The Science of Screening in Relation to Pregnancy

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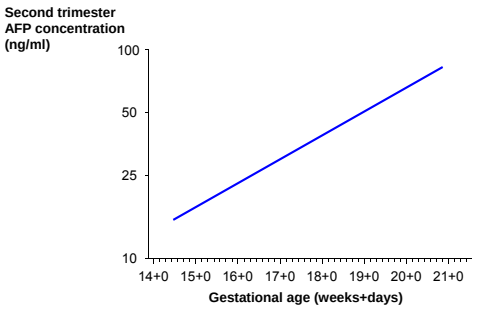
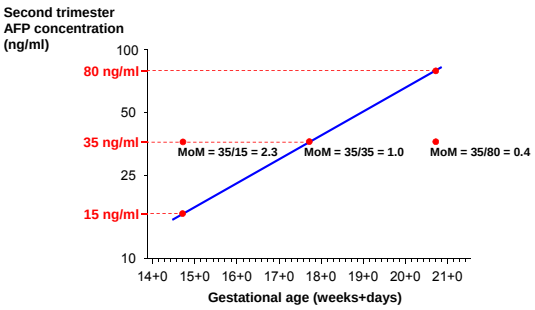
Central to prenatal screening is the multiple of the median (MoM)

Multiple of the median (MoM)

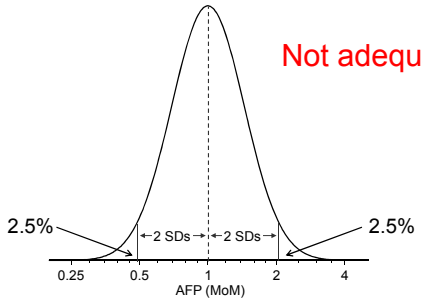


Multiple of the median (MoM)



Maternal serum AFP

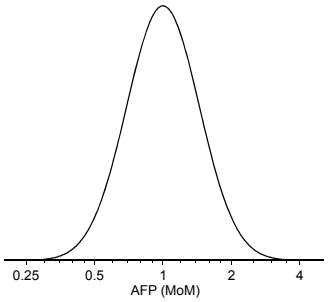
- AFP "normal" range



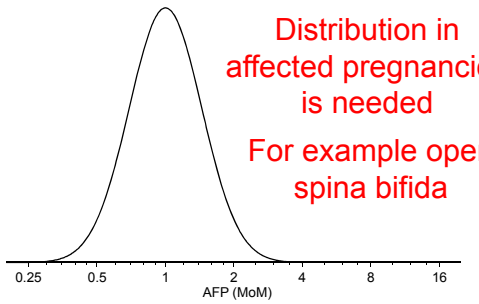
SD, standard deviation

Maternal serum AFP

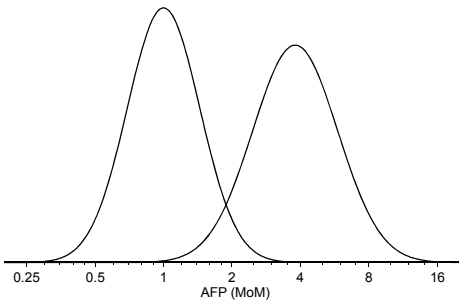
- Reflects distribution in unaffected pregnancies



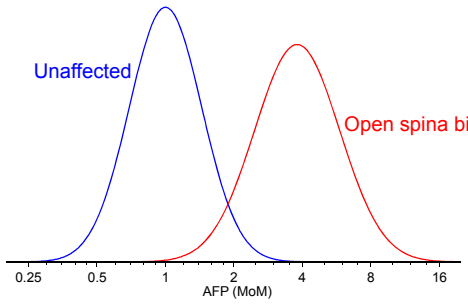
Maternal serum AFP in unaffected and affected pregnancies



Maternal serum AFP in unaffected and affected pregnancies

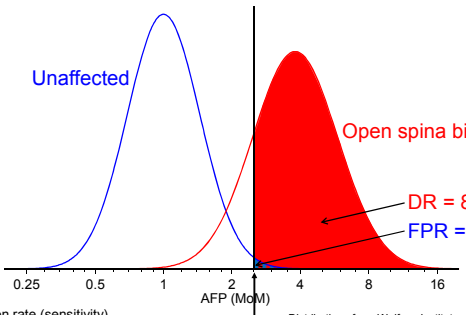


Maternal serum AFP in unaffected and open spina bifida pregnancies



Distributions from Wolfson Institute and Wald et al. *J Epidemiol Community Health* 1982;**36**:87-95

Maternal serum AFP in unaffected and open spina bifida pregnancies

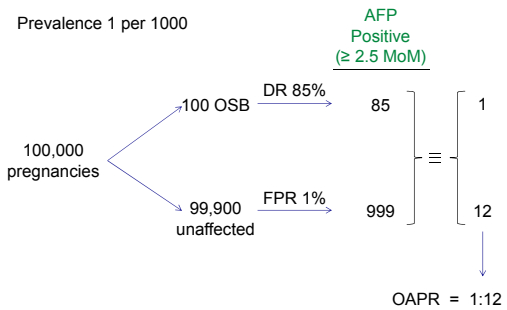


DR, detection rate (sensitivity)
FPR, false-positive rate (1-specificity)

Distributions from Wolfson Institute and Wald et al. *J Epidemiol Community Health* 1982;**36**:87-95

Estimating the odds of being affected given a positive result (OAPR)

- Prevalence 1 per 1000

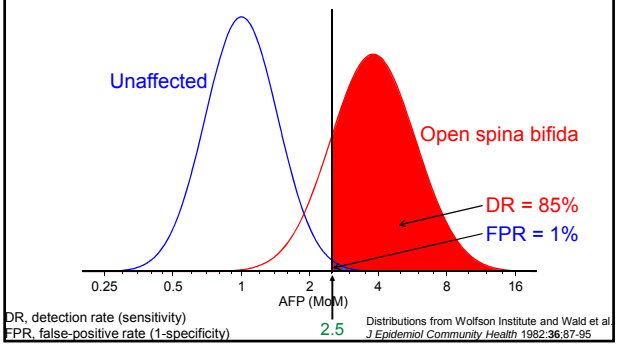


OSB, open spina bifida

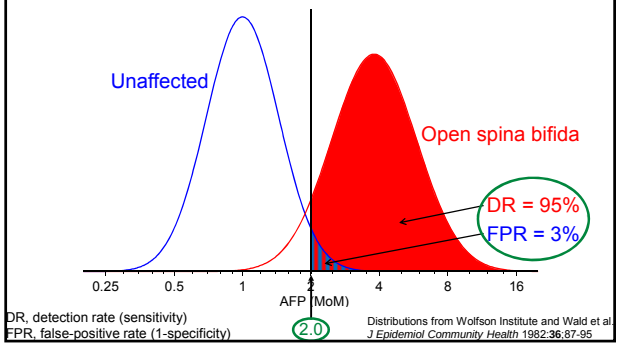
Detection rate and false-positive rate

- There is a trade-off between the detection rate and the false-positive rate
- Specifying one without the other is meaningless

Maternal serum AFP in unaffected and open spina bifida pregnancies

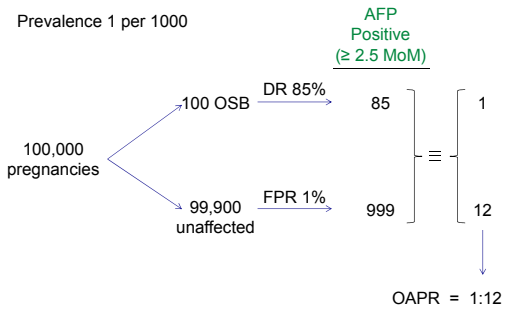


Maternal serum AFP in unaffected and open spina bifida pregnancies



Estimating the odds of being affected given a positive result (OAPR) using 2.0 MoM

- Prevalence 1 per 1000



OSB, open spina bifida

Estimating the odds of being affected given a positive result (OAPR) using 2.0 MoM

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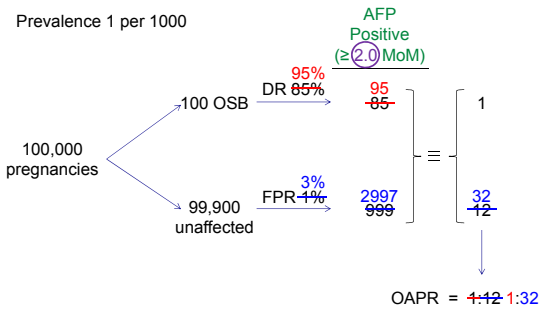


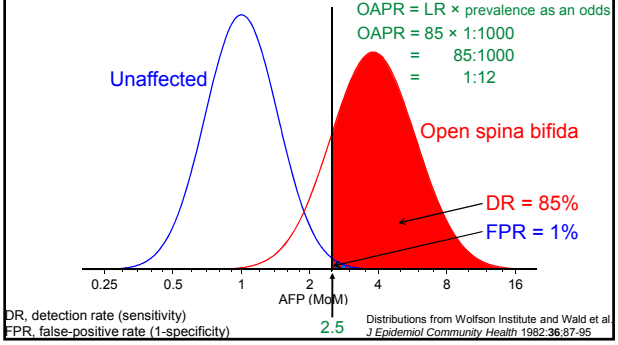
OSB, open spina bifida

Calculating the odds of being affected given a positive results (OAPR) using the likelihood ratio (LR)

$LR = \frac{DR}{FPR} = \frac{85}{1} = 85$

OAPR = LR × prevalence as an odds

OAPR = 85 × 1:1000
= 85:1000
= 1:12

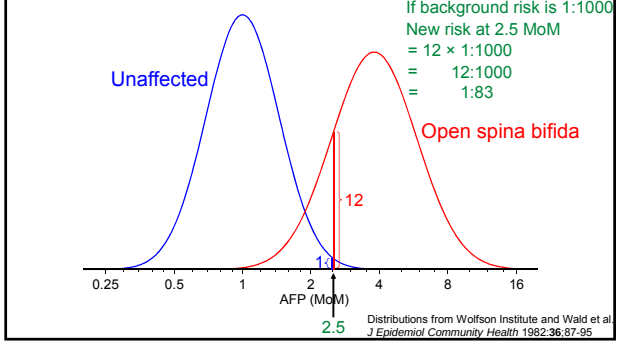


Calculating a woman's individual risk at 2.5 MoM

$LR = \frac{12}{1} = 12$

If background risk is 1:1000

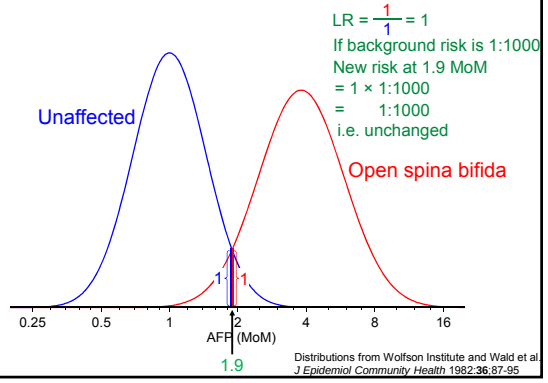
New risk at 2.5 MoM
= 12 × 1:1000
= 12:1000
= 1:83



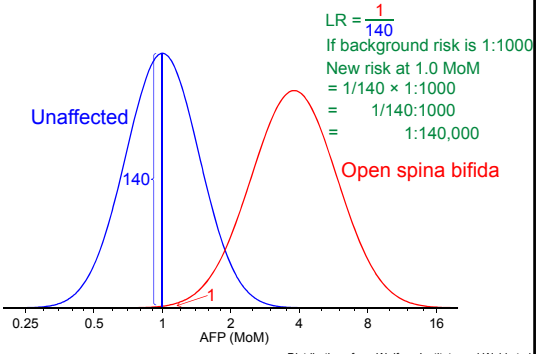
Problem

If a woman is told that her risk of having an open spina bifida pregnancy is quite 'normal', that is, the risk is no higher and no lower than the risk for women in general, what is her maternal serum AFP level?

AFP MoM value for woman with risk no higher or lower than for women in general



AFP MoM value for woman with risk no higher or lower than for women in general



Multiple marker screening

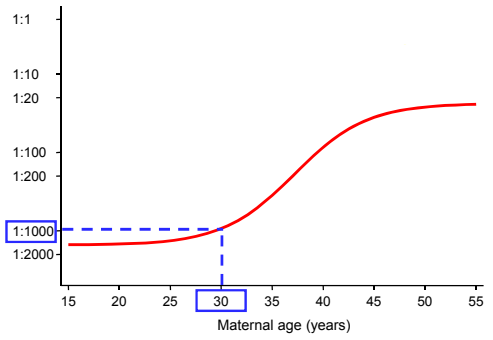
Multiple marker screening

- A "common currency" is needed that can combine the screening effect of different markers
- Not mass units or MoMs
- It is the risk of having an affected pregnancy

Example

Estimating the risk of a 30 year old woman with a serum AFP value of 0.50 MoM having a pregnancy with Down's syndrome

Risk of Down's syndrome and maternal age



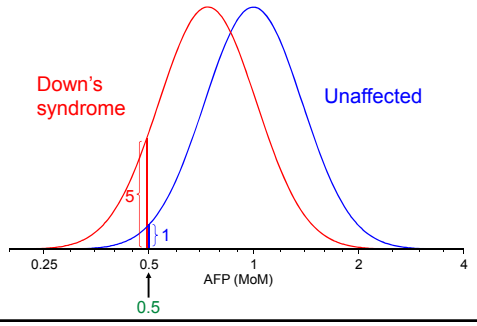
Morris et al *J Med Screen* 2002; **9**,2-6

Example

Estimating the risk of a 30 year old woman with a serum AFP value of 0.50 MoM having a pregnancy with Down's syndrome

Likelihood ratio (LR) for AFP MoM of 0.5

LR = 5 (approximately)



Risk for 30 year old woman with a serum AFP of 0.5 MoM is:-

= LR_{AFP} × Age specific risk

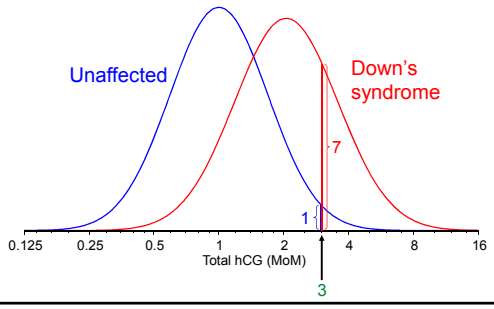
= 5 × 1:1000 = 1:200

The woman also has an hCG level of 3 MoM.

What is the new risk?

Likelihood ratio (LR) for hCG MoM of 3

LR = 7 (approximately)



Likelihood ratio (LR) for AFP MoM of 0.5 and hCG MoM of 3

If the woman has LR's of

5 for AFP and

7 for hCG then

Combined LR = 5 × 7 = 35

(If the two tests are independent)

Risk for 30 year old woman with a serum AFP of 0.5 MoM and a hCG of 3 MoM is:-

$= LR_{AFP\ \&\ hCG} \times$ Age specific risk

= 35 × 1:1000 = 1:30

Independence of the screening markers

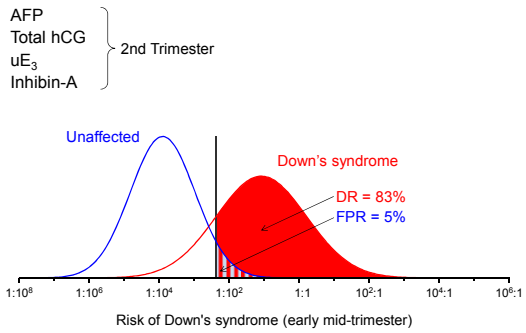
- In practice the markers are not independent. For AFP and hCG the following formula is used

$$LR = \frac{\frac{1}{SD_{AFP_{DS}} \times SD_{hCG_{DS}} \times 2\pi \times \sqrt{\det(R_{DS})}} \times e^{-0.5 \times Z_{DS}^{T} \times R_{DS}^{-1} \times Z_{DS}}}{\frac{1}{SD_{AFP_{Un}} \times SD_{hCG_{Un}} \times 2\pi \times \sqrt{det(R_{Un})}} \times e^{-0.5 \times Z_{Un}^{T} \times R_{Un}^{-1} \times Z_{Un}}}$$

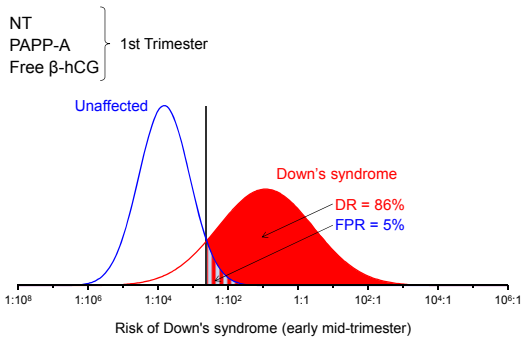
- But if correlations are small, product of LRs will be approximately correct

DS, Down's syndrome; Un, unaffected
SD, standard deviation
R, correlation matrix; det(R), determinant of correlation matrix; R^{-1}, inverse of correlation matrix
Z, matrix of AFP and hCG MoMs minus means, divided by SD; Z^{T}, transpose of matrix

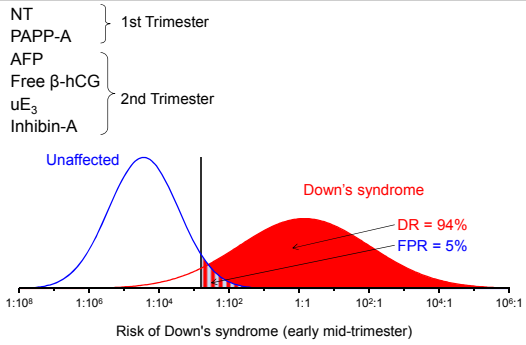
Distributions of risk estimates using the Quadruple test



Distributions of risk estimates using the Combined test



Distributions of risk estimates using the Integrated test



Free DNA measurement as a screening marker

DNA from chromosome 21 in maternal plasma

1.3% of total DNA is on chromosome 21

About 15% of the DNA in maternal plasma is fetal

Chromosome 21 DNA fragments in maternal plasma

	From mother	From fetus	Total
Unaffected	85	15	100
Down's syndrome	85	x 1.5 22.5	107.5

Relative amount in a Down's syndrome pregnancy = 107.5/100 = 1.075

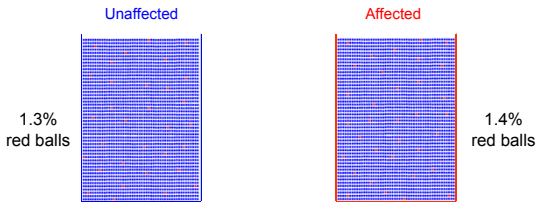
DNA from chromosome 21 in maternal Plasma

Unaffected pregnancy 1.3%

Down's syndrome pregnancy 1.3% x 1.075 = 1.4%

The conceptual challenge: Distinguishing 1.4% from 1.3%

Imagine 2 bins

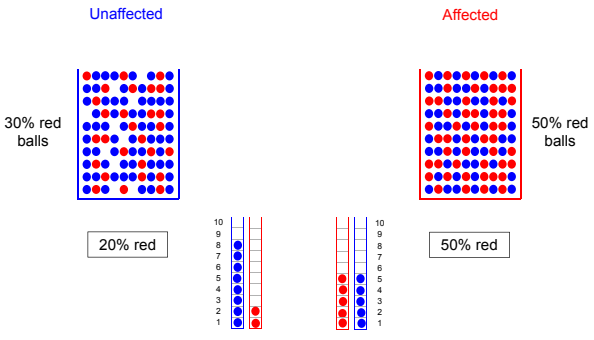


If all balls were counted there would be no distribution – the result would be the correct answer

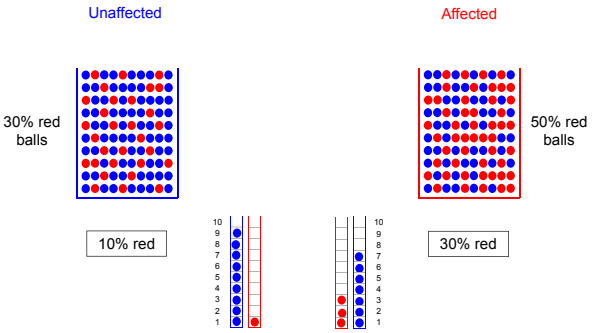
Distinguishing 1.4% from 1.3%

- The distributions arise from sampling
- To illustrate this assume a bigger difference
  - 50% and 30% instead of 1.4% and 1.3%
  - Reduce number of balls to 100
  - Sample 10 balls

Sample 10 balls (DNA fragments)



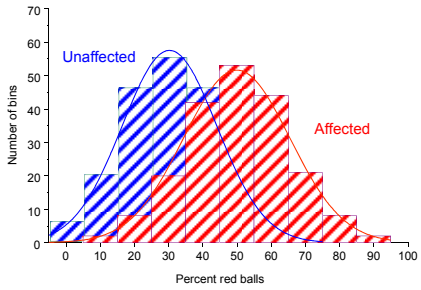
Sample 10 balls (DNA fragments)



100 balls in an 'affected' bin and 100 balls in an 'unaffected' bin: results from sampling 10 balls from each bin 200 times

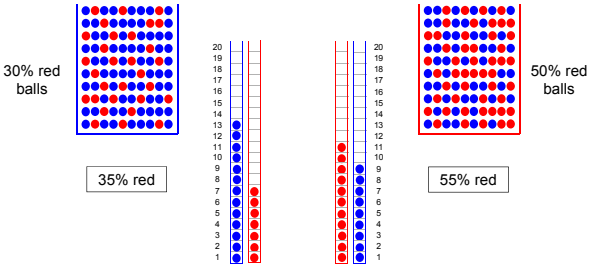
	Percent red balls										
	0	10	20	30	40	50	60	70	80	90	100
Unaffected (200 samples)	6	20	46	55	46	20	5	2	0	0	0
Affected (200 samples)	0	2	8	20	42	53	44	21	8	2	0

Converting this into 2 histograms



The overlap between the 2 distributions can be decreased by sampling more balls eg 20 instead of 10 balls

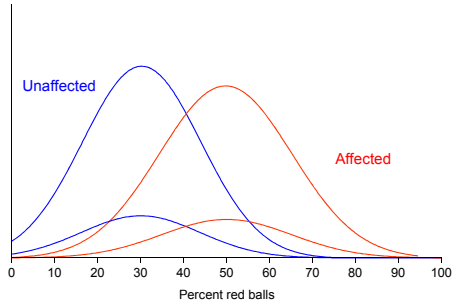
Sampling 20 instead of 10



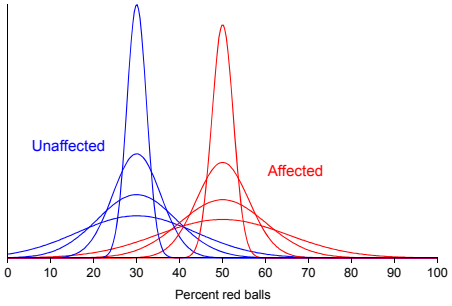
100 balls in an 'affected' bin and 100 balls in an 'unaffected' bin: results from sampling 20 balls from each bin 200 times

	Percent red balls																				
	0	5	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100
10 balls drawn from 100 balls																					
Unaffected (200 samples)	6	-	20	-	46	-	55	-	46	-	20	-	5	-	2	-	0	-	0	-	0
Affected (200 samples)	0	-	2	-	8	-	20	-	42	-	53	-	44	-	21	-	8	-	2	-	0
20 balls drawn from 100 balls																					
Unaffected (200 samples)	0	4	5	16	18	38	46	34	18	13	6	2	0	0	0	0	0	0	0	0	0
Affected (200 samples)	0	0	0	0	2	3	6	16	27	22	40	25	30	17	7	4	1	0	0	0	0

100 balls in an 'affected' bin and 100 balls in an 'unaffected' bin: results from sampling 10 balls from each bin 200 times



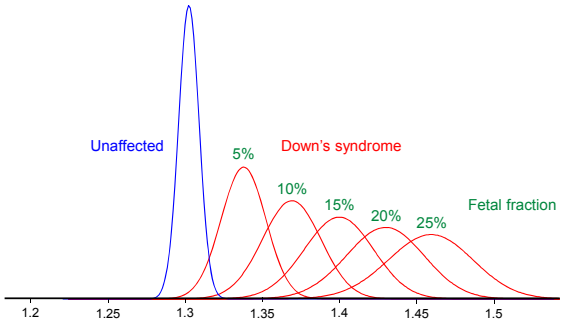
100 balls in an 'affected' bin and 100 balls in an 'unaffected' bin: results from sampling 20 balls from each bin 200 times



From balls to DNA

- Instead of balls; DNA fragments
- Instead of bins; pregnancies
- Percent DNA on Ch21; 1.3% in unaffected
  1.4% in affected
- Very large number of DNA fragments need to be sampled – about 10 million

Distributions of DNA fragments from chromosome 21 in affected and unaffected pregnancies



Canick et al. *Prenat Diagn* 2013;33:667-74

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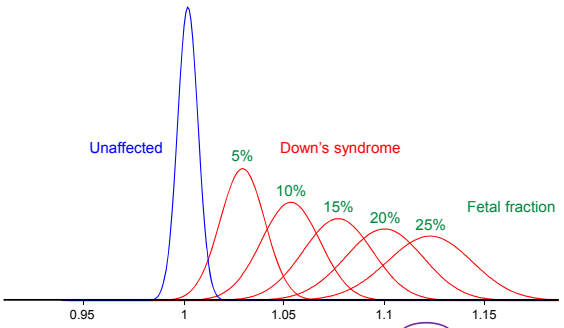

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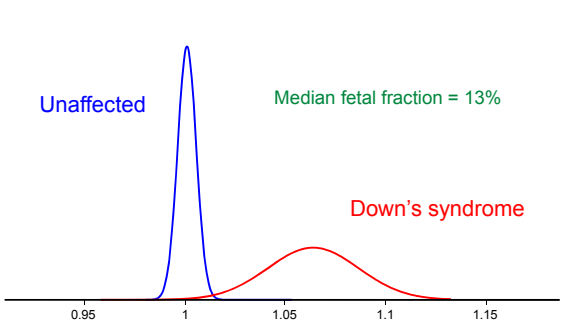
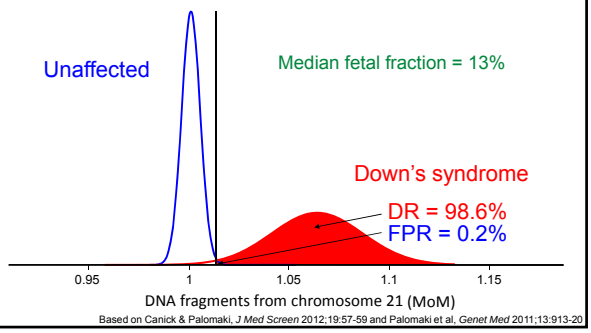
Distributions of DNA fragments from chromosome 21 Counting about 10 million fragments



Based on Canick & Palomaki, *J Med Screen* 2012;19:57-59

Distributions of DNA fragments from chromosome 21 Counting about 10 million fragments



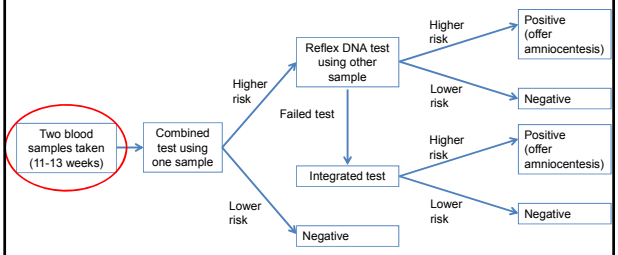
Based on Canick & Palomaki, *J Med Screen* 2012;19:57-59 and Palomaki et al, *Genet Med* 2011;13:913-20

Main issues with DNA testing

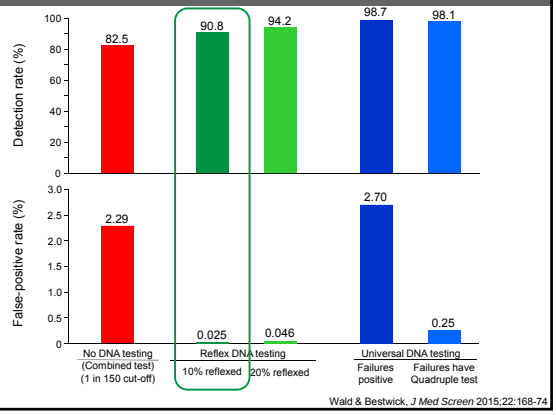
- Test failures – about 2-9%.
  - Could be reduced by at least half with a 2nd sample
- High cost
- Time taken to complete test – 7 to 14 days

An approach that can overcome these issues

Reflex DNA testing



Screening performance according to policy

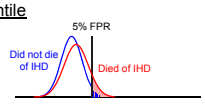
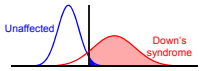
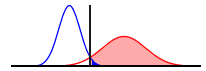
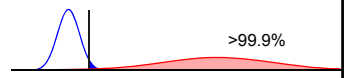


Wald & Bestwick, *J Med Screen* 2015;22:168-74

Cautionary note

Risk factors with relative risks less than 50 do not translate into good screening markers

Examples

Screening test	Relative risk: top vs bottom quintile		DR for 5% FPR
Cholesterol / ischaemic heart disease	3		15%
Combined test / Down's syndrome	660		88%
Integrated test / Down's syndrome	6,700		96%
DNA test / Down's syndrome	2,600,000		>99.9%

Wald NJ, Morris JK. *Arch Intern Med* 2011:171;286-291
http://www.wolfson.qmul.ac.uk/cepm/rsc

Key Points

7. In DNA screening "reflexing" avoids causing needless worry

8. Causal risk factors usually make poor screening tests

1. Avoid "normal" ranges

6. The high performance of DNA screening is due to counting many molecules rather than measuring concentrations that naturally overlap in affected and unaffected pregnancies

The application of science to screening in pregnancy has much to offer

2. Specify distributions in affected and unaffected pregnancies separately

5. Risk is the common currency for multiple marker screening

4. Distinguish between LR for population and LR for individuals

3. DR without FPR is meaningless