# FETAL CELLS & FF DNA IN MATERNAL BLOOD:

## *the new era of prenatal diagnosis*

**GC DI RENZO, MD, PhD, FRCOG, FACOG**

*University of Perugia, Italy*

# Prevalence of Trisomies 21, 18, 13

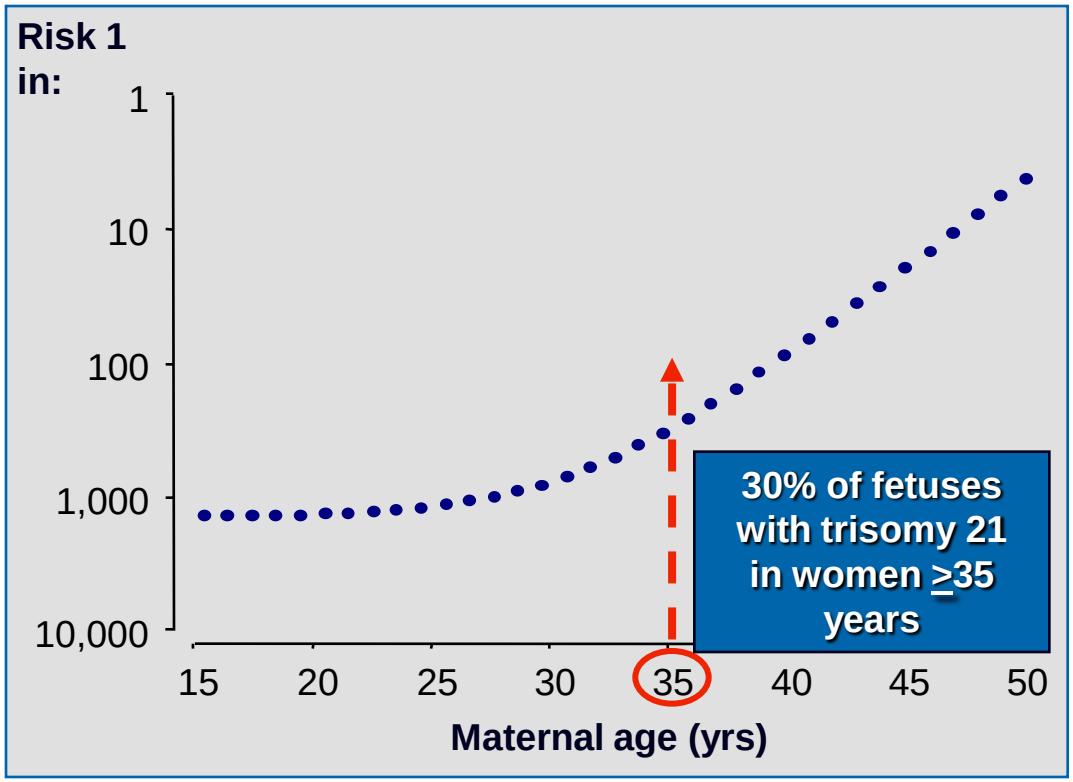
| Trisomy Type | Condition Name | Frequency |
|---|---|---|
| Chromosome 21 | Down syndrome | 1 in 700 live births |
| Chromosome 18 | Edwards syndrome | 1 in 5,000 live births |
| Chromosome 13 | Patau syndrome | 1 in 16,000 live births |

U.S. National Library of Medicine. Genetics Home Reference. Down Syndrome: http://ghr.nlm.nih.gov/condition/downsyndrome, Trisomy 18: http://ghr.nlm.nih.gov/condition/trisomy-18, Trisomy 13: http://ghr.nlm.nih.gov/condition/trisomy-13. Accessed July 12, 2012.

# 1970s

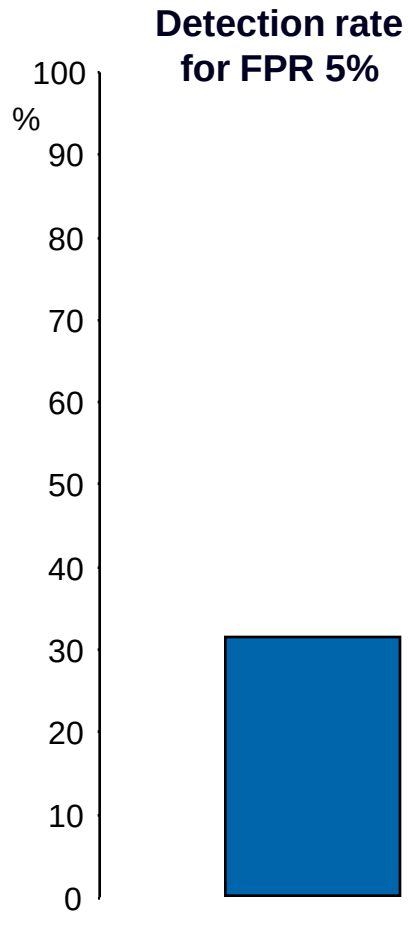
# Screening for aneuploidies

## Maternal age

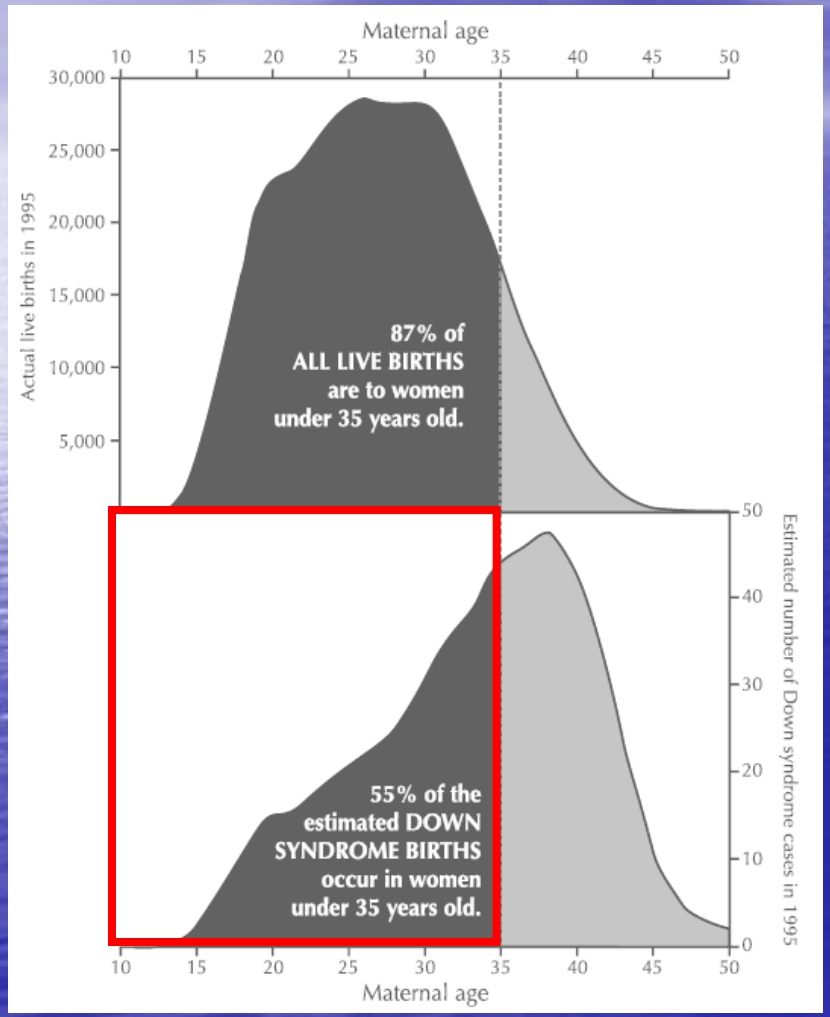



**In one-third of the Mongolian imbeciles in institutions the mothers were at the time of gestation approaching the climacteric period.**

*Shuttleworth GE. Mongolian imbecility. Br Med J **1909**;2:661–5*



# Importance of Screening All Pregnant Women



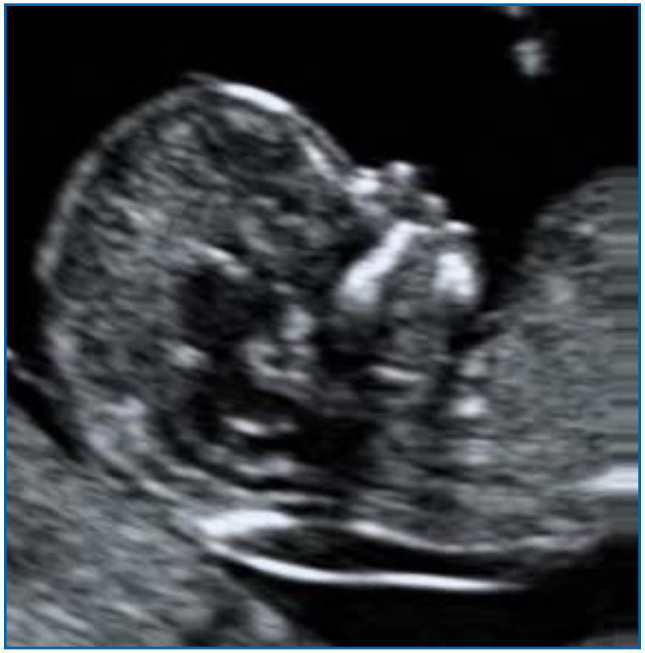

**Majority of babies born with Down syndrome are in women under 35 years old**

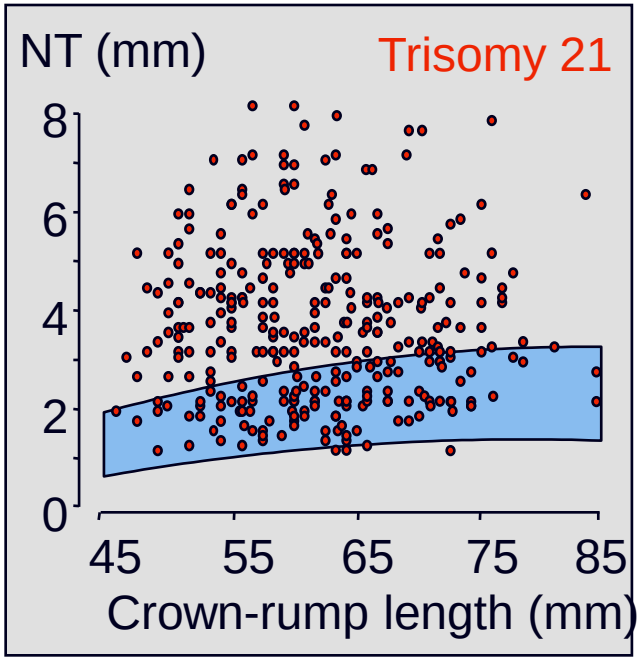
Provider handbook for The California Prenatal Screening Program 2009.

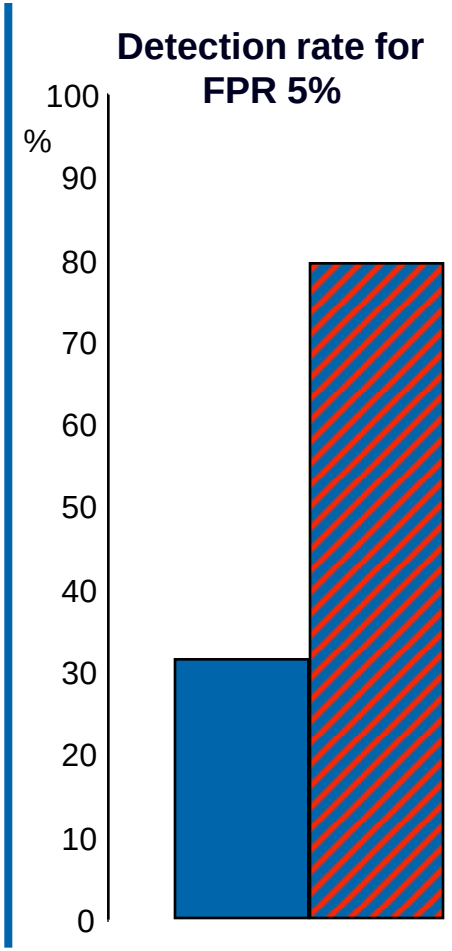
# 1990s

# Screening for aneuploidies

## Maternal age and fetal nuchal translucency





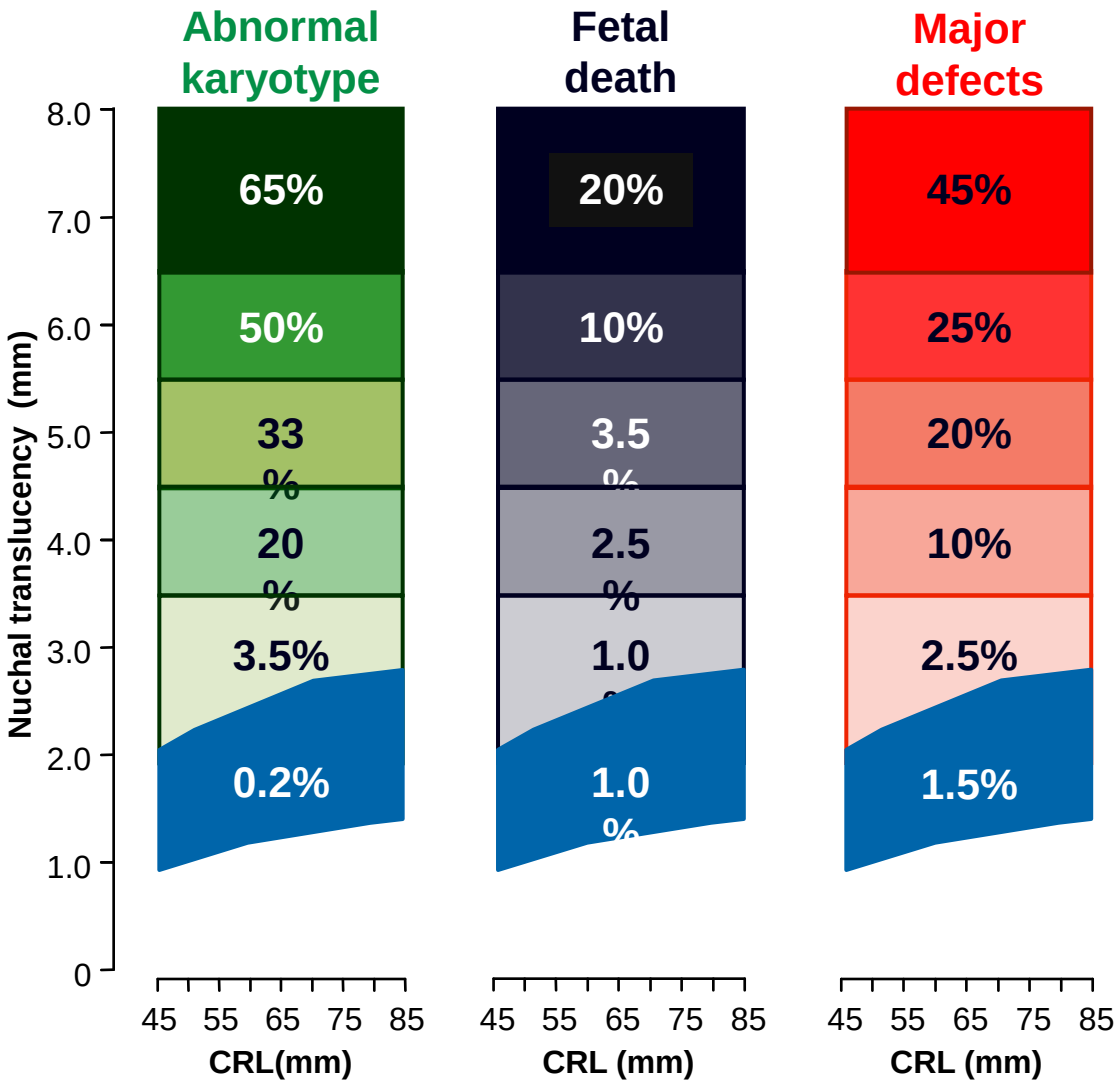

THE LANCET **1998;352:343-6.**

Snijders RJ, Noble P, Sebire N, Souka A, Nicolaides KH

**Assessment of risk of trisomy 21 by maternal age and fetal nuchal-translucency thickness**

96,127 singleton pregnancies, including 326 cases of trisomy 21: DR 77% for FPR 5%

# Implications of increased NT




Cardiac defects
Lethal skeletal dysplasias
Diaphragmatic hernia
Exomphalos
Megacystis

Akinesia deformation sequence
Spinal muscular atrophy

Treacher-Collins syndrome
Jarcho-Levin syndrome
Beckwith-Wiedemman syndrome Smith-Lemli-Opitz syndrome
Zellweger syndrome
Noonan syndrome
di George syndrome
Congenital lymphedema

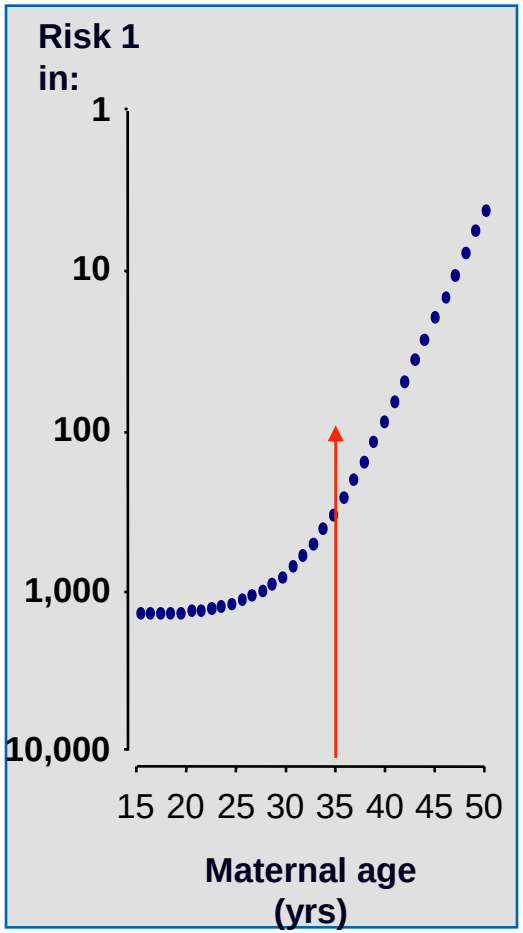
Dyserythropoietic anaemia
Thalassaemia-a
Parvovirus B19 infection

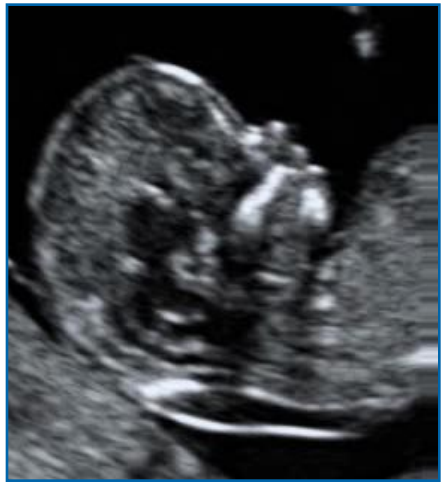
Souka *et al.* Am J Ob Gyn 2004

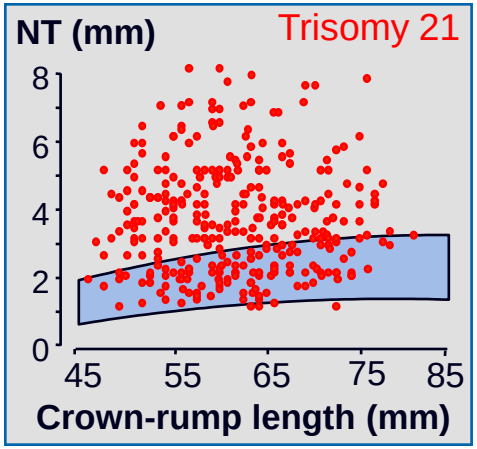
# 2000

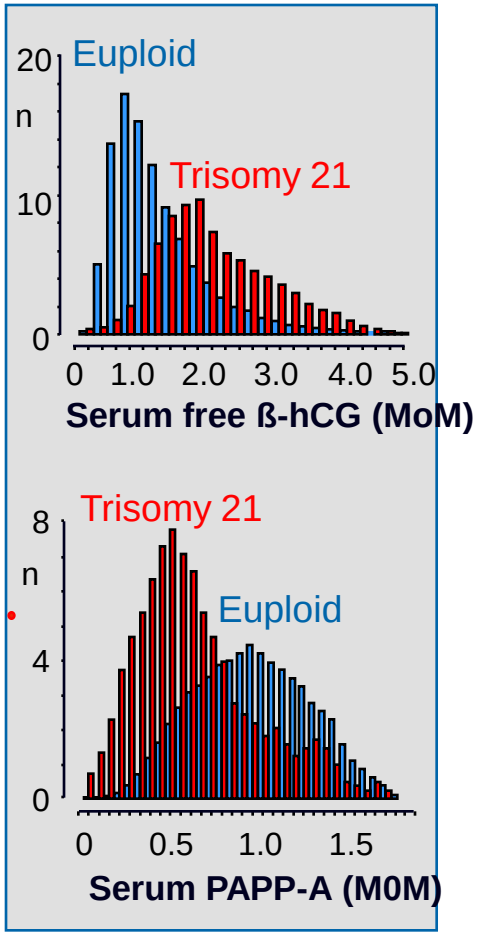
# Screening for aneuploidies

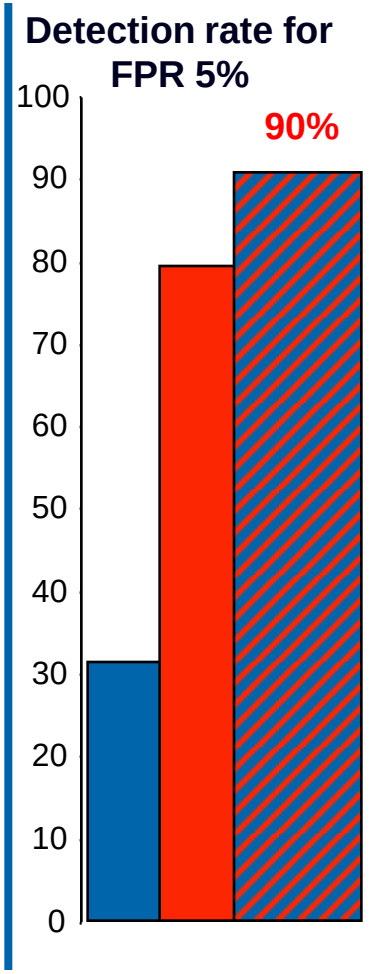
## 1st trimester combined test





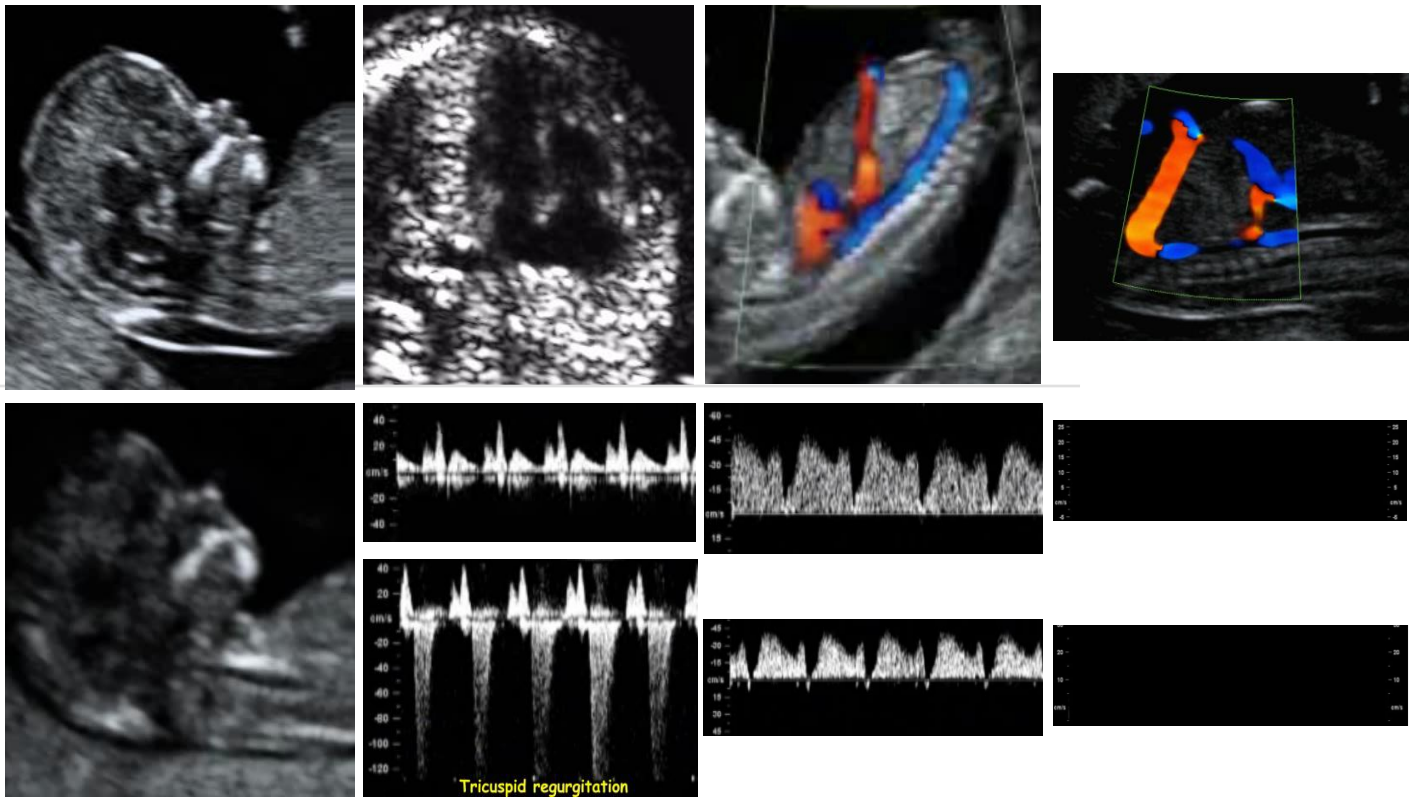



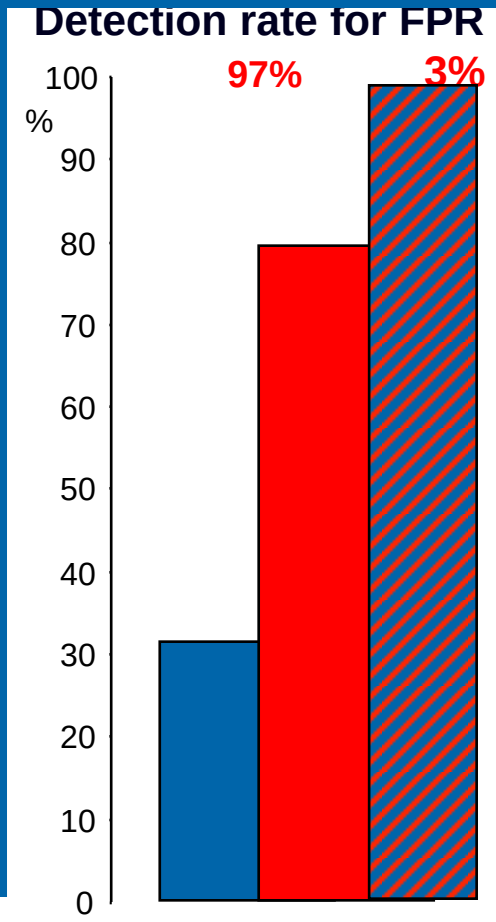



# Screening for aneuploidies

## 2000-10

## 1st trimester combined test and additional US markers



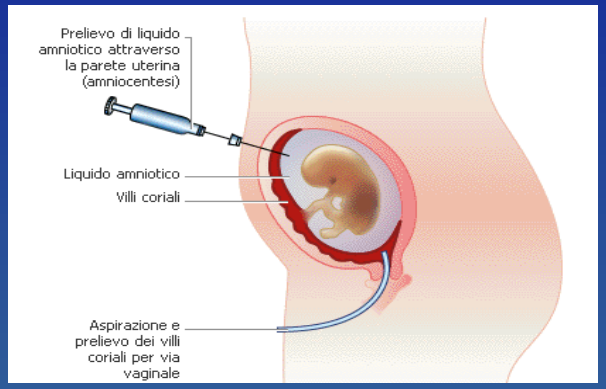
# CURRENT PRENATAL DIAGNOSIS TOOLS

## INVASIVE

Villocentesis

Amniocentesis

Cordocentesis



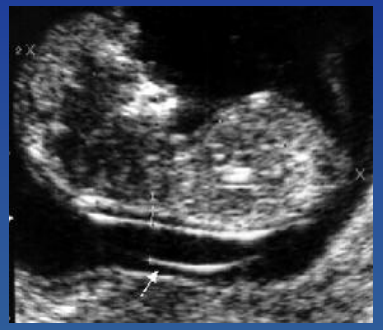

**Offered to women at risk for:**

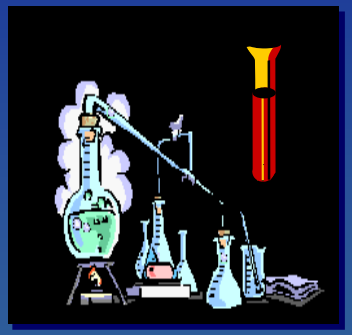
- maternal age
- positive screening test
- chromosomal abnormalities
- previous affected child

## NON INVASIVE

Ultrasound screening

Biochemical screening





Low sensitivity and specificity (< 100%)

# Potential Limitations of Current Screening Tests

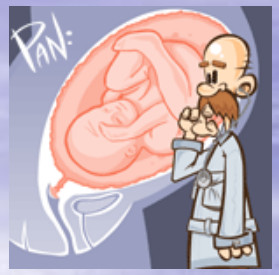
**High false positive rate** (5%)

**Inconvenient**
**Multiple visits**
**Specialized ultrasound**

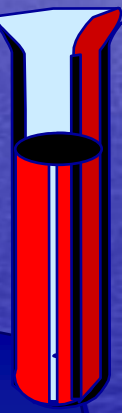
**Late information**
**Prolonged uncertainty**

**Safety concerns**

# NEED TO DEVELOP NEW NON INVASIVE PRENATAL DIAGNOSTIC TESTS

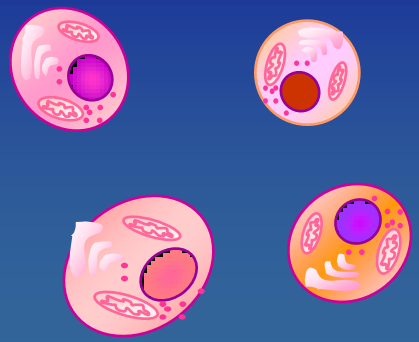



- SIMPLE
- EASY
- LEAST AGGRESSIVE
- LEAST ANXIOUS
- MORE SENSITIVE
- MORE SPECIFIC

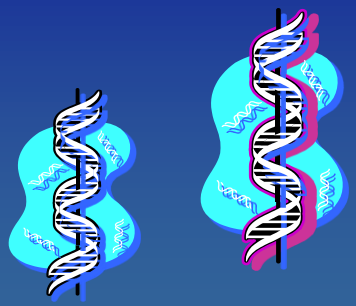


## NEW APPROACHES

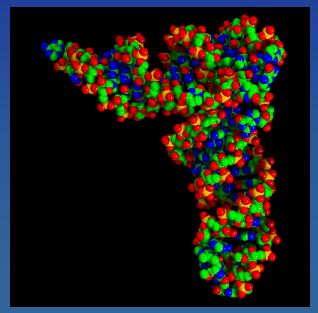
### FETAL CELLS IN MATERNAL BLOOD



### FREE FETAL DNA IN MATERNAL BLOOD

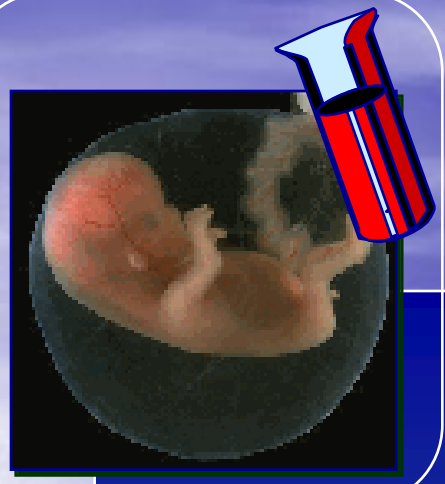


### FREE FETAL RNA IN MATERNAL BLOOD



# KEY BIOLOGICAL QUESTIONS

- ✓ Which is the ideal fetal cell type for a non invasive prenatal diagnosis?
- ✓ Which is the frequence of fetal cells in maternal blood?
- ✓ Which are suited laboratory approaches to enrich and to purify fetal cells in maternal blood?
- ✓ Are fetal cells always present in maternal blood during gestation?
- ✓ Are the fetal cells, isolated from maternal blood, sufficient for genetic diagnosis?
- ✓ Which is the best timing to retrieve fetal cells from maternal blood?



# FETAL CELL TYPES IN MATERNAL BLOOD DURING GESTATION

Studies on fetal blood obtained by cordocentesis have been able to strengthen the knowledge of the composition and development of fetal blood component throughout pregnancy

- ✓ LYMPHOCYTES
- ✓ ERYTHROBLASTS
- ✓ TROPHOBLASTS
- ✓ HEMATOPOIETIC STEM PROGENITOR CELLS
- ✓ MESENCHYMAL STEM CELLS

# NUMBER OF FETAL CELLS

- 1 fetal cell/ $10^5$ – $10^8$ maternal cells
  (Price JO et al., 1991; Hamada H et al., 1993; Langlois S et al., 1993)
- 1 fetal cell/ ml of maternal blood
  (Bianchi D et al., 1997)
- 2 –6 fetal cells/ml maternal blood
  (Krabchi et al., 2001)
- 0 – 2 fetal progenitor cells/ ml maternal blood
  (Guetta et al., 2003)

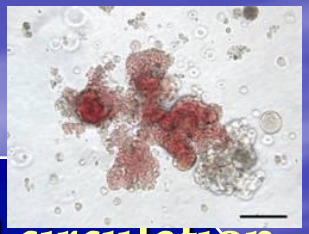
✓ Numerous studies demonstrated that in women carrying fetus with trisomy 21 or 13 and in pregnancies complicated by preeclampsia, the mean number of fetal cells increase in respect to normal pregnancies.

(Holzgreve W et al., 2007)

# TIME OF APPEARENCE OF FETAL CELLS IN MATERNAL CIRCULATION

- ✓ After 40 days of gestation
  (Holzgreve W. et al., 1993)
- ✓ From 4th week of gestation
  (Peault B et al., 2003; Lo YMD et al., 1996)
- ✓ 11 - 16 weeks of gestation
  ( Ideal time for isolating fetal cells from maternal blood )

# HEMATOPOIETIC STEM PROGENITOR CELLS (HSPCs)



✓ **Presence in maternal blood**: HSPCs are present in maternal circulation from 4th weeks of gestation whereas their concentration decrease after 20 weeks.

✓ **Identification**: CD34, CD133 monoclonal antibodies;

✓ **In vitro culture expansion** has been studied and proposed by Lo et al. (Lancet, 1994), Little et al. (Blood 1997) and Di Renzo et al. (Journal of Hematotherapy & Stem Cell Research 2000).

✓ **Frequency:** fetal/maternal cell ratio is 1 per $4.75 \times 10^6$- $1.6 \times 10^7$ cells.

✓ **Advantages:**

- Clonogenicity;
- Increased clonogenicity in fetal blood during early 2nd trimester;
- Versatility to culture and to proliferate extensively in vitro.

✓ **Work in progress**:

- persistence in maternal blood after pregnancy: *solved*
- new fetal HSPCs markers : *working on*

# A NEW METHODOLOGY OF FETAL STEM CELL ISOLATION, PURIFICATION, AND EXPANSION: PRELIMINARY RESULTS FOR NON INVASIVE PRENATAL DIAGNOSIS

Tilesi, Coata, Di Renzo et al.

Journal of Hematotherapy & Stem Cell Research 2000; 9: 583-590

# RESULTS

An enrichment of

33 times of BFU-E/CFU-E and

16 times of CFU-GM colonies after

miniMACS CD34+ HSPCs purification

was obtained

# RESULTS

## Results of FISH analysis with X and Y, 21 chromosome fluorescent probes in cultured cells

| Slide Identific. | Fetal karyotype | Number examined cells by FISH | Number cells with XY signals | Number cells with Y signals | Number cells with trisomy 21 signals | Fetal/maternal cell ratio |
|---|---|---|---|---|---|---|
| *27 | 46, XY | 669 | 5 | - | - | 1/133 |
| 19 | 46, XY | 1433 | 6 | - | - | 1/238 |
| †3 | 46, XY | 570 | - | 11 | - | 1/52 |
| 4 | 46, XY | 1050 | - | 4 | - | 1/262 |
| °18 | 47, XX+21 | 659 | - | - | 19 | 1/34 |
| 15 | 47, XY+21 | 100 | - | - | 8 | 1/12 |

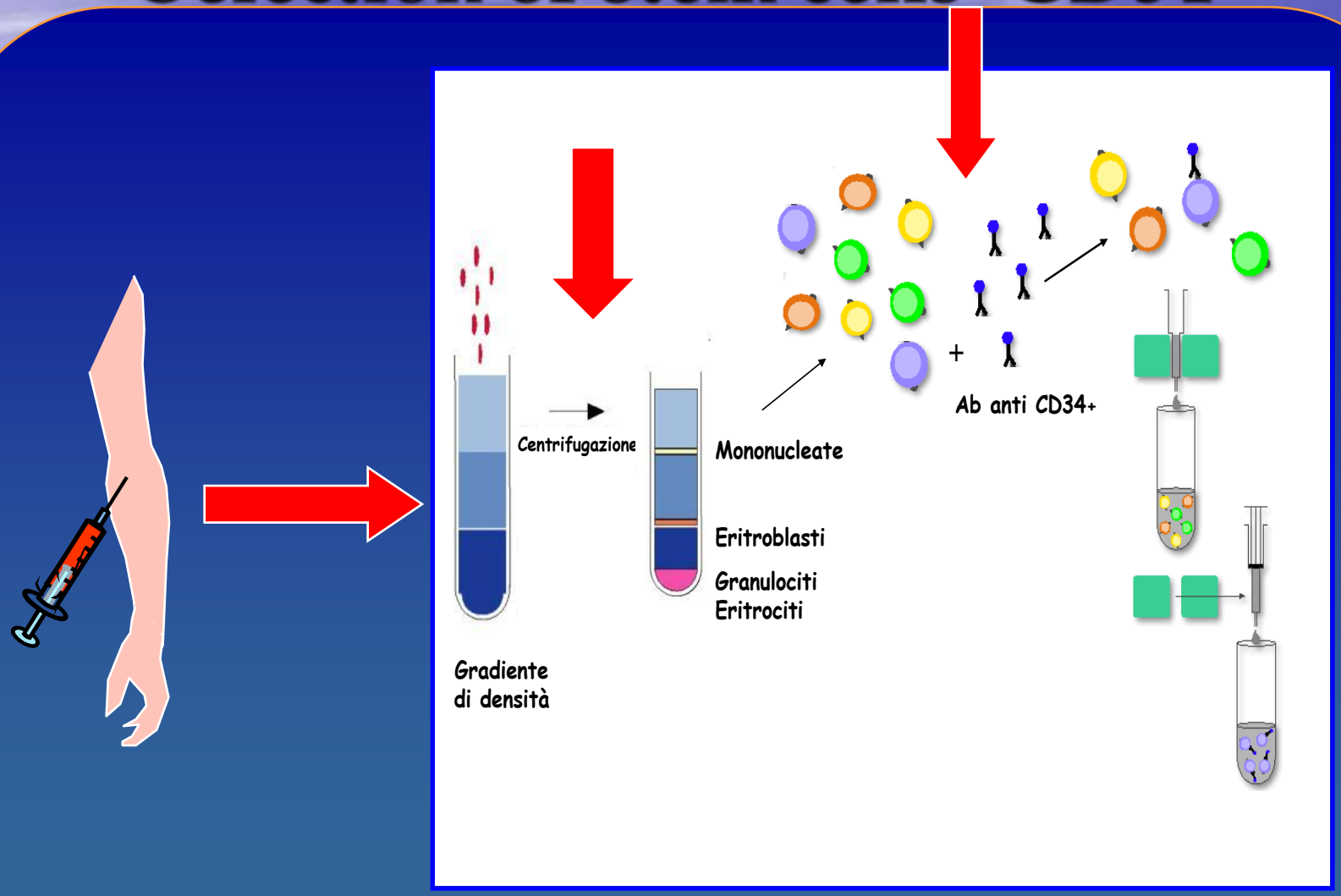
# IN SUMMARY…..

THE **SAFE** ( ***SANGUE FETALE: FETAL BLOOD***) TEST

IS COMPRISING THREE STEPS

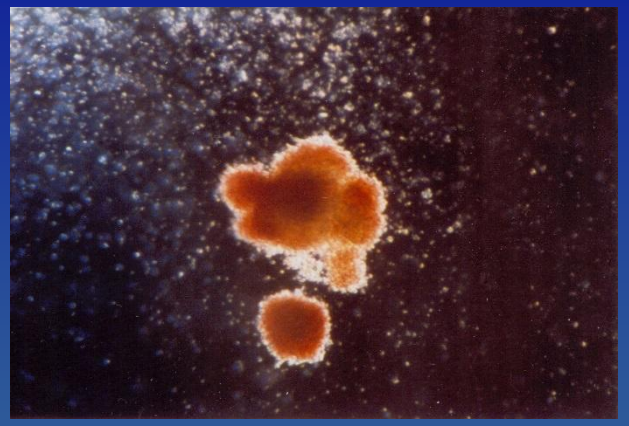
# METHODOLOGY

## Selection of stem cells CD34+



# METHODOLOGY

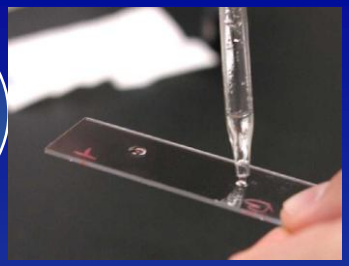
## Expansion in vitro of CD 34+



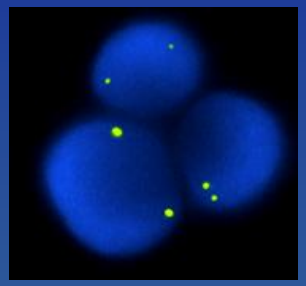
# METHODOLOGY

## Preparation of nuclei by dropping and FISH

**Lisi cellulare mediante soluzione ipotonica e semina dei nuclei mediante dropping**



**FISH**



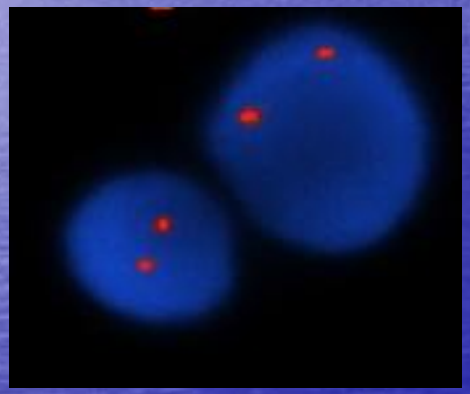
Reading

# MOTORIZED MYCROSCOPE WITH AUTOMATED ACQUISITION SYSTEM

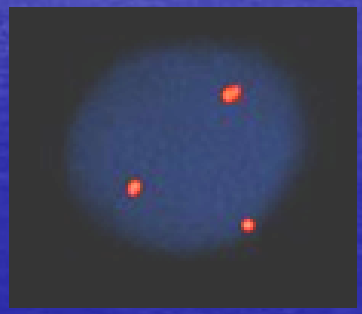
**Microscope BX-61 Olympus with software BX-UCB Olympus**

# FISH PERFORMED BY USING LSI 21 PROBE FOR THE NON INVASIVE DIAGNOSIS OF FETAL TRISOMY 21

A



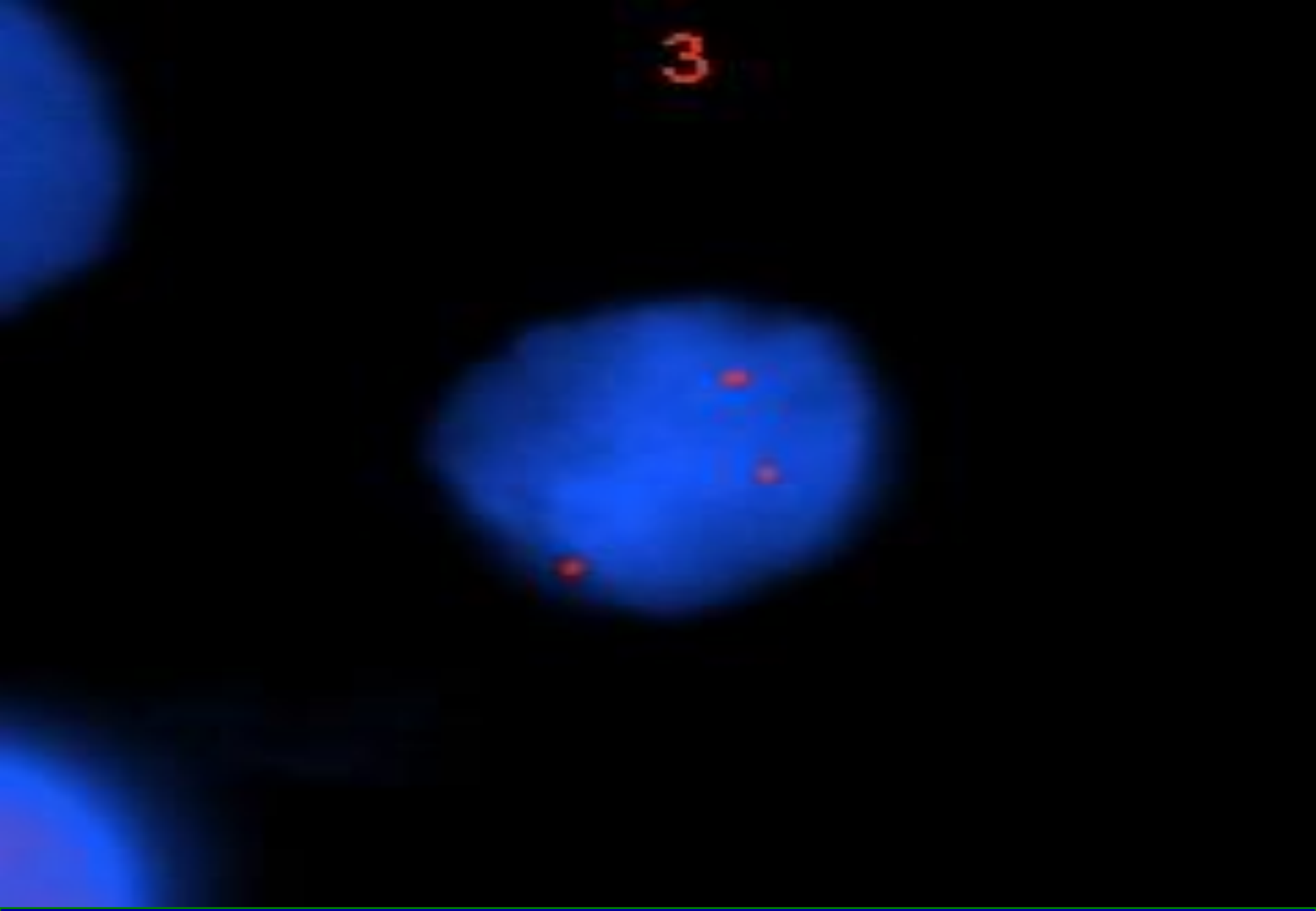
B



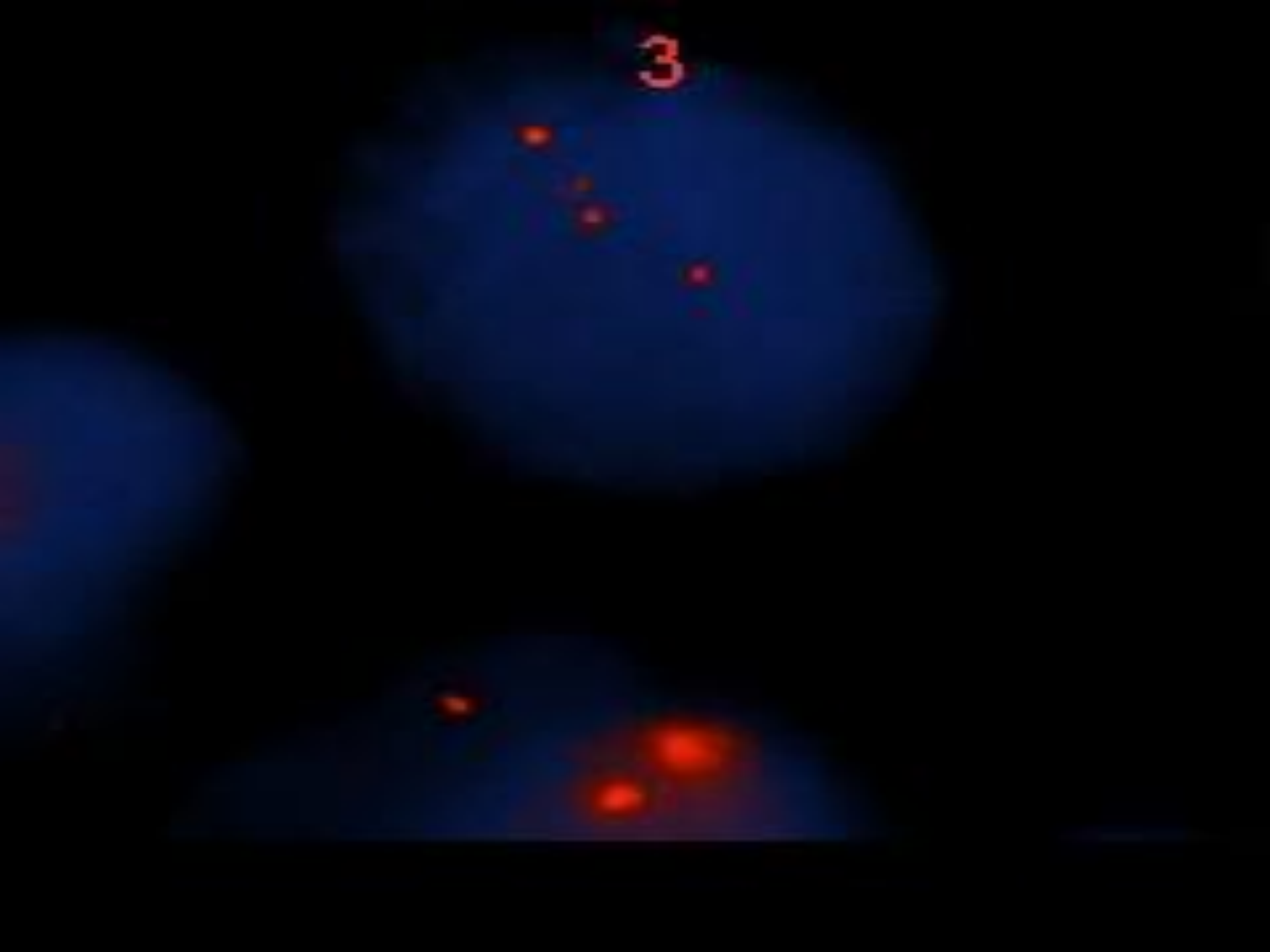
A and B: Frames obtained by using the automated mycroscope

A: Two disomic nuclei for the chromosome 21

B: Fetal trisomic nucleus for the chromosome 21




SAFE TEST: TRISOMY 21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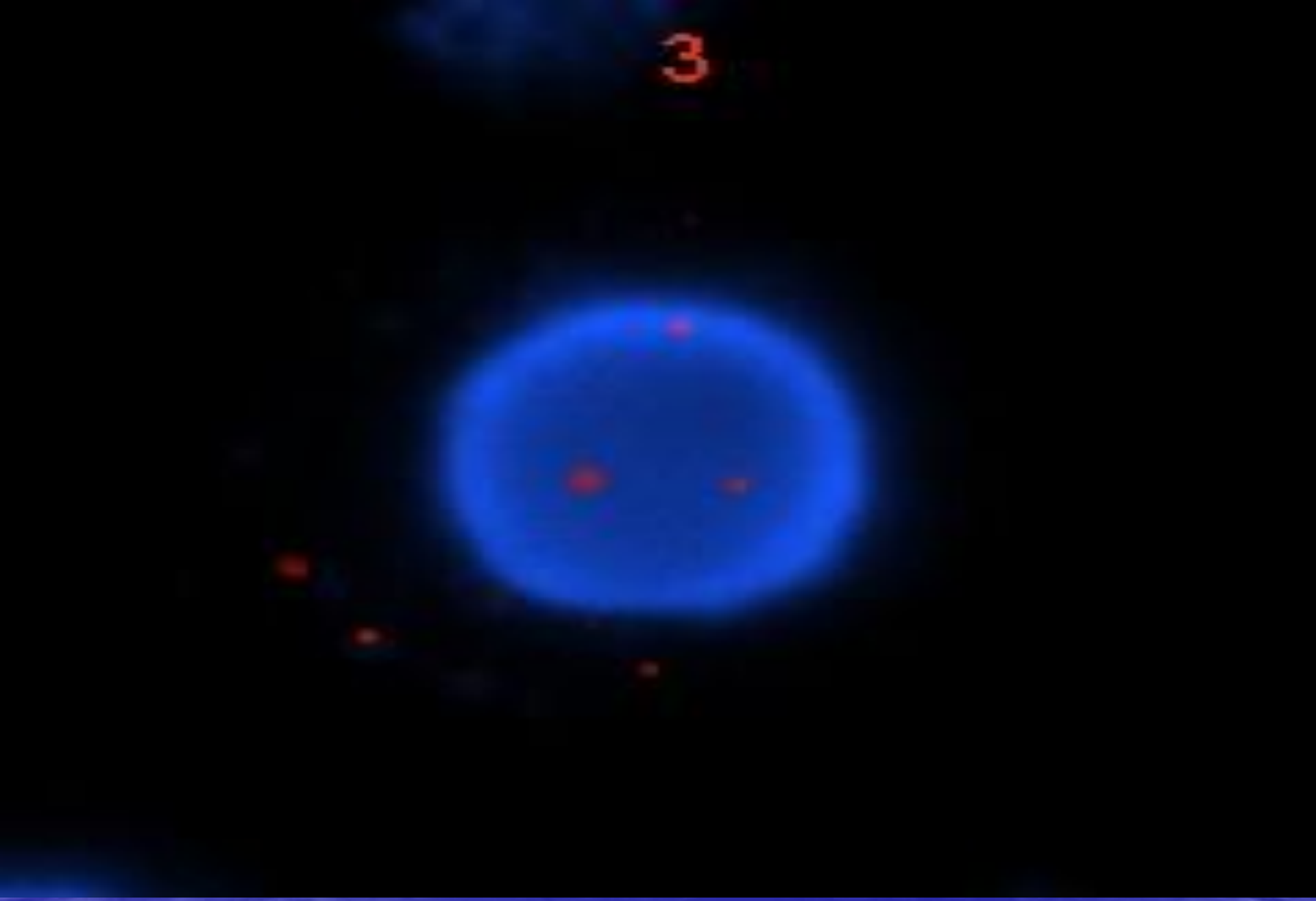
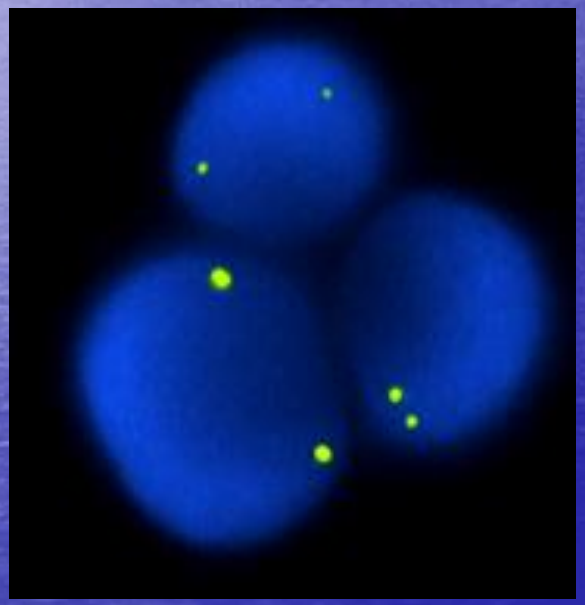


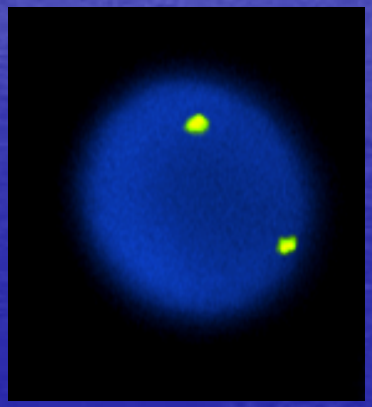

SAFE TEST: TRISOMY 21

# FISH PERFORMED BY USING LSI 13 PROBE FOR THE NON INVASIVE DIAGNOSIS OF FETAL TRISOMY 13

A

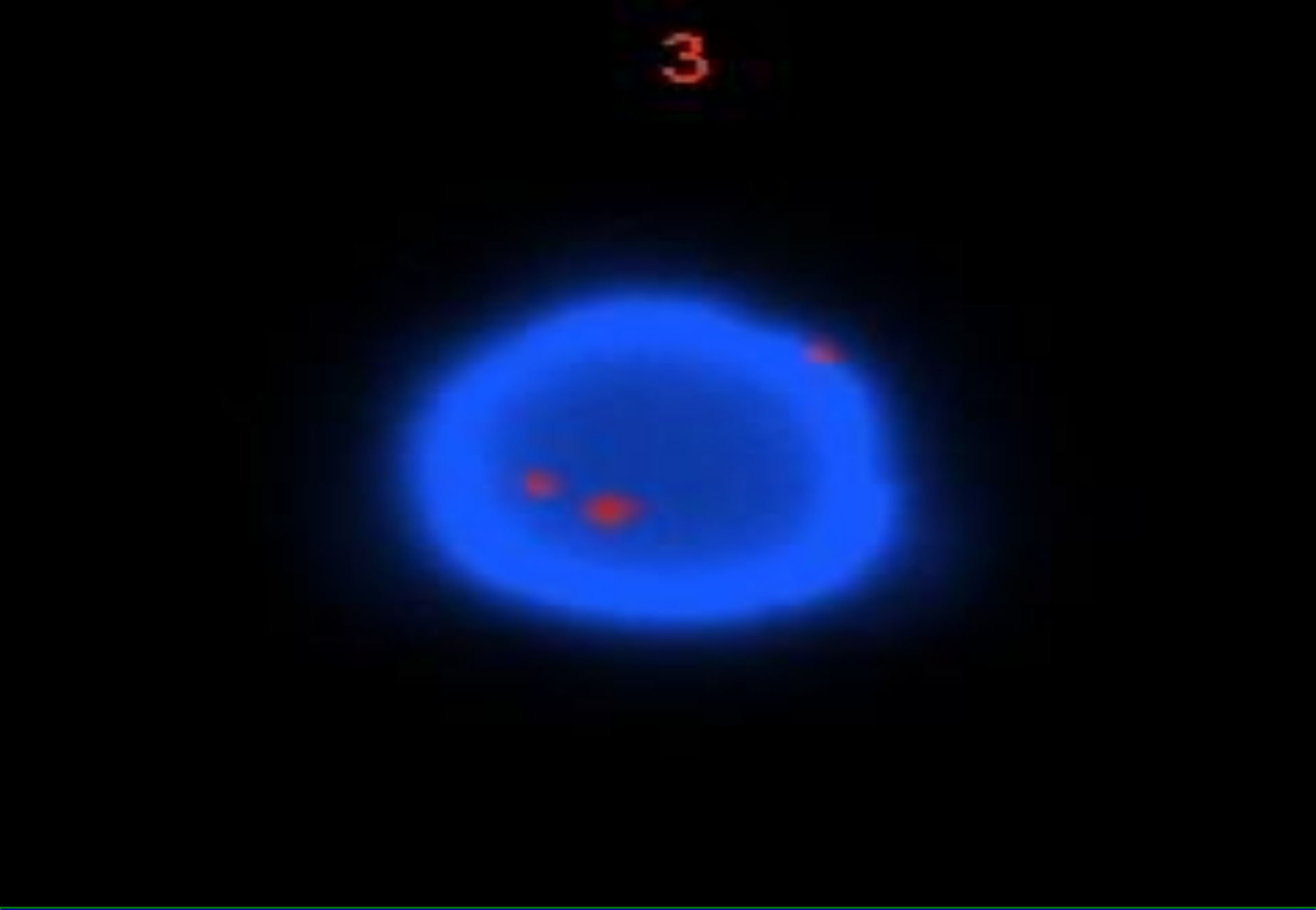


B



A and B: Frames obtained by using the automated mycroscope

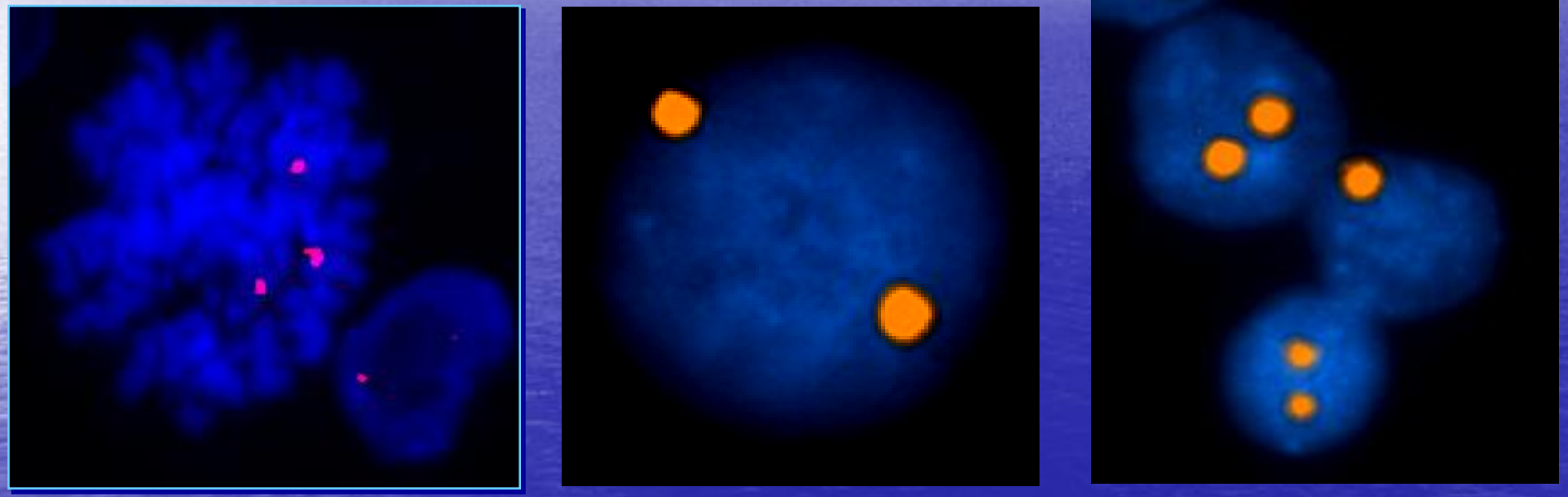
A and B: Each nucleus showed is disomic for the chromosome 13




SAFE TEST: TRISOMY 13

# FISH PERFORMED BY USING CEP 18 PROBE FOR THE NON INVASIVE DIAGNOSIS OF FETAL TRISOMY 18

A B C

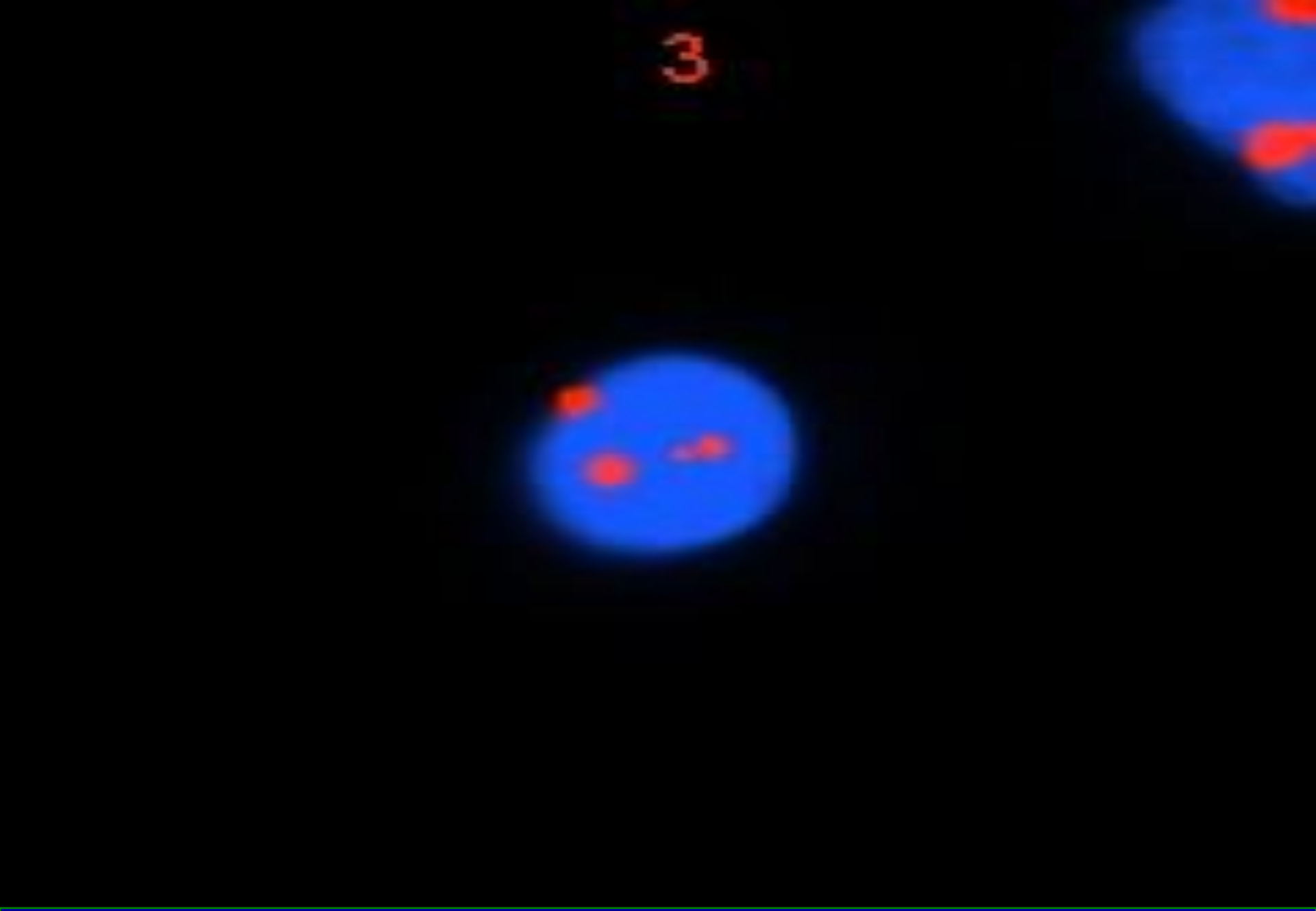


A: Fetal metaphase shows three 18 orange spots and a maternal nuclei with two 18 orange spots

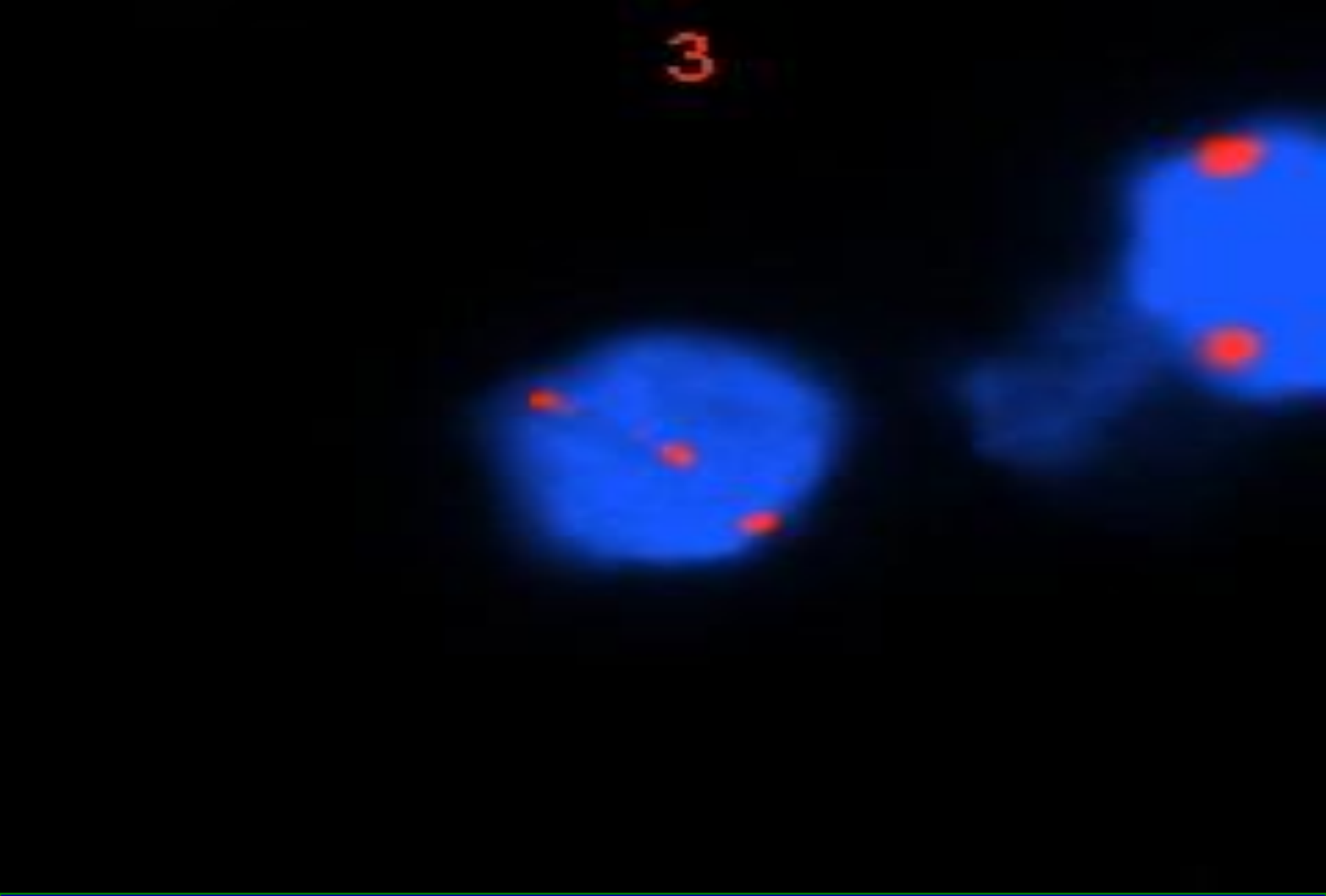
B and C: Frames obtained by using the automated mycroscope

B: Disomic nucleus for the chromosome 18

C: Two disomic nuclei and one monosomic nucleus for the chromosome 18



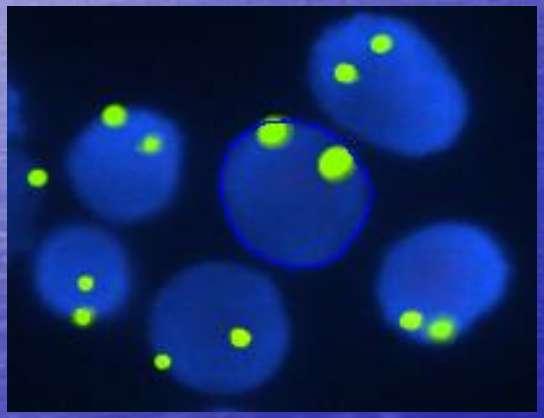

SAFE TEST: TRISOMY 18



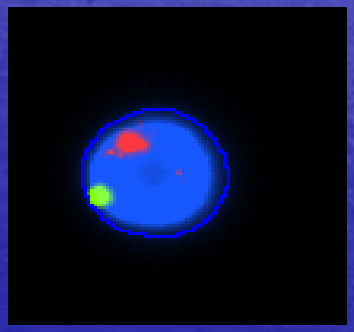

SAFE TEST: TRISOMY 18

# FISH PERFORMED BY USING CEP XY PROBE FOR THE NON INVASIVE DIAGNOSIS OF FETAL GENDER

A



B



A and B: Frames obtained by using the automated mycroscope

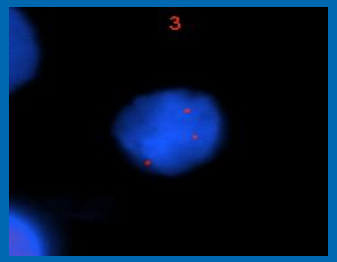
A: XX nuclei

B: Fetal XY nucleus

# SAFE TEST – FETAL CELLS

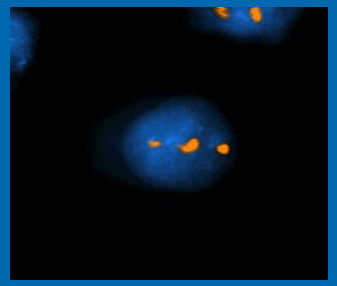
- 21 Chromosome analysis

  Diagnostic accuracy 97.9%



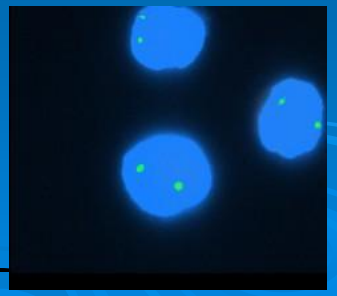

- 18 Chromosome analysis

  Diagnostic accuracy 98.9%



- 13 Chromosome analysis

  Diagnostic accuracy 98.9%



# RESULTS SAFE TEST 2006-2010

- 1782 tests: checked by CVS, amniocentesis, birth genetic map
- 18 trisomy 21
- 6 trisomy 18
- 1 trisomy 13
- 1 Klinefelter

- Detection rate 100%
  Sensitivity 100% Specificity 94%

- Chr 21 sens 100% spec 91%
- Chr 18 sens 100% spec 92%
- Chr 13 sens 100% spec ND
- Chr X & Y sens 100% spec 100%

# NEW POSSIBILITIES FROM FREE FETAL DNA

# 1992

# Cell-free fetal DNA in maternal blood

THE LANCET

**Presence of fetal DNA in maternal plasma and serum**

Dennis Lo *et al.* 1997;350:485

5% of total maternal plasma cfDNA is fetal

**Fetal sex determination (X-linked disease)**

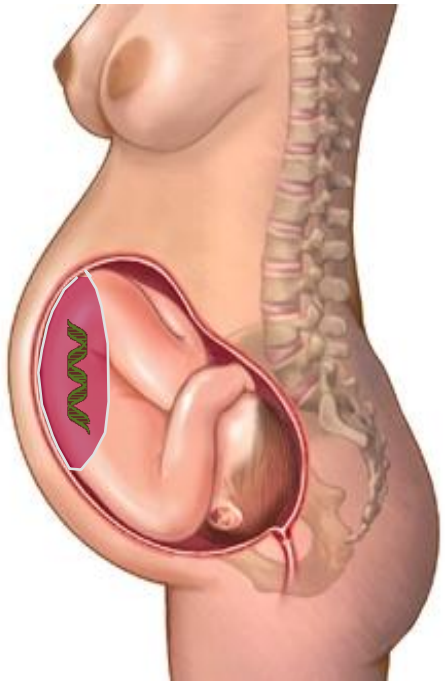
- Y chromosome in male fetuses

**Hemolytic disease**

- RHD gene in Rh D negative women

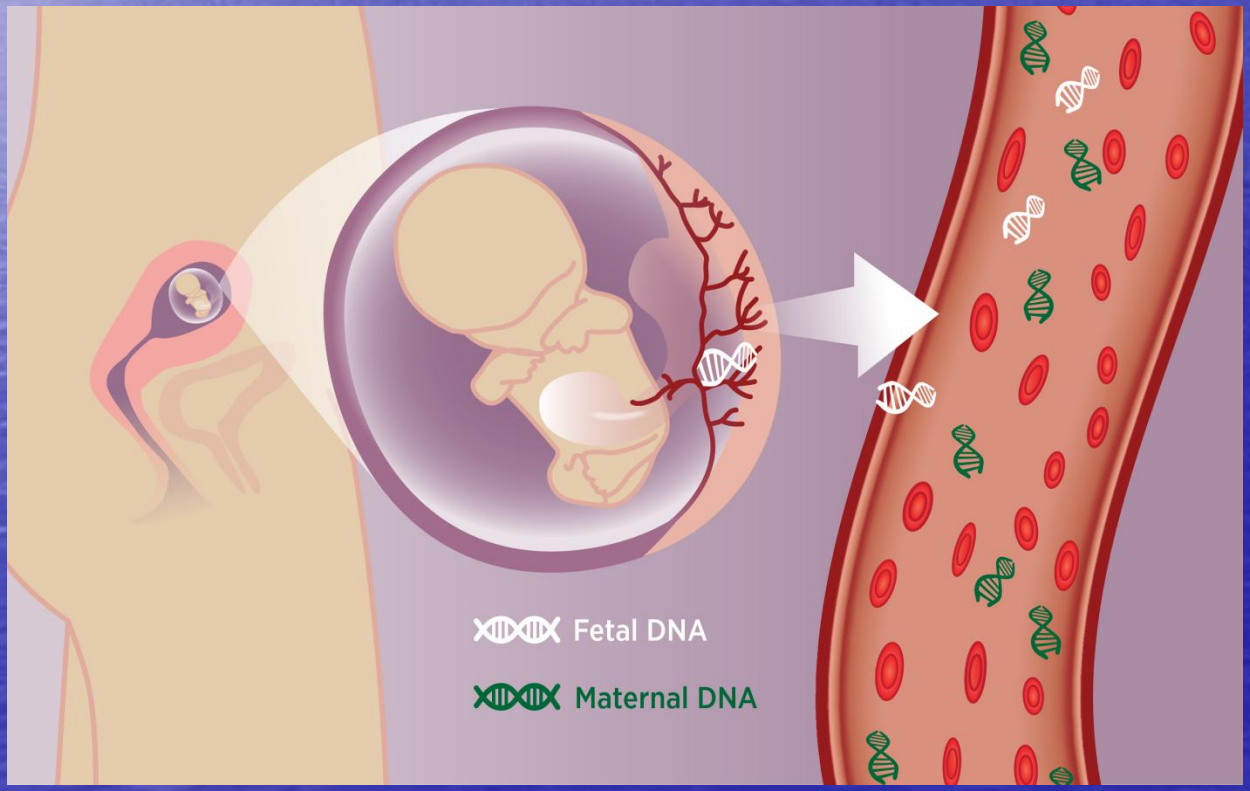
**Autosomal dominant disease**

- Achondroplasia, Myotonic dystrophy, Huntington's disease

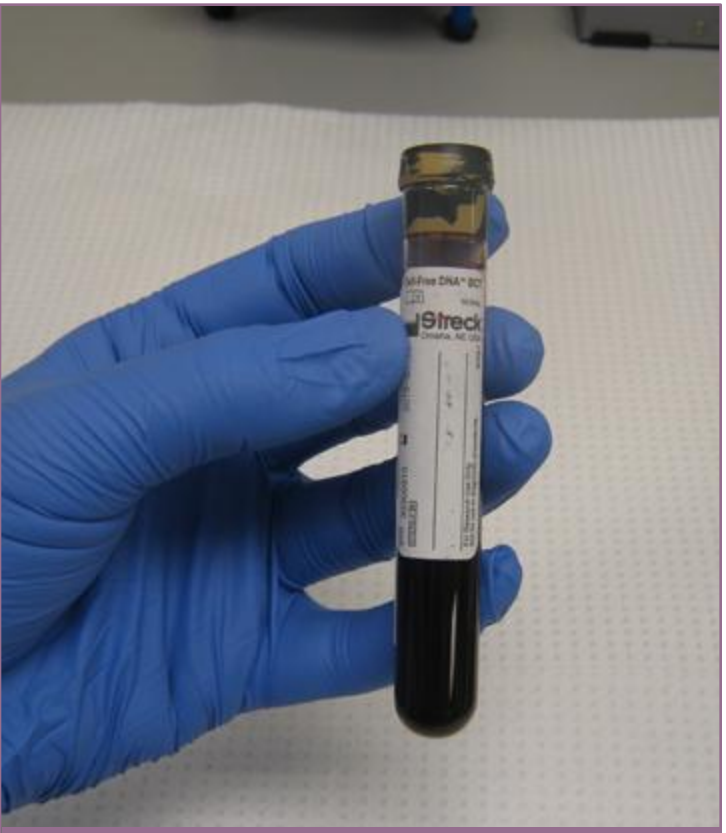


# Cell-free DNA in Maternal Blood

- Cell-free DNA (cfDNA) are short DNA fragments
- In pregnancy, cfDNA from both the mom and fetus are in maternal blood
- Amount of fetal cfDNA present is a small fraction of the maternal cfDNA



# New possibilities: NIPT



Standard Blood Draw

- Simpler clinical protocol
- As early as 10 weeks gestation
- Higher detection rate
- 30-50x lower false positive rate

The Benefits

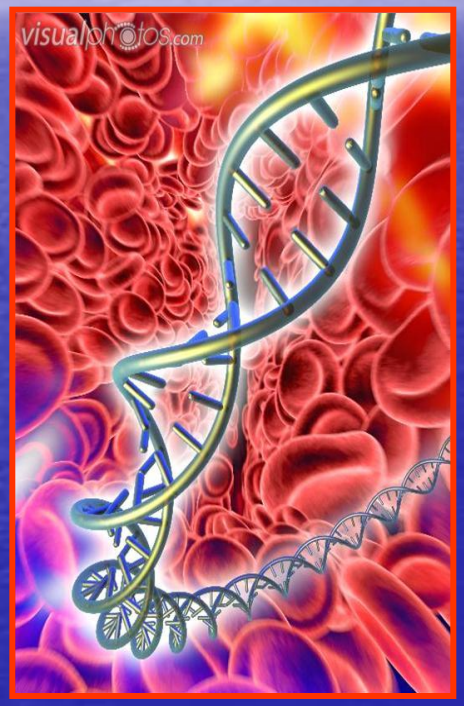
# CELL-FREE FETAL DNA

**Presence of cell-free fetal DNA in the maternal circulation**

**Fetal gender determination**

**Time:**
10-13 weeks of gestation

**Target population:**
pregnant women at risk of ambiguous genitalia, X-linked conditions and single gene disorders such as congenital adrenal hyperplasia




**Fetal RhD genotyping**

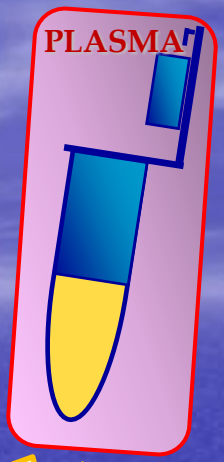
**Time:**
from the 13th week of gestation

**Target population:**
RhD-negative pregnant women

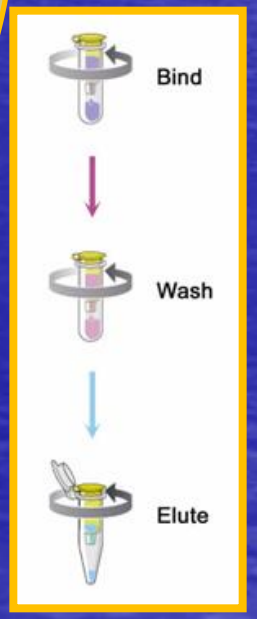
# CELL-FREE FETAL DNA

Peripheral blood sampling

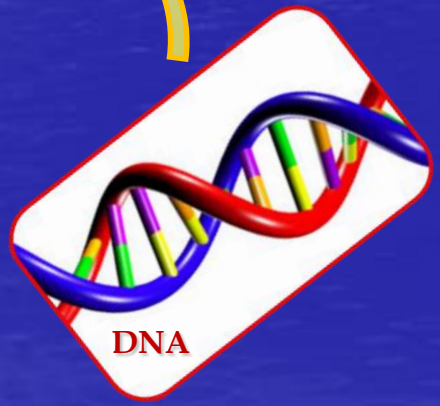

Density gradient centrifugation




## METHODS




DNA extraction




Analysis by real time PCR
(Polymerase Chain Reaction)

# Non invasive fetal gender determination

**Di Renzo et al.**

Prenat Diagn 2008
Am J Ob Gyn 2009
Clin Genet 2011

# PRENATAL ASSESSMENT OF FETAL GENDER

1. So far, the test has been performed on 912 pregnant women .
2. The use of our interpretation criteria allowed us to improve the test by reducing false positive results.
3. The test is functional in clinical routine practice of non invasive prenatal diagnosis since it is easy, rapid and automated. After about 4 hours from the blood sampling it is possible to obtain the results of 20 samples simultaneously.

| | |
|---|---|
| SENSITIVITY (%) | 99.9 |
| SPECIFICITY (%) | 99.5 |
| VPP (%) | 99.5 |
| VPN (%) | 100 |
| EFFICIENCY (%) | 99.7 |

# COMPARISON OF TWO DNA EXTRACTION METHODS FOR THE DEVELOPMENT OF A PRENATAL NONINVASIVE GENETIC TEST FOR FETAL STATUS RhD DIAGNOSIS

Fanetti, Coata, Di Renzo et al.

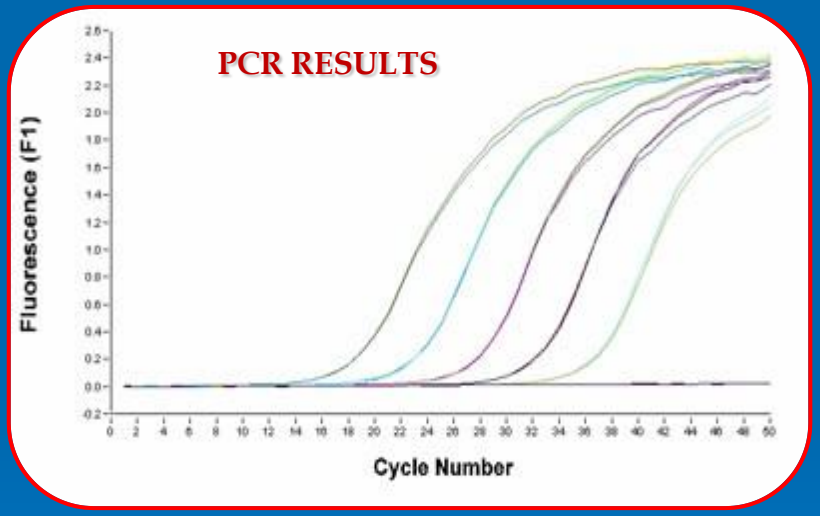
PRENAT DIAGN 2010

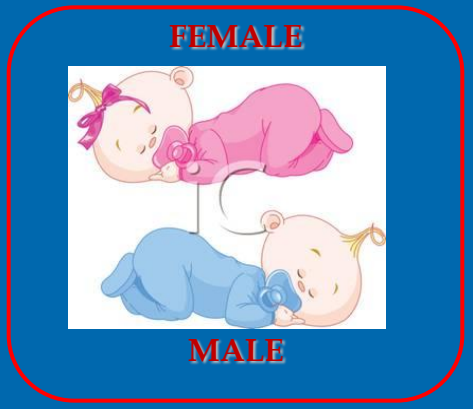
safenetwork

# CELL-FREE FETAL DNA

## Fetal gender determination

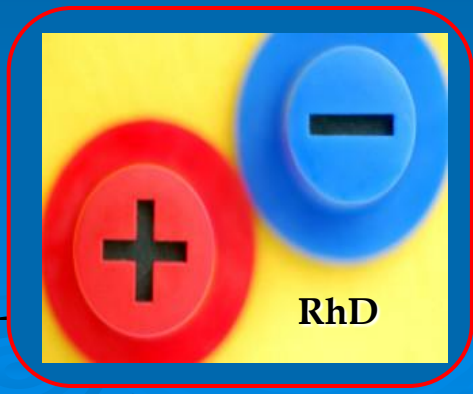
Diagnostic accuracy: 99,8%




## Fetal RhD genotyping

Diagnostic accuracy: 97,5%



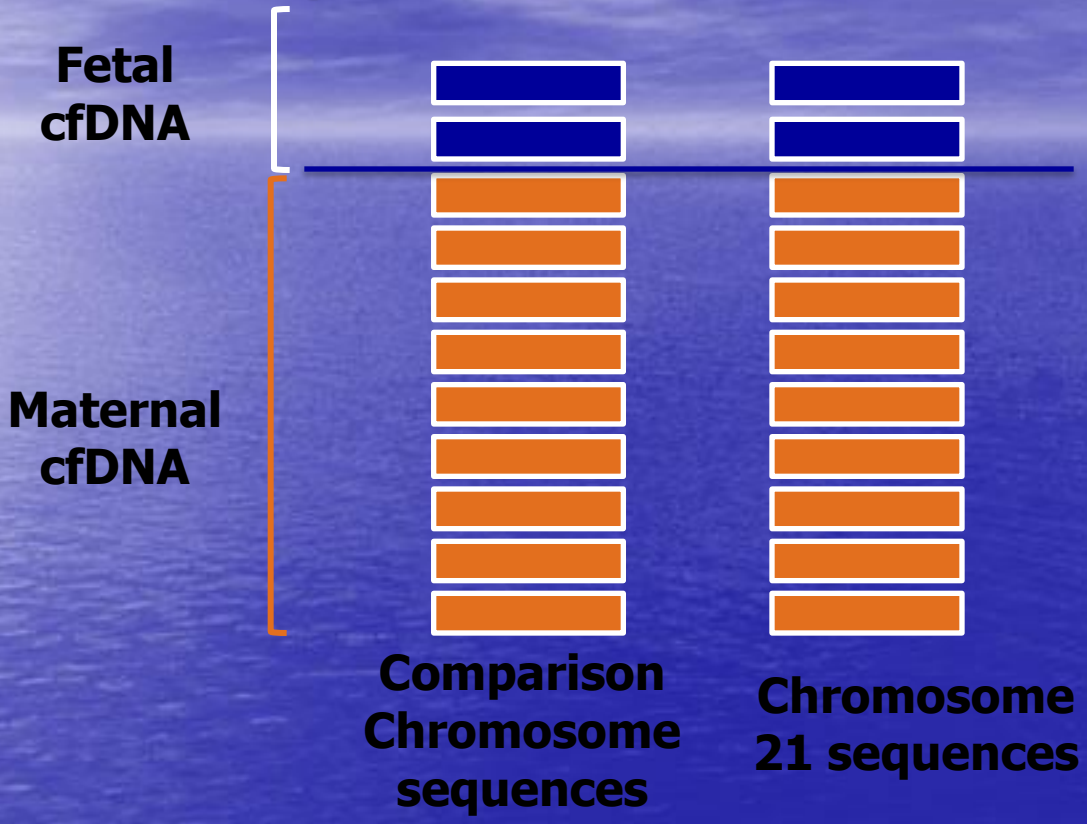
# PRENATAL ASSESSMENT OF FETAL RhD STATUS

1. So far, the test has been performed on 166 pregnant women

2. The test is functional in clinical routine practice of non invasive prenatal diagnosis since it is easy, rapid and automated. After about 4 hours from the blood sampling it is possible to obtain the results of 20 samples simultaneously.

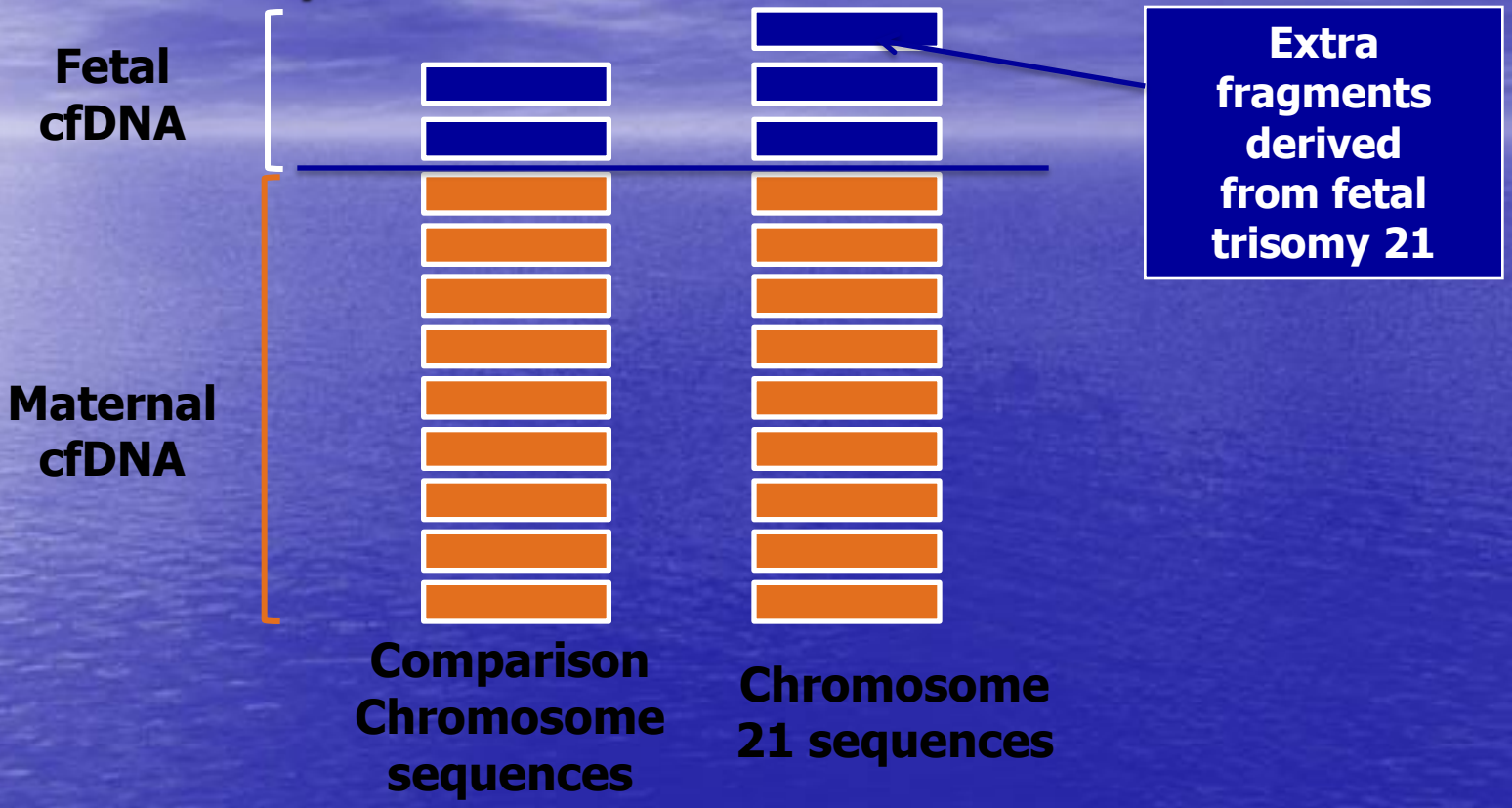
| | |
|---|---|
| SENSITIVITY (%) | 97.7 |
| SPECIFICITY (%) | 100 |
| VPP (%) | 100 |
| VPN (%) | 96.3 |
| EFFICIENCY (%) | 99.8 |

# Fetal Trisomy Detection With cfDNA



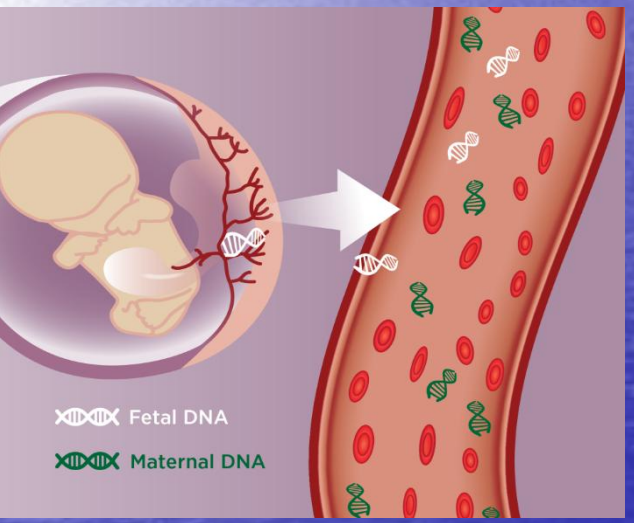

- Each bar represents hundreds of cfDNA fragments
- Counting of chromosome cfDNA fragments done by DNA sequencing

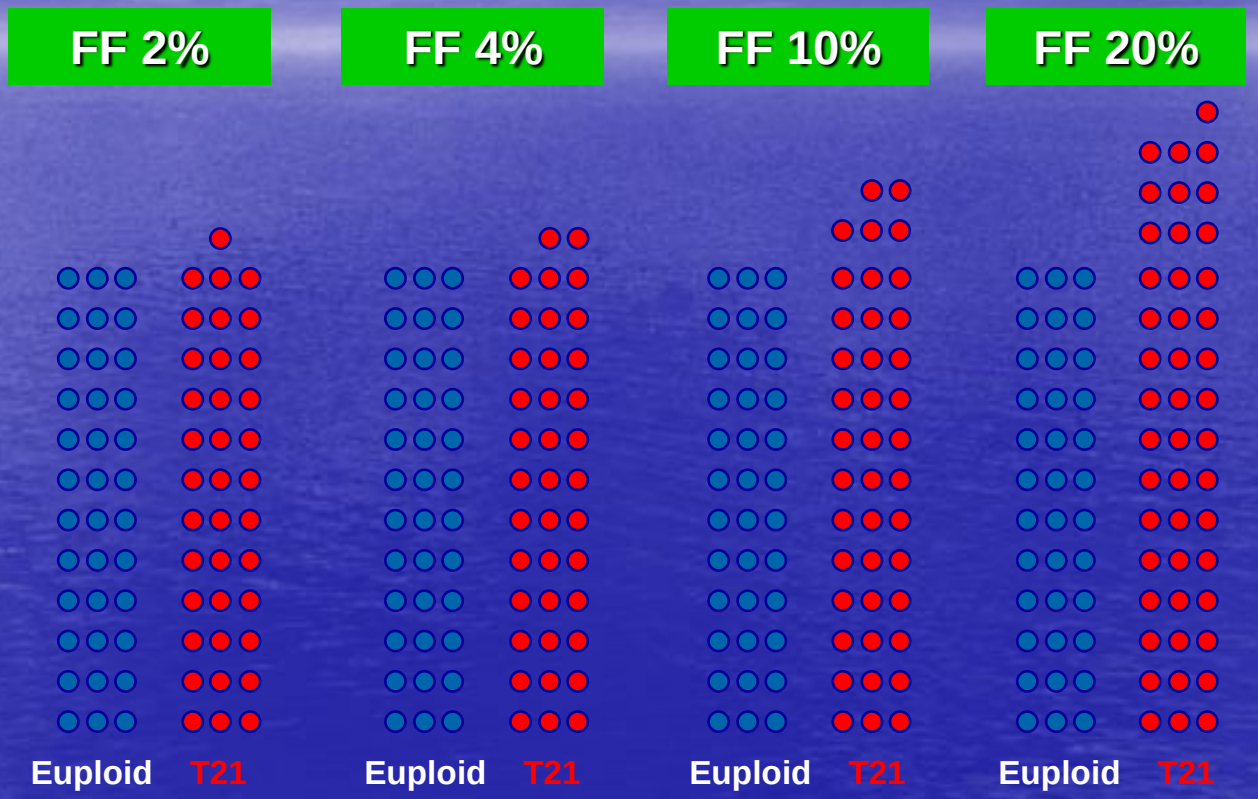
# Fetal Trisomy Detection With cfDNA




* Overabundance of chromosome 21 cfDNA fragments in T21, although small, can be measured with DNA sequencing

# Cell-free DNA in maternal blood
# Screening for aneuploidies




- Cell-free DNA (cfDNA) are short DNA fragments
- In pregnancy, cfDNA from both the mother and fetus are in maternal blood
- Amount of fetal cfDNA present is a small fraction of the maternal cfDNA

# NIPT Technology

***Different approaches to cfDNA analysis***

Massively Parallel Shotgun Sequencing (MPSS)

Directed Approach (e.g. Harmony Test)

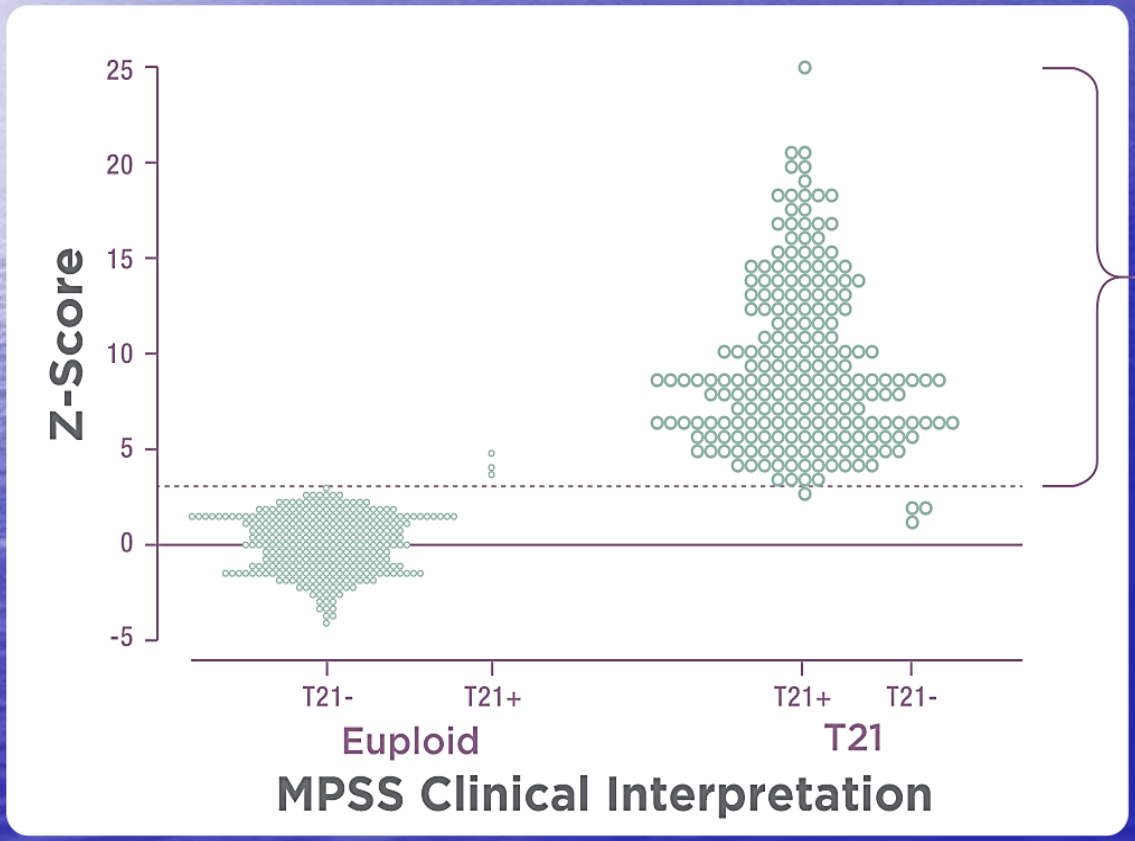
**Key differences**

Binary +/- result based on z-score

**Risk classification and risk score**

# Massively Parallel Shotgun Sequencing (MPSS)

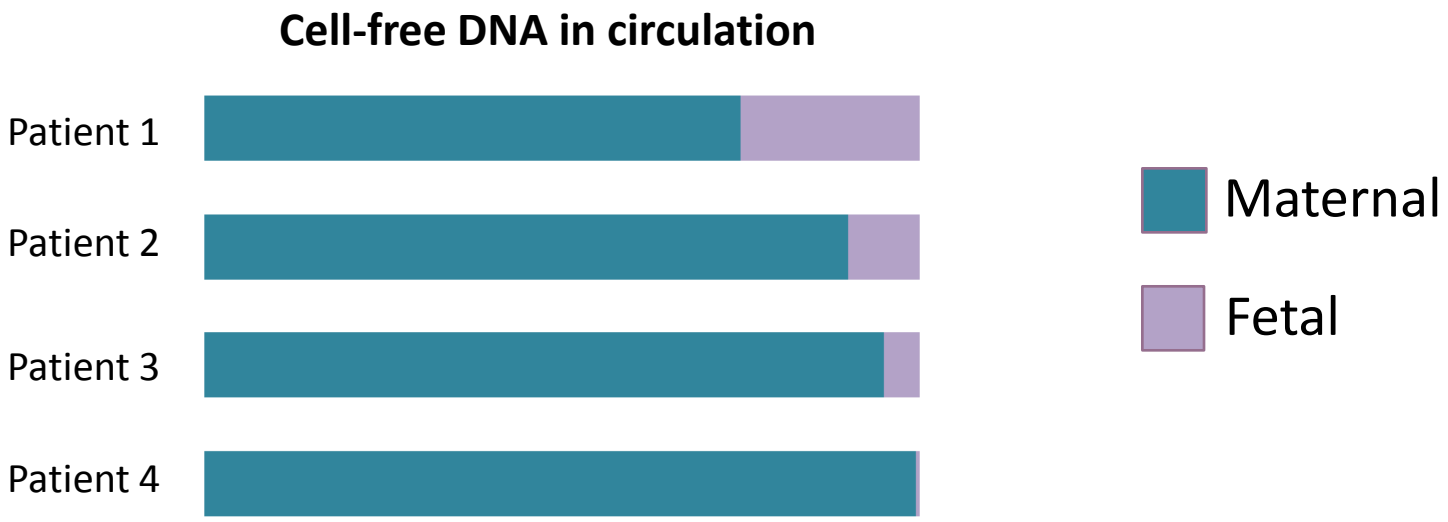
- **MPSS is a random sampling of cfDNA fragments**
- **An arbitrary z-score cut-off is used to determine trisomy**




All classified as "positive" with no distinction between extremely high values and those just above the cut-off

Palomaki GE et al., Genet Med. 2011 Nov;13(11):913-20.

# Importance of measuring fetal DNA amount

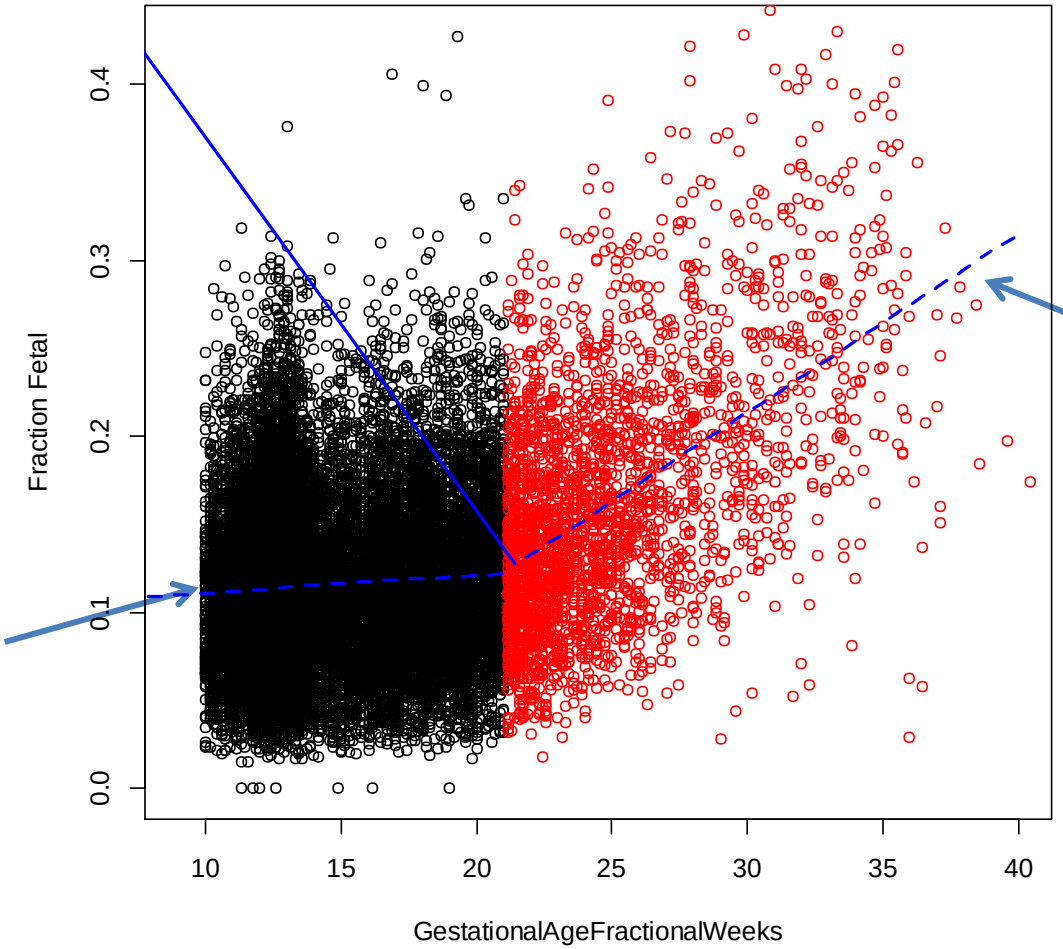



- Percentage of maternal to fetal DNA in circulation can vary from woman to woman, and changes throughout gestation[1]
- In some samples, there is very little or no detectable fetal DNA
- **Important to choose a lab that measures fetal fraction**
  - CAP accreditation program recommends that NIPT labs measure and report fetal fraction[2]

1. Wang E et al, Prenat Diagn. 2013 Jul;33(7):662-6. 2. College of American Pathologists Molecular Pathology Checklist. MOL.34927. 7/29/2013

# Fetal Fraction – Gestational Age Relationship

**Prior to 21 wks GA:** fetal fraction increases by 0.11% per week




**Post 21 wks GA:** fetal fraction increases by 1.1% per week

Wang E et al, Prenat Diagn. 2013 Jul;33(7):662-6.

# Consequences of NOT measuring fetal DNA

If very little fetal DNA is present, result is based on maternal DNA

- Male fetuses may be called as "female"
- **PATIENTS CARRYING FETUS WITH TRISOMY MAY RECEIVE FALSE REASSURANCE** (*increased risk of **"false negative"** results)*

➢ If fetal DNA percent is not measured and reported, validity of individual result is not known

*"Can deeper sequencing alleviate the need to measure fetal DNA?"*

- A recent independent study[1] evaluated various depths of sequencing of chromosome 21 at varying levels of fetal fraction
  - **Conclusion**: Detection rates will suffer if fetal DNA amount is 3% or less *at any depth of sequencing*

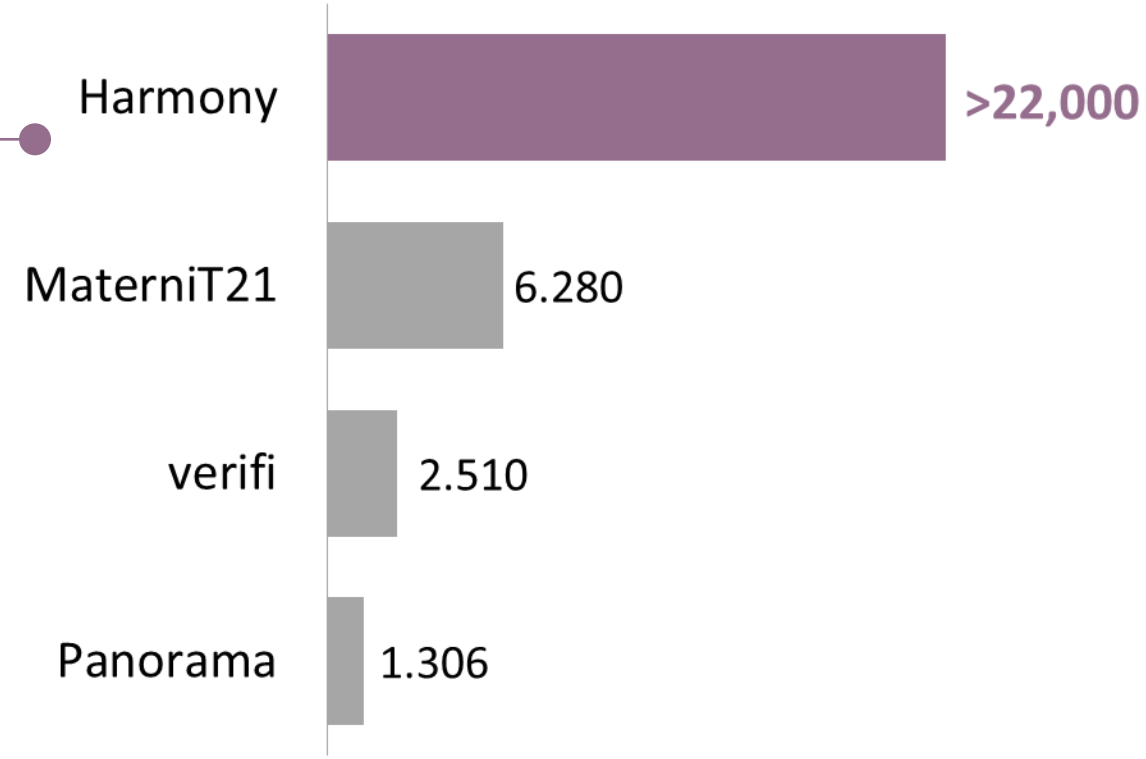
1. Benn and Cuckle. Prenat Diagn. 2014 Aug;34(8):778-83.

# Comparison of NIPT Tests

| | Harmony | MaterniT21+ *(Sequenom)* | verifi *(Verinata)* | NIFTY *(BGI)* | PraenaTest *(Lifecodexx)* | Panorama *(Natera)* |
|---|---|---|---|---|---|---|
| **Technology** | **Directed** | **MPSS (random)** | **MPSS (random)** | **MPSS (random)** | **MPSS (random)** | **Directed** |
| **Fetal fraction measured for proper testing** | + | + | − | − | + | + |
| **Test success rate** | + | + | + | + | + | − |
| **Individualized risk score** | + | − | − | − | − | + |
| **Low cost** | + | − | − | + | − | − |
| **Robust clinical studies** | + | + | − | − | − | − |

Data collected as of 11/06/2013

# Harmony is backed by extensive evidence




| | # of clinical studies | # of independent studies |
|---|---|---|
| Harmony | 10 | 3 |
| MateriniT21 | 6 | 0 |
| verifi | 3 | 0 |
| Panorama | 2 | 1 |

As of October 2014

# Extensive Clinical Data

**Clinical Validity and Use**

| Study | Subjects | Reference |
|---|---|---|
| NEXT – General pregnancy, 1st trimester | 18,955 | *NEJM 2015* |
| NICE - Cohort validation study | 3,228 | Norton M et al., AJOG 2012 |
| Clinical experience in Belgium & Netherlands | 3,000 | Willems et al, FVV 2014 |
| General pregnancy population, 1st trimester | 2,049 | Nicolaides et al, AJOG 2012 |
| Trisomy 13 | 1,949 | Ashoor et al., Ultra Obstet Gyn 2013 |
| Kypros Nicolaides clinical implementation | 1,005 | Mar Gil et al, Ultra Obstet Gyn 2013 |
| EU-NITE - European study | 520 | Verweij et al., Prenatl Diag, 2013 |
| High-risk population, 1st trimester | 400 | Ashoor et al., AJOG 2012 |
| FORTE | 338 | Sparks et al., AJOG 2012 |
| DANSR | 298 | Sparks et al., Prenat Diagn 2012 |
| Ob/Gyn real world experience | 289 | Fairbrother et al., Prenat Diagn 2013 |
| Twins study | 275 | Mar Gil et al., Fetal Diagn Ther 2013 |
| Sex chromosome aneuploidies, study 1 | 177 | Nicolaides et al., Fetal Diagn Ther 2013 |
| Sex chromosome aneuploidies, study 2 | 432 | Hooks et al., Prenat Diagn 2014 |

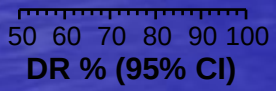
**Fetal Fraction**

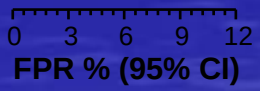
| | | |
|---|---|---|
| Maternal weight effects - commercial data | 22,000 | Wang et al., Prenat Diagn 2013 |
| Consistent in high and low-risk women | 3,007 | Brar et al, J Mat Fet Neonat Med 2013 |
| Fetal cfDNA and pregnancy complications | 1,949 | Poon et al., Fetal Diagn Ther 2013 |
| Maternal weight and fetal factors, study 2 | 1,949 | Ashoor et al. Ultras Obstet Gyn 2013 |
| Maternal weight and fetal factors, study 1 | 400 | Ashoor et al., Fetal Diagn Ther 2012 |
| Fetal fraction in twins | 70 | Struble et al., Fetal Diagn Ther 2013 |

# Cell free DNA test

## Trisomy 21

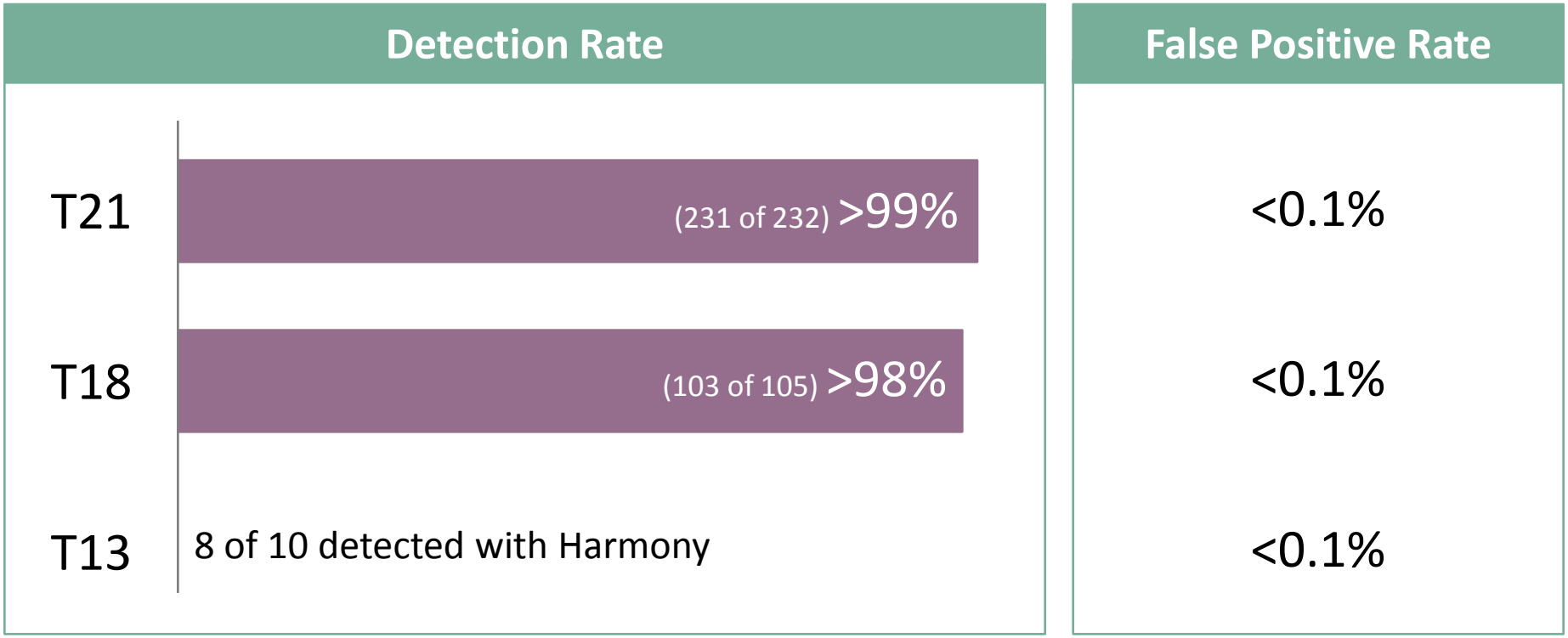
| Author | DR (95% CI) | Wt (%) | FPR (95% CI) | Wt (%) |
|---|---|---|---|---|
| Chiu *et al.*, 2011 [86] | 100.0 (95.8 to 100) | 10.52 | 2.06 (0.43 to 5.9) | 1.52 |
| Ehrich *et al.*, 2011 [39] | 100.0 (91.0 to 100) | 4.84 | 0.24 (0.01 to 1.4) | 4.02 |
| Palomaki *et al.*, 2011 [212] | 98.6 (95.9 to 99.7) | 25.76 | 0.20 (0.04 to 0.6) | 11.87 |
| Sehnert *et al.*, 2011 [13] | 100.0 (75.0 to 100) | 1.69 | 0.00 (0.00 to 10.3) | 0.37 |
| Ashoor *et al.*, 2012 [50] | 100.0 (92.9 to 100) | 6.17 | 0.00 (0.00 to 1.1) | 3.45 |
| Bianchi *et al.*, 2012 [89] | 100.0 (95.9 to 100) | 10.88 | 0.00 (0.00 to 0.9) | 3.97 |
| Jiang *et al.*, 2012 [16] | 100.0 (79.4 to 100) | 2.06 | 0.00 (0.00 to 0.4) | 7.93 |
| Lau *et al.*, 2012 [11] | 100.0 (71.5 to 100) | 1.45 | 0.00 (0.00 to 3.7) | 1.02 |
| Nicolaides *et al.*, 2012 [8] | 100.0 (63.1 to 100) | 1.09 | 0.00 (0.00 to 0.2) | 14.54 |
| Norton *et al.*, 2012 [81] | 100.0 (95.6 to 100) | 9.92 | 0.04 (0.00 to 0.2) | 18.89 |
| Sparks *et al.*, 2012 [36] | 100.0 (90.3 to 100) | 4.47 | 0.00 (0.00 to 2.8) | 1.37 |
| Zimmerman *et al.*, 2012 [11] | 100.0 (71.5 to 100) | 1.45 | 0.00 (0.00 to 2.7) | 1.40 |
| Guex *et al.*, 2013 [30] | 100.0 (88.4 to 100) | 3.75 | 0.00 (0.00 to 2.5) | 1.52 |
| Liang *et al.*, 2013 [37] | 100.0 (90.5 to 100) | 4.60 | 0.00 (0.00 to 1.0) | 3.63 |
| Nicolaides *et al.*, 2013 [25] | 100.0 (86.3 to 100) | 3.14 | 0.00 (0.00 to 1.8) | 2.09 |
| Song *et al.*, 2013 [8] | 100.0 (63.1 to 100) | 1.09 | 0.00 (0.00 to 0.2) | 13.41 |
| Stumm *et al.*, 2013 [39] | 97.4 (86.5 to 99.9) | 4.84 | 0.00 (0.00 to 0.9) | 4.20 |
| Verweij *et al.*, 2013 [18] | 94.4 (72.7 to 99.9) | 2.30 | 0.00 (0.00 to 0.7) | 4.83 |
| **Pooled analysis [809]** | **99.0 (98.2 to 99.6)** | **100.0** | **0.08 (0.03 to 0.14)** | **100.0** |




Gil et al., 2013

# High detection rate; low false positive rate




Studied in over 6,000 patients, including >2,000 average-risk women

Mosaicism


1. Sparks AB et al., Am J Obstet Gynecol. 2012 Apr;206(4):319.e1-9.
2. Ashoor G et al., Am J Obstet Gynecol. 2012 Apr;206(4):322.e1-5.
3. Sparks AB et al., Prenat Diagn. 2012 Jan;32(1):3-9.
4. Norton M et al., Am J Obstet Gynecol. 2012 Aug;207(2):137.e1-8.
5. Nicolaides KH et al., Am J Obstet Gynecol. 2012 Nov;207(5):374.e1-6.
6. Ashoor G et al., Ultrasound Obstet Gynecol. 2013 Jan;41(1):21-5.
7. Data on file

# Screening for trisomy 21 1960-2013

| | 100,000 pregnancies | | | |
|---|---|---|---|---|
| | Trisomy 21 N=200 | | 99,800 normal | |
| METHOD OF SCREENING | DR | Detected | False positive | |
| Maternal age | 30% | 60 | 5% | 4990 |
| Serum biochemistry at 16 wks | 70% | 140 | 5% | 4990 |
| Combined test at 12 wks | 90% | 180 | 5% | 4990 |
| Combined test PLUS at 12 wks | 97% | 194 | 3% | 2994 |
| **Cell-free DNA** | 99% | 198 | 0.08% | 80 |


***International Federation of Gynecology and Obstetrics***
***Working Group on Best Practice in Maternal-Fetal Medicine***

**Chair: G C Di Renzo**

**Expert members:**
**E Fonseca, Brasil**
**S Hassan, USA**
**M Kurtser, Russia**
**M T Leis, Mexico**
**K Nicolaides, UK**
**N Malhotra, India**
**H Yang, China**

**Expert members ex officio:**
**S Arulkumaran, FIGO**
**M Hod, EAPM**
**C Hanson, SM Committee**
**L Cabero, CBET Committee**
**Y Ville, ISUOG**
**M Hanson, DOHaD**
**PP Mastroiacovo, Clearinghouse**
**JL Simpson, March of Dimes**
**D Bloomer, GLOWM**

# Screening for chromosomal abnormalities and non invasive prenatal diagnosis and testing

- **Maternal age has a low performance as a screening for fetal chromosomal abnormalities with a DR of 30-50% for FPR of 5-20%. Therefore, invasive testing for diagnosis of fetal aneuploidies should not be carried out by taking into account only maternal age.**

- **First-line screening for trisomies 21, 18 and 13 should be achieved by the combined test, which takes into account maternal age, fetal nuchal translucency (NT) thickness, fetal heart rate (FHR) and maternal serum free β-human chorionic gonadotropin (β-hCG) and pregnancy-associated plasma protein-A (PAPP-A). The combined risk test has a DR of 90% for trisomy 21 and 95% for trisomies 18 and 13, at FPR of about 5%.**

- **The combined test could be improved by assessing additional ultrasonographic markers, including the fetal nasal bone and Doppler assessment of the fetal ductus venosus flow and tricuspid flow. If all those markers are included the DR is increased to more than 95% and the FPR decreased to less than 3%.**

- **Screening by analysis of cfDNA in maternal blood has a DR of 99% for trisomy 21, 97% for trisomy 18 and 92% of trisomy 13, at a total FPR of 0.4%.**

- **Clinical implementation of cfDNA testing should preferably be in a contingent strategy based on the results of first-line screening by the combined test at 11-13 weeks' gestation. In this case, we recommend the strategy below:**
  - Combined test risk over **1 in 100**: the patients can be offered the options of cfDNA testing or invasive testing.
  - Combined test risk between **1 in 101 and 1 in 2,500**: the patients can be offered the option of cfDNA testing
  - Combined test risk lower than **1 in 2,500:** there is no need for further testing.

# FETAL CELLS and ffDNA: CONCLUSIONS AND FUTURE PERSPECTIVES

- Although new methodologies based on SNPs and free fetal nucleic acids are arising, we believe that the use of fetal cells is still a good approach for non invasive prenatal diagnosis of fetal trisomies, because it allows us to visualize directly the fetal nuclei and their chromosomes. *In this respect, our SAFE test is up to know the only one offered at clinical level.*

- ffDNA can be utilised with high specificity and sensitivity for the determination of fetal sex and fetal RhD status as early as 9 wks gestation. Moreover recently its applicability for the diagnosis of trisomies has been clearly validated.


THANK YOU
GRAZIE
GRACIAS