Prenatal Screening for Down Syndrome: Past, Present and Emerging Practices

Glenn E. Palomaki, PhD

March 20, 2014
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- Assistant Professor of Pathology and Laboratory Medicine, Women & Infants Hospital / Alpert Medical School at Brown University
- Member of the CAP Biochemical and Molecular Biology Resource Committee
- Authored/co-authored over 250 peer-reviewed articles
- Current research interests include: evaluation of screening and diagnostic tests, focusing on genetic/genomic applications; evidence synthesis in structured evidence-based reviews; and issues relation to screening test implementation
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Disclosures

• Employee of Women & Infants Hospital of Rhode Island, with honorarium and consultant fees paid to WIH

• **Grant / Research Support:**
  2008/12: Co-PI of InFANet Study - Sequenom
  2013/15: PI of DNAFirst Study - Natera

• **Salary / Consultant Fees (paid to WIH):**
  Beckman Coulter, Ansh Labs, Celula, PerkinElmer

• **Honorarium / Expenses:**
  Beckman Coulter, Ansh Labs, Natera

• **Stocks / Bonds:** None

• **IP / Royalty:** None
Defining ‘Medical screening’

• Screening is the systematic application of a test or inquiry, to identify individuals at sufficient risk of a specific disorder to benefit from further investigation or direct preventive action, among persons who have not sought medical attention on account of symptoms of that disorder.

Wald, NJ. J Med Screen 1994
**Down syndrome screening: Age**

- The first screening test was the question ‘How old are you?’
- If answered “35 or older”, the woman was offered invasive testing (amniocentesis or CVS) and diagnostic testing (karyotype).

‘Screen Positives’ located to the right of the red line at 35 years
Detection rate 50%
False positive rate 15%
Down syndrome screening: Quad

- Maternal age was inexpensive, reliable and available early in pregnancy, but
- Had low detection and high false positive rates
- Today, 2\textsuperscript{nd} trimester ‘quadruple’ testing is common (maternal age, AFP, uE3, hCG and Inh-A)

‘Screen Positives’ located to the left of the red line at risk of 1:270
  - Detection rate 80%
  - False positive rate 5%
Down syndrome screening: Integrated

- 1\textsuperscript{st} trimester ‘combined’ testing (maternal age, NT, PAPP-A and hCG) has similar performance to ‘quadruple testing, but
- ‘combined’ + ‘quadruple’ = an ‘integrated’ test

‘Screen Positives’ located to the left of the red line at risk of 1:100
Detection rate 90%
False positive rate 2%
# Prenatal screening in the US in 2012

<table>
<thead>
<tr>
<th>Type of test</th>
<th>Labs</th>
<th>Median N</th>
<th>Number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1(^{st}) trimester</td>
<td>34</td>
<td>3,000</td>
<td>565,692 (19)</td>
</tr>
<tr>
<td>2(^{nd}) trimester</td>
<td>122</td>
<td>2,538</td>
<td>1,770,024 (60)</td>
</tr>
<tr>
<td>Integrated</td>
<td>30</td>
<td>4,176</td>
<td>583,416 (21)</td>
</tr>
<tr>
<td>All</td>
<td>123</td>
<td>3,660</td>
<td>2,963,592 (100)</td>
</tr>
</tbody>
</table>

Palomaki GE et al., Archives Path Lab Med 2013

Represents about 70% of all US pregnancies
Current status of prenatal screening

- Combinations of maternal age and:
  - serum markers (AFP, uE3, hCG, PAPP-A, inhibin-A)
  - ultrasound markers (nuchal translucency or NT)

- But, these tests
  - are complex
  - still miss 10-20% of Down syndrome cases
  - require invasive diagnostic tests to 2-6% of women
  - most positive tests are false positives
  - can only identify
    - Down syndrome
    - and to some extent, trisomy 18 and trisomy 13

- Would like even better performance, and for a wider range of prenatal disorders
Circulating cell free (ccf) DNA

- Different than identifying whole fetal cells in circulation (Bianchi Prenat Diagn 2002)
- First reported in 1997 by Dr. Dennis Lo, Chinese University of Hong Kong (Lancet, 350:485)
  - Used Y chromosome probes in maternal plasma (and serum) to identify male fetuses
  - Both maternal and fetal (actually placental) DNA are found in maternal circulation
  - DNA already fragmented (150 to 200 bp)
  - Represents entire genome of the mother and fetus
  - Fetal/placental ccfDNA quickly cleared after birth
  - Ratio of fetal to total ccf DNA is 10% (range <4% to 40%)
Commercial companies offering clinical testing

Lifecodexx AG
Konstanz, Germany

PrenaTest

Beijing Genomics Institute
Shenzhen, China

NIFTY Test

Berry Genomics
Beijing, China

BambniTest

Sequenom Ctr for Mol Med
San Diego, California

Verinata Health
Redwood City, California

Ariosa Diagnostics
San Jose, California

Natera Inc.
San Carlos, California
# Commercial methodologies

<table>
<thead>
<tr>
<th>Company (Country)</th>
<th>Sequencing</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Berry Genomics (China)</td>
<td>Shotgun</td>
<td>Counting</td>
</tr>
<tr>
<td>BGI (China)</td>
<td>Shotgun</td>
<td>Counting</td>
</tr>
<tr>
<td>LifeCodexx (Germany)</td>
<td>Shotgun</td>
<td>Counting</td>
</tr>
<tr>
<td>Sequenom (US)</td>
<td>Shotgun</td>
<td>Counting</td>
</tr>
<tr>
<td>Verinata (US)</td>
<td>Shotgun</td>
<td>Counting</td>
</tr>
<tr>
<td>Ariosa (US)</td>
<td>Targeted</td>
<td>Counting</td>
</tr>
<tr>
<td>Natera (US)</td>
<td>Targeted</td>
<td>Pattern matching</td>
</tr>
</tbody>
</table>
Shotgun vs targeted sequencing

• Shotgun sequencing: randomly selected small fragments sequenced without regard to their location in the genome.
• Targeted sequencing: a preliminary enrichment step selects ‘regions of interest’ in the genome and then sequencing focuses on fragments from those regions.
Chromosome-specific contribution

![Bar graph showing chromosome sizes and contributions.]

- Chromosomes 1 and 2 contribute significantly, with chromosome 1 contributing 8% and chromosome 2 contributing 1.5%.
- The graph illustrates the size distribution of different chromosomes in megabases (MBases) and their percentage contributions in the genome.
Counting method (10% of free DNA is fetal)

Relative amount of chromosome 21

<table>
<thead>
<tr>
<th>Normal Mother</th>
<th>Normal Fetus</th>
</tr>
</thead>
<tbody>
<tr>
<td>18 copies</td>
<td>+</td>
</tr>
<tr>
<td>20 copies</td>
<td></td>
</tr>
</tbody>
</table>

Relative amount of chromosome 21

<table>
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<th>Normal Mother</th>
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<tbody>
<tr>
<td>18 copies</td>
<td>+</td>
</tr>
<tr>
<td>21 copies</td>
<td></td>
</tr>
</tbody>
</table>

Need to distinguish 21 copies from 20 copies, a 5% difference.
Counting method (10% of free DNA is fetal)

Relative amount of chromosome 21

Normal Mother   Normal Fetus
18 copies       +        2 copies  
20 copies

Relative amount of chromosome 21

Normal Mother   DS Fetus
18 copies       +        3 copies  
21 copies

Need to distinguish 21 copies from 20 copies, a 5% difference.
Counting: Detecting Down syndrome

Chiu R W K et al. PNAS 2008;105:20458
Counting: Down syndrome

Chiu R W K et al. PNAS 2008;105:20458
## Counting: Down syndrome

### To test an individual:

Determine the % of sequenced fragments that map to chromosome 21 for that person and compare that to the mean %, as a z score.

<table>
<thead>
<tr>
<th>% sequences mapped to chromosome in euploid</th>
<th>in test sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.51</td>
<td>1.59</td>
</tr>
<tr>
<td>1.49</td>
<td>1.49</td>
</tr>
<tr>
<td>1.48</td>
<td>1.48</td>
</tr>
<tr>
<td>1.50</td>
<td>1.50</td>
</tr>
<tr>
<td>1.53</td>
<td>1.53</td>
</tr>
<tr>
<td>1.49</td>
<td>1.49</td>
</tr>
<tr>
<td><strong>1.50 ± 0.02</strong></td>
<td><strong>1.50 ± 0.02</strong></td>
</tr>
</tbody>
</table>

### z score calculation

\[
z = \frac{(1.59 - 1.50)}{0.02} = +4.5
\]
Counting: Down syndrome

Chiu R W K et al. PNAS 2008;105:20458
Z-score vs fetal fraction: Euploid

Three of 1,471 with z-scores above 3 (false positives?)

Palomaki GE, GIM 2011
Z-score vs fetal fraction: Down syndrome

Four of 212 with z-scores below 3 (false negatives?)

Palomaki GE, GIM 2011
Study conclusions: Down syndrome

• Detection rate is 98.6% (209/212)
  
  “99 of 100 fetuses with Down syndrome will have a positive DNA test.”

• False positive rate is 0.2% (3/1471)

  “About 1 in 500 normal fetuses will have a positive DNA test.”

• Failure rate is 0.8% (13/1696).

  “In about 1 in 125 tests, no results will be reported (failed DNA test).”
The ‘genotyping’ interpretation

- 20,000 SNPs targeted on chromosomes 21, 18, 13, X and Y
- Genotype the mother using plasma buffycoat (optional genotyping of father)
- Observe the pattern of ‘informative’ SNPs
- If fetal trisomy, unexpected patterns are observed
- The relative position of patterns can be used to estimate fetal fraction
- Unique patterns can identify twins, triploidy
- Method can’t be applied to: twins, surrogate mothers, bone marrow transplants, egg donor
## External clinical validation studies (US)

<table>
<thead>
<tr>
<th>Study</th>
<th>FPR (%)</th>
<th>No-calls</th>
<th>DR (%)</th>
<th>No-call</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palomaki 2011</td>
<td>3/1,471 (0.2)</td>
<td>13/1,697 (0.8)</td>
<td>209/212 (98.6)</td>
<td>0</td>
</tr>
<tr>
<td>Ashoor 2012</td>
<td>0/300 (0)</td>
<td>1/400 (0.7)</td>
<td>50/50 (100)</td>
<td>0</td>
</tr>
<tr>
<td>Bianchi 2012</td>
<td>0/311 (0)</td>
<td>23/532 (4.3)</td>
<td>89/89 (100)</td>
<td>1</td>
</tr>
<tr>
<td>Norton 2012</td>
<td>1/2,887 (0.1)</td>
<td>148/3,228 (4.6)</td>
<td>81/81 (100)</td>
<td>3</td>
</tr>
<tr>
<td>Nicolaides 2013</td>
<td>0/204 (0)</td>
<td>13/242 (5.4)</td>
<td>25/25 (100)</td>
<td>2</td>
</tr>
<tr>
<td><strong>All</strong></td>
<td>4/4,173 (0.1)</td>
<td><strong>454/457 (99.3)</strong></td>
<td><strong>6</strong></td>
<td></td>
</tr>
</tbody>
</table>

Technology is advancing and ‘real world’ experience is being gained. The performance of current commercial testing may differ.
Down syndrome screening: ccf DNA

- ccf DNA testing of maternal plasma
- Tests involve next generation sequencing (NGS)

'Screen Positives' located to the left of the red line at risk of 1:100
Detection rate 99%
False positive rate 0.2%
## Detection of trisomy 18

<table>
<thead>
<tr>
<th>Study</th>
<th>FPR (%)</th>
<th>DR (%)</th>
<th>T18 No-call</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palomaki 2012</td>
<td>5/1,688 (0.3)</td>
<td>59/59 (100)</td>
<td>3</td>
</tr>
<tr>
<td>Ashoor 2012</td>
<td>0/300 (0.0)</td>
<td>48/50 (96)</td>
<td>0</td>
</tr>
<tr>
<td>Bianchi 2012</td>
<td>1/461 (0.2)</td>
<td>35/37 (95)</td>
<td>2</td>
</tr>
<tr>
<td>Norton 2012</td>
<td>2/2,888 (0.1)</td>
<td>37/38 (97)</td>
<td>4</td>
</tr>
<tr>
<td>Nicolaides 2013</td>
<td>0/192 (0.0)</td>
<td>3/3 (100)</td>
<td>0</td>
</tr>
<tr>
<td><strong>All</strong></td>
<td>8/5,509 (0.1)</td>
<td>182/187 (97.3)</td>
<td>9</td>
</tr>
</tbody>
</table>
## Detection of trisomy 13

<table>
<thead>
<tr>
<th>Study</th>
<th>FPR (%)</th>
<th>DR (%)</th>
<th>T18 No-call</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palomaki 2012</td>
<td>16/1,688 (0.9)</td>
<td>11/12 (92)</td>
<td>0</td>
</tr>
<tr>
<td>Bianchi 2012</td>
<td>3/488 (0.2)</td>
<td>11/14 (79)</td>
<td>2</td>
</tr>
<tr>
<td>Ashoor 2013</td>
<td>2/1,838 (0.1)</td>
<td>8/10 (80)</td>
<td>0</td>
</tr>
<tr>
<td>Nicolaides 2013</td>
<td>0/192 (0)</td>
<td>1/1 (100)</td>
<td>0</td>
</tr>
<tr>
<td><strong>All</strong></td>
<td>21/4,307 (0.5)</td>
<td>31/37 (83.8)</td>
<td>2</td>
</tr>
</tbody>
</table>
Practice guidelines

- Professional organizations agree on the following
  - Sequencing of cell free DNA is sensitive and specific for the common autosomal trisomies (21, 18, 13)
  - Testing should be offered to ‘high risk’ pregnancies
  - Patient and provider education is important
  - Insufficient data for testing of twin pregnancies
  - Positive results followed up by offer of invasive testing
  - Testing should not be offered to the general pregnancy population (‘low risk’) until more information is available

- Reimbursement is improving for ‘high risk’ patients

American Congress of Obstetricians and Gynecologists (ACOG)
American College of Medical Genetics and Genomics (ACMG)
International Society of Prenatal Diagnosis (ISPD)
National Society of Genetic Counselors (NSGC)
Society of Obstetricians and Gynecologists of Canada (SOGC)
ccfDNA testing in ‘high risk’ women

Down syndrome: DR = 98%, FPR = 0.2% , ‘No call’ = 1%

6,000
‘High risk’
(1:19)

300
DS

5,700
Euploid

284
pos

3
neg

3
fail

11
pos

5,632
neg

57
fail

295 (4.9%)
Offer Dx testing
26:1 (284:11)

60 (1.0%)
Offer Dx testing
1:19 (3:57)

5,635 (93.9%)
Routine care
1:1,900 (3:5,632)
False positive / false negative results

- Some FP/FN results are due to technology limitations (low fetal fraction, setting cut-offs)
- Others FP/FN results are analytically correct, but clinically incorrect
  - False positive results can be caused by
    - Confined placental mosaicism (placental abnormal)
    - Vanishing twin
    - Maternal sex aneuploidy or mosaicism
    - Maternal cancer
  - False negative results can be caused by
    - Confined placental mosaicismism (placenta normal)
Commercial LDTs: further disorders

- **Sex chromosome aneuploidies**
  - Provided by most commercial LDTs
  - 45X, 47XXY, 47XXX, 47,XYY and fetal sex
  - Weakest performance, (>90% detection)

- **Twin pregnancies**
  - Two commercial LDTs will test known twin pregnancies
  - Limited data; performance appears to be good (100% detection)

- **Deletion/duplication syndromes**
  - Available for two commercial LDT’s
  - Validation data not yet published (2/2014)

- **Screening in the general pregnancy population**
  - Lower prevalence / predictive value
  - Offered by primary care providers
  - Issue of ‘no-calls’, costs, reimbursement
Selected Resources

Upcoming Free Webinars
register at www.cap.org/webinars

• IHC Assays – New Evidence-Based Guideline for Analytic Validation
  - April 1 at 11 am Central presented by Jeffery Goldsmith, MD, FCAP
## CAP Learning – Testing Maternal Plasma DNA for Down Syndrome

<table>
<thead>
<tr>
<th>Course</th>
<th>Learning Objectives</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>FP-C 2013: Method Comparisons in Maternal Serum Screening for Down Syndrome</strong>&lt;br&gt;CE= 1.0</td>
<td>As a result of participating in this activity, you will be able to:&lt;br&gt;• Describe why performing a method comparison when a new kit lot is being implemented can help ensure quality prenatal screening.&lt;br&gt;• Describe how to interpret the results of a matched scatterplot, the first step in a Bland-Altman method comparison.&lt;br&gt;• Assess Bland-Altman plots to identify proportional and non-proportional changes.&lt;br&gt;• Apply best practices relevant to the CAP Checklist question CHM.31900 for assessing and recomputed medians when new reagent lots are introduced.</td>
</tr>
<tr>
<td><strong>Rapid Methods for Targeted Prenatal Diagnosis of Common Chromosome Aneuploidies</strong>&lt;br&gt;CE/CME/SAM= 0.0</td>
<td>Improvements in non-invasive screening methods for trisomy 21 (Down syndrome) and other aneuploidies during the first and second trimester of pregnancy have radically changed the indications for prenatal diagnosis over the last decade. Consequently, there was a need for rapid tests for the detection of common chromosome aneuploidies resulting in the development of molecular methods for the rapid, targeted detection of (an)euploidies of the chromosomes 13, 18, 21 and the sex chromosomes. The analysis of large series of prenatal samples has shown that such tests can detect the great majority of chromosome abnormalities in prenatal diagnosis. This resulted in lively discussions on whether conventional karyotyping should remain the standard method for the majority of prenatal cases or can be replaced by rapid tests only. This review gives an overview of different aspects of the three most common tests for rapid, targeted prenatal detection of (an)euploidies, i.e. interphase fluorescence in-situ hybridisation (iFISH), quantitative fluorescent polymerase chain reaction (QF-PCR) and multiplex ligation-dependent probe amplification (MLPA).</td>
</tr>
</tbody>
</table>
# CAP Learning – Testing Maternal Plasma DNA for Down Syndrome

<table>
<thead>
<tr>
<th>Course</th>
<th>Learning Objectives</th>
</tr>
</thead>
<tbody>
<tr>
<td>NACB Guidelines: Maternal-Fetal Risk Assessment and Reference Values in Pregnancy CE/CME/SAM – 0.0</td>
<td>When this version was first posted in 2003, the committee believed it was important to include and address the expanded role of the clinical laboratory in the assessment of fetal and maternal risk. This includes guidelines for laboratory testing and risk assessment that truly encompass the total time period from the confirmation of the new pregnancy through a healthy delivery for both mother and child, and the early detection of hidden health problems through newborn screening.</td>
</tr>
</tbody>
</table>
The CAP Learning Portal includes content and tools designed to support the learning needs of pathologists. A user must login to cap.org in order to access the portal. In the portal, you will find:

- Learning Options search/catalog
- Competency Model for Pathologists
- Personal Progress Check
- My Learning Plan
- Help Center (Guides, Video, FAQs)

Benefits
- Increase effectiveness to plan and manage learning
- Increase efficiency to target learning needs and identify premium learning solutions
- Increase satisfaction with learning solutions that meet specific learner needs
- Increase capability to maintain professional certifications
To learn more...

- For more details and to register for/access educational offerings:
  1. Log in to the cap.org website
  2. Click on the “Learning Portal” tab.
  3. Click on the “Browse Our Learning Catalog” tab
  4. Type your desired topic in the “Search” box or make a selection from the list provided.
    A list of available learning options displays

![CAP Learning Options](image_url)
Short Presentations on Emerging Concepts (SPECS)

- Pathology SPECs are:
  - short PowerPoint presentations, created for pathologists, focused on selected diseases where molecular tests play a key role in patient management.
  - valuable resource for your discussions with Tumor Boards or other physician colleagues.

- Now Available:
  - Emerging Concepts in the Diagnosis of Respiratory Viruses (NEW)
  - Emerging Concepts in Molecular Testing in Breast Cancer (NEW)
  - Emerging Concepts in the Workup of Colorectal Cancer
  - Emerging Concepts in Therapeutic Guidance for Metastatic Melanoma
  - Emerging Concepts in the Diagnosis and Workup of Thyroid Cancer
  - Emerging Concepts in Colorectal Cancer Hereditary Non-Polyposis Cancer (Lynch Syndrome)
  - Emerging Concepts in the Workup of Polycythemia and Thrombocytosis: JAK2

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**Molecular Pathology (single gene, small panel)**  
**Genomic Analysis (large panels, exome, genome)**  
**Digital Pathology**  
**In Vivo Microscopy**

Register or download the order from through the [CAP member tab](#). Once registered, you will be notified when a new issue is released.

Questions? Contact [capguides@cap.org](mailto:capguides@cap.org).
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- HPV vaccination of adolescents
- Pathologists creating value
- Genomic analysis

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- Digital pathology
- Residency
- Informatics and genomics
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“Prenatal Screening for Down syndrome: Past, Present and Emerging Practices”
by Glenn E. Palomaki, PhD

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Jill Kaufman, PhD,
Director of Personalized Health Care at jkaufma@cap.org

NOTE: There is no CME/CE credit available for today’s free webinar.