Prenatal Screening for Fetal Defects

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Disclosures

- Beckman Coulter, Inc.
  - Research support

- AFP, hCG, uE3, DIA, and PAPP-A tests are not FDA-cleared for aneuploidy screening.

Objectives

- *Describe* how biochemical and ultrasound markers are used to screen for open neural tube defects and determine aneuploidy risk

- *Explain* new molecular-based approaches for aneuploidy screening

Screening for Which Defects?

Method	Fetal defect	Incidence (live births, approximate)
Biochemical screening only	Open neural tube defects (ONTD)	1 in 1,000
Biochemical & DNA-based screening	Trisomy 21 (Down syndrome)	1 in 700
Biochemical & DNA-based screening	Trisomy 18 (Edwards syndrome)	1 in 5,000
DNA-based screening only	Trisomy 13 (Patau syndrome)	1 in 16,000

Biochemical Screening Test Choices

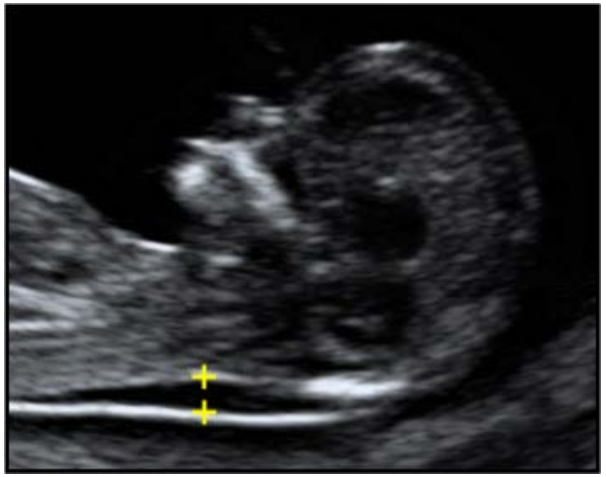
Test Name	ONTD	DS	T18	Trimester
AFP Only	✔			2nd
Combined		✔	✔	1st
Triple	✔	✔	✔	2nd
Quad	✔	✔	✔	2nd
Integrated	✔	✔	✔	1st & 2nd
Serum Integrated	✔	✔	✔	1st & 2nd
Sequential	✔	✔	✔	1st & 2nd

Biochemical Screening Markers

Marker	Source
Alpha-fetoprotein (AFP)	Fetus
Human chorionic gonadotropin (hCG)	Placenta
Unconjugated estriol (uE3)	Fetus/Placenta
Dimeric inhibin A (DIA)	Placenta
Pregnancy-associated plasma protein A (PAPP-A)	Placenta
Nuchal translucency	Fetus

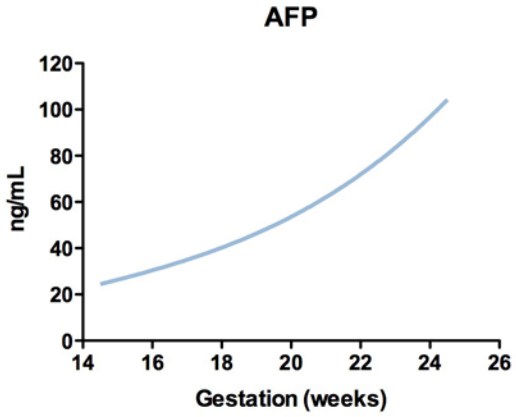
Nuchal Translucency (NT)

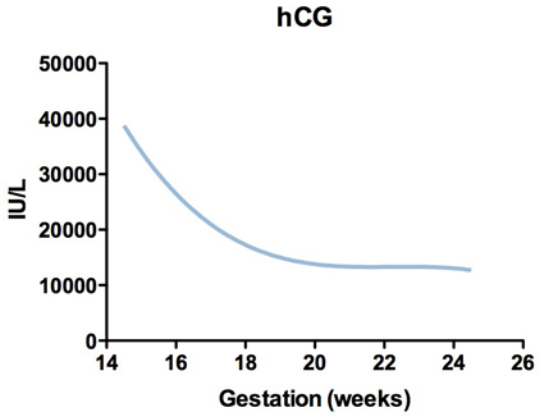
- The space that can be visualized between the fetal skin and the soft tissues covering the cervical spine
- Performed on fetuses at 10 – 14 wks gestation
  - Measurement requires specific training and extended practice
- Increased thickness strongly associated with fetal aneuploidy
  - Cardiac defects with over-perfusion of head and neck; abnormal lymphatics?
- Not specific for aneuploidies

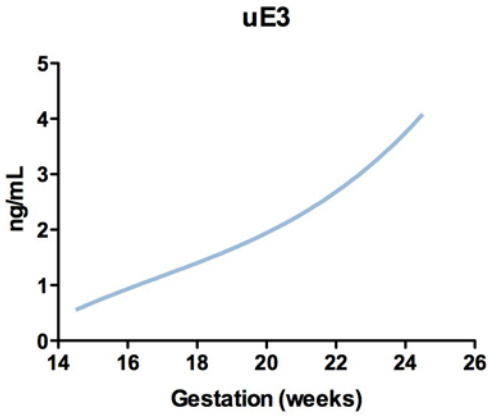


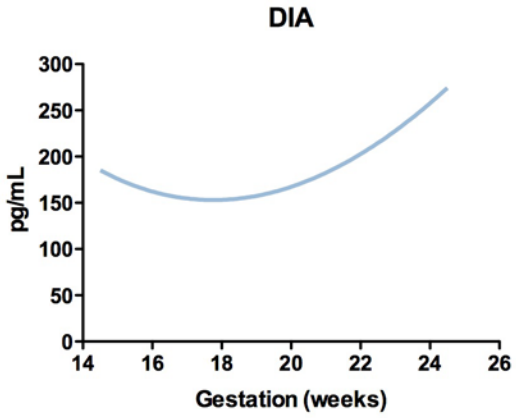
www.fetalmedicine.com

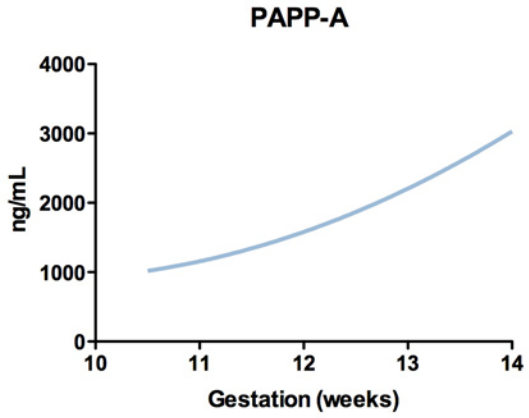
Marker Concentrations by Gestational Age











Multiple of the Median (MoM)

- Ratio between the patient's result and the median result appropriate for the gestational age of fetus

$$\text{MoM} = \frac{\text{Patient's result}}{\text{Median result}}$$

- Medians determined by the laboratory for each marker across all gestational ages required for a given test strategy

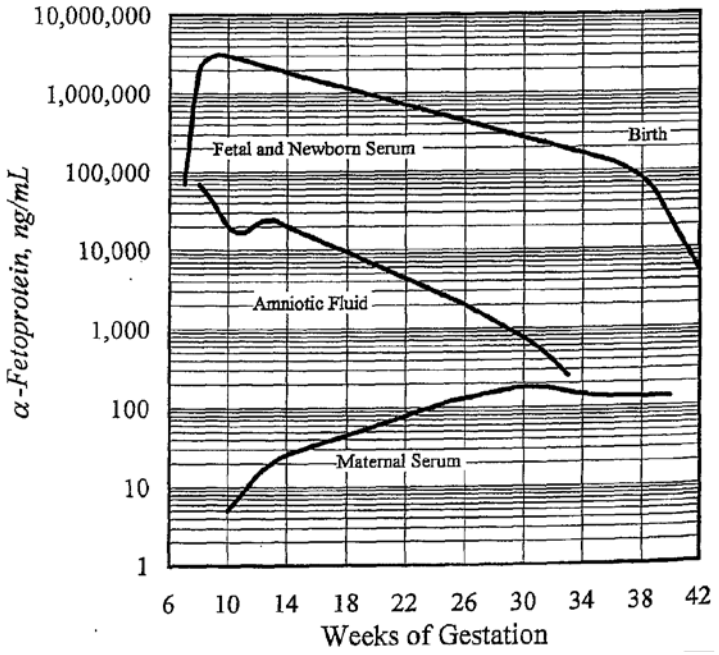
Multiple of the Median (MoM)

Gestational age (weeks)	Patient's AFP concentration (ng/mL)	Median AFP concentration (ng/mL)	Ratio	MoM
16	15	30	15/30	0.5
16	30	30	30/30	1.0
18	30	40	30/40	0.75

SCREENING FOR OPEN NEURAL TUBE DEFECTS

Open NTD Screening with AFP

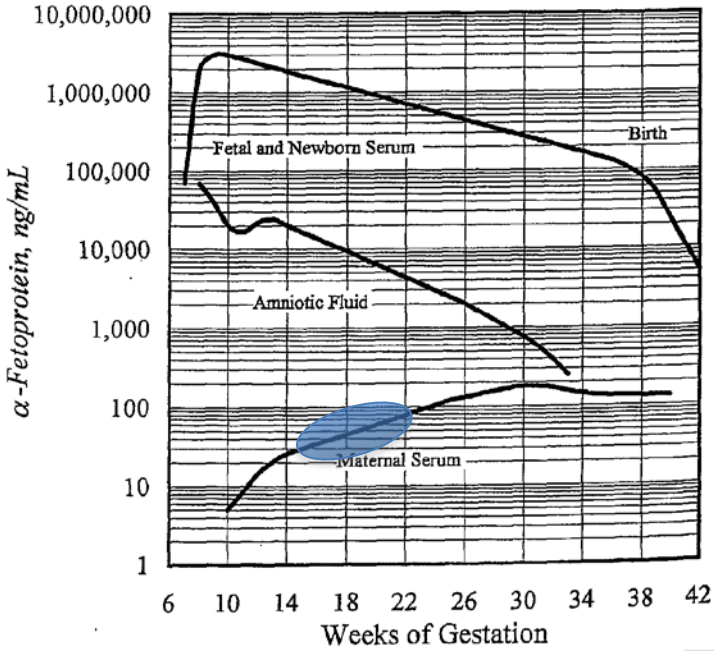
- Peaks in fetus at 9 weeks (~$3x10^6$ ng/mL) then steadily declines
- AF-AFP mirrors that of serum but concentration is ~100x lower
- MS-AFP detected at ~10 weeks (~10,000x lower)



Tietz, 4th ed, 2005

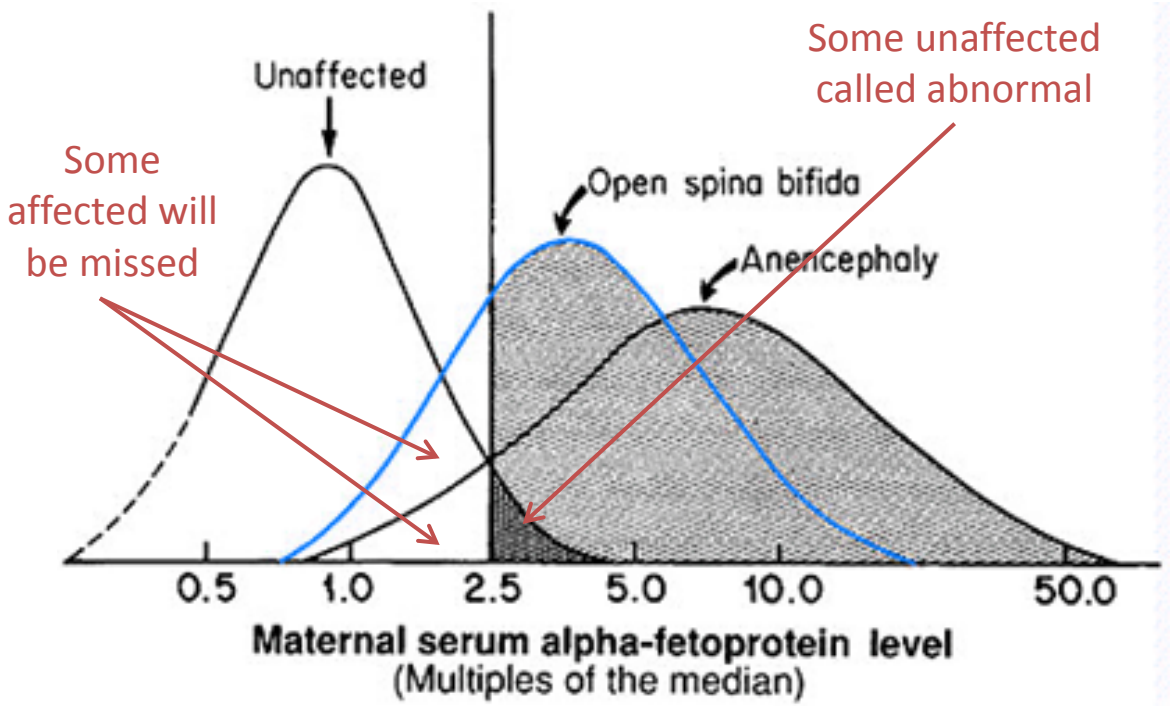
Open NTD Screening with AFP

- ONTD in direct contact with amniotic fluid
  - AF-AFP increases followed by MS-AFP
- Ideal screening time is 16-18 weeks
  - AFP MoM distributions of affected and unaffected are maximally different
  - Sufficient time for follow-up tests
- Can be done at 15-22 weeks



Tietz, 4th ed, 2005

ONTD Screening Performance



- MS-AFP interpretation based on AFP MoM (2.5 is common)
- 70-85% sensitive for open spina bifida; >95% for anencephaly
- Most positive screening tests are false-positive (2% PPV)

Other Causes of Abnormal NTD Screens

- Underestimation of GA (most common)
- Multifetal gestations
- Fetal demise
- Ventral wall defects
- Urinary tract abnormalities

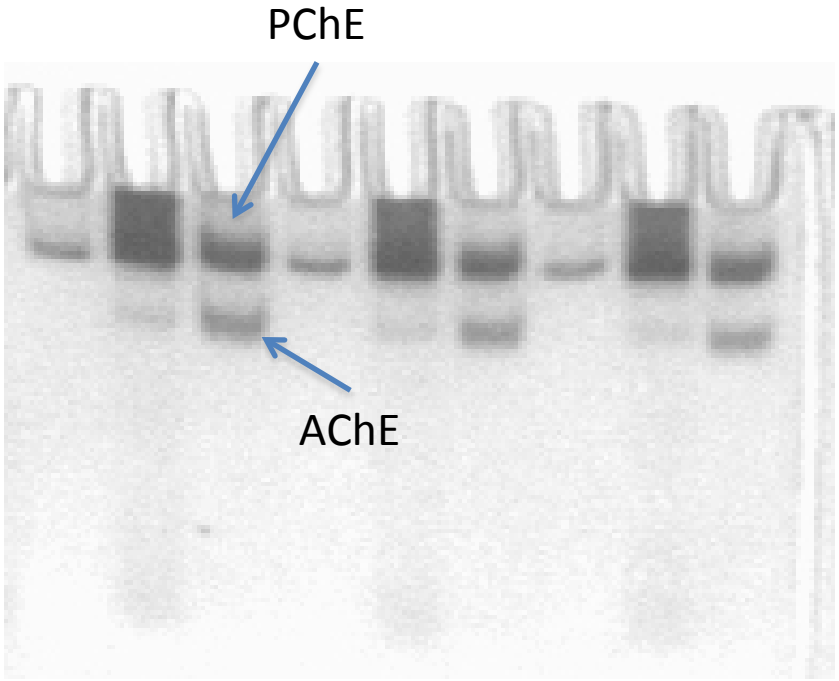
When ONTD Screen Is Abnormal

- Perform targeted ultrasound
  - Confirm GA
  - Rule out multiple gestations or fetal demise
  - Observe fetal head and spine for defects
- If AFP MoM 2.5 – 2.9 then may repeat screen from a new specimen to sort out false-positive results
  - ~40% of false-positives become true-negatives
  - 2-3% increase in false-negatives
- Amniocentesis to obtain amniotic fluid

- Measure AF-AFP
- Qualitative detection of acetylcholinesterase (AChE)

Amniotic Fluid AFP and AChE

- AF-AFP
  - More powerful indicator of ONTD than MS-AFP
- AChE
  - Present in nerve tissue
  - Hydrolyzes acetylcholine
  - Not normally present in AF (pseudocholinesterase is)
  - Electrophoretic detection is 98% sensitive and >99% specific for ONTD
- Evaluate for fetal blood when AChE positive
  - Contains both AFP and AChE



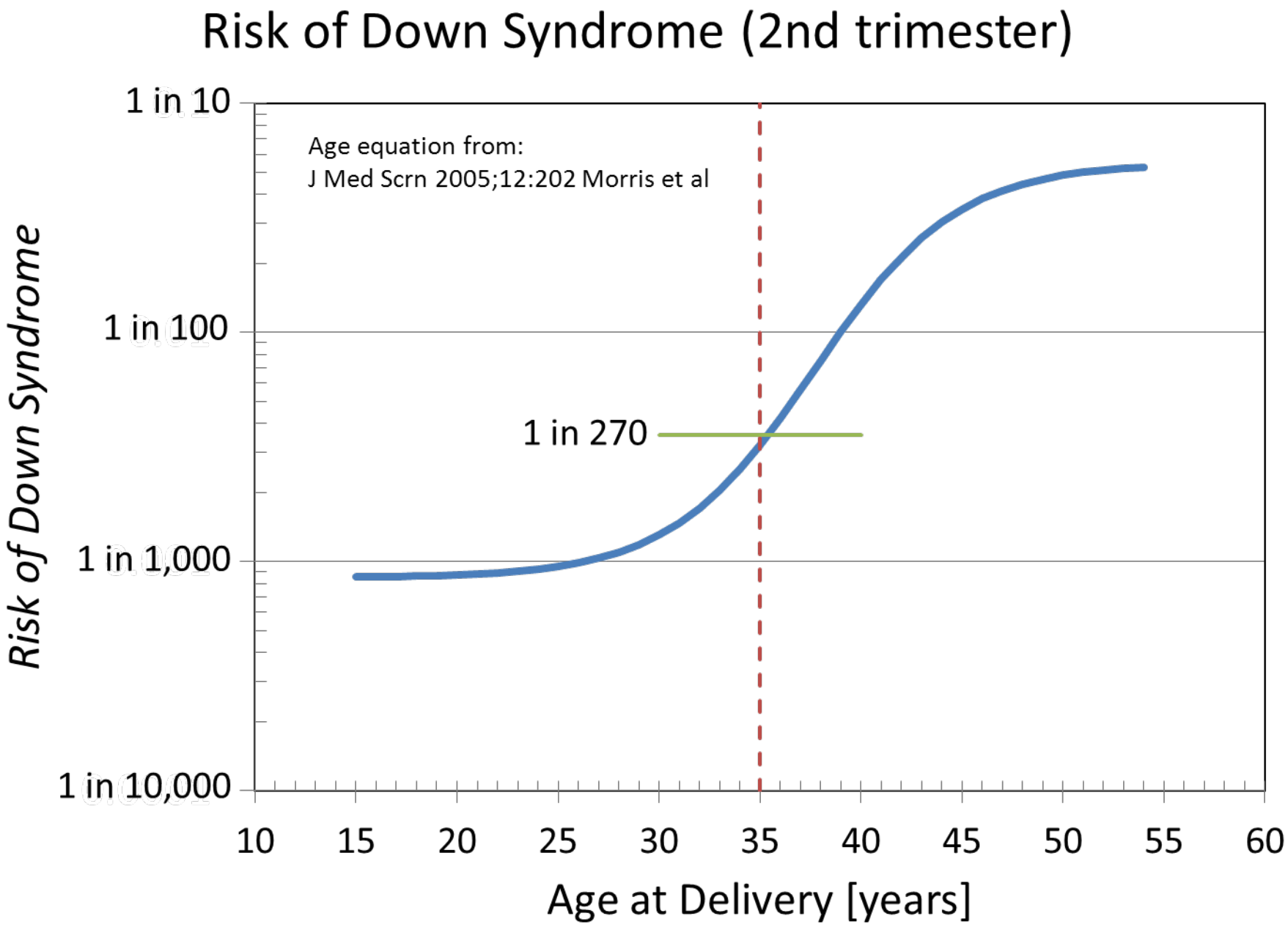
Interpreting Abnormal ONTD Tests

MS-AFP	AF-AFP	AF-AChE	Interpretation
↑	↑	Present	Very likely ONTD or ventral wall defect
↑	↑	Absent	Other fetal defect (ventral wall, demise, chromosome, urinary tract, cleft palate, nephrosis, others)
↑	N	Absent	Excludes nearly all cases of open structural defects

BIOCHEMICAL SCREENING FOR ANEUPLOIDIES

Biochemical Screening Test Choices

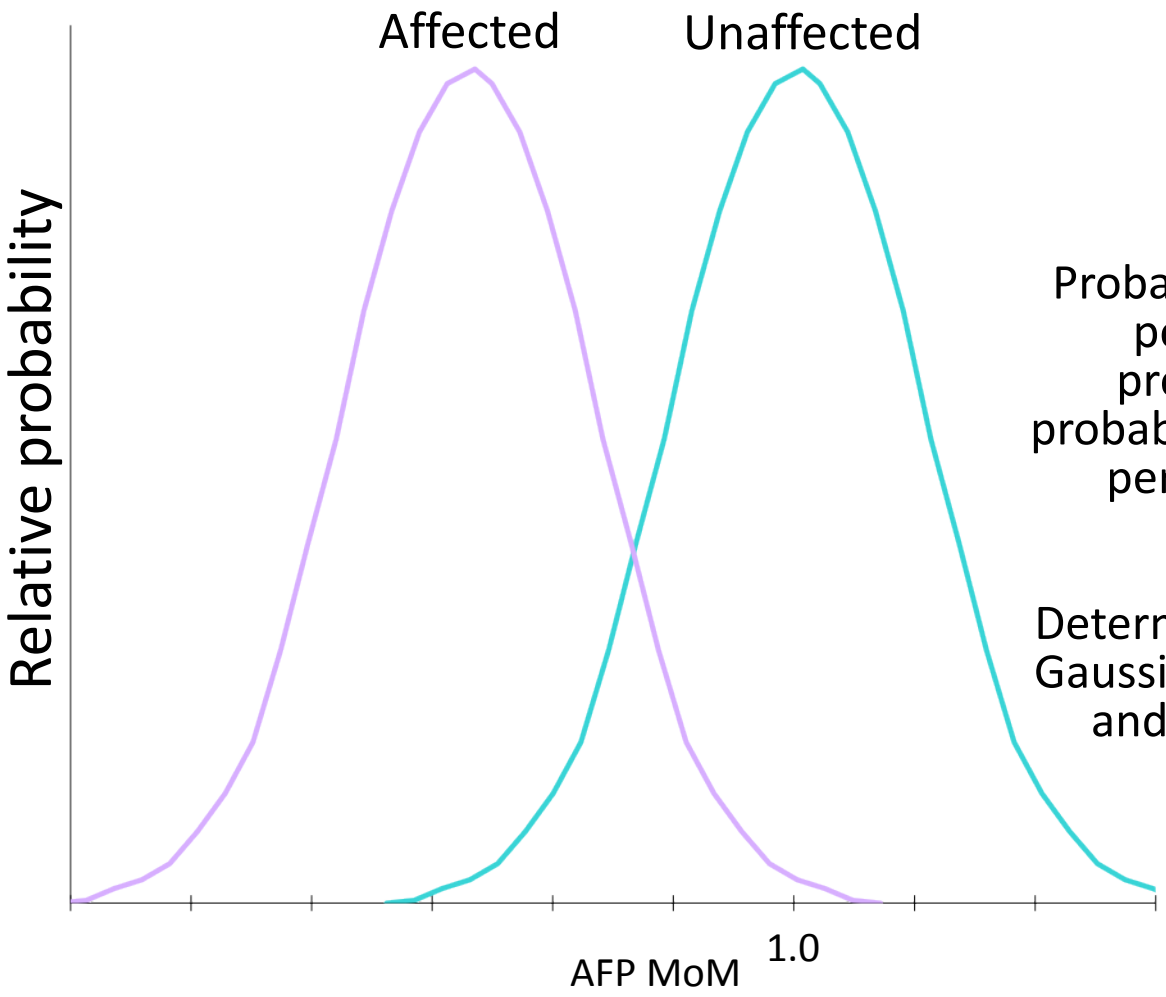
Test Name	AFP	hCG	uE3	DIA	PAPP-A	NT	Trimester
Combined		✔			✔	✔	1st
Triple	✔	✔	✔				2nd
Quad	✔	✔	✔	✔			2nd
Integrated					✔	✔	1st
	✔	✔	✔	✔			2nd
Serum Integrated					✔		1st
	✔	✔	✔	✔			2nd
Sequential		✔			✔	✔	1st
	✔	✔	✔	✔			2nd


Risk of Down Syndrome (2nd trimester)
Age equation from:
J Med Scrn 2005;12:202 Morris et al
1 in 270
1 in 10
1 in 100
1 in 1,000
1 in 10,000
Risk of Down Syndrome
10
15
20
25
30
35
40
45
50
55
60
Age at Delivery [years]

Biochemical Screening

1. Determine the Pre-test odds (age-based)
2. Measure marker concentrations in maternal serum
3. Calculate MoM of each marker using GA-specific medians
4. Determine the likelihood ratio for each marker at the patient's MoM
5. Multiply the pre-test odds by the likelihood ratios to determine the post-test odds

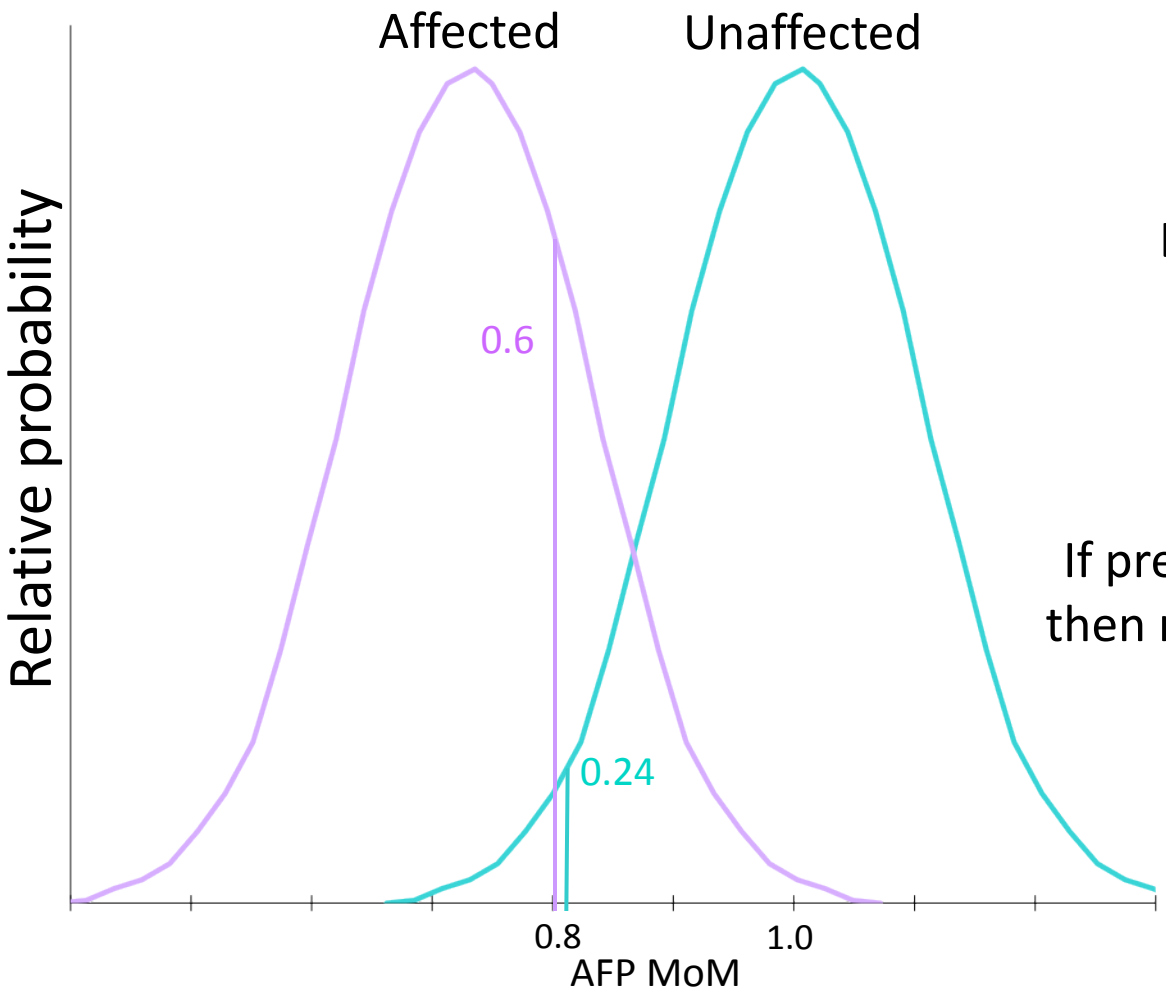
Likelihood Ratio



Probability of a given result in a person with an affected pregnancy divided by the probability of the same result in a person with an unaffected pregnancy

Determined by the heights of the Gaussian distributions in affected and unaffected pregnancies

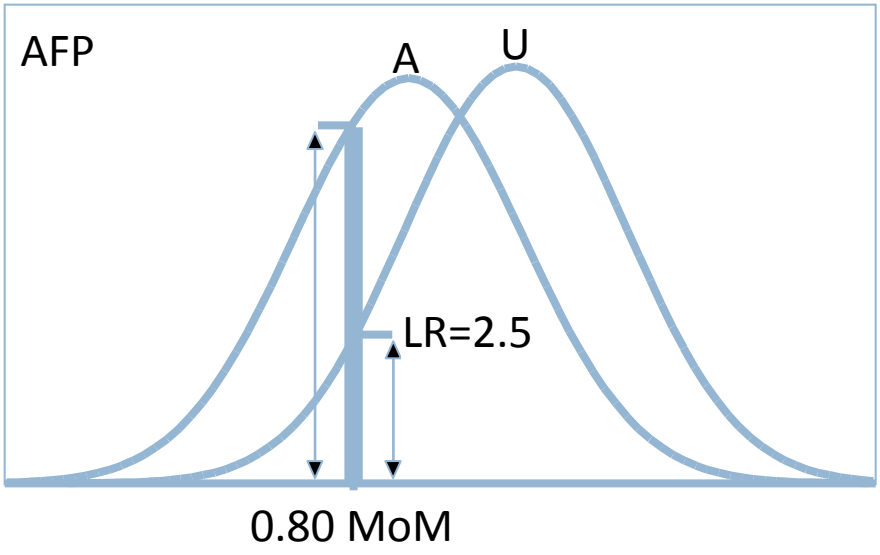
Likelihood Ratio

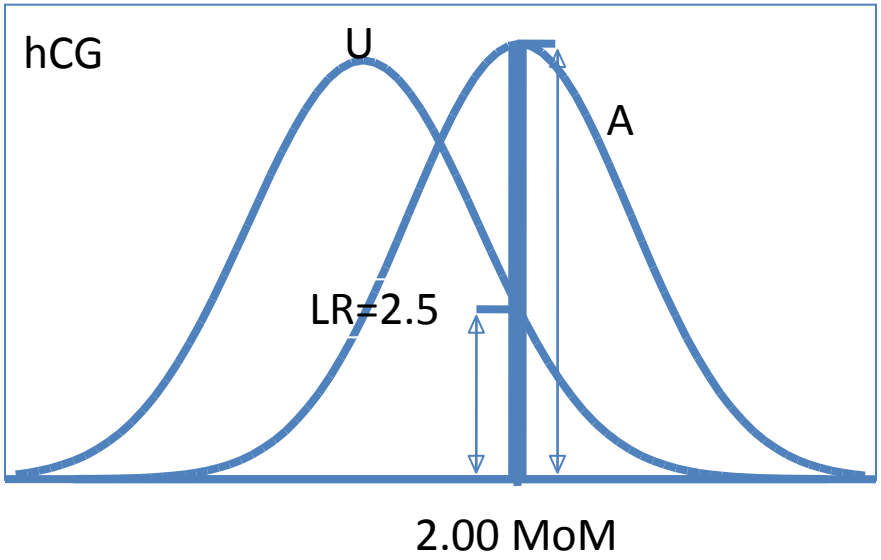


LR = Prob of affected / Prob of unaffected

LR = 0.6/0.24 = 2.5

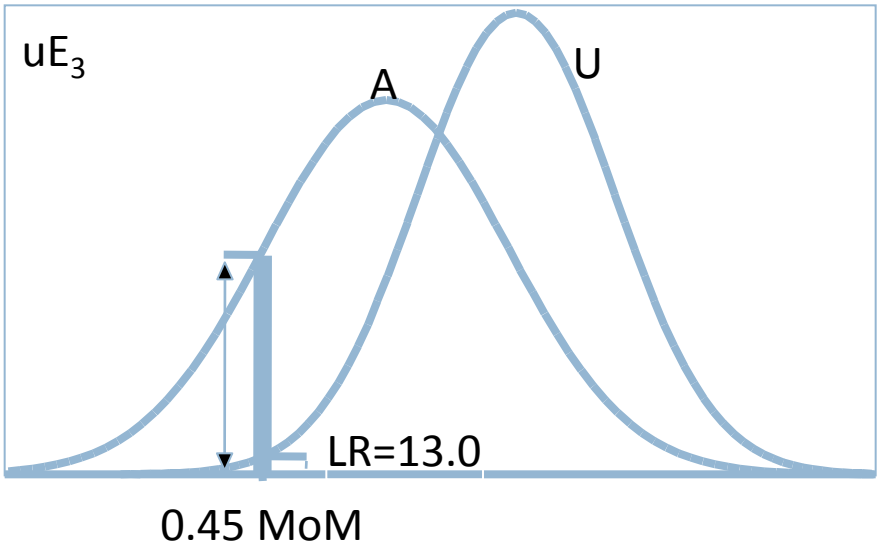
If pre-test odds were 1 to 900 then new odds are 2.5x greater or 1 to 360

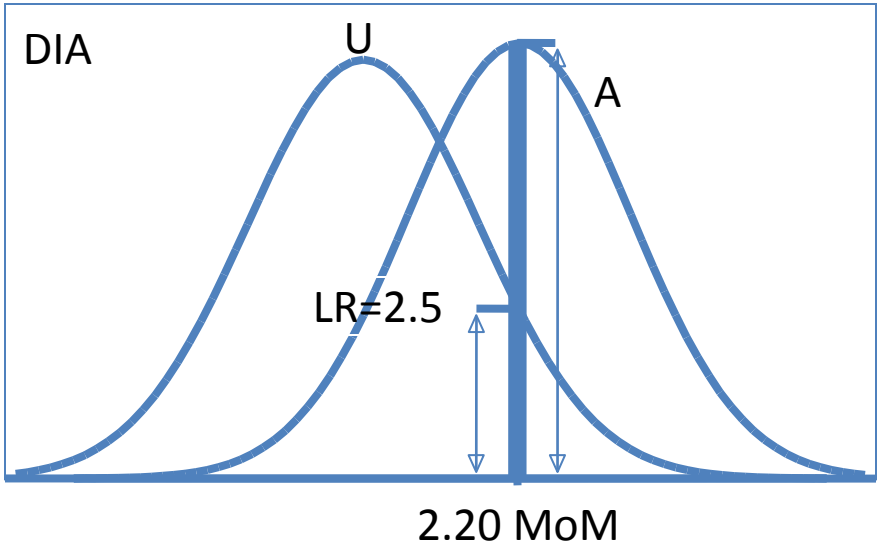




LR = 2.5 × 2.5 × 13.0 x 2.5 = 203

Post-test Risk = 1 in 270 × 203 = 1 in 1.3





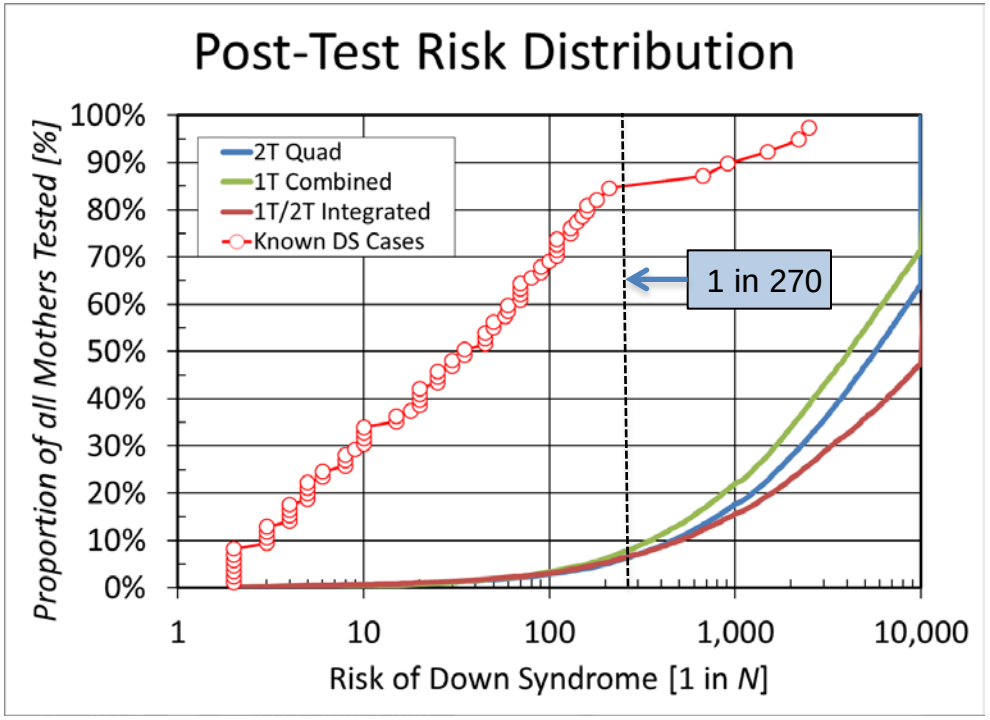
Biochemical Screening

1. Determine the Pre-test odds (age-based)
2. Measure marker concentrations in maternal serum
3. Calculate MoM of each marker using GA-specific medians
4. Determine the likelihood ratio for each marker at the patient's MoM
5. Multiply the pre-test odds by the likelihood ratios to determine the post-test odds
6. Interpret the post-test odds….what is abnormal?

Selecting a Cutoff: What is Abnormal?

Two philosophies among U.S. laboratories

1. Use 1 in 270 (DS risk for a 35 yo) regardless of test panel



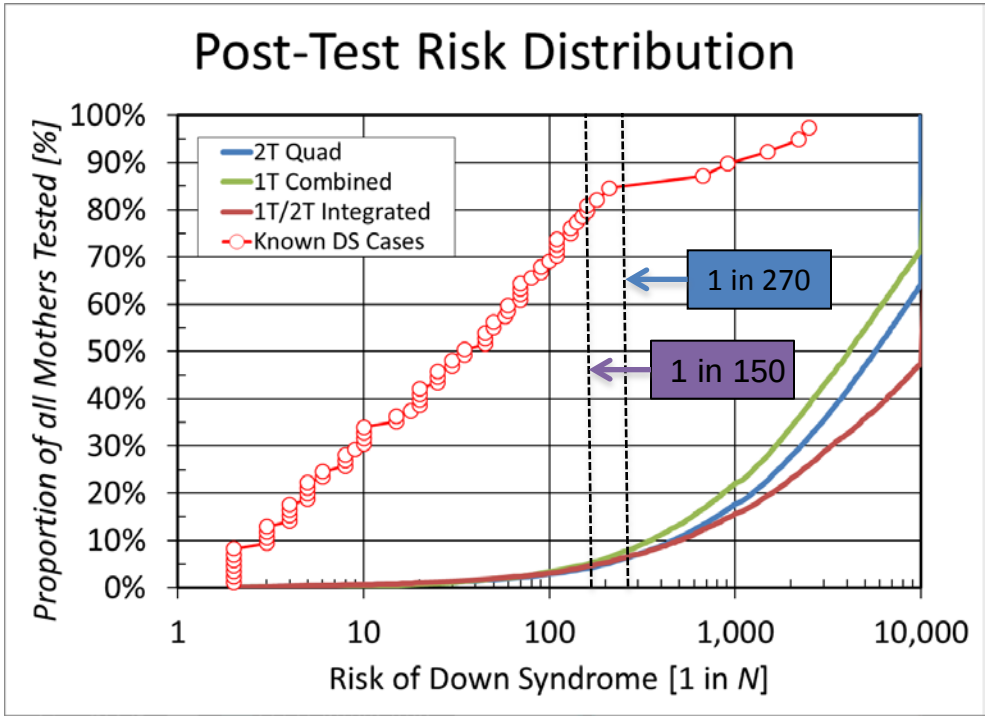
Cutoff	Initial positive rate (Quad test)	Detection rate (Quad test)
1 in 270	6.6%	86%

Selecting a Cutoff: What is Abnormal?

Two philosophies among U.S. laboratories

1. Use 1 in 270 (DS risk for a 35 yo) regardless of test panel

2. Use different cutoff for each test panel to lower the initial positive rate



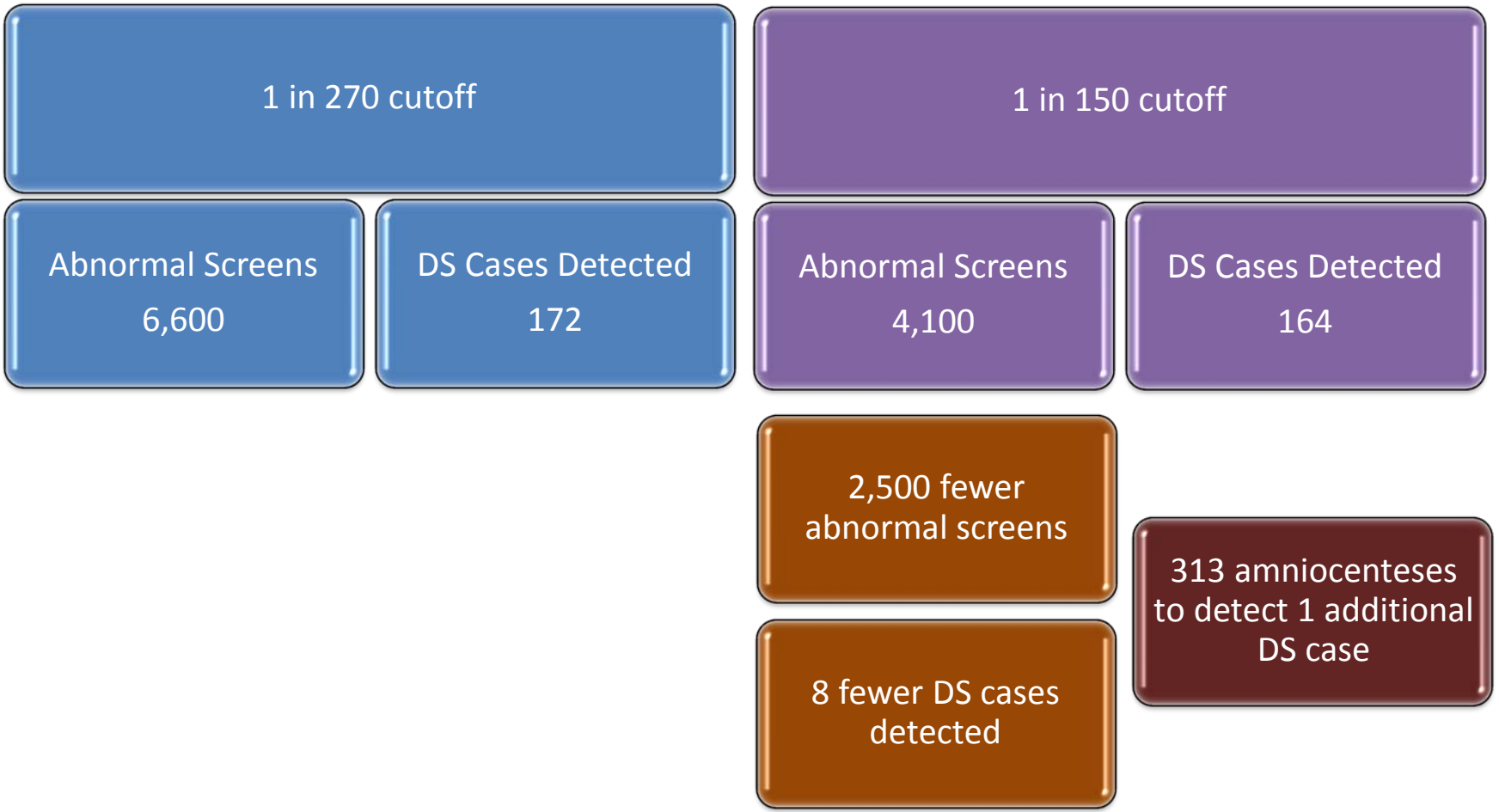
	Cutoff	Initial positive rate (Quad test)	Detection rate (Quad test)
Higher detection rate; more false-positives →	1 in 270	6.6%	86%
Lower detection rate; fewer false-positives →	1 in 150	4.1%	82%

Selecting a Cutoff: What is Abnormal?

100,000 women screened 200 DS cases expected (1 in 500 prevalence)		100,000 women screened 200 DS cases expected (1 in 500 prevalence)	
1 in 270 cutoff		1 in 150 cutoff	
Initial Positive Rate 6.6%	Detection Rate 86%	Initial Positive Rate 4.1%	Detection Rate 82%
Abnormal Screens 6,600	DS Cases Detected 172	Abnormal Screens 4,100	DS Cases Detected 164

Ashwood ER, unpublished data (2012)

Selecting a Cutoff: What is Abnormal?



Ashwood ER, unpublished data (2012)

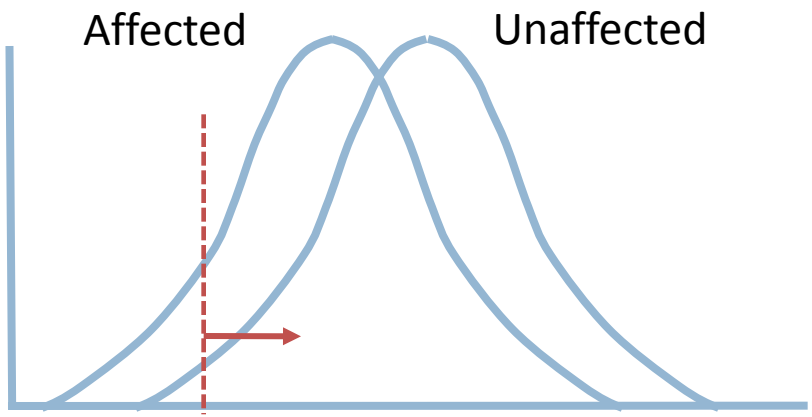
When Biochemical Screen is Abnormal: DO

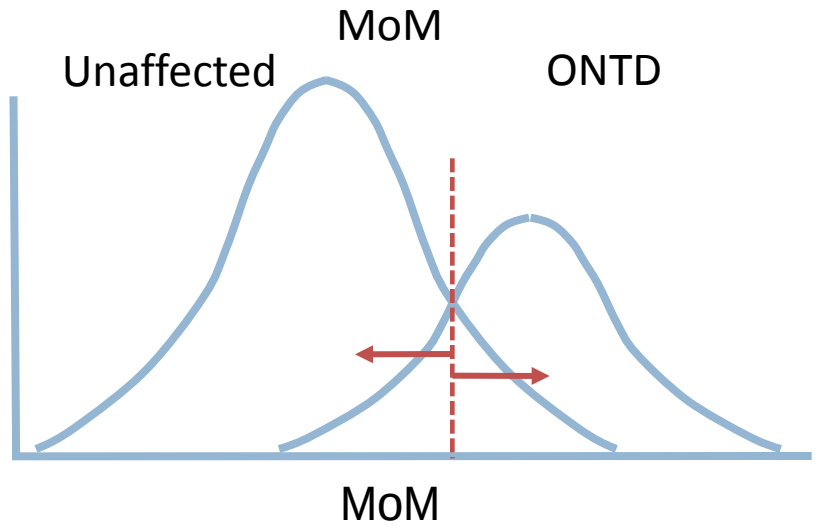
- Targeted ultrasound
  - Confirm GA (overestimated gives DS pattern)
  - Evaluate fetus for anomalies consistent with aneuploidy

- Offer diagnostic testing (fetal chromosomes)
  - 1st trimester: CVS
  - 2nd trimester: amniocentesis
  - Fetal loss rates vary (0.5-1.0%) and lowest in institutions that perform the frequently

When Biochemical Screen is Abnormal: DON'T

- Do not re-test! Regression towards the mean
  - Repeated measurements at tails of distribution return results closer to the population mean
  - Repeat testing will increase false-negative screens
- Okay to repeat if sample collected at <11 weeks (1st tri) or <14 weeks (2nd tri)





Which Biochemical Screening Test is Best?

Test Name	AFP	hCG	uE3	DIA	PAPP-A	NT	Trimester
Combined		✔			✔	✔	1st
Triple	✔	✔	✔				2nd
Quad	✔	✔	✔	✔			2nd
Integrated					✔	✔	1st
	✔	✔	✔	✔			2nd
Serum Integrated					✔		1st
	✔	✔	✔	✔			2nd
Sequential		✔			✔	✔	1st
	✔	✔	✔	✔			2nd

Which Biochemical Screening Test is Best?

First and second trimester antenatal screening for Down's syndrome: the results of the Serum, Urine and Ultrasound Screening Study (SURUSS)

N J Wald, C Rodeck, A K Hackshaw, J Walters, L Chitty, A M Mackinson

J Med Screen 2003; **10**:56–104

The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 NOVEMBER 10, 2005 VOL. 353 NO. 19

First-Trimester or Second-Trimester Screening, or Both, for Down's Syndrome

Fergal D. Malone, M.D., Jacob A. Canick, Ph.D., Robert H. Ball, M.D., David A. Nyberg, M.D., Christine H. Comstock, M.D., Radek Bukowski, M.D., Richard L. Berkowitz, M.D., Susan J. Gross, M.D., Lorraine Dugoff, M.D., Sabrina D. Craigo, M.D., Ilan E. Timor-Tritsch, M.D., Stephen R. Carr, M.D., Honor M. Wolfe, M.D., Kimberly Dukes, Ph.D., Diana W. Bianchi, M.D., Alicja R. Rudnicka, Ph.D., Allan K. Hackshaw, M.Sc., Geralyn Lambert-Messerlian, Ph.D., Nicholas J. Wald, F.R.C.P., and Mary E. D'Alton, M.D., for the First- and Second-Trimester Evaluation of Risk (FASTER) Research Consortium*

Which Biochemical Screening Test is Best?

PPVs at 85% DR

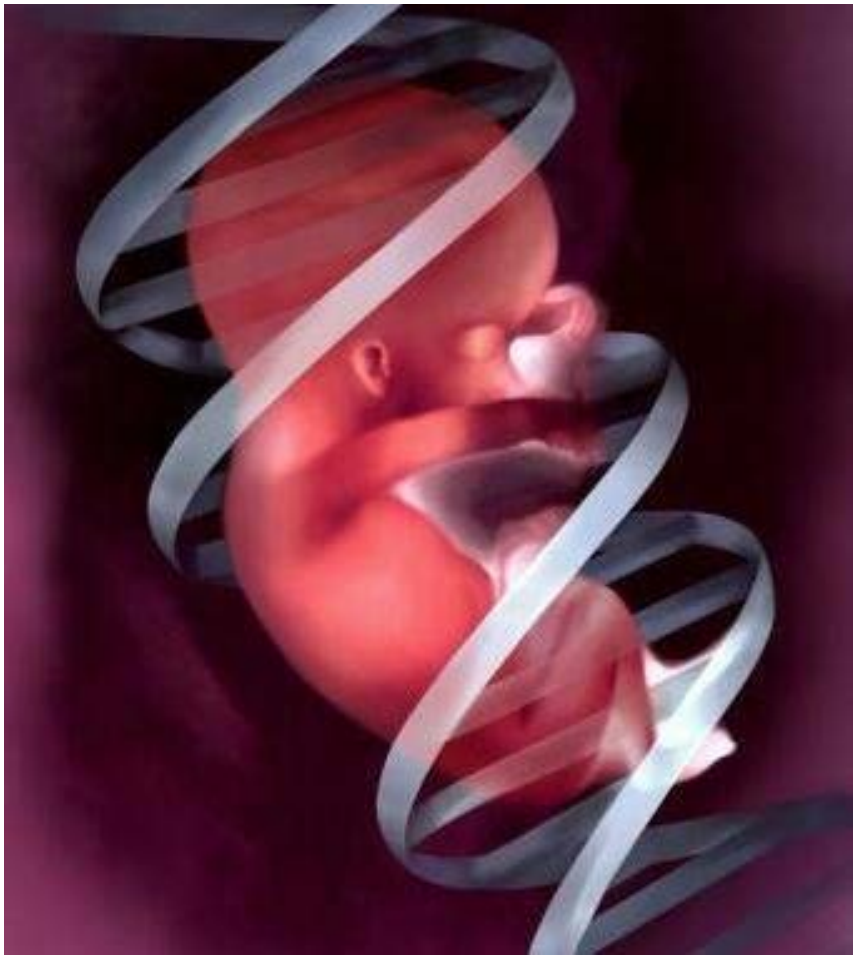
Integrated	17%
Serum Integrated	7%
Combined	3%
Quad	3%
Triple	2%

J Med Screen 2003;10:56-104

DNA-BASED SCREENING FOR ANEUPLOIDIES

Cell Free Fetal DNA in Maternal Blood

- Reported by Lo, et al. in 1997
- Derived primarily from the placenta and represents ~10% of total DNA circulating in maternal blood
- Screening tests that identify molecular pathology of aneuploidies



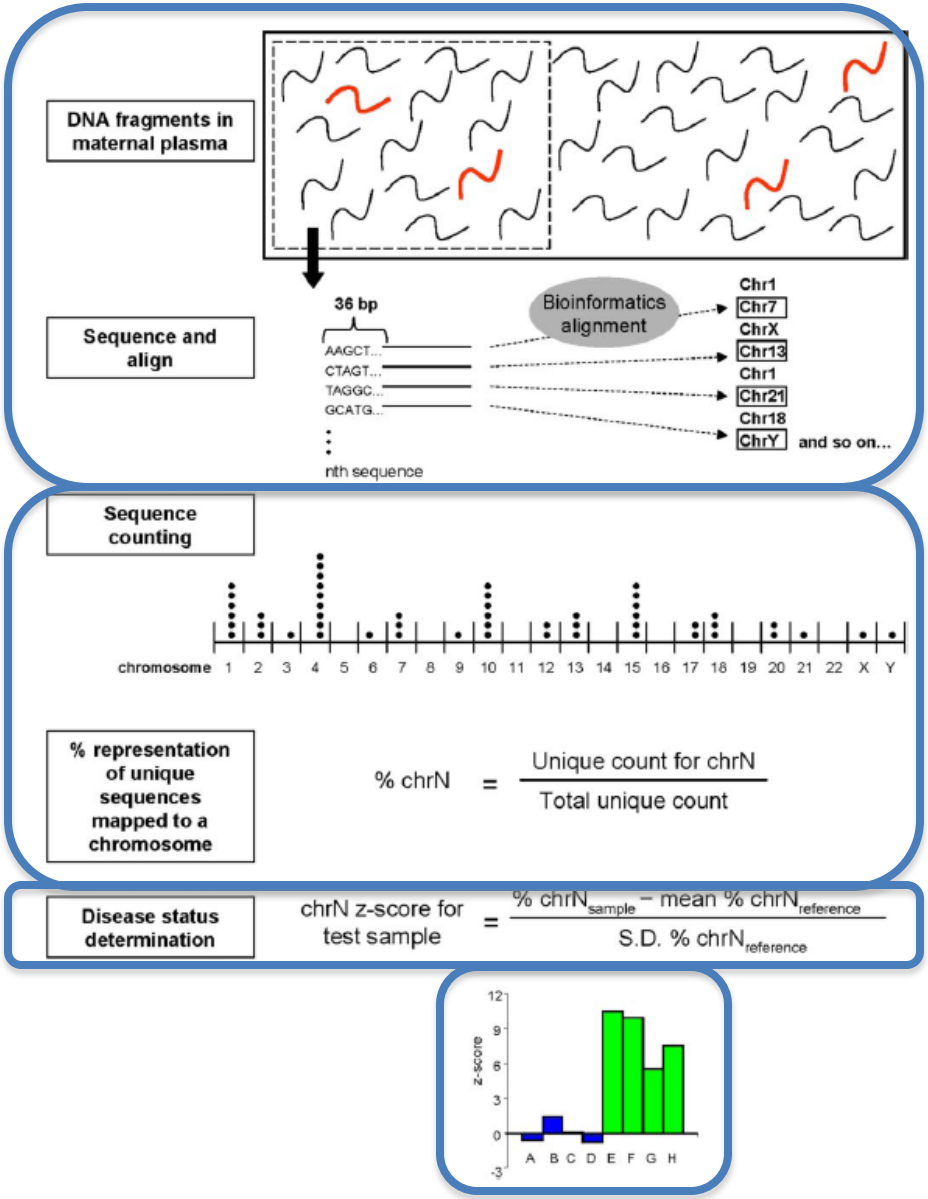
Commercially Available DNA-based Screening Tests

Company	Location	Product	Method
Ariosa Diagnostics, Inc.	San Jose, CA	Harmony™ Prenatal Test	Targeted SNPs
Natera, Inc.	San Carlos, CA	Parental Support™	Targeted sequencing
Sequenom Center for Molecular Medicine	San Diego, CA	MaterniT21™ Plus	MPSS
Verinata Health, Inc.	Redwood City, CA	Verifi® Prenatal Test	MPSS
MPSS: Massively parallel shotgun sequencing			

- Methods may differ but goal is the same
  - Identify extra copies of a specific chromosome

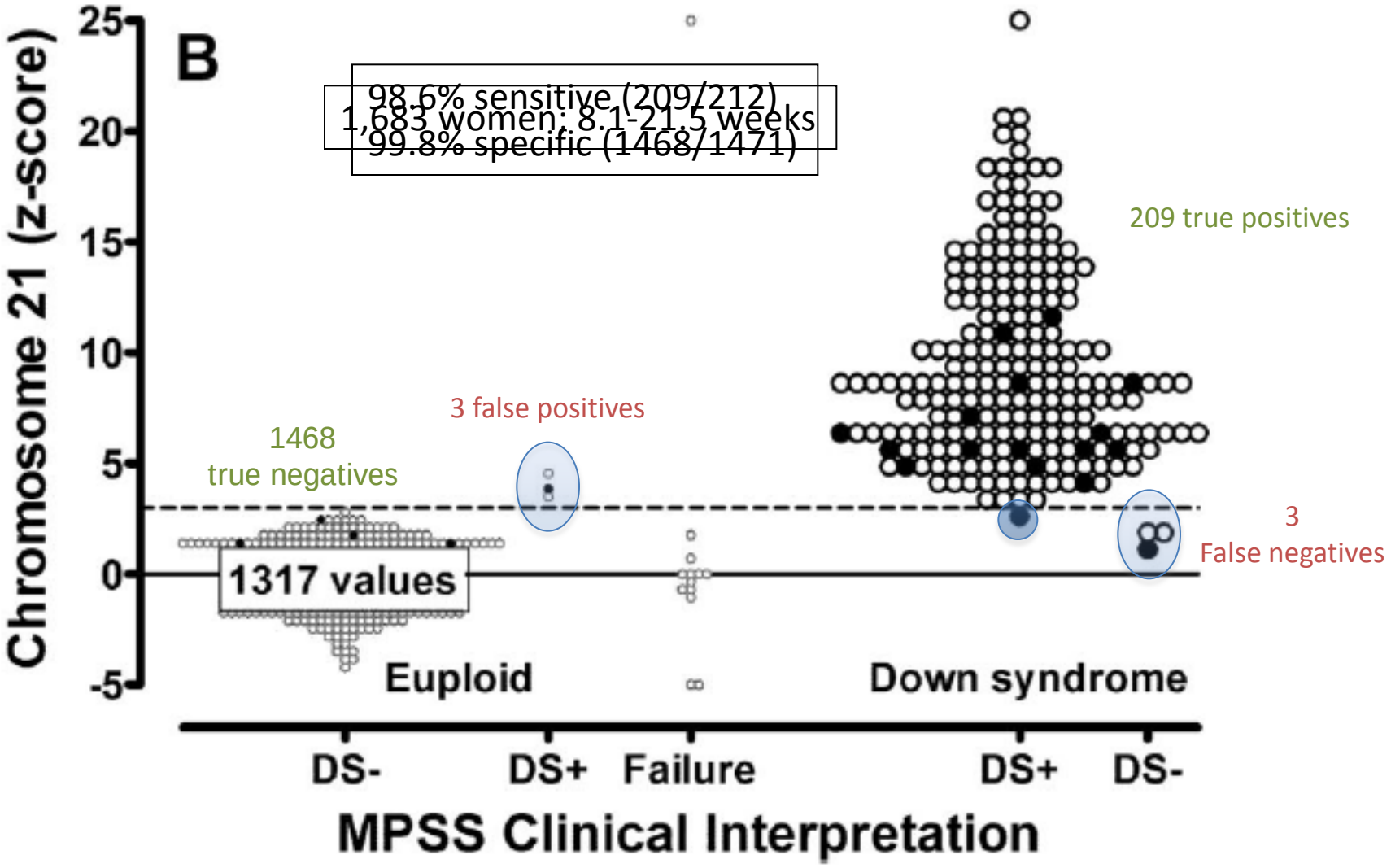
Massively Parallel Shotgun Sequencing

- 1st 36 bases of each DNA fragment sequenced and mapped to a specific chromosome
- Number of unique sequences are counted and expressed as percentage of all unique sequences (% chrN)
- Z-scores for each chromosome calculated and evaluated against a cutoff Z-score of +3



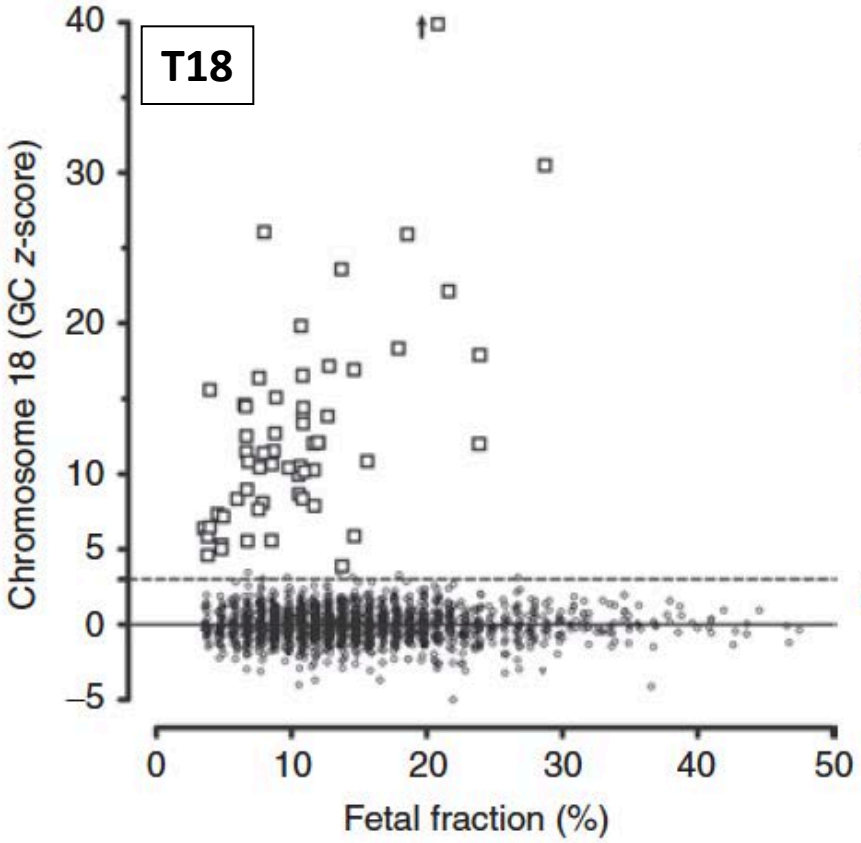
PNAS 2008;105:20458-20463

MPSS Clinical Performance (T21)

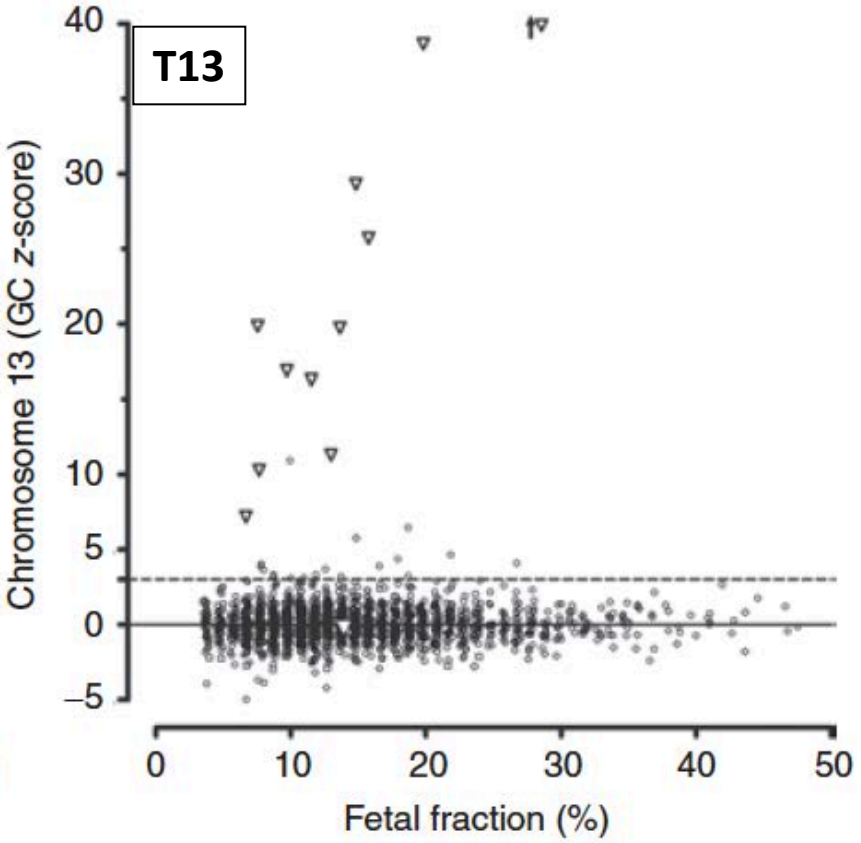


Genet Med 2011;13:913-920

MPSS Clinical Performance (T18 & 13)



100% sensitive (59/59)
99.7% specific (1683/1688)



92.3% sensitive (12/13)
99.1% specific (1672/1688)

Genet Med 2012;14:296-305

Clinical Performance of Commercially Available DNA-based Screening Tests

Company	Product	Detection Rate (%)			False-positive rate (%)
		T21	T18	T13	
Ariosa Diagnostics, Inc.	Harmony™ Prenatal Test	100	100	NA	<0.1
Natera, Inc.	Parental Support™	100	100	100	0
Sequenom Center for Molecular Medicine	MaterniT21™ Plus	99	100	92	0.3-1.0
Verinata Health, Inc.	Verifi® Prenatal Test	100	97	79	0

AJOG 2012;206:319.e1-319.e9
Genet Med 2011;13:913-920
Genet Med 2012;14:296-305
Obstet Gynecol 2012;119:890-901
Prenat Diagn 2012;32:1233-1241

If DNA-based testing is so good, should it be the primary screening test?

Clinical Scenario: General Population Screening

- Offer DNA-based testing to all pregnant women as the primary screening test

- Consider:
  - 100,000 women from the general pregnant population
  - T21 prevalence of 1 in 500

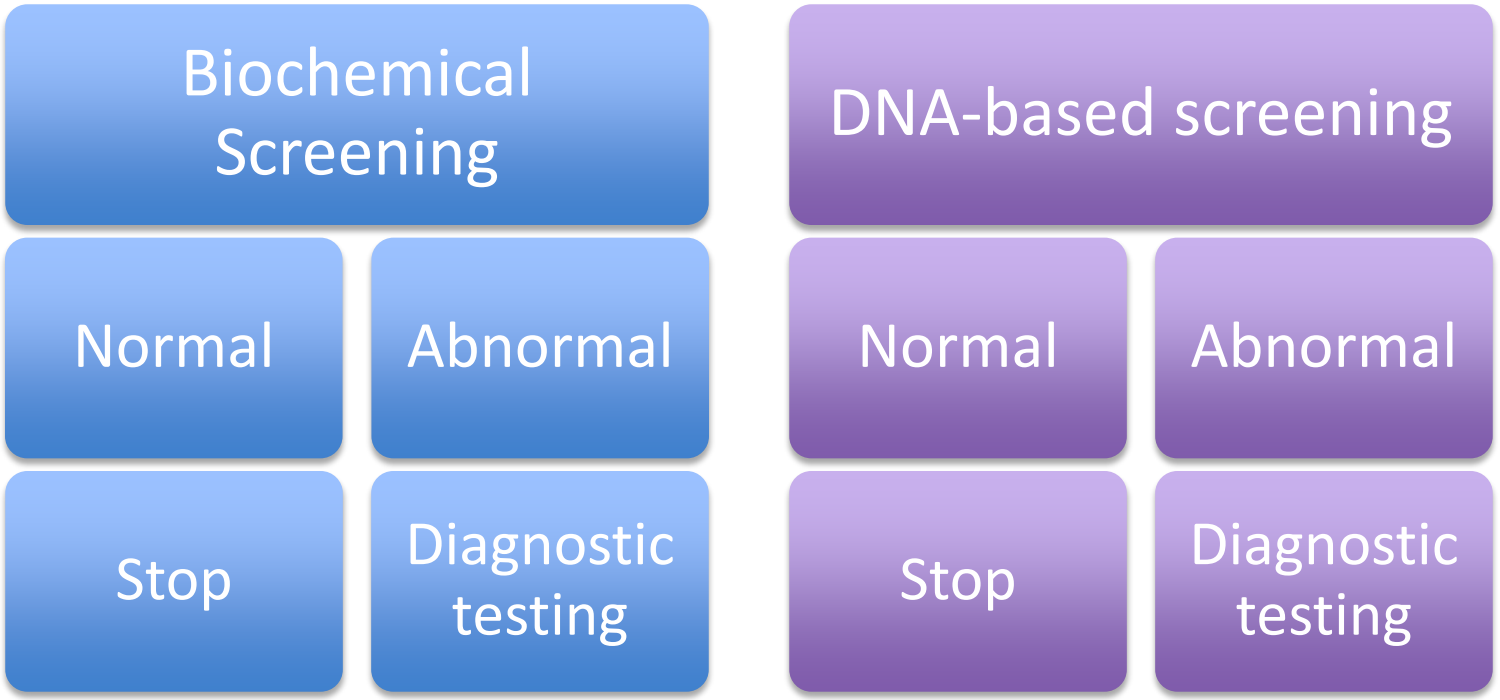
Biochemical vs. DNA-based Test as 1° Screen

	Quad
Number screened	100,000
T21 prevalence	1 in 500 (N=200)
Detection rate (%)	80
False positive rate (%)	5
T21 identified (N)	160 (out of 200)
False-positives (N)	4,990 (out of 99,800)
PPV (%)	3.1
Odds	1 to 31

Biochemical vs. DNA-based Test as 1° Screen

	Quad	DNA
Number screened	100,000	100,000
T21 prevalence	1 in 500 (N=200)	1 in 500 (N=200)
Detection rate (%)	80	99
False positive rate (%)	5	0.2
T21 identified (N)	160 (out of 200)	198 (out of 200)
False-positives (N)	4,990 (out of 99,800)	200 (out of 99,800)
PPV (%)	3.1	49.7%
Odds	1 to 31	1 to 1

Which Approach is Best?

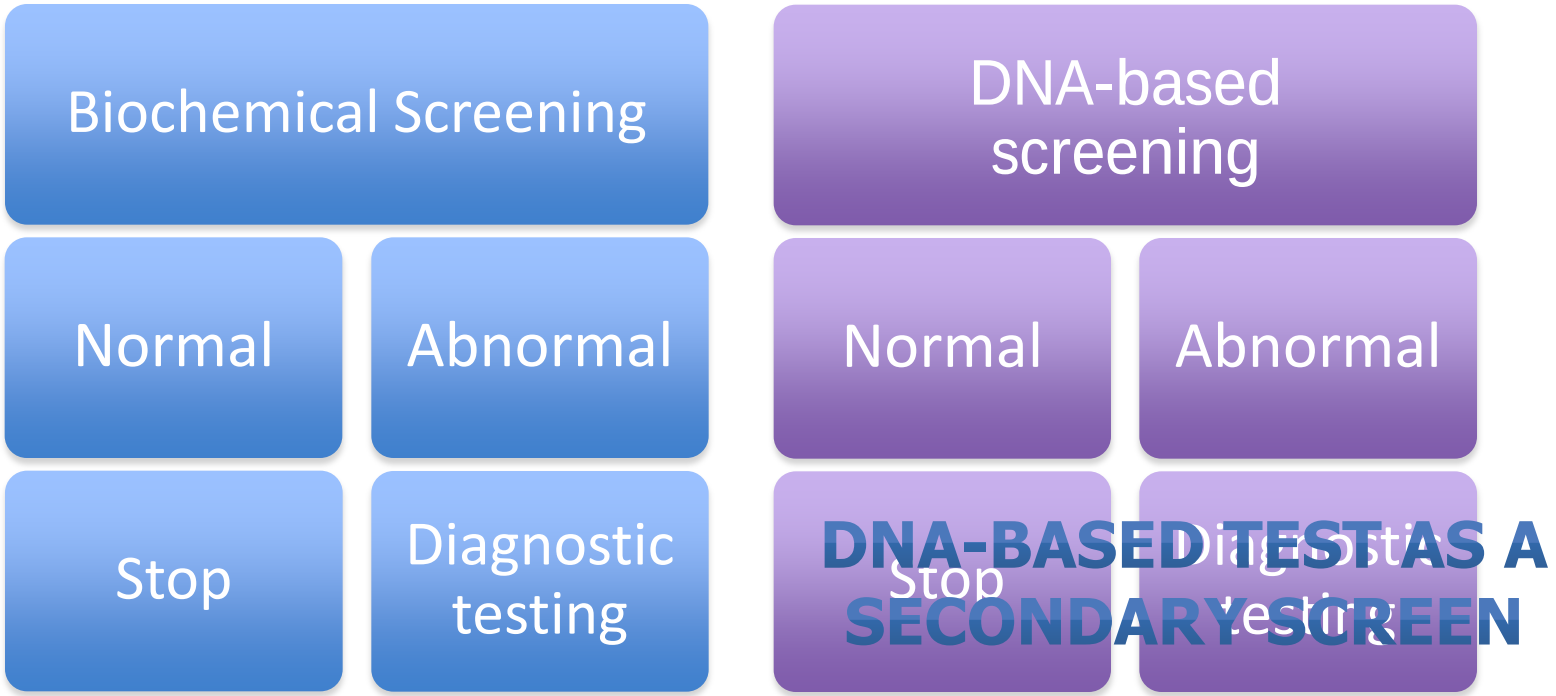


DNA-based Test as 1° Screen Dilemmas

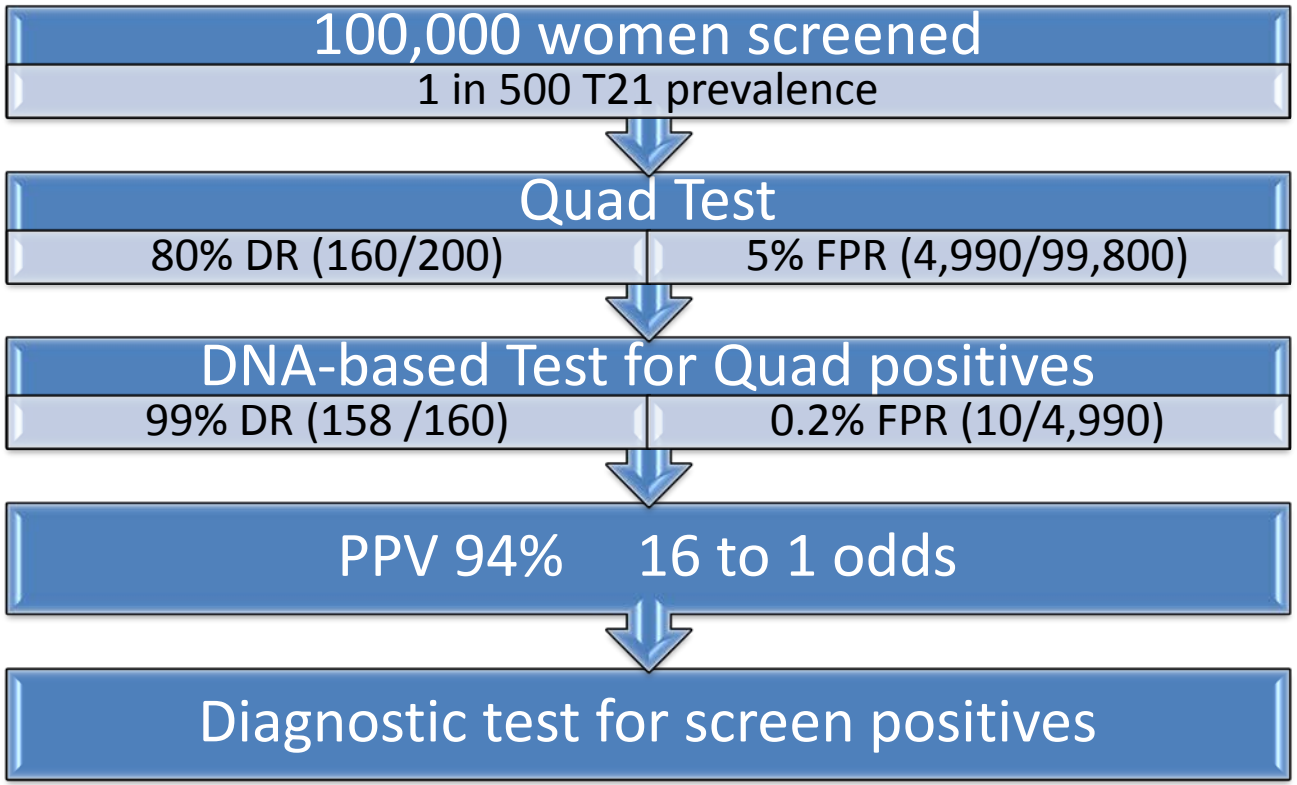
- All published studies have been performed in "high-risk" populations
  - Advanced maternal age
  - Prior affected pregnancy
  - High NT
  - Abnormal biochemical screening test

- Practical considerations
  - Limited availability
  - Longer TAT compared to biochemical screening
  - High costs (>$1,000)
  - Lack of insurance coverage

Which Approach is Best?



Quad First then DNA



Is this likely to change over time and with more evidence?

YES

The American College of Obstetricians and Gynecologists
WOMEN'S HEALTH CARE PHYSICIANS

The Society for Maternal-Fetal Medicine

COMMITTEE OPINION

Number 545 • December 2012

Noninvasive Prenatal Testing for Fetal Aneuploidy

Box 1. Indications for Considering the Use of Cell Free Fetal DNA ⇦

- Maternal age 35 years or older at delivery
- Fetal ultrasonographic findings indicating an increased risk of aneuploidy
- History of a prior pregnancy with a trisomy
- Positive test result for aneuploidy, including first trimester, sequential, or integrated screen, or a quadruple screen.
- Parental balanced robertsonian translocation with increased risk of fetal trisomy 13 or trisomy 21.

Summary

- Classic prenatal screening combines biochemical and US markers to identify pregnant women at increased risk for having a baby with an open neural tube defect, Down syndrome, or trisomy 18

- More conservative cutoffs decrease the number of abnormal biochemical screening tests and results in fewer unnecessary diagnostic tests

- DNA-based screening tests have excellent aneuploidy detection rates and can enhance the value of biochemical testing

Self-Assessment Questions

1. What is the AFP MoM at 20 weeks of gestation (median 40 ng/mL) in a woman with a serum AFP concentration of 120 ng/mL?
   A. 0.3
   B. 0.5
   C. 2.0
   D. 3.0

2. Changes to the Down syndrome risk cutoff has the most dramatic effect on what parameter?
   A. Detection rate
   B. Number of abnormal screen results
   C. Percentage of normal screen results
   D. Biomarker MoM

3. A primary advantage of DNA-based aneuploidy screening tests compared to biochemical screening tests is:
   A. They can be performed in the first trimester
   B. They do not rely on the NT measurement
   C. They are widely available
   D. They have a higher detection rate

Marker Dependent LR

- Determine the "marker dependent" likelihood ratio by calculating H_{DS} and $H_{Unaffected}$

$$H = \frac{1}{\Pi\sigma(2\pi)^{p/2} \cdot \det(\mathbf{R})^{1/2}} \exp\left[-\frac{\mathbf{Z}^T\mathbf{R}^{-1}\mathbf{Z}}{2}\right]$$

- LR = H_{DS} divided by $H_{unaffected}$