy detected. Results consistent with trisomy for chromosome 18
- **Chromosome 13**: No aneuploidy detected. Results consistent with diploid chromosome 13
- **Sex Chromosomes**: No aneuploidy detected. Results consistent with two sex chromosomes (XX)

**Comments**: Genetic counseling is recommended. Clinical correlation with ultrasound findings and other screening tests is indicated. If definitive diagnosis is desired, chorionic villus sampling and/or amniocentesis is necessary.
# Prenatal Aneuploidy Test Report

**Verifi™ Prenatal Test**

**Report Date and Time:**
- Date: 09/04/2012
- Time: 11:18 AM

## PROVIDER INFORMATION

- **Name:** Timothy Hurley
- **Address:** 915 Camino De Salud NE MSC 10-5580
- **City:** Albuquerque, NM 87131
- **Phone:** (505) 272-8913
- **Fax:** (505) 272-1311

## PATIENT INFORMATION

- **Name:**
- **DOB:**
- **Medical Record/Patient ID:** 4961781
- **Gestational Age at Draw:** 23 weeks
- **Ordering Physician:** Timothy Hurley
- **Client Sample ID:** 4961781

## Prenatal Aneuploidy Test Results

<table>
<thead>
<tr>
<th>Chromosome</th>
<th>Test Results</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromosome 21</td>
<td>No aneuploidy detected</td>
<td>Results consistent with diploid Chromosome 21</td>
</tr>
<tr>
<td>Chromosome 18</td>
<td>Unclassifiable</td>
<td>Result falls in zone that is equivocal for determination of aneuploidy status</td>
</tr>
<tr>
<td>Chromosome 13</td>
<td>No aneuploidy detected</td>
<td>Results consistent with diploid Chromosome 13</td>
</tr>
</tbody>
</table>

**Comments:**

Genetic counselling is recommended. Clinical correlation with ultrasound findings and other screening tests is indicated. If definitive diagnosis is desired, chorionic villus sampling and/or amniocentesis is necessary.

## Sample Information

- **Order ID:** 20501079
- **Sample ID:** 2050513
- **Draw Date:** 08/23/2012
- **Draw Time:** 12:04 PM
- **Receipt Date:** 08/27/2012

## Test Claims

### Sensitivity

<table>
<thead>
<tr>
<th>Chromosome</th>
<th>Somen</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromosome 21*</td>
<td>100.0</td>
<td>92.5-100.0 (N=489)</td>
</tr>
<tr>
<td>Chromosome 18*</td>
<td>87.2</td>
<td>83.0-92.0 (N=489)</td>
</tr>
<tr>
<td>Chromosome 13*</td>
<td>78.8</td>
<td>68.4-90.0 (N=489)</td>
</tr>
</tbody>
</table>

### Specificity

<table>
<thead>
<tr>
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<th>Somen</th>
<th>95% Confidence Interval</th>
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<td>99.0</td>
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<td>99.0-99.0 (N=489)</td>
</tr>
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</table>

*Data from Verifi Health Inc.*

**Disclaimer:**

This material in which the information is used to guide patient care is the responsibility of the healthcare provider, including advising for the need for genetic counseling or additional diagnostic testing. Any diagnostic test should be interpreted in the context of all available clinical findings. The test was developed and its performance characteristics were determined by Verifi Health, Inc. It has not been cleared or approved by the U.S. Food and Drug Administration. Although laboratory-developed tests in our laboratory have not been subject to U.S. FDA regulation, certification of the laboratory is required under the Clinical Laboratory Improvement Amendments (CLIA) to ensure the quality and validity of the tests. Our laboratory is CLIA-accredited and certified under CLIA as qualified to perform high complexity clinical laboratory testing.

*Unclassifiable result*
Professional Guidelines

• National Society of Genetic Counselors Position Statement 2012
  
  • Supports NIPT as an option for patients whose pregnancies are considered to be at an increased risk for certain chromosome abnormalities.

  • Should be offered in the context of informed consent, education, and counseling by a qualified provider

  • Patients with abnormal results should receive genetic counseling and be given the option of standard confirmatory diagnostic testing

Professional Guidelines

- **ACOG NIPT Committee Opinion December 2012**

  - Recognizes NIPT as an advanced screening test in high risk populations

  - Women should receive detailed counseling that explains the benefits and limitations of the test prior to undergoing testing

  - **Should not** be offered to low risk population or women with multiple gestation pregnancies

  - While NIPT is highly sensitive and specific, it **does not replace** diagnostic testing such as CVS or amniocentesis
Professional Guidelines

• International Society for Prenatal Diagnosis, position Statement, November, 2012

• Offer NIP testing to “high risk” patients

• Detailed counseling to review limitations and benefits should be provided

• Should not be considered a diagnostic test

• Offer invasive testing to patients with a positive result

• Insufficient evidence to know how test will perform for multiple gestations

• Efficacy of sex chromosome aneuploidy testing is “unacceptably low.”
Who is high risk?

• Advanced maternal age: 35y or more at delivery

• Abnormal screening test results

• Ultrasound abnormalities

• History of a previous child with chromosomal aneuploidy
Cost of NIPT

- Harmony (Ariosa/Integrated Genetics) $795
- MaterniT-21-Plus (Sequenom) $2762
- Panorama (Natera) $2967 insurance, $799 self pay
- Verifi (Verinata) $1500 insurance
- Labs offer various financial arrangements, discounts
Counseling: Standard screening vs NIPT

• **Standard Screening**
  Pretty high detection rate for Down syndrome, trisomy 18, may or may not detect other aneuploidy
  Initial positive rate of 5% using stepwise sequential protocol
  Non-invasive
  Available 11-21 weeks
  Available for multiple gestations (with limitations)
  Years of experience with test and performance
  Moderately pricey

• **NIPT**
  High detection rate for trisomy 21, 13, 18, sex chromosome abn.
  Low false positive rate
  Non-invasive
  Available any time after 10 weeks
  Not available for multiple gestations
  New test. Limited data
  Expensive, insurance may or may not cover
  Not a diagnostic test
Counseling: NIPT vs diagnostic test

**NIPT:**
- Non-invasive, no risk to baby
- Identifies most cases of common trisomies
- Not as accurate as diagnostic test
- Not as complete as diagnostic test
- Expensive, insurance may or may not cover

**CVS/Amniocentesis**
- Invasive procedure with a small risk
- “For sure” results, though small risk of placental mosaicism with CVS
- Complete karyotype, can do additional genetic testing
- Expensive, insurance likely covers
Standard Screening vs NIPT

• Positive quad or sequential screen:
  • Baby probably DOES NOT have Down syndrome

• Positive NIPT result:
  • Baby probably DOES have Down syndrome
  • Not a diagnostic test. Would not endorse termination without confirmation
Nuchal Translucency Ultrasound

• Nuchal translucency ultrasound recommended for patients choosing NIPT or diagnostic testing

• Increased NT can be an indicator of structural malformation or single gene disorder
NIPT Performance: Prevalence 5%

Sensitivity – 99%
Specificity – 99%

<table>
<thead>
<tr>
<th>Trisomy 21</th>
<th>Disease (+)</th>
<th>Disease (-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test (+)</td>
<td>4,950</td>
<td>1,000</td>
</tr>
<tr>
<td>Test (-)</td>
<td>50</td>
<td>94,000</td>
</tr>
<tr>
<td></td>
<td>5,000</td>
<td>95,000</td>
</tr>
</tbody>
</table>

PPV: $\frac{4950}{5950} = 83.2\%$

NPV: $\frac{94,000}{95,000} = 99.47\%$
NIPT Performance: Prevalence 0.1%

Sensitivity – 99%
Specificity – 99%

<table>
<thead>
<tr>
<th>NIPT Result</th>
<th>Disease (+)</th>
<th>Disease (-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test (+)</td>
<td>99</td>
<td>1,000</td>
</tr>
<tr>
<td>Test (-)</td>
<td>1</td>
<td>98,900</td>
</tr>
</tbody>
</table>

100       99,900       100,000

PPV: 99/1,099 = 9%
NPV: 98,900/98,901 = 99.99%
NIPT in Low Risk Population

- Research in process about test performance in low-risk population

- 2013 paper suggests that universal screening by NIPT is not cost-effective*

- WHEN IT BECOMES AVAILABLE

- Informed consent will be even more critical than it is now
  
How to do NIPT?

• All kit-based

• Can get kits directly from reference lab, blood drawn in any lab

• Harmony available through Integrated Genetics

• Panorama available through Quest Diagnostics

• Strongly recommend patient consultation with specialist familiar with this testing (i.e. genetic counselor)
Practical Issues-Arranging NIPT at UNM

- Refer to a genetic counselor for discussion and ordering
  - Risk assessment and discussion of risk factors
  - Discussion of all testing options
  - Discussion of finances
  - GC will order test, contact patient with results, contact referring provider with results
NIPT-Benefits

• Highly sensitive and highly specific screening test

• Excellent option for high risk patients who want information but do not want the risk of an invasive procedure

• Can get results as early as 9-10 weeks

• Low false positive rate

• Yes/No answer easier to understand than risk ratio
NIPT-Caveats

- Not a diagnostic test
- Yes/No answer may be misleading
- New test, data about performance are limited (e.g. twins)
- Limited number of chromosome conditions detected
- Possibility of "unclassifiable" or complex result
- May add another step, and time, to testing process
Near Future Directions for NIPT

Potential for expanded use in prenatal testing

• Expanding patient eligibility:
  • Multiple gestations
  • General population testing

• Expanding test menu:
  • Other whole chromosome aneuploidy
  • Mosaic conditions
  • Triploidy
  • Microdeletions/duplications
  • Single gene disorders
  • Whole genome testing
Case examples

Putting this new technology into practice
Current Use of NIPT-Case 1

Maria is a 37 year old G3P3002 who presents for prenatal care at 11 weeks’ gestation. She is healthy, her prior obstetric history is unremarkable, and she has no family history of birth defects or genetic disorders. She would like to avoid an invasive test.

What test(s) do you offer her?
Current Use of NIPT- Case 1

Maria would like to have a non-invasive aneuploidy test.

How would you arrange for the testing to be done?

Results are consistent with diploidy for chromosomes 21, 13, and 18. Results are consistent with two sex chromosomes, XX.

What do you say to her about these results? What words would you use?
Current Use of NIPT-Case 1

Maria’s nuchal translucency measurement is within normal limits at 1.2 mm

Do you need to do any other screening or testing for birth defects or chromosome abnormalities? If yes, what?
Current use of NIPT: Case 2

• Andrea is a 20 year old with a positive quad screen. Down syndrome risk is 1 in 20. She is 17 weeks.

• What additional testing options do you offer?
Current Use of NIPT-Case 2

- Andrea wants to know if her baby has DS.
- She is unwilling to take the risk of an amnio
- She chooses NIPT.

- Results are consistent with trisomy of chromosome 21

- What do you tell her about these test results?
- What follow-up services do you offer?
- Will your pregnancy management change with these results?
Current Use of NIPT-Case 2

- Amniocentesis necessary for definite diagnosis
- Andrea declines amniocentesis
- She will continue her pregnancy
- She is counseled extensively about Down syndrome
- She is referred for a fetal echo by cardiology
- Prenatal care includes antenatal testing due to risk of stillbirth
Current Use of NIPT-Case 3

- Lydia is 32 years old. She has a first trimester screen showing a 1 in 10 risk of trisomy 18. She is 14 weeks pregnant by the time you talk to her about the results.

What follow-up services and testing do you offer her?
Current Use of NIPT-Case 3

• Lydia wants to know for sure if her baby has trisomy 18 because she would terminate the pregnancy.

• She schedules amniocentesis at 16 weeks

• “Could I have that other test just to get some information in the meantime?”

• What do you think about that?
• How do you respond?
Current Use of NIPT-Case 3

- NIPT is consistent with diploidy for chromosomes 21, 13 and 18.
- Lydia is very reassured, cancels amnio appt.
Current Use of NIPT-Case 3

• Lydia comes for a follow-up US at 17 weeks

• Multiple abnormalities on US:
  • Echogenic bowel, echogenic cardiac focus, possible heart defect, single umbilical artery, growth lagging 1-2 weeks.

• What additional testing do you offer? Why?
Current Use of NIPT-Case 3

- Lydia opts to have amniocentesis
- Karyotype shows 69, XXY, triploidy
- Lydia doesn’t understand why the NIPT did not detect this

- What do you tell her?
- What follow-up services/options do you offer her?
Current Use of NIPT- Case 4

Jane is a 42 year old G3P2 seen at 11 weeks’ gestation due to advanced maternal age

She is offered CVS, amnio, NIPT. She chooses NIPT

Results are consistent with trisomy for chromosome 18

What do you tell Jane?
Do you offer her additional testing? If so, what?
Jane opts to confirm NIPT results with CVS

Preliminary CVS results (FISH/PAT) show evidence of trisomy 18

What do you tell Jane?
Do you discuss options (i.e. continue vs terminate pregnancy) now, or wait til final results? Why?
Current Use of NIPT-Case 4

Jane schedules termination of pregnancy, but after the time final results are expected.

- Final CVS results show mosaicism
  - 47,XY, +18 (15 cells)
  - 46, XY (5 cells)

- Now what do you tell Jane?
- How do you advise her regarding termination?
- Would you offer her any more testing before termination?
- If so, what?
Current Use of NIPT-Case 4

• Jane cancels her appointment for termination

• She has amniocentesis at 16 weeks

• Results are non-mosaic 46, XY

• How do you counsel Jane?
• Do you recommend any follow-up testing?
• If yes, what and why?
Current Use of NIPT-Case 4

• Jane opts to continue the pregnancy

• Follow-up ultrasound exams are done to re-evaluate fetal anatomy and growth.

• As of 29 weeks, fetal growth is normal, no malformations are seen
Current Use of NIPT Case 4

- Cell-free fetal DNA is largely extra-embryonic in origin
- Chorionic villi are extra-embryonic
- Confined placental mosaicism is a well-known phenomenon, detectable by CVS
- NIPT may identify placental mosaicism that is not present in the fetus
Current Use of NIPT-Case 5

- Felicia is a 30 year old veterinarian who is about 15 weeks pregnant. She came for a first trimester screen, but was already 14 ½ weeks. She has read about NIPT and would like to have it done.

- What do you tell her?
- Do you arrange the testing for her? Why or why not?
Current Use of NIPT-Case 5

- NIPT has not been validated in a “low risk” population.

- Positive predictive value will be lower in a population with lower disease prevalence.

- ACOG guidelines do not endorse use in this population.
Eva is a 37 year old G3P2 with a di/di twin pregnancy. She is about 14 weeks pregnant when you meet her.

Eva intended to have a sequential screen. She had an ultrasound for nuchal translucency measurement at 12 weeks, both twins have NTs within normal limits. She failed to go in for the blood draw to complete the first part of the screen, and now it’s too late.

She has heard about NIPT and asks if she can do that.

What do you say to her?
Let’s review
Maria—what, again?

- Maria is a 37 year old G3P3002 who presents for prenatal care at 11 weeks’ gestation. She is healthy, her prior obstetric history is unremarkable, and she has no family history of birth defects or genetic disorders. She would like to avoid an invasive test.
Maria

Which tests is it appropriate to offer Maria?

A. Chorionic villus sampling
B. Sequential screen
C. Amniocentesis
D. Non-invasive prenatal aneuploidy testing
E. All of the above
You tell Maria that NIPT is superior (higher detection rate, lower false positive rate) to which of these tests?

A. Sequential screen
B. CVS
C. 20 week ultrasound
D. Amniocentesis
E. A and C
You tell Maria that NIPT is best at detecting:

- A. Down syndrome
- B. Trisomy 18
- C. XXY
- E. Trisomy 13
- F. Depends on which laboratory does the testing.
Maria

- Maria thinks she wants NIPT. When can you do it?

- A. After 10 menstrual weeks
- B. Between 10 and 20 weeks
- C. Between 15 and 21 weeks.
- D. Only after 16 weeks.
Maria

Where would you send Maria to get this testing done?

A. Your local Tricore laboratory
B. SED labs
C. Your local genetic counselor
D. Sequenom
E. You could draw the blood in your office.
F. C and E
• Thank you

• Anything else?