
NATIONAL REFERENCE LABORATORY

UNIVERSITY OF UTAH
SCHOOL OF MEDICINE

Department of Pathology

# Non-Invasive Prenatal Testing (NIPT) using Cell-Free Fetal DNA for Prenatal Assessment

Edward Ashwood
President and CEO, ARUP Laboratories
Tenured Professor of Pathology, University of Utah
18 December 2014

# Disclosures

1. Ed Ashwood receives a professor's salary from University of Utah.
2. His administrative duties include being President and CEO of ARUP Laboratories.
3. His clinical duties include directing Maternal Serum Screening for ARUP.
4. ARUP Laboratories is a non-profit enterprise of the University of Utah having no private ownership.

# Objectives

1. *Describe* how circulating cell-free DNA in maternal plasma is a mixture of maternal and placental DNA, and how ccfDNA can be tested to determine the risk of fetal aneuploidy.
2. *Discuss* the effect of fetal fraction of ccfDNA on the 'no-call' rate.
3. Following an abnormal traditional serum screen, *recommend* the optimal follow-up test.
4. *Discuss* the impact of cell-free DNA testing on the evolution of maternal serum testing, both confirmatory testing and general population screening.

# Topics

1. History of prenatal screening

2. Circulating cell-free DNA
   - Discovery
   - Use for predicting fetal Down syndrome risk
3. Using ccfDNA in high risk women

4. Anticipated general population screening

# Acknowledgement

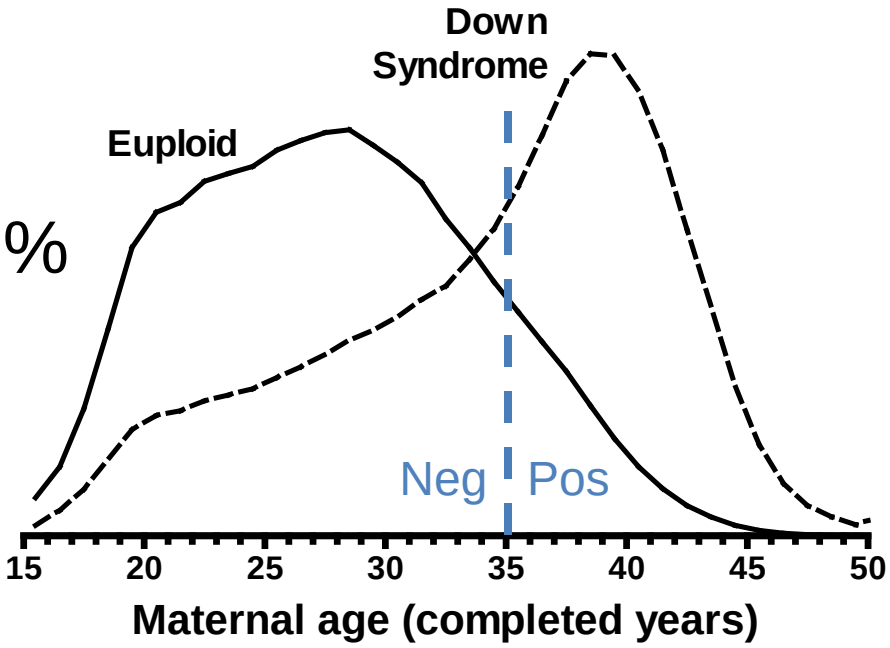

- Dr. Glenn Palomaki, Associate Director, *Institute for Preventive Medicine and Medical Screening (IPMMS)*
  - graciously contributed many slides for this webinar
  - has challenged my thinking on this subject with many exciting discussions over the years


# History of Down syndrome screening

# Down syndrome screening

- Before 1984, the ‘screening test’ for DS was the question, “How old are you?”
- If the woman was 35 or older, she was offered amniocentesis to obtain fluid for fetal karyotype.

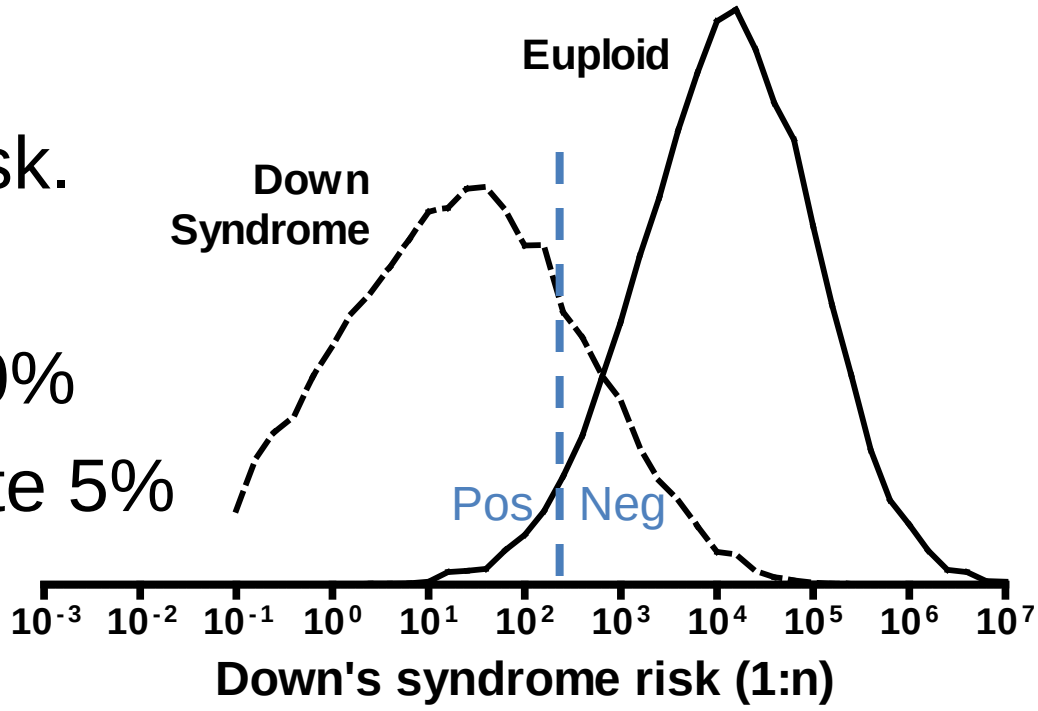
- Detection rate 50%
- False positive rate 15%



# Down syndrome screening

- Discovery that DS cases had low second trimester (2T) AFP in 1984 lead to laboratory screening -- more 2T markers were added.
- Currently, the most common test in the US is the Quad.
- Result is a DS risk.

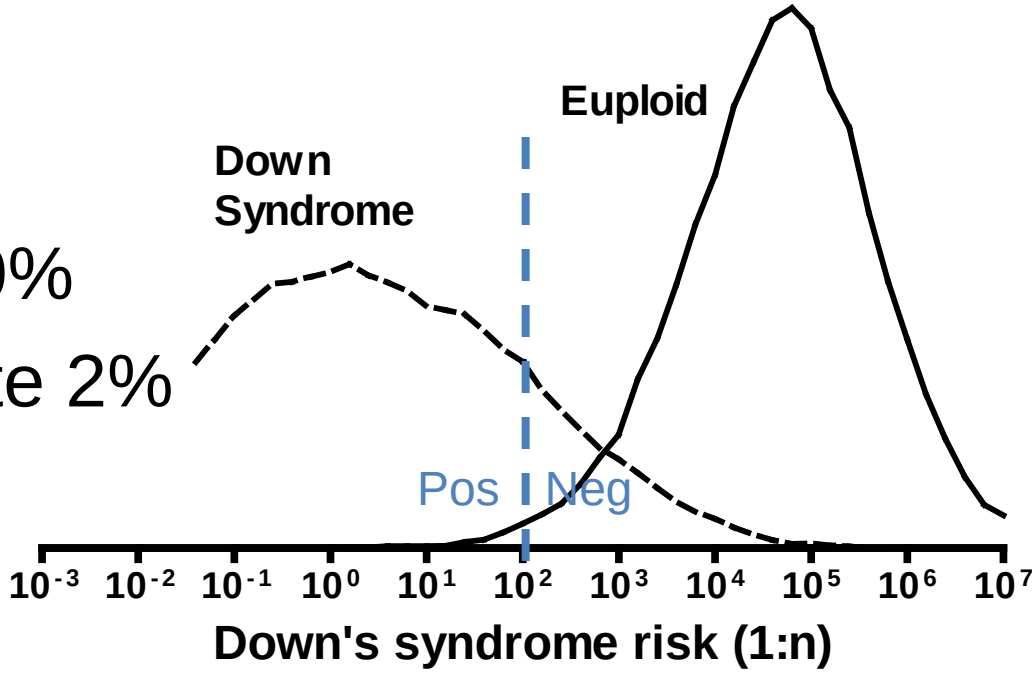
- Detection rate 80%
- False positive rate 5%



# Down syndrome screening

- First trimester ‘combined’ testing has similar performance to Quad testing.
- Both 1T and 2T can be combined to produce an ‘integrated screen.’

- Detection rate 90%
- False positive rate 2%



# Screening for Down Syndrome in the United States

## Results of Surveys in 2011 and 2012

*Glenn E. Palomaki, PhD; George J. Knight, PhD; Edward R. Ashwood, MD; Robert G. Best, PhD; James E. Haddow, MD*

● *Context.*—Participants in a College of American Pathologists external proficiency testing program for first and second trimester Down syndrome screening.

*Objectives.*—To determine the number of women screened for Down syndrome in the United States, along with the type of test received and to compare those results to earlier surveys in 1988 and 1992.

*Design.*—Questionnaires regarding the type and number of Down syndrome tests performed per month were completed by participants in early 2011 and again in early 2012.

*Results.*—After accounting for some of the missing responses, data from up to 131 laboratories indicated that 67% (2 764 020 of 4 130 000) to 72% (2012: 2 963 592 of 4 130 000) of US pregnancies received prenatal screening for Down syndrome. Second trimester tests were most common (2012: 60%; 1 770 024 of 2 963 592), followed by integrated (2012: 21%; 627 876 of 2 963 592), and first trimester (2012: 19%; 565 692 of 2 963 592). The 6 largest laboratories tested 61% of screened pregnancies and offered the widest array of tests, while the smallest 32 tested 1% and almost always offered only second trimester tests.

*Conclusions.*—The current population estimate of 72% pregnancies screened annually is higher than estimates from 1988 (25%) and 1992 (50%). Available testing choices are also more varied, and all testing methods perform better than those methods available 10 years ago. Clinicians should ensure that women are offered tests that follow recommended best-practice testing protocols, and screening laboratories should assess whether patient needs are being met.

(*Arch Pathol Lab Med.* 2013;137:921–926; doi: 10.5858/arpa.2012-0319-CP)

# Screening for Down syndrome in US

| Type of Test | Number of Labs | Number of screens (%) |
|---|---|---|
| First Trimester | 34 | 566,000 (19%) |
| Second Trimester | 122 | 1,770,000 (60%) |
| Both | 30 | 583,000 (21%) |
| All | 123 | 2,964, 000 (100%) |

Thus, about 70% of all US pregnancies are screened.

From Palomaki, Knight, Ashwood, Best, Haddow. Arch Pathol Lab Med 2013

# Current screens are not optimal

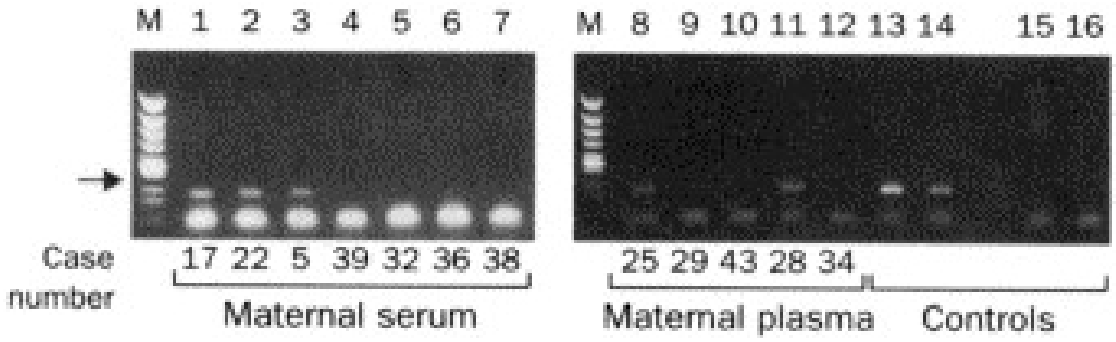
# Circulating cell-free (ccf) DNA

THE LANCET

**Early report**

## Presence of fetal DNA in maternal plasma and serum

*Y M Dennis Lo, Noemi Corbetta, Paul F Chamberlain, Vik Rai, Ian L Sargent, Christopher W G Redman, James S Wainscoat*




Dr. Y M Dennis Lo, Chinese University of Hong Kong

The Lancet, 350:9076, 1997, 485-7

# Circulating cell-free (ccf) DNA

- Both maternal and fetal (mostly placental) DNA are found in maternal circulation
- DNA in small fragments (150 to 200 bp)
- ccfDNA represents the entire genome of the mother and fetus
- Fetal ccfDNA is undetectable 1 day after birth
- Ratio of fetal to total ccfDNA is 10% (ranging from <4% to 40%)

# Early ccfDNA Methods

- SRY gene on the Y chromosome
- SERPINEB2 mRNA for C18
- placenta-specific 4 (PLAC4) for C21
- Many others

# Next Generation Sequencing

## Noninvasive diagnosis of fetal aneuploidy by shotgun sequencing DNA from maternal blood

H. Christina Fan*, Yair J. Blumenfeld†, Usha Chitkara†, Louanne Hudgins‡, and Stephen R. Quake*§

*Department of Bioengineering, Stanford University and Howard Hughes Medical Institute, 318 Campus Drive, Clark Center, Room E300, Stanford, CA 94305; †Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, Stanford University, 300 Pasteur Drive, Room HH333, Stanford, CA 94305; and ‡Division of Medical Genetics, Department of Pediatrics, Stanford University, 300 Pasteur Drive, Stanford, CA 94305

PNAS, October, 2008

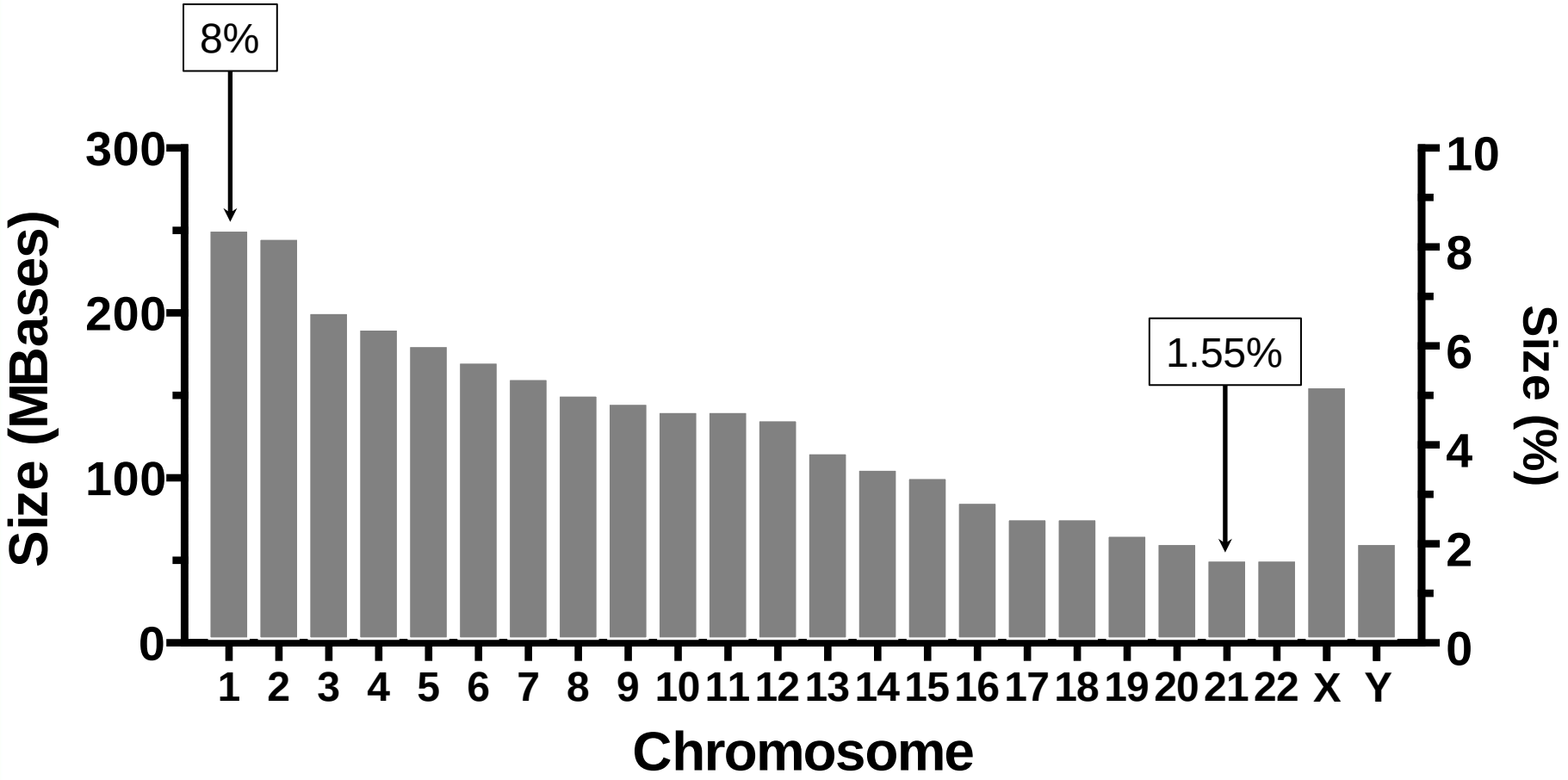
## Noninvasive prenatal diagnosis of fetal chromosomal aneuploidy by massively parallel genomic sequencing of DNA in maternal plasma

Rossa W. K. Chiu[a,b], K. C. Allen Chan[a,b], Yuan Gao[c,d], Virginia Y. M. Lau[a,b], Wenli Zheng[a,b], Tak Y. Leung[e], Chris H. F. Foo[f], Bin Xie[c], Nancy B. Y. Tsui[a,b], Fiona M. F. Lun[a,b], Benny C. Y. Zee[f], Tze K. Lau[e], Charles R. Cantor[g,1], and Y. M. Dennis Lo[a,b,1]

[a]Centre for Research into Circulating Fetal Nucleic Acids, Li Ka Shing Institute of Health Sciences, Departments of [b]Chemical Pathology and [e]Obstetrics and Gynaecology, and [f]Centre for Clinical Trials, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong SAR, China; [c]Center for the Study of Biological Complexity and [d]Department of Computer Science, Virginia Commonwealth University, Richmond, VA 23284; and [g]Sequenom, Inc., San Diego, CA 92121

PNAS, December, 2008

# Base Pair (BP) proportion of genome by chromosome



# ccfDNA NGS Counting Method

- Sequence ccfDNA fragments randomly
- Choose any fragment
- Match first 36 bp to chromosome
- If unique match exists, increment counter
- If not, disregard this sequence
- Repeat millions of times

# ccfDNA Counting Method

CTTACCGTAATTCGGTCTAAAGTTCCAATAGGGGAG
Matches chromosome 12
Increment count

TACCGTATATTCGGTCTAGCAGTTCCAATAGGTGAC
Matches chromosomes 1 and 6
Discard

CCAGTATATTCGGTCTAGCAGTTCCAATAGGTGACT
Matches chromosome 3
Increment count

ACCGTAATTCGGTCTAAAGTTCCAATAGGGGAGCCT
Matches chromosome 12
Increment count

| | Counts | | Counts | | Counts |
|---|---|---|---|---|---|
| 1 | | 9 | | 17 | |
| 2 | | 10 | | 18 | |
| 3 | 1 | 11 | | 19 | |
| 4 | | 12 | 2 | 20 | |
| 5 | | 13 | | 21 | |
| 6 | | 14 | | 22 | |
| 7 | | 15 | | X | |
| 8 | | 16 | | Y | |

# ccfDNA Counting Method

3 Million matches later

Chromosome 21 has 51,740 counts (1.67%)

Should be 1.55%

Counts are about 8 SD higher than expected (Z score = 8)

Probable karyotype is 47,XY,+21

| | Counts | | Counts | | Counts |
|---|---|---|---|---|---|
| 1 | 249,250 | 9 | 141,213 | 17 | 81,195 |
| 2 | 243,199 | 10 | 135,534 | 18 | 78,077 |
| 3 | 198,022 | 11 | 135,006 | 19 | 59,128 |
| 4 | 191,154 | 12 | 133,851 | 20 | 63,025 |
| 5 | 180,915 | 13 | 115,169 | 21 | 51,740 |
| 6 | 171,115 | 14 | 107,349 | 22 | 51,304 |
| 7 | 159,138 | 15 | 102,531 | X | 155,270 |
| 8 | 146,364 | 16 | 90,354 | Y | 59,373 |

ARTICLE

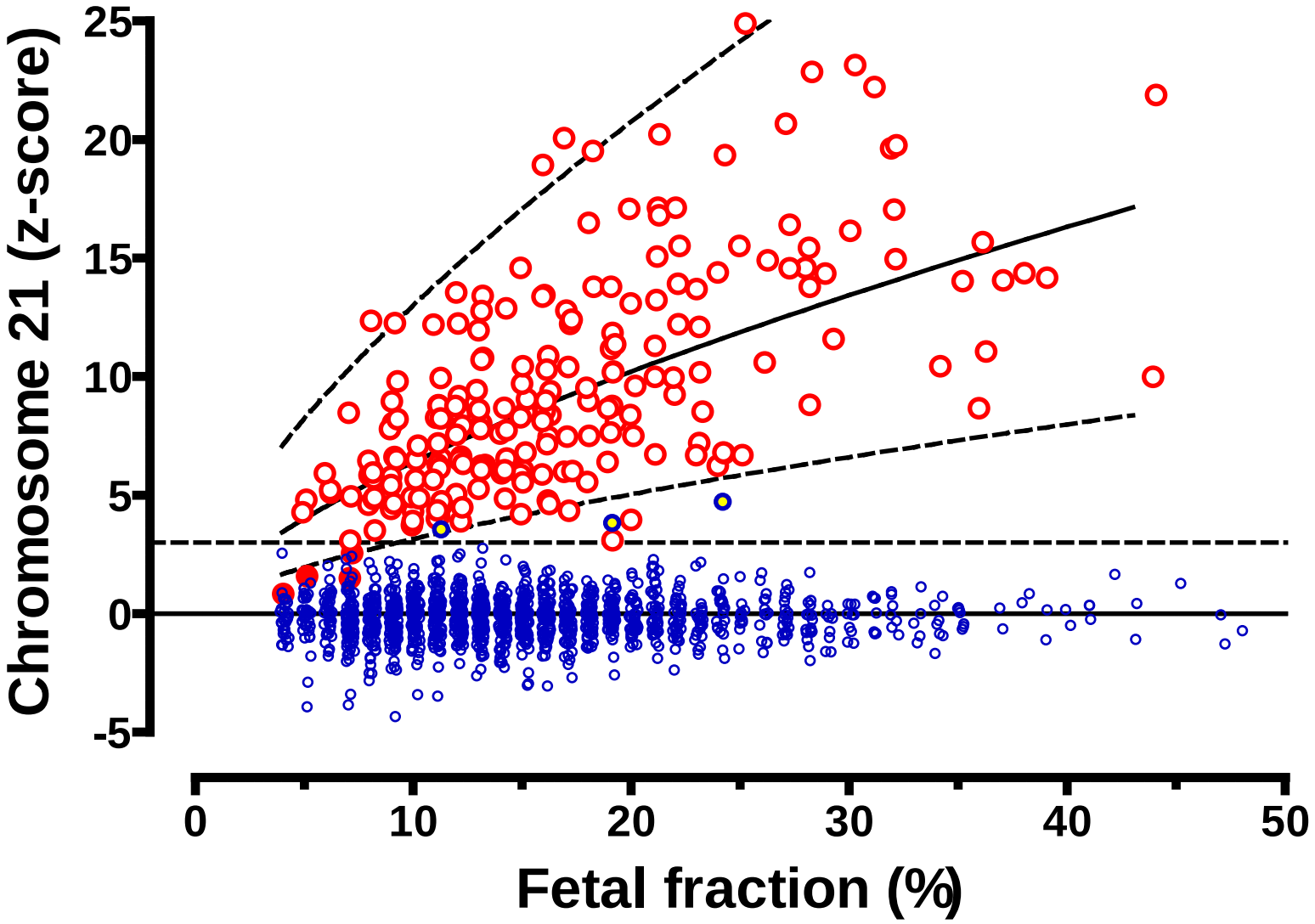
# DNA sequencing of maternal plasma to detect Down syndrome: An international clinical validation study

*Glenn E. Palomaki, PhD[1], Edward M. Kloza, MS[1], Geralyn M. Lambert-Messerlian, PhD[1], James E. Haddow, MD[1], Louis M. Neveux, BA[1], Mathias Ehrich, MD[2], Dirk van den Boom, PhD[2], Allan T. Bombard, MD, MBA[2,3,4], Cosmin Deciu, MSc[3], Wayne W. Grody, MD, PhD[5], Stanley F. Nelson, MD[6], and Jacob A. Canick, PhD[1]*

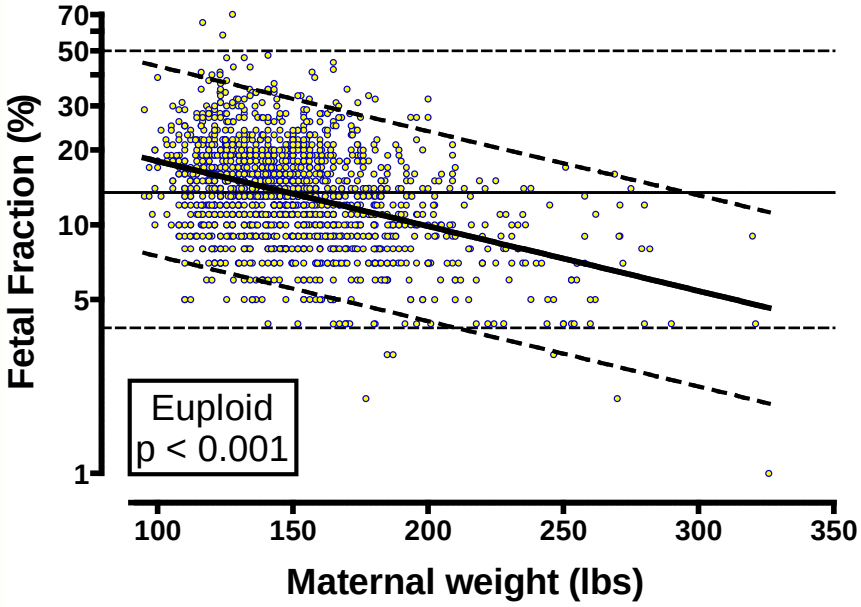
Genet Med. 2011 Nov;13(11):913-20

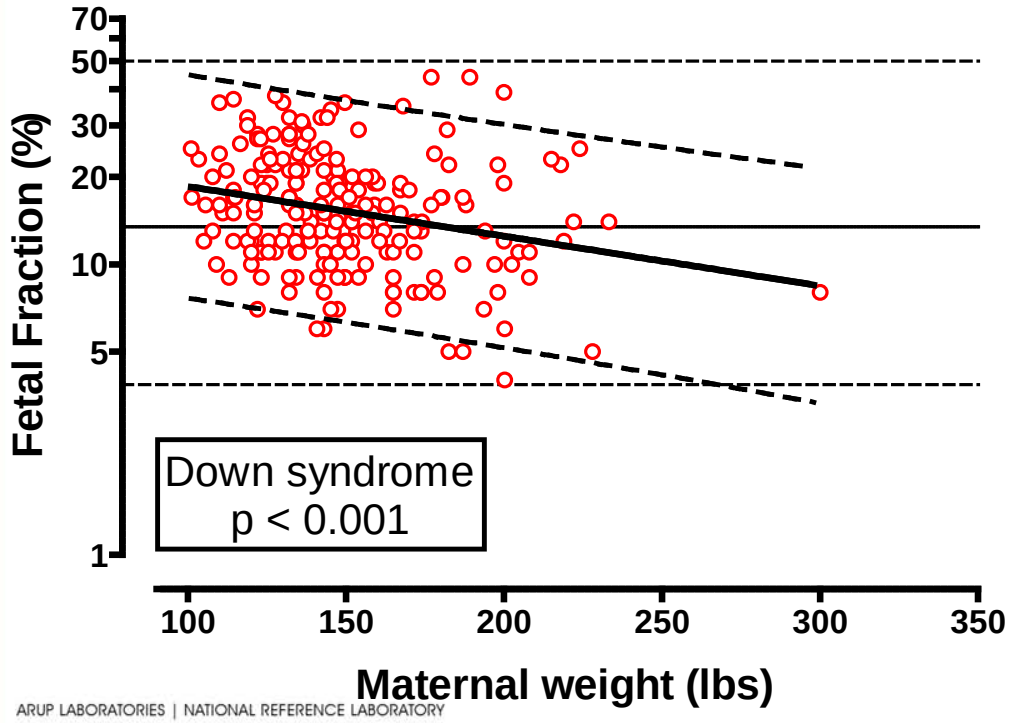
- Document the performance (sensitivity and specificity) of a laboratory-developed test (LDT) for Down syndrome.
- Document subsequent improvements in the LDT, including the identification of other aneuploidies (e.g., trisomy 18).

# Z-score versus fetal fraction



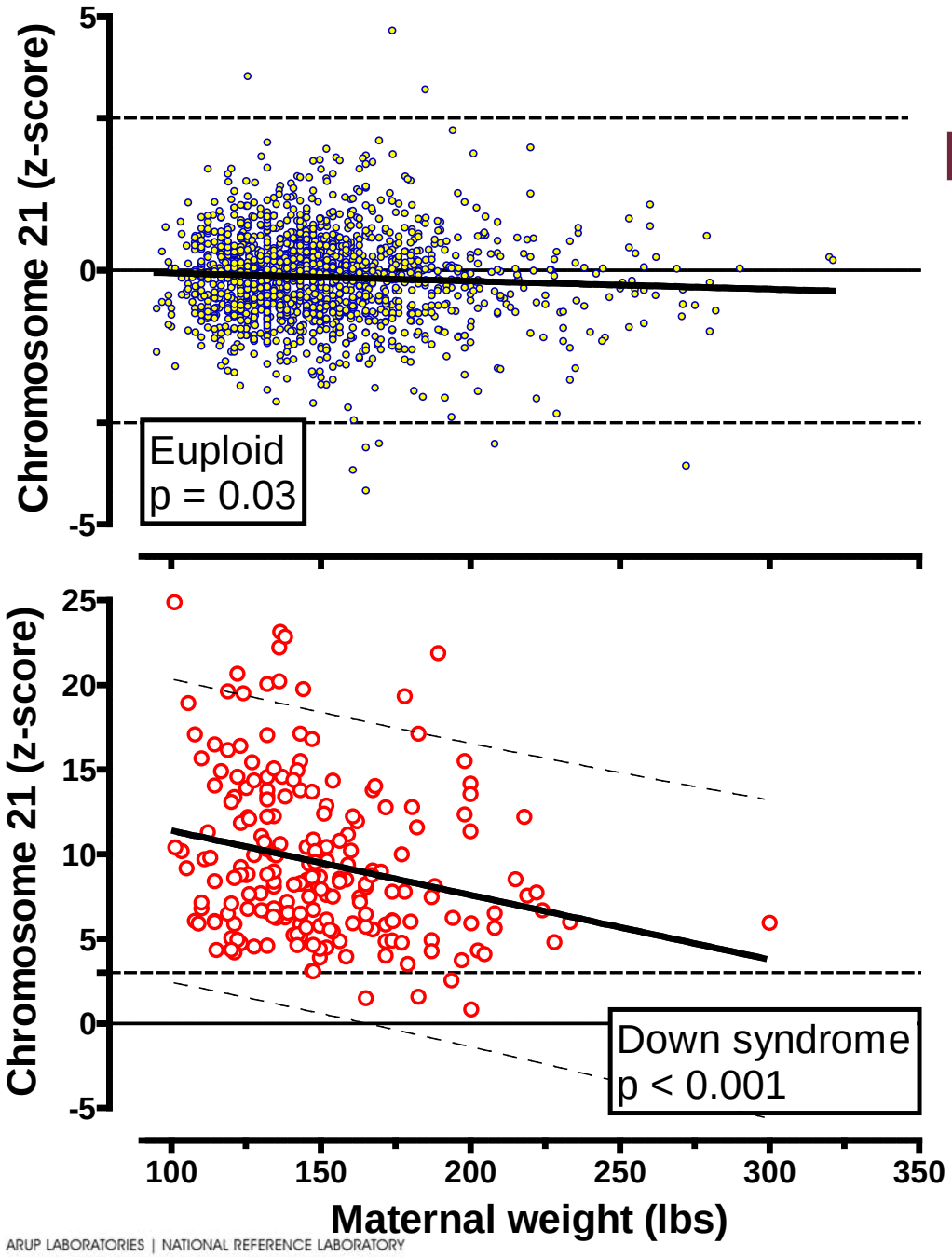
# Importance of maternal weight




| Maternal Wt (lbs) | Expected FF (%) |
|---|---|
| 100 | 17.8 |
| 150 | 13.2 |
| 200 | 9.8 |
| 250 | 7.3 |

# Importance of maternal weight




| Maternal Wt (lbs) | Expected z-score |
|---|---|
| 100 | 11.4 |
| 150 | 9.5 |
| 200 | 7.6 |
| 250 | 5.7 |

# Four Current Methods

| | Shotgun | Targeted |
|---|---|---|
| **Counting** | Sequenom<br>Verinata | |
| **Genotyping** | | Natera |
| **Both** | | Ariosa |

*Sequencing method*: any fragment (shotgun) versus selectively amplified sequences (targeted)

*Interpretation*: comparing observed percentage of aligned fragments from chromosome of interest to expected (counting) versus modeling observed SNP genotype to specific models (genotyping)

# Detection of Down syndrome (T21): Summary of published US studies

| | | All | | T21 |
|---|---|---|---|---|
| **Study** | FPR (%) | No-calls | DR (%) | No-call |
| | | | | |
| Palomaki 2011 | 3/1,471 (0.2) | 13/1,697 (0.8) | 209/212 (98.6) | 0 |
| Ashoor 2012 | 0/ 300 (0 ) | 1/ 400 (0.7) | 50/ 50 (100) | 0 |
| Bianchi 2012 | 0/ 311 (0 ) | 23/ 532 (4.3) | 89/ 89 (100) | 1 |
| Norton 2012 | 1/2,887 (0.1) | 148/3,228 (4.6) | 81/ 81 (100) | 3 |
| Nicolaides 2013 | 0/ 204 (0 ) | 13/ 242 (5.4) | 25/ 25 (100) | 2 |
| | | | | |
| **All** | 4/4,173 (0.1) | | **454/457 (99.3)** | **6** |

**Accounting for 'no-calls' DR = 454/463 or 98.0%**

# Performance of ccfDNA

- Detection rate is about 98%

  "*98 of 100 Down syndrome fetuses tested will have a positive result; one will be missed and another will be a no-call*."

- False positive rate is about 0.2% or less

  *"Only 1 in 500 normal fetuses will have a positive DNA test."*

- Failure rate ranges from ~1% to 5%

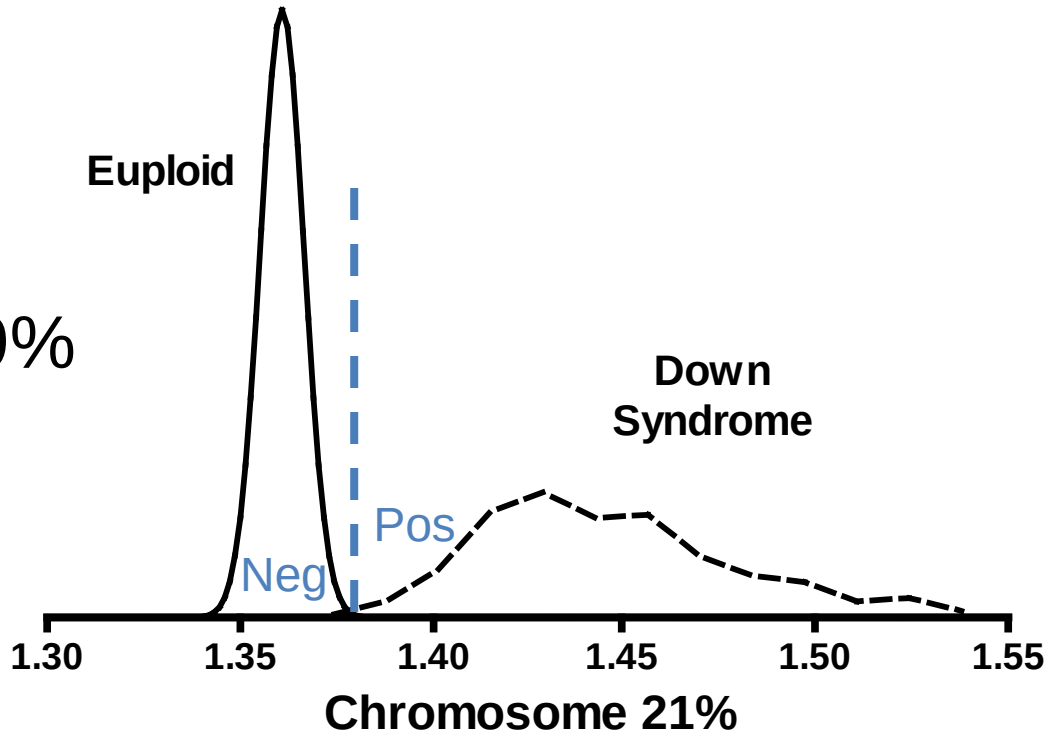
  *"Depending on the test, between 1 and 5 of every 100 women will have a test result that does not provide useful information about the woman's Down syndrome risk."*

Palomaki, Ashwood. NEJM 2014

# Down syndrome screening

- ccfDNA testing of maternal plasma
- Tests involve shotgun or targeted next generation sequencing

- Detection rate 99%
- FPR 0.2%



# ccfDNA for Other Disorders

- Trisomy 18: DR ≈ 90%, higher no-calls
- Trisomy 13: DR ≈ 88%
- Turners 45,X: DR ≈ 95%
- Triple X 47,XXX: DR ≈ 89%
- Klinefelter 47,XXY: DR ≈ 100%
- 22q deletion (DiGeorge)
- 5p minus (Cri-du-chat)
- 15q (Prader-Willi/Angleman)
- 1p36 deletion

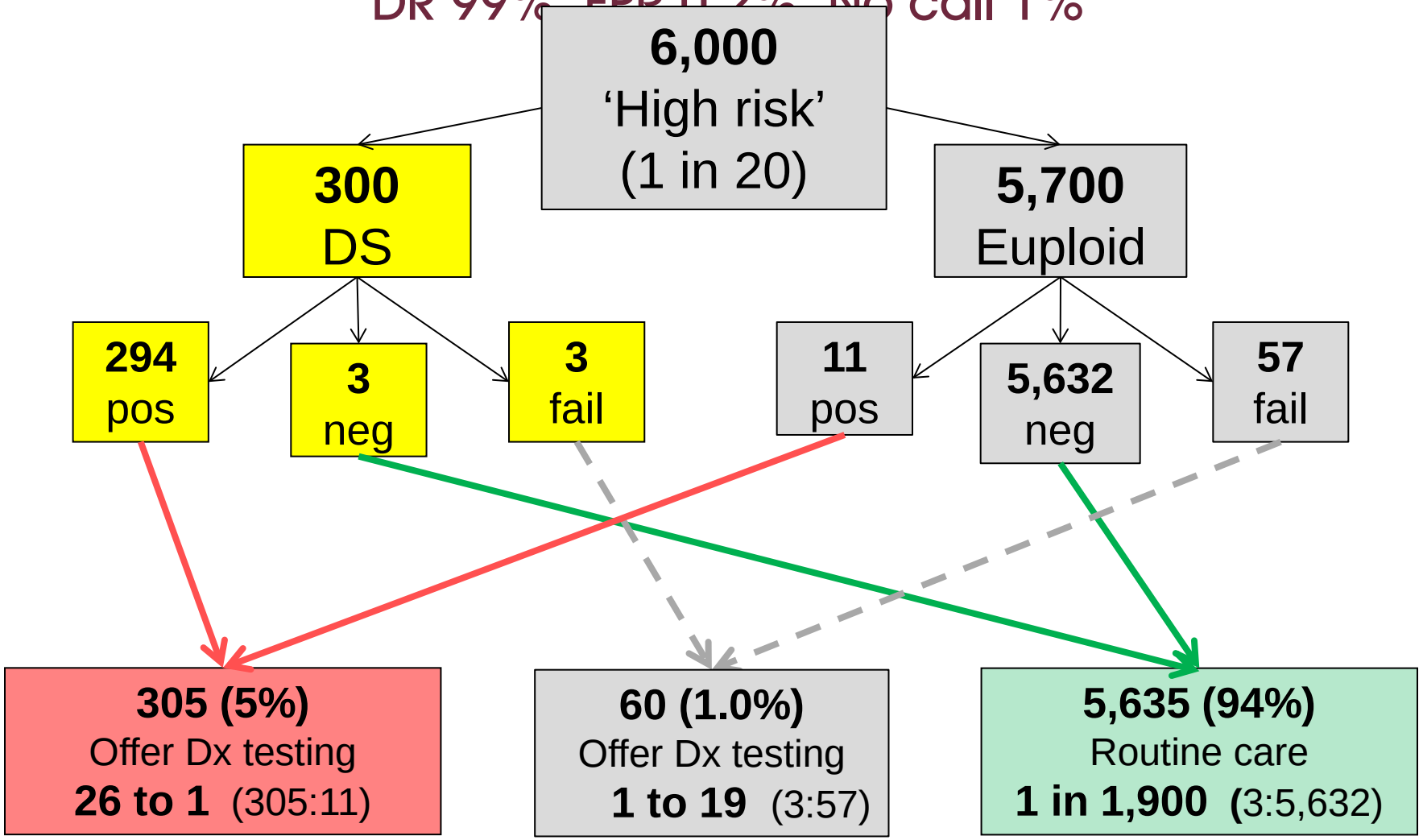
# Professional Practice Guidelines

- Generally agree that
  - Sequencing of cell-free DNA is sensitive and specific for trisomies of chromosome 21, 18, and 13
  - Testing should be offered to ‘high risk’ pregnancies
  - Patient and provider education is important
  - Insufficient data for twins
  - 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
  - ACMG’s guideline allows general population testing

ACOG, NSGC, ISPD, ACMG, SOGC

# ccfDNA testing in 'High risk' women

DR 99%, FPR 0.2%, No call 1%



ARUP Consult

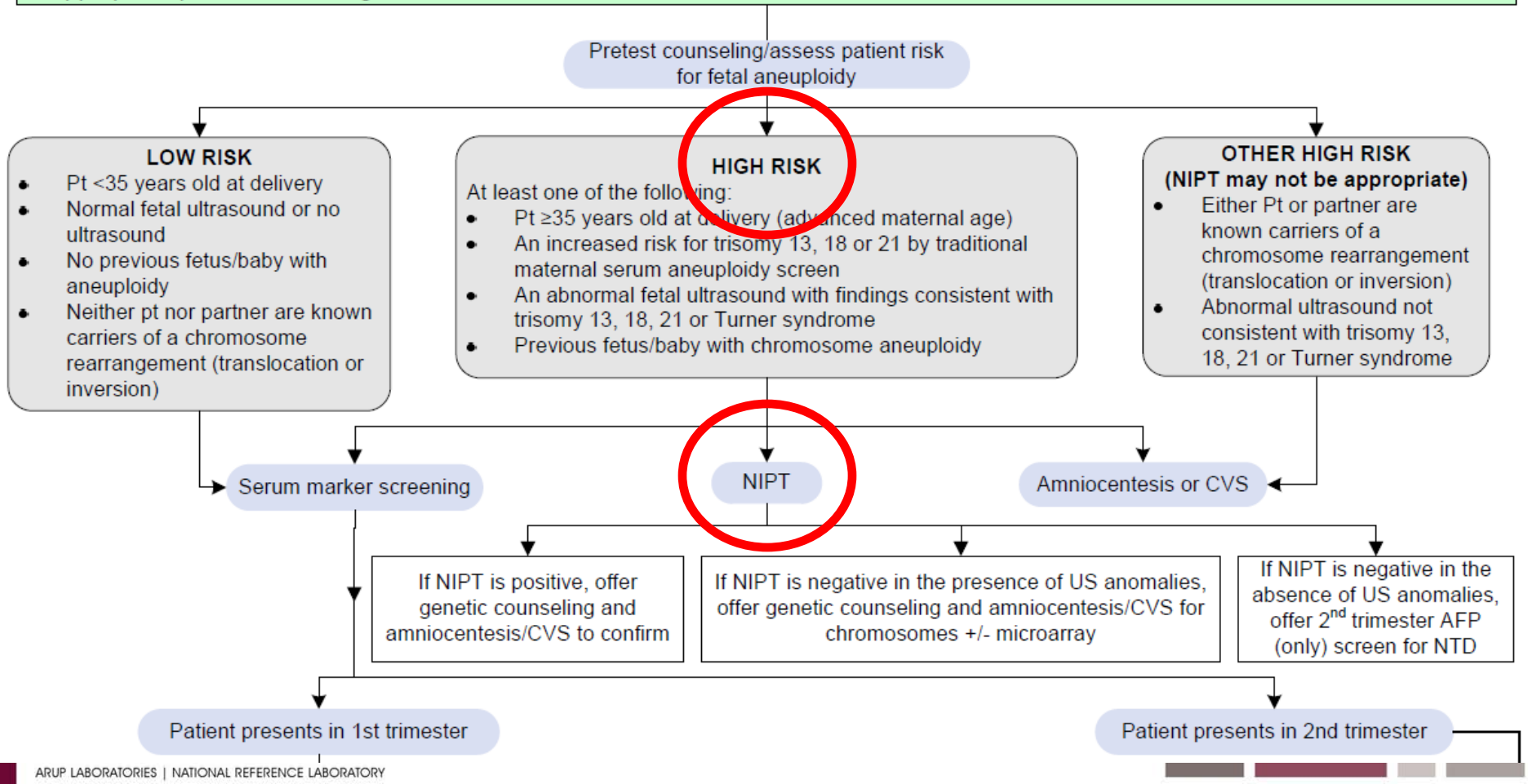
# Prenatal Screening and Diagnosis

**(Based on ACOG screening recommendations, 2007;
ACOG Committee Opinions Recommendations, 2012)**

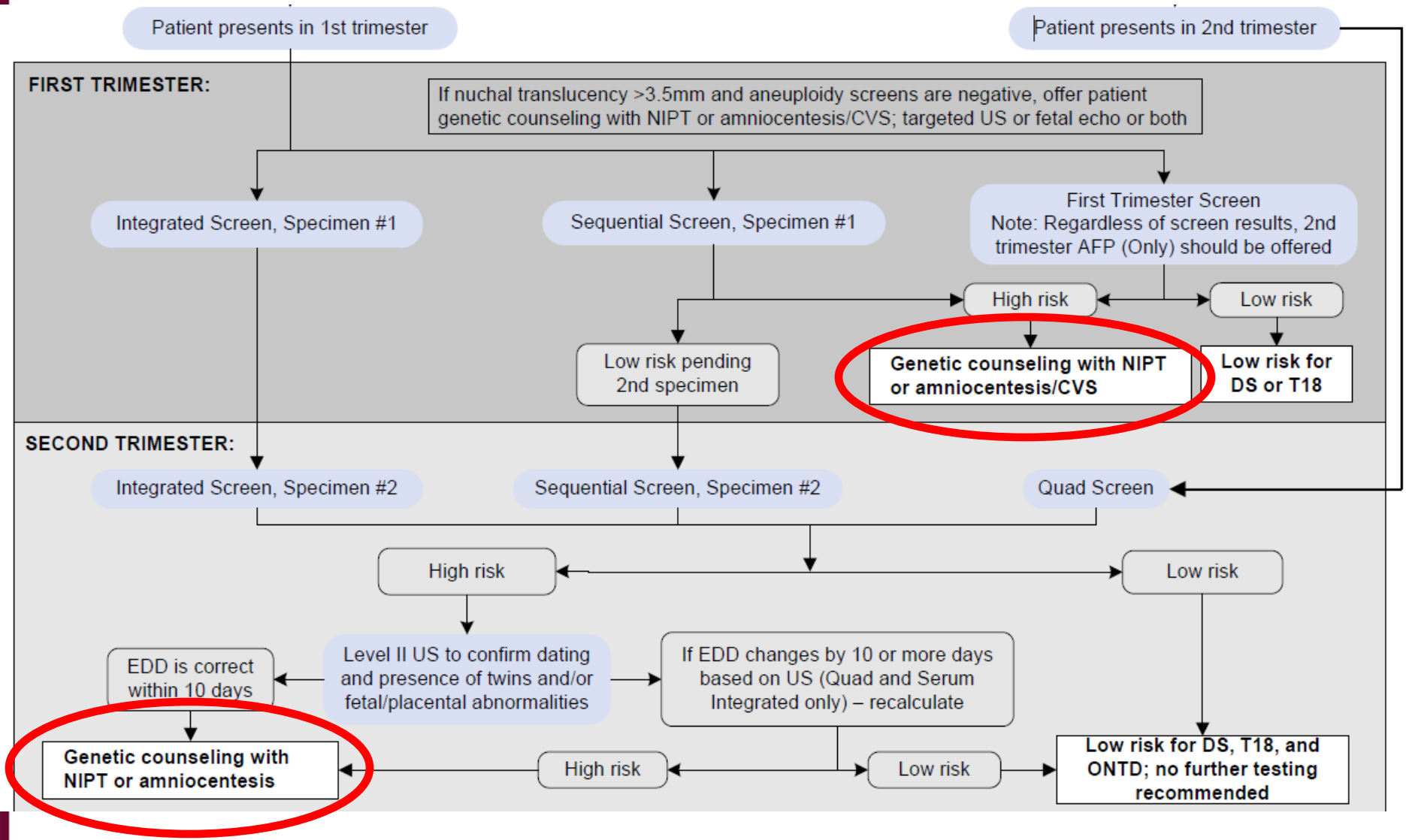
Click here for topics associated with this algorithm

## Screening Recommendations

- All women, regardless of age, should have the option of invasive testing
- Maternal age of 35 years alone should not be used as a cutoff to determine who is offered screening versus who is offered invasive testing, however maternal age does play a role in determining a priori risk for certain fetal abnormalities
- This algorithm provides a guideline. Women may choose screening options alternate to what is recommended by their risk category after appropriate pretest counseling

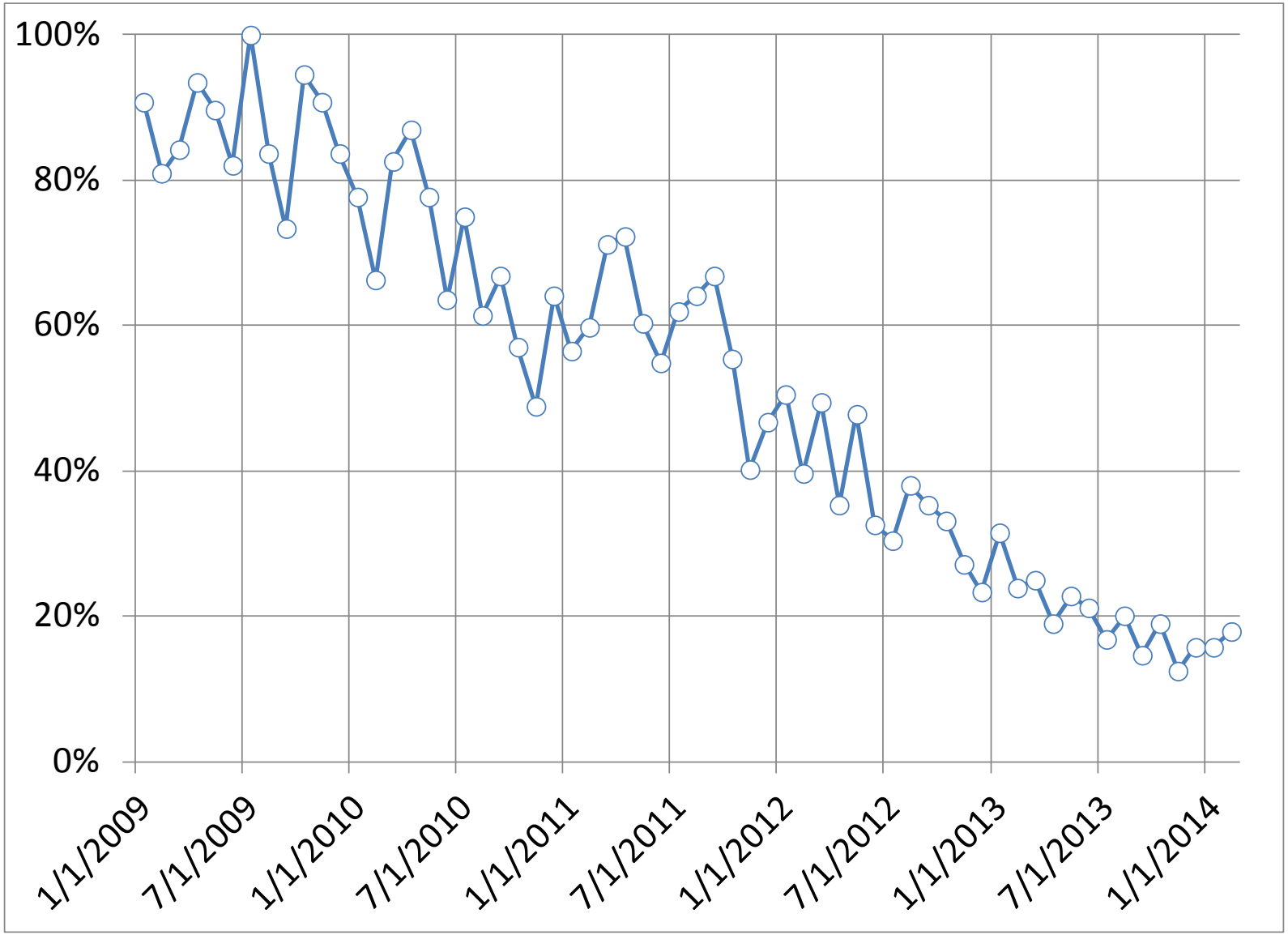


# ARUP Testing Algorithm, cont.

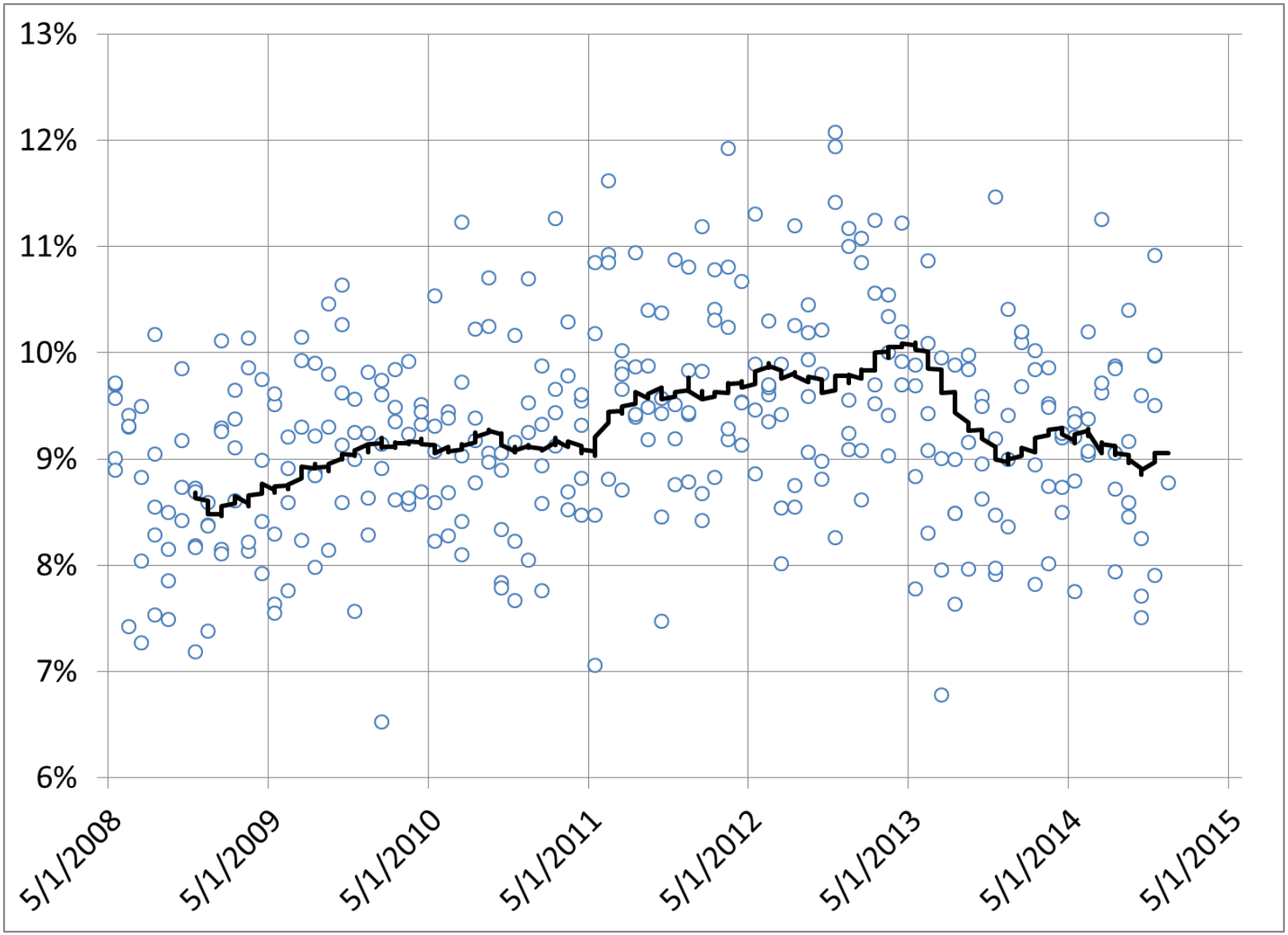


# Impact of uptake rates of confirmatory cell-free DNA testing

# Aminocentesis Decline (AF AFP)



# Percent AMA for Quad Tests



# Legal Issue – Intellectual Property

- Patents (total of 32 US patents)
  - ccfDNA, first patent, US 6,258,540
    - 10/30/2013: US District Court finds ’540 patent invalid
    - ccfDNA a ‘product of nature’
    - Litigation to higher court pending
- IP Lawsuits
  - Every company has/had lawsuits with the others
  - 12/3/2014: Illumina (Verinata) and Sequenom announce settlement of  infringement claims for $$$
- Most labs are reluctant to begin ccfDNA testing

# Is ccfDNA ready for general population screening?

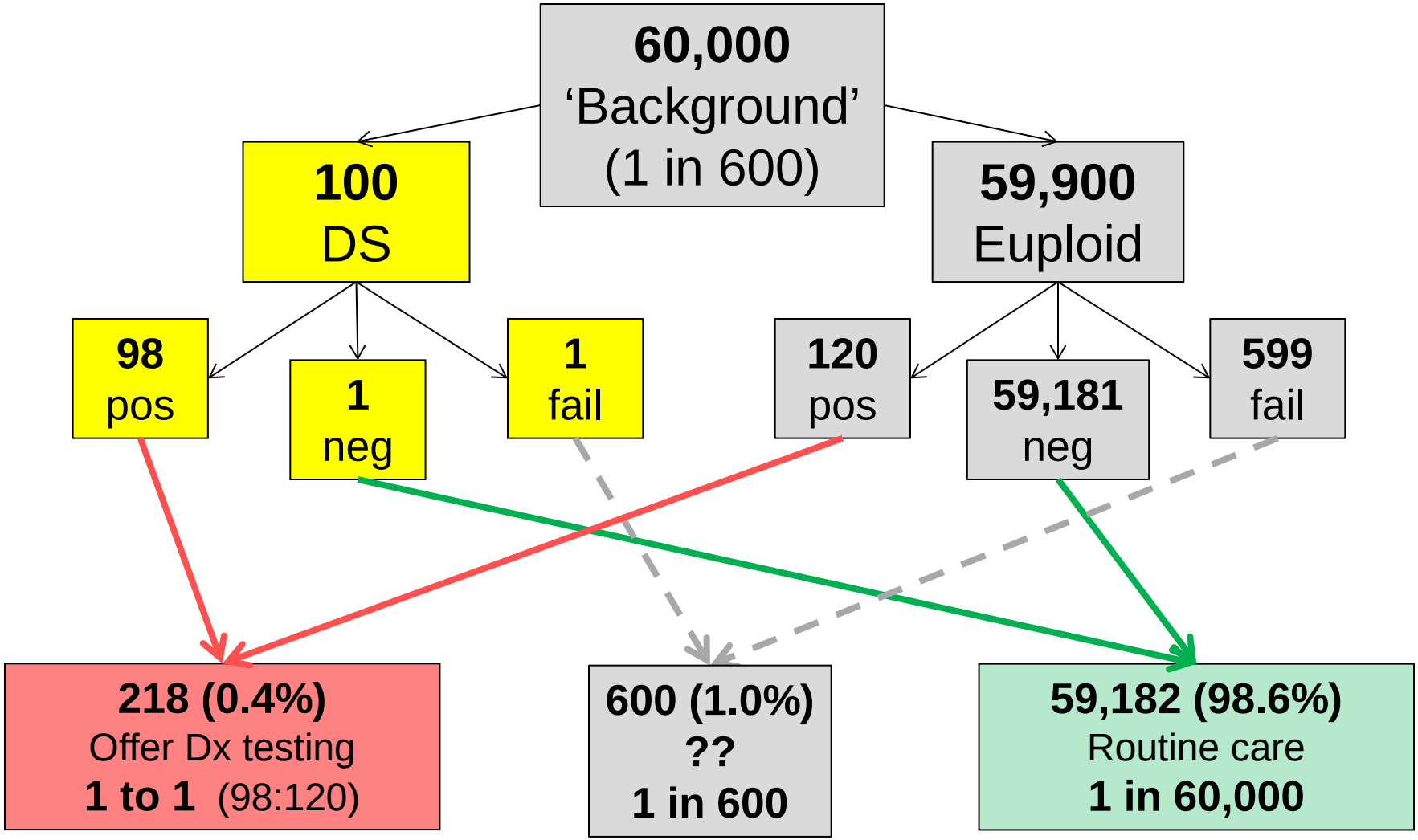
# Professional Practice Guidelines

- Generally agree that
  - Sequencing of cell-free DNA is sensitive and specific for trisomies of chromosome 21, 18, and 13
  - Testing should be offered to 'high risk' pregnancies
  - Patient and provider education is important
  - Insufficient data for twins
  - 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**
  - ACMG's guideline allows general population testing

ACOG, NSGC, ISPD, ACMG, SOGC

# ccfDNA testing in general population

## DR 99%, FPR 0.2%, No call 1%



# General Screening versus High Risk Testing

- Positive and Negative predictive values
  - High risk: many true positives compared to FP
  - Low risk:  equal numbers of TP and FP
- Impact on test failures / no calls
  - High risk: offered diagnostic testing
  - Low risk:  needs some other options (e.g., repeat ccfDNA, serum screening, ultrasound)
- Counseling/education
  - High risk: genetic counseling
  - Low risk:  too few genetic counselors, so education would need to come from primary care providers

# Cost Effectiveness

DOI: 10.1002/pd.4511

PRENATAL **DIAGNOSIS**

ORIGINAL ARTICLE

## A cost-effectiveness analysis of cell free DNA as a replacement for serum screening for Down syndrome

Brandon S. Walker, Brian R. Jackson, Danielle LaGrave, Edward R. Ashwood and Robert L. Schmidt

- Current NIPT cost can be $1000 or more
- NIPT can save money for society at $549/test
- Cost effective for payers at $216/test

# Conversion to General Screening

- ccfDNA conversion is aided by
  - Existing serum screening program embedded in routine care
  - Reimbursement for serum screening of ~$300
  - Demonstration of ccfDNA clinical validity
- ccfDNA conversion is hindered by
  - high cost / charge for ccfDNA testing
  - Recommendations against ccfDNA general screening
  - Lack of reimbursement from some payers
  - IP issues deterring many labs from performing
  - Studies showing how to implement ccfDNA
- Conversion to general pregnancy population screening will take several years

# Breaking News

- The Boston Globe 12/14/2014

"Oversold prenatal tests spur some to choose abortions"

  - Claims that patients and physicians confuse detection rate with positive predictive value
  - Claims that Sequenom, Natera, and Ariosa make misleading marketing claims

  - Doesn't give a balanced comparison of current screening to ccfDNA testing

# Conclusions

- ccfDNA testing is dramatically and positively affecting the pregnancies of high risk women
- For women with abnormal traditional maternal serum screens, ccfDNA is the best secondary test
- General population screening using ccfDNA will take several years
- The no-call rate, especially for obese women, complicates the workup algorithm

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