
بنام خدا

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Fetal Cell Free DNA in Maternal Blood

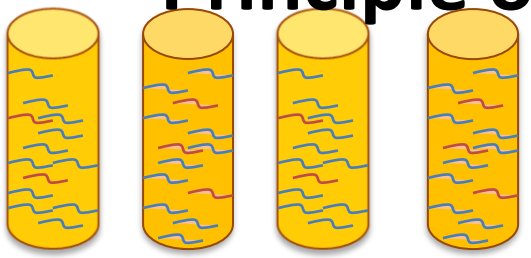
Cell-free DNA testing in maternal blood provides the most

effective method of screening for trisomy 21, with a reported

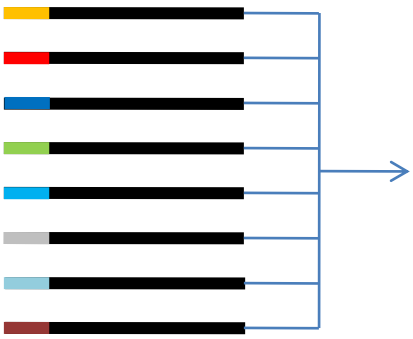
detection rate of 99% and a false positive rate of less than 0.1%.

The combined test (nuchal translucency measurement with serum protein markers) has been the recommended NHS method of screening for chromosomal abnormalities, with a Down syndrome detection rate of nearly 90% for a 3% positive rate.

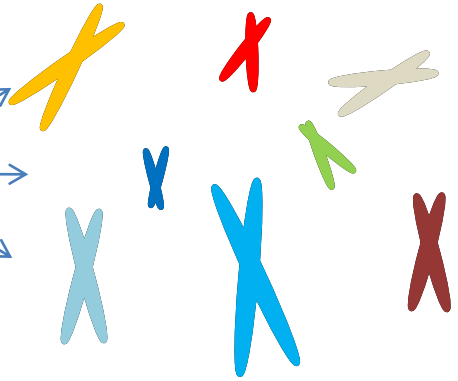
Principle of NIFTY

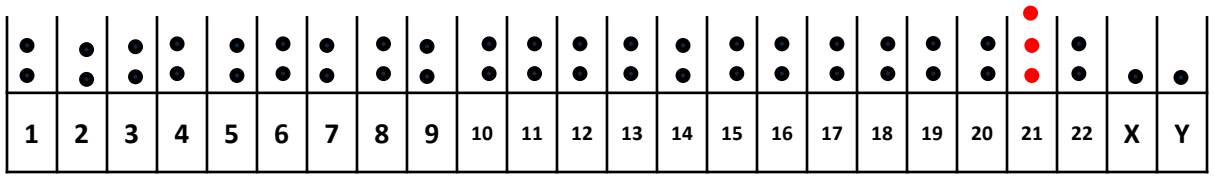


50 bp



Bioinformatics





$$\%chrN = \frac{\text{fragments for chrN}}{\text{total fragments}}$$

Plasma cf-DNA extraction

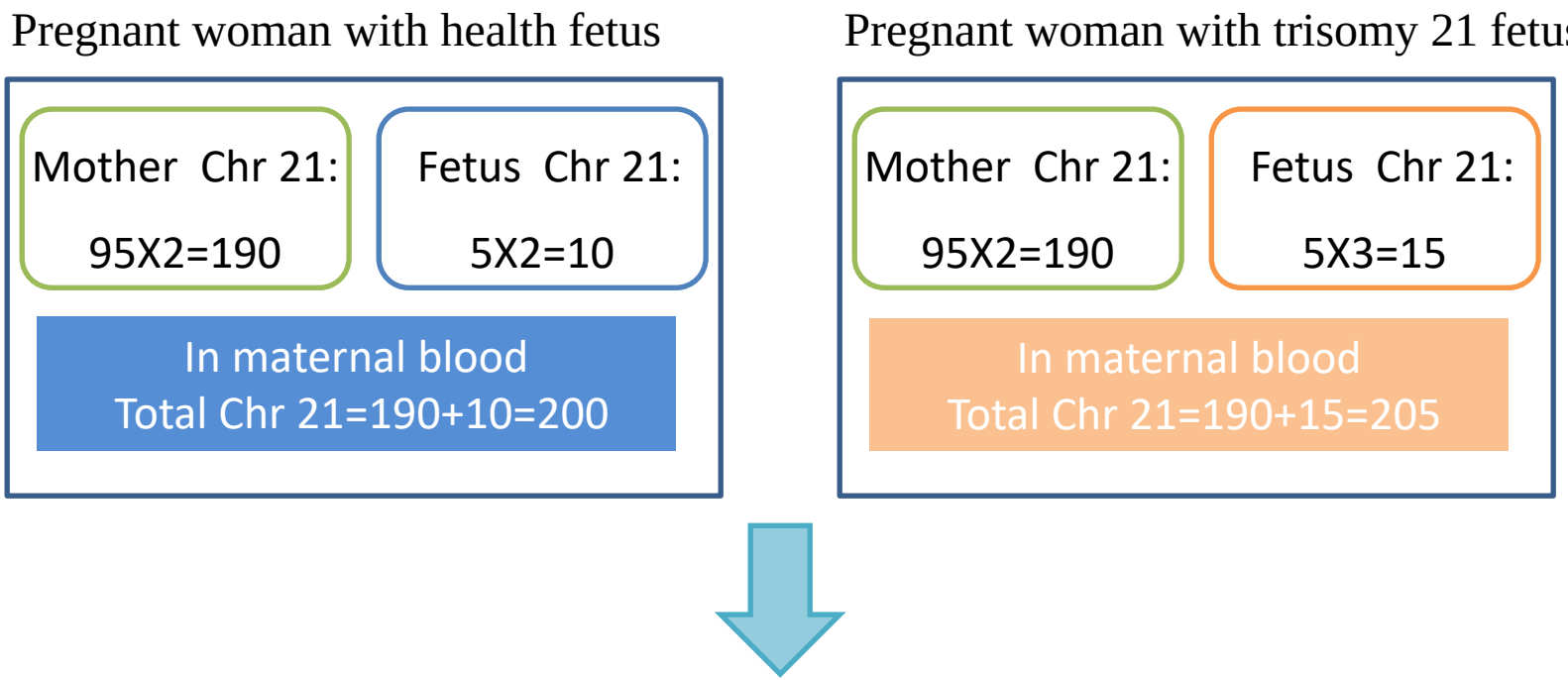
Sequencing & alignment

Chromosomes counting

Coverage computation

Statistic Model

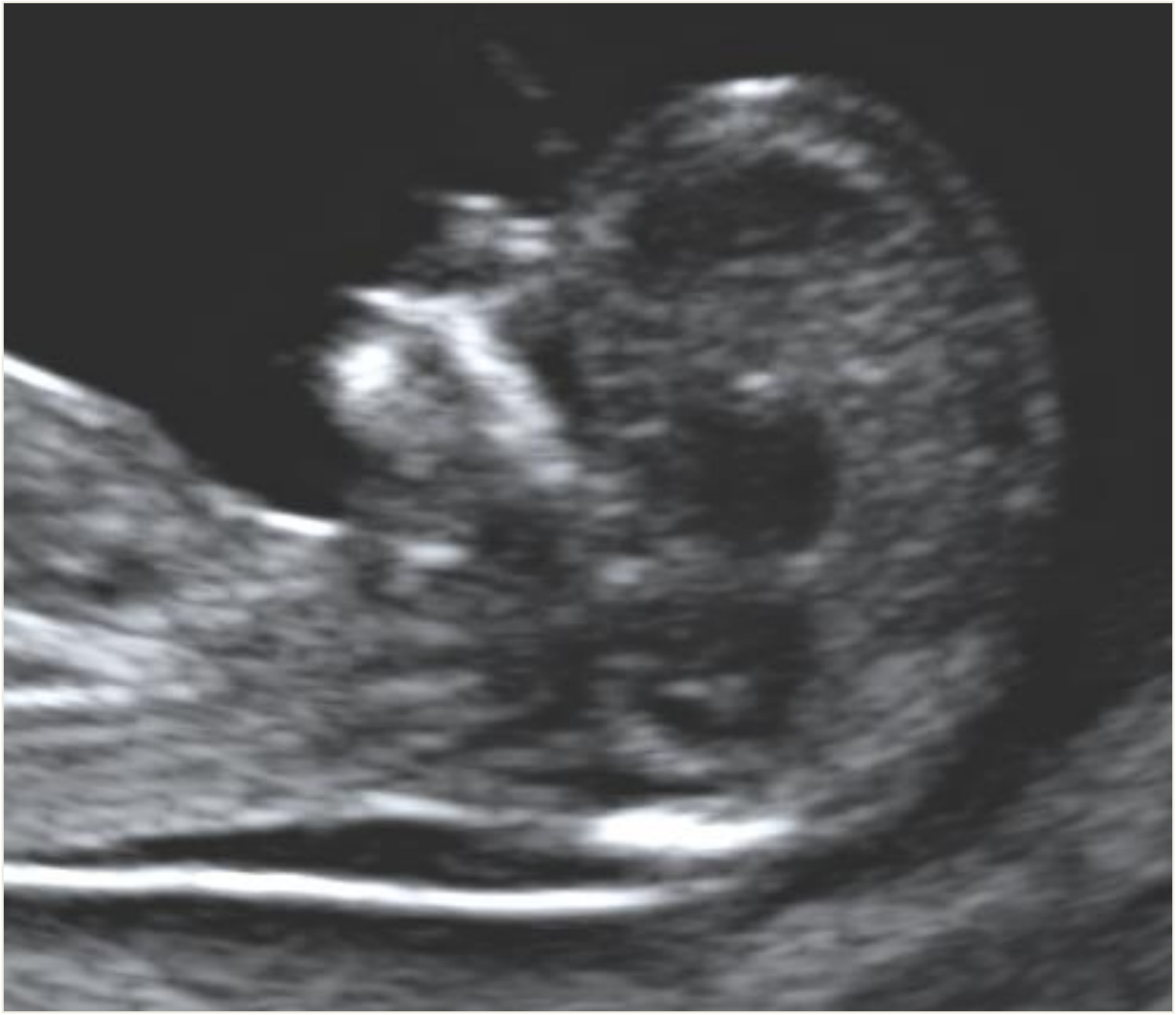
- 100 cell-equivalent free DNA /ml plasma: 95 from mother, 5 from fetus.

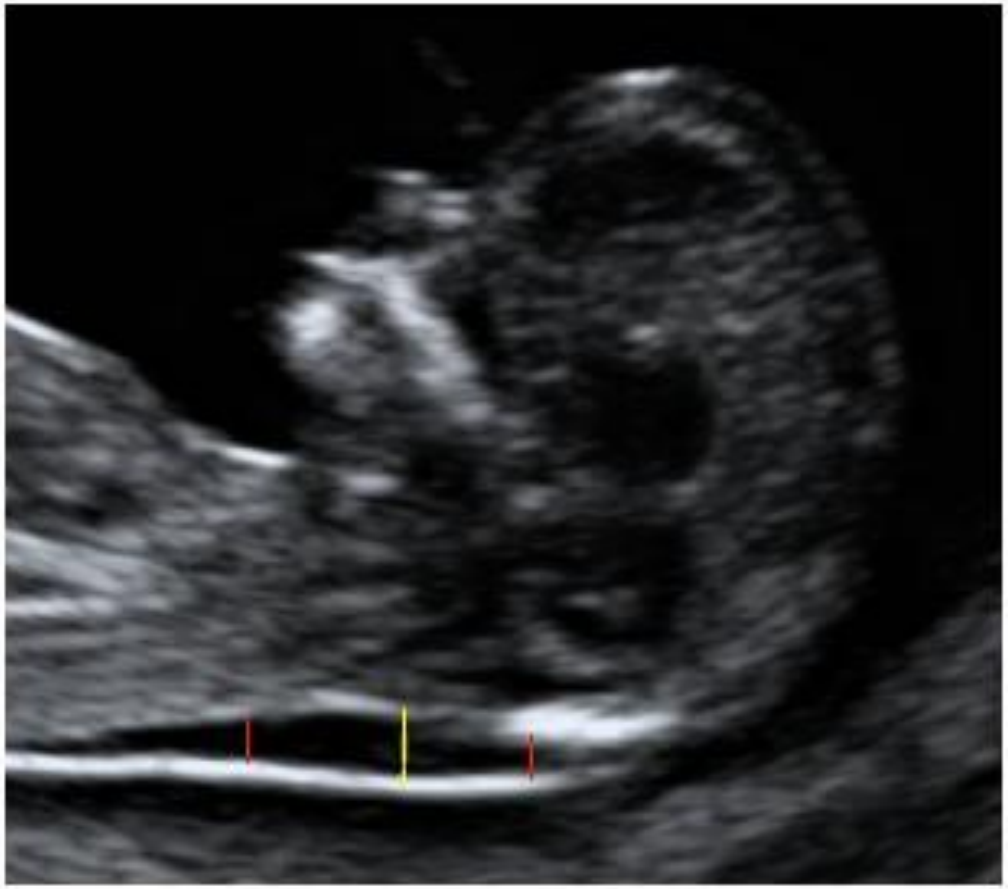


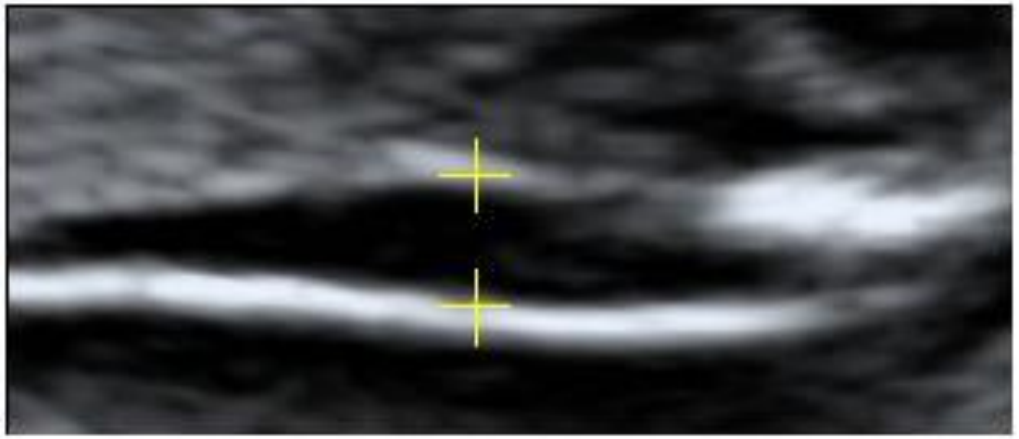
Statistics for t score and L score

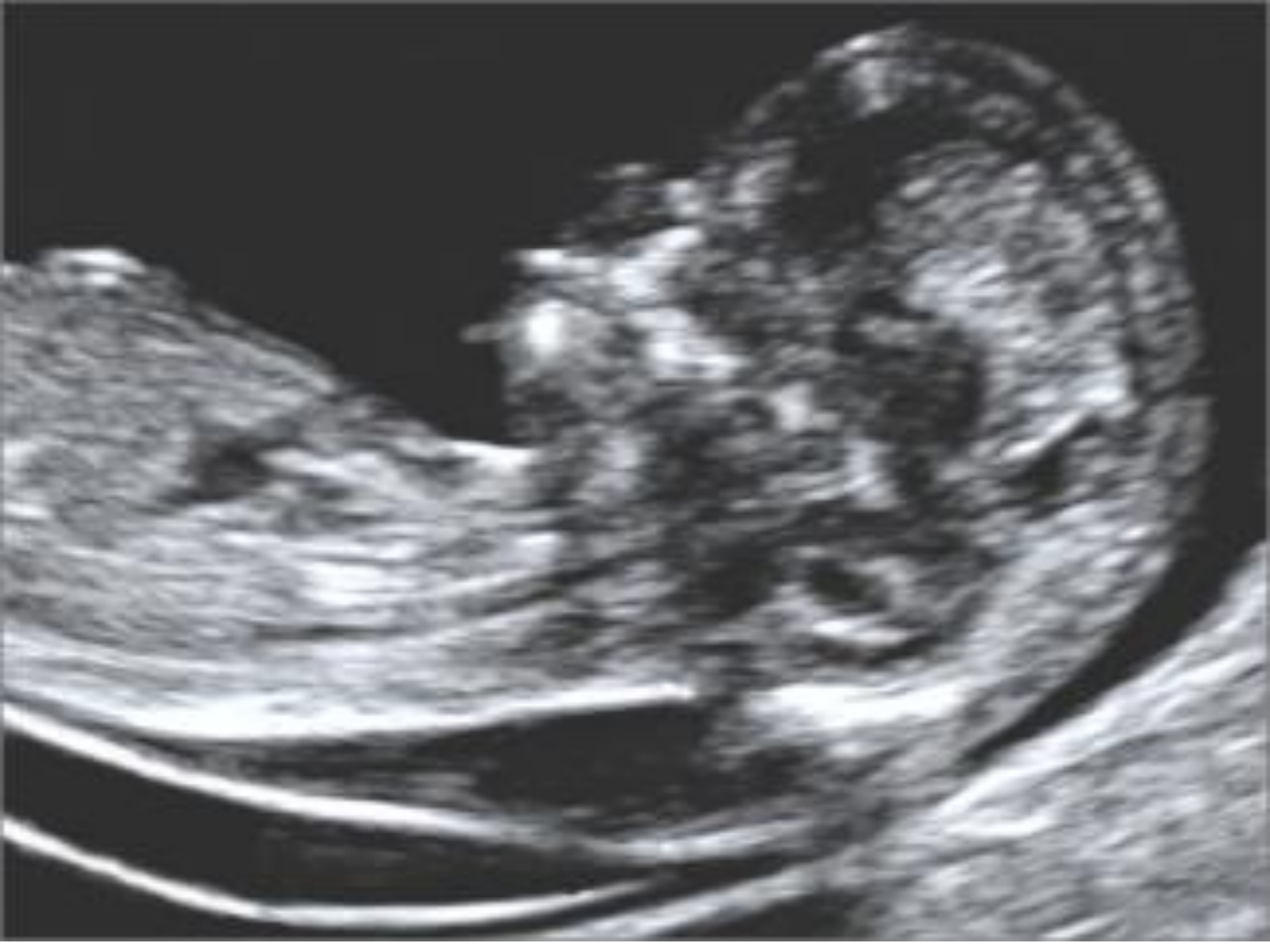
- **GC correction**
- **Binary hypothesis statistics analysis**

Nuchal Translucency

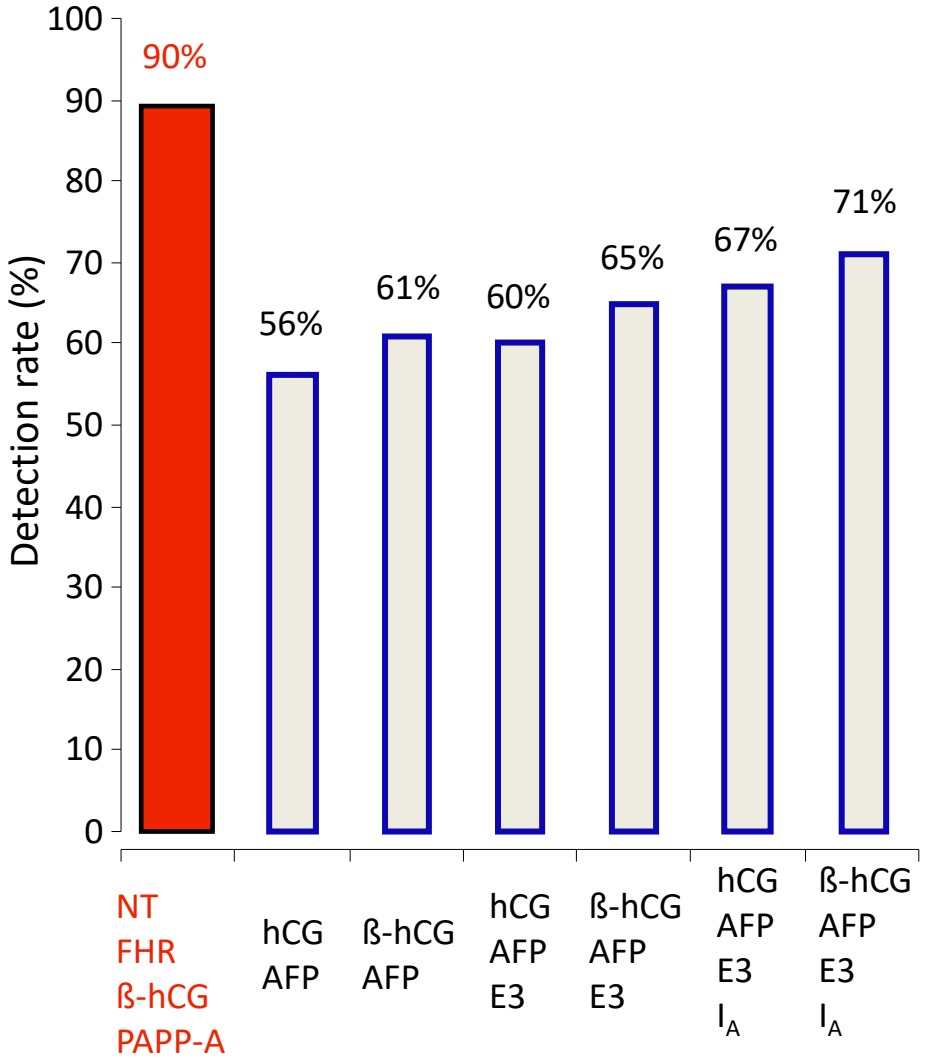








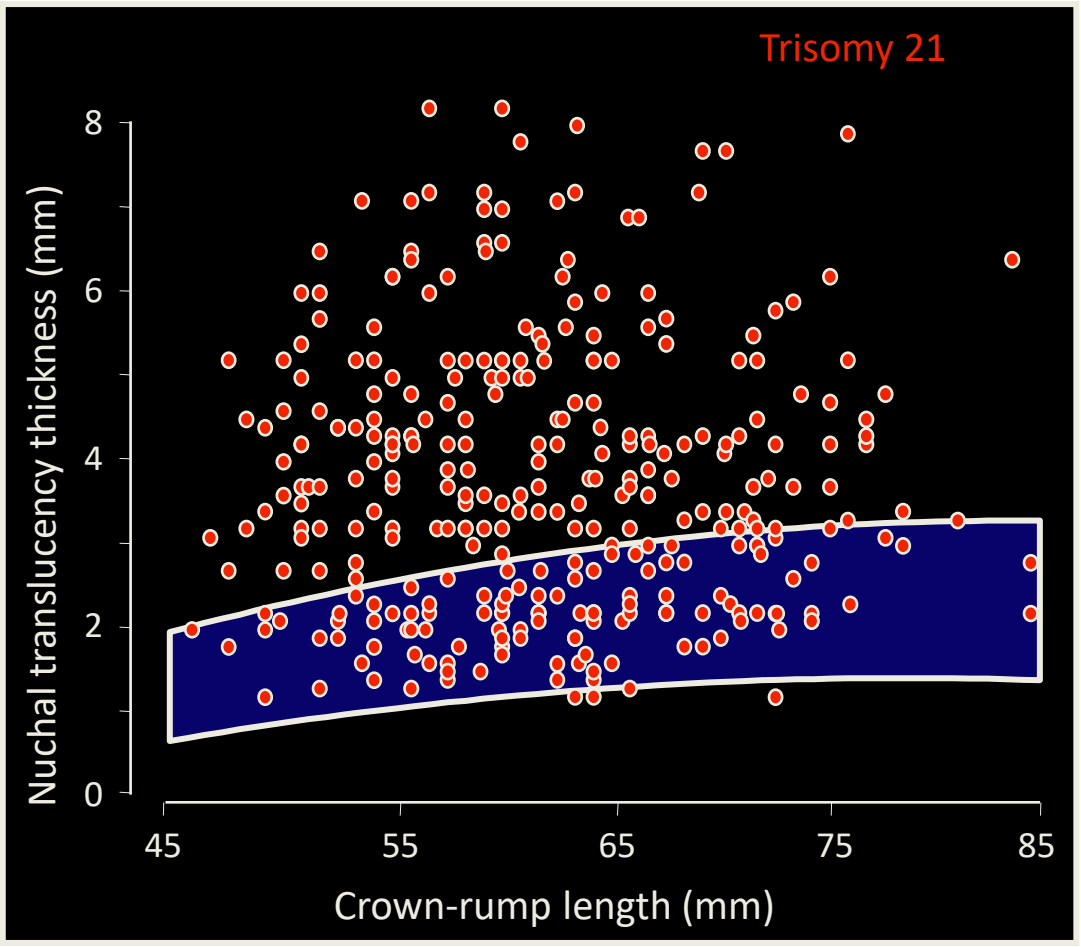
2nd trimester biochemical screening



Trisomic pregnancies are associated with altered maternal serum concentrations of various feto-placental products.

- Screening in the second trimester by maternal age and various combinations of total or free ß-hCG, AFP, uE3 and Inhibin A can identify 56-71% of trisomy 21 pregnancies for a false positive rate of 5% (Meta-analysis by H Cuckle, P Penn and D Wright, Semin Perinatol 2005;29:252-7).

- Screening in the first trimester by a combination of maternal age, fetal NT, FHR and serum free ß-hCG and PAPP-A identifies about 90% of trisomy 21 pregnancies for a false positive rate of 3%


Trisomy 21
Nuchal translucency thickness (mm)
0
2
4
6
8
45
55
65
75
85
Crown-rump length (mm)

In 1997 the presence of cell-free fetal DNA (cffDNA) in the maternal circulation was reported.

Theoretical Basis of NIFTY

THE LANCET

Early report

Lancet 1997; **350:** 485–87

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

Fundamental Features of Cell-Free Fetal DNA

① The content is 970 times greater than the DNA level of fetal cells in maternal blood.

② The content changes on a regular basis. Can be detected in maternal plasma from the 5th week of gestation. The concentration increases with the gestation and there are some individual differences.

③ Disappears soon after childbirth. The average half-life of cell-free fetal DNA is 16.3 minutes (4 to 30 minutes) and it is undetectable in 2 hours.

Fetal DNA comes from the placenta,can be detected from the first trimester of pregnancy onwards and is rapidly cleared from the maternal circulation after delivery.

- maternal plasma MPS offers a much more efficient method than digital PCR for maximising the amount of diagnostic information that can be obtained from a plasma sample.

- To date, published data indicate extremely good results for trisomy 21 and trisomy 18 prediction when sequencing is successful. However, there is typically a single-digit percentage chance of no result due to the samples or sequencing results not meeting certain quality control criteria (which can vary from approximately 1–10% depending on the service provider), although a repeat sample will produce a result in the majority of such cases.

- The amount of cffDNA(fetal fraction) in maternal blood increases with gestational age and decreases with maternal BMI.

- Increased maternal weight and fetal aneuploidy is associated with lower fetal DNA fraction.

- When the test can be performed:
- From 9 weeks up to delivery

The reason could be high adipose cell turnover

increasing maternal plasma DNA or increasing blood volume and so a dilutional effect.

This should be mentioned in counselling or patient information literature.

- When a twin pregnancy is monochorionic (and so monozygotic), both fetuses will be affected or unaffected.

Monochorionic twins

- Since the amount of cffDNA is approximately double that of a singleton pregnancy,51 cffDNA aneuploidy
- testing will not only be possible but probably more effective than in singletons.

Therefore

- invasive testing confirmation will be required before termination of pregnancy.

The complexity introduced by twin pregnancies suggests

that, prior to cffDNA testing, a good quality ultrasound scan would be a valuable first step in all pregnancies,

to detect empty pregnancy sacs, for example, with fetal medicine counselling when one is suspected.

- There is good evidence that the source of the cffDNA is the placenta.
- Abnormal cell lines can be present in the placenta that are not present in the fetus (in approximately 1% of CVS samples), a phenomenon often called 'confined placental mosaicism'.

- The widespread use of cffDNA is limited by cost

- And

- Another major disadvantage of screening nonspecifically for chromosomal aneuploidies (including but not limited to Down syndrome) is that women who are pregnant will frequently be informed of findings of uncertain significance.

Table 1. Cell-free DNA Test Performance Characteristics in Patients Who Receive an Interpretable Result*

			Age 25 years	Age 40 years
	Sensitivity (%)	Specificity (%)	PPV (%)	PPV (%)
Trisomy 21	99.3	99.8	33	87
Trisomy 18	97.4	99.8	13	68
Trisomy 13	91.6	99.9	9	57
Sex chromosome aneuploidy	91.0	99.6	--†	--

- In general population traditional methods may indirectly identify a fetus with an unbalanced translocation etc which cannot be detected by cffDNA

Maternal plasma MPS will be much

better than maternal serum screening in the second trimester, which is still offered for women who book late.

- MPS cannot detect triploidy but it can be detected by SNP-based techniquie

- BUT

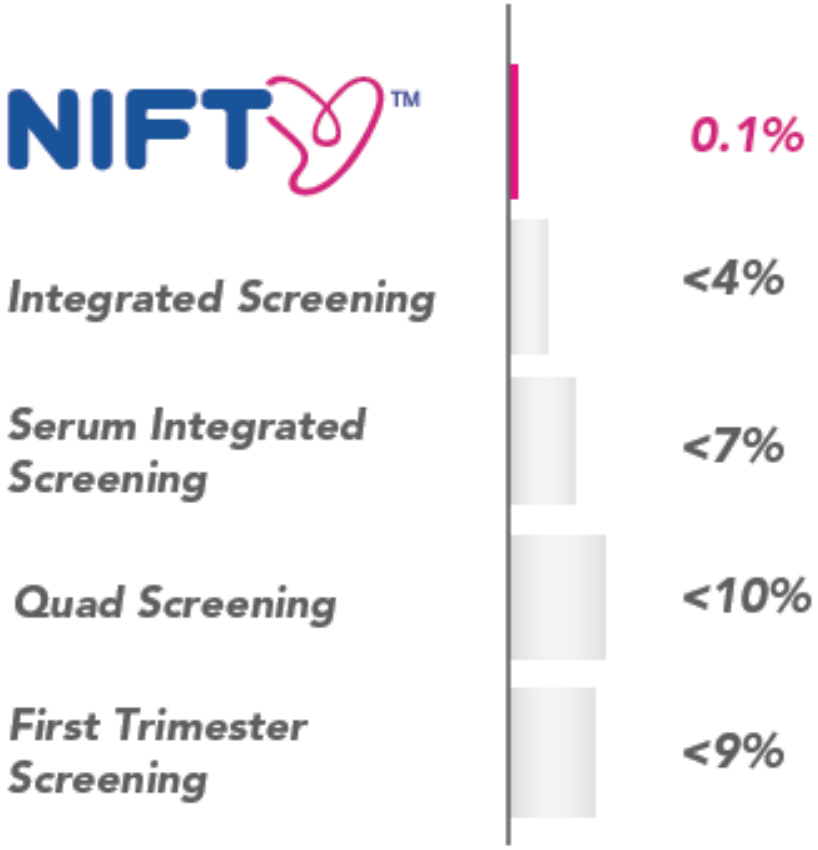
- It can never replace NT scan but is an ecellent complementary test

- cffDNA in all protocols is a test added to 1st trimester screening

The changes will also mean the workload of trained fetal medicine practitioners undertaking amniocentesis/CVS will reduce and the ratio will shift towards more CVS and away from amniocentesis.

- However, the previous abandonment of the use of what used to be called 'soft markers' to adjust screening risk will be further consolidated.

A Comparison of False Positive Rates (FPR)



265,000 tests performed

5 False Positive*

0 False Negative

*False positive is due to insufficient sequencing data, which has been optimized by increasing sequencing depth, false positive rate has been dramatically reduced.

Data Summary (Mar 2009- Nov 2014)

428,154 tests performed

	Number	Specificity	Sensitivity
T21	2933	99.99%	99.95%
T18	907	99.98%	99.96%
T13	342	99.98%	100%

*Samples volume reach 700 per day

ACOG Recommendations September 2015:

- Proper patient councelling
- Traditional screenings are the 1st line choice
- Any patient can choose cffDNA regardless of her risk status if sh eis carefully coumcelled
- cffDNA only screens for common trisomies
- All positive results should have an invasive test
- Multiple screenings is not cost effective and should not be performed

- Termination of pregnancy should not be based on cffDNA result
- No call test result should have genetic councelling , detailed ultrasound scan and a diagnostic test if needed based on risk status
- Routine cffDNA for microdeletions is not recommended yet

- Routine cffDNA for multiple gestations is not recommended but there is emerging evidence that it is useful in twins
- In case of fetal anomaly the mother should be offered a diagnottic test
- A negative cffDNA test does not ensure an unaffected pregnancy
- cffDNA does not screen for NTD

Thank you very much for your attention