NIPT para Aneuploidías

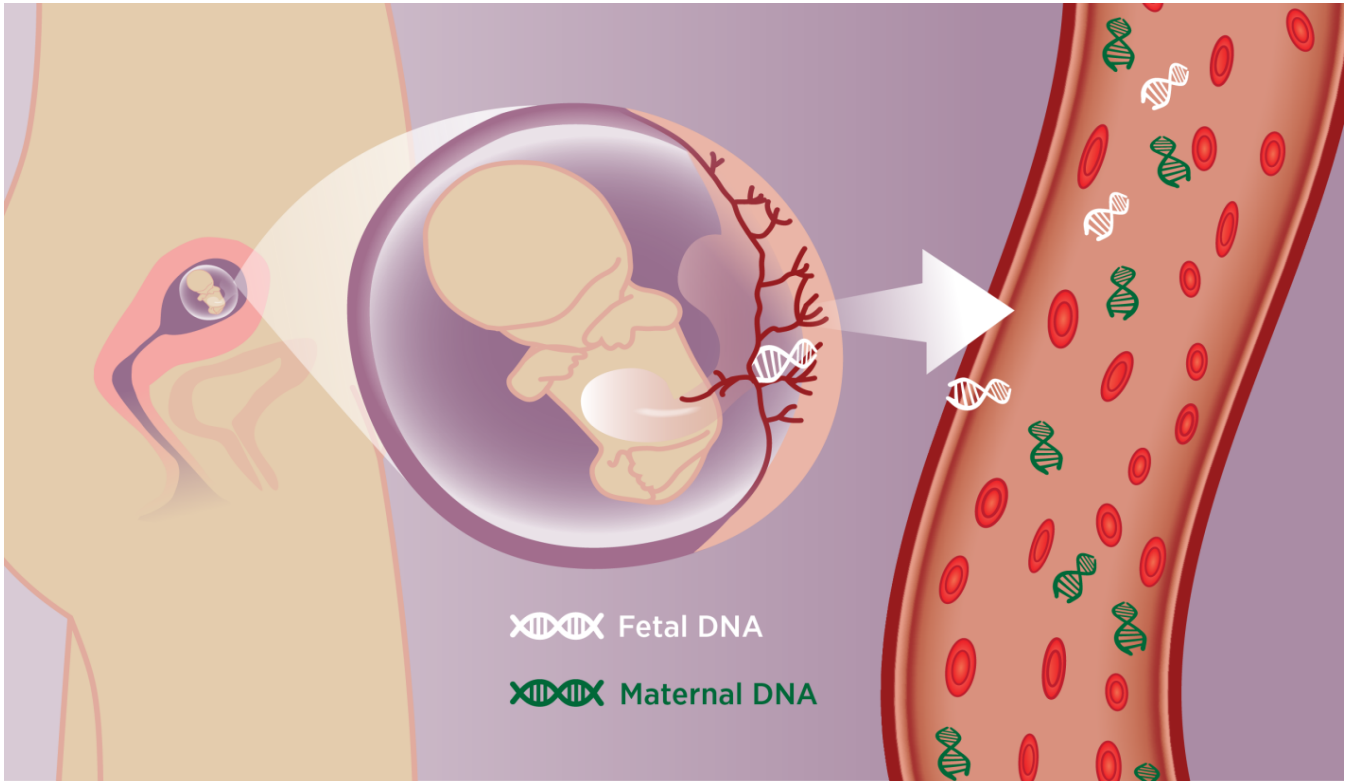
En Alto o Bajo Riesgo?

Vincenzo Cirigliano PhD

Genética Molecular
Labco, Barcelona
vincenzo.cirigliano@labco.eu

Cell-free DNA en Sangre Materna

- **Cell-free DNA (cfDNA) es presente en fragmentos muy cortos**
- **En todas las gestaciones hay cfDNA de madre y feto en circulación materna**
- **La cantidad de cfDNA fetal solo es una pequeña fracción del cfDNA materno**
- **cfDNA desaparece en pocas horas despues del parto**



LABCO
Quality Diagnostics

Aplicación Clinica

- **Enfermedades X-linked**

  El Cromosoma Y ha sido el primer marcador de cfDNA

  100% a partir de 8-10 semanas

- **Genotipaje Rh**

  Lo et al, 1998: La detección del genotipo RhD fetal es posible en todos los casos a partir del segundo trimestre

  Confirmado en los últimos años en varios laboratorios

  Primera Aplicación Rutinaria de Diagnóstico Prenatal No Invasivo (British National Blood Service 2001)

LABCO
Quality Diagnostics

Genotipaje Rh Fetal

	PRENATAL RHD	POSTNATAL RhD
RhD+	**184**	**184**
RhD + variants	**3 ***	**2**
RhD-	**91**	**91**
RhD -Variants	**4 ****	**5*****
Total	**282**	**282**

- ✓ Concordancia 100%
- ✓ 34% fetus RhD-

  (No anti-D)

* compatible with RHDVI type 1 or 4
** compatible RHD-CE-DS
*** compatible with de novo mutation

Grande *et al* 2012 Prenatal Diagnosis 32, 1–6

LABCO Quality Diagnostics

Detección de Mutaciones Paternas

- Distrofia Miotónica
- Acondroplasia
- Fibrosis Quística
- ß Talasemia
- Hiperplasia Adrenal Congénita

Recesivas padre y madre con misma mutación

- Anomalías Cromosómicas

LABCO
Quality Diagnostics

SEQUENCES OF PRENATAL TESTS

COUNSELLING (MATERNAL AGE/PREVIOUS HISTORY)

↓

Non Invasive Screening
1st / 2nd SERUM - ULTRASOUND

↓

CVS / AMNIOCENTESIS

↓

QF-PCR | aCGH | CYTOGENETICS

LABCO
Quality Diagnostics

Limitaciones del Cribado Actual

- Falsos Positivos

  Técnicas invasivas innecesarias, angustia

- Tiempo

  Pueden extenderse al segundo trimestre

- Conveniencia

  Múltiples visitas y ecografía pueden limitar acceso/eficacia

- Seguridad

  Rechazo a técnicas invasivas por el riesgo de perdida fetal

LABCO
Quality Diagnostics

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

Communicated by Leonard A. Herzenberg, Stanford University School of Medicine, Stanford, CA, August 22, 2008 (received for review July 13, 2008)

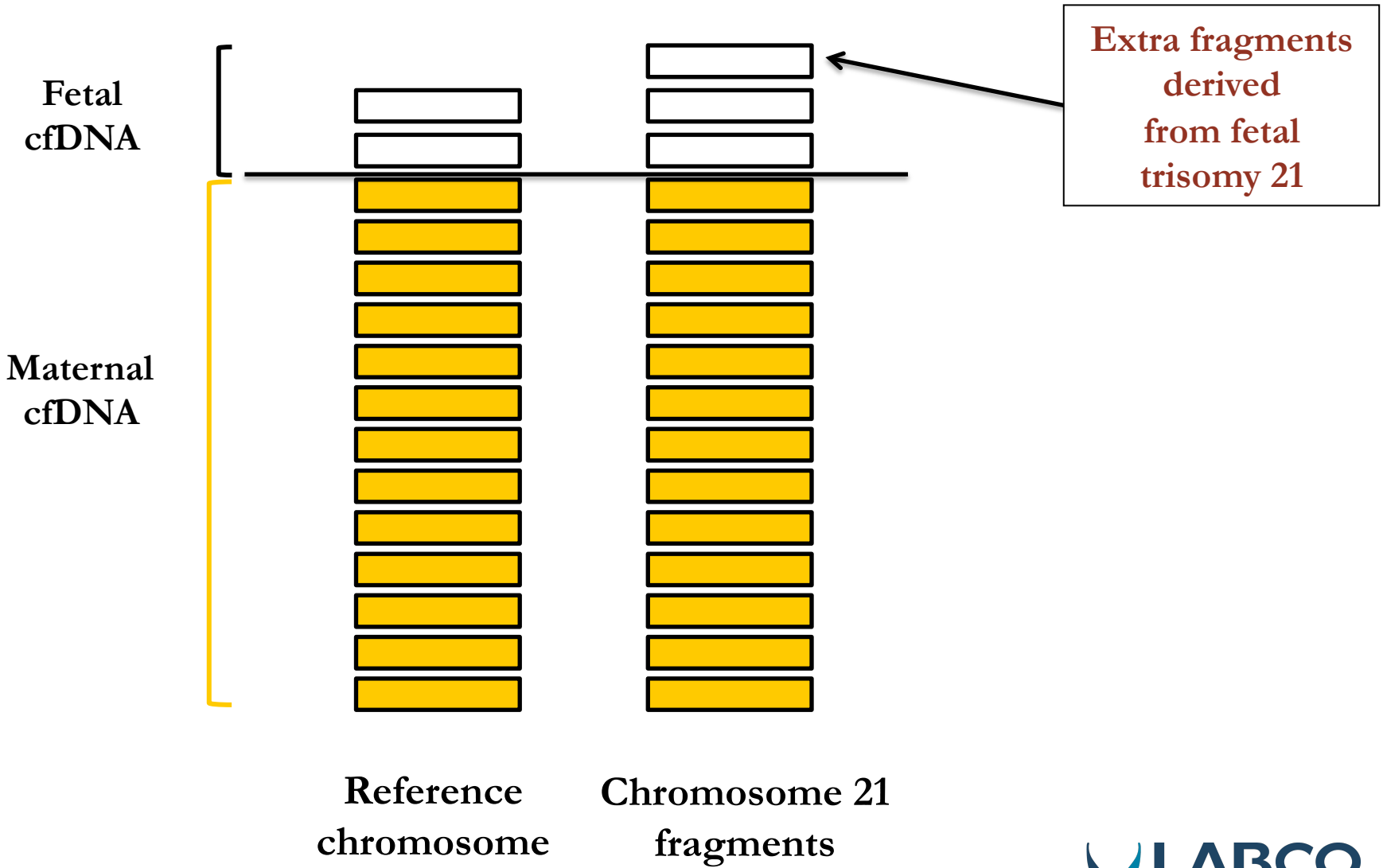
Noninvasive prenatal diagnosis of fetal chromosomal aneuploidy by massively parallel genomic sequencing of DNA in maternal plasma

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

Contributed by Charles R. Cantor, October 22, 2008 (sent for review September 29, 2008)

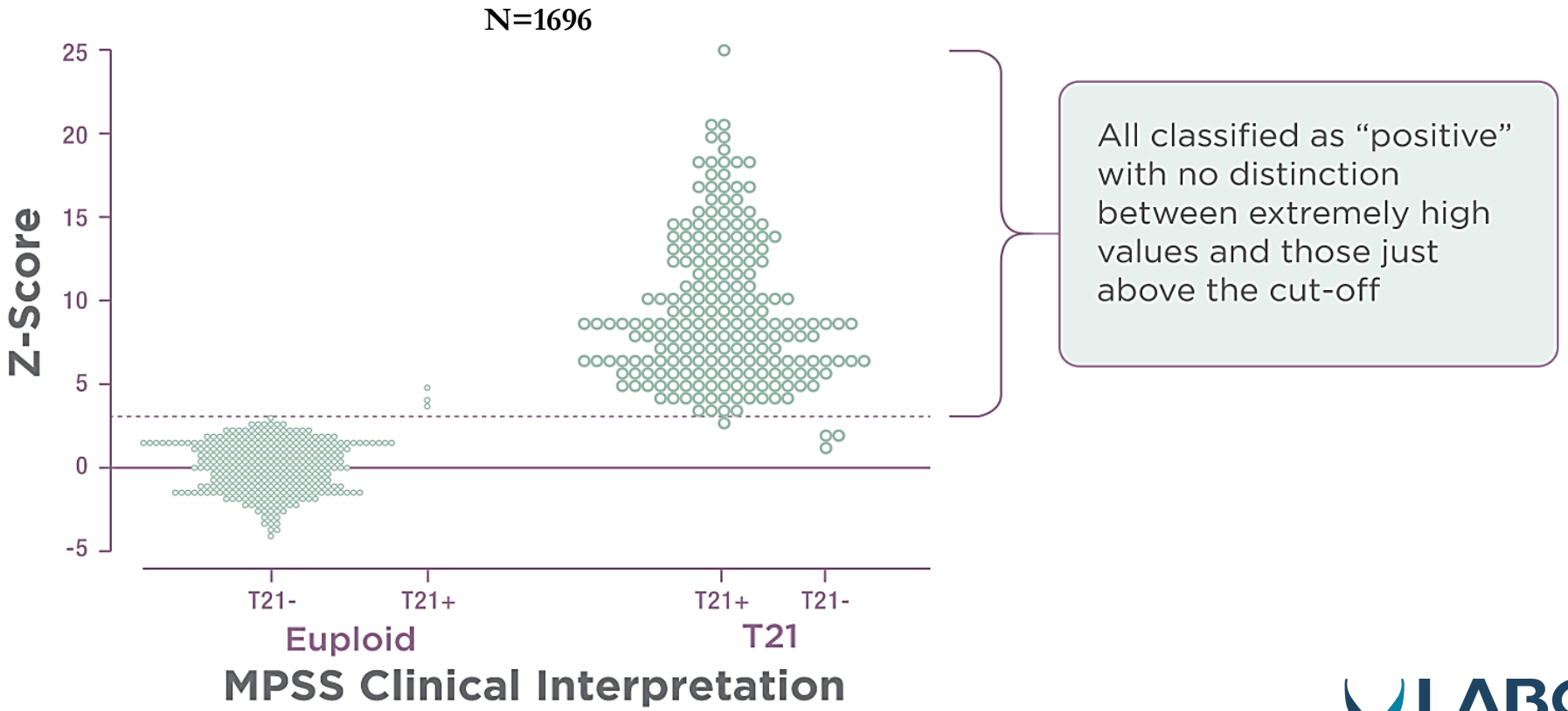
Fetal Trisomy Detection with cfDNA



LABCO
Quality Diagnostics

Massively Parallel Shotgun Sequencing (MPSS)

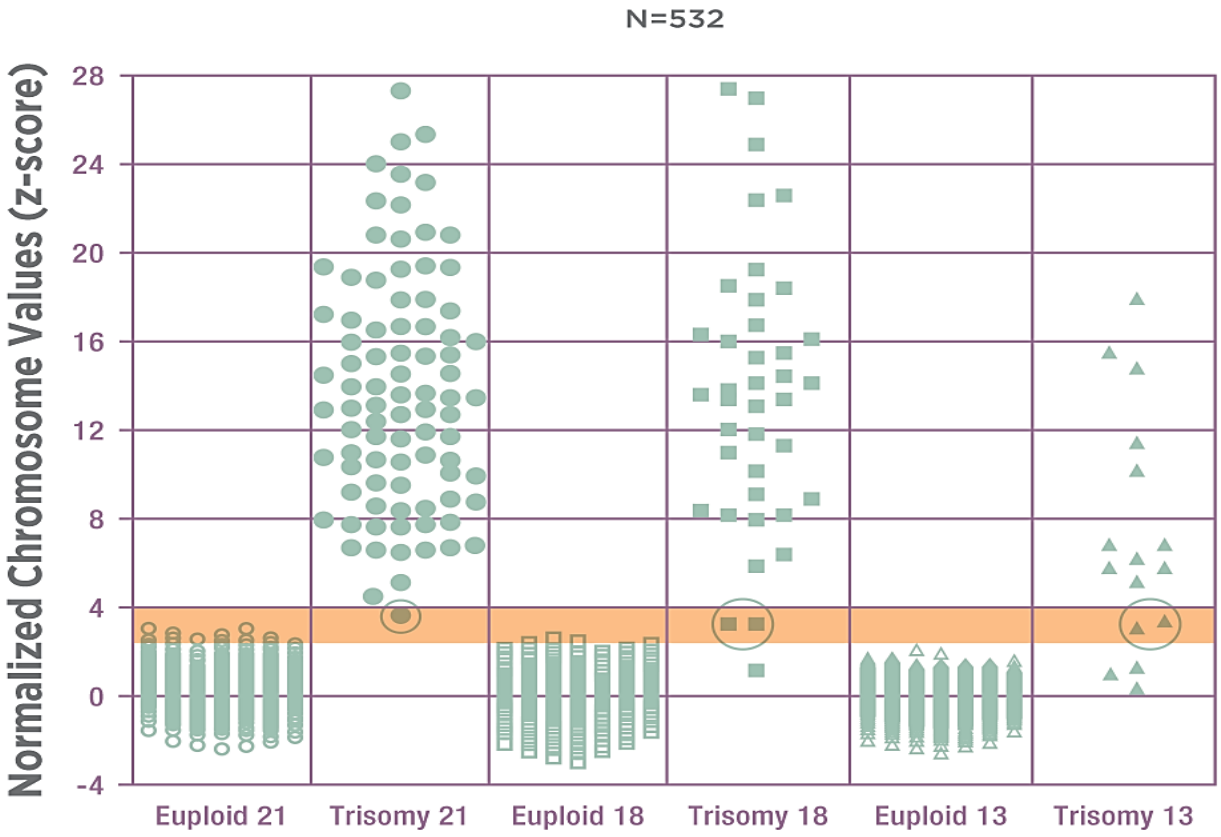
- **MPSS is a random sampling of cfDNA fragments**
- **An arbitrary z-score value is used as a cut-off for trisomy**



LABCO Quality Diagnostics

Palomaki et al. DNA sequencing of maternal plasma to detect Down syndrome: an international clinical validation study. Genet Med. 2011 Nov;13(11):913-20.

MPSS Unclassified Values

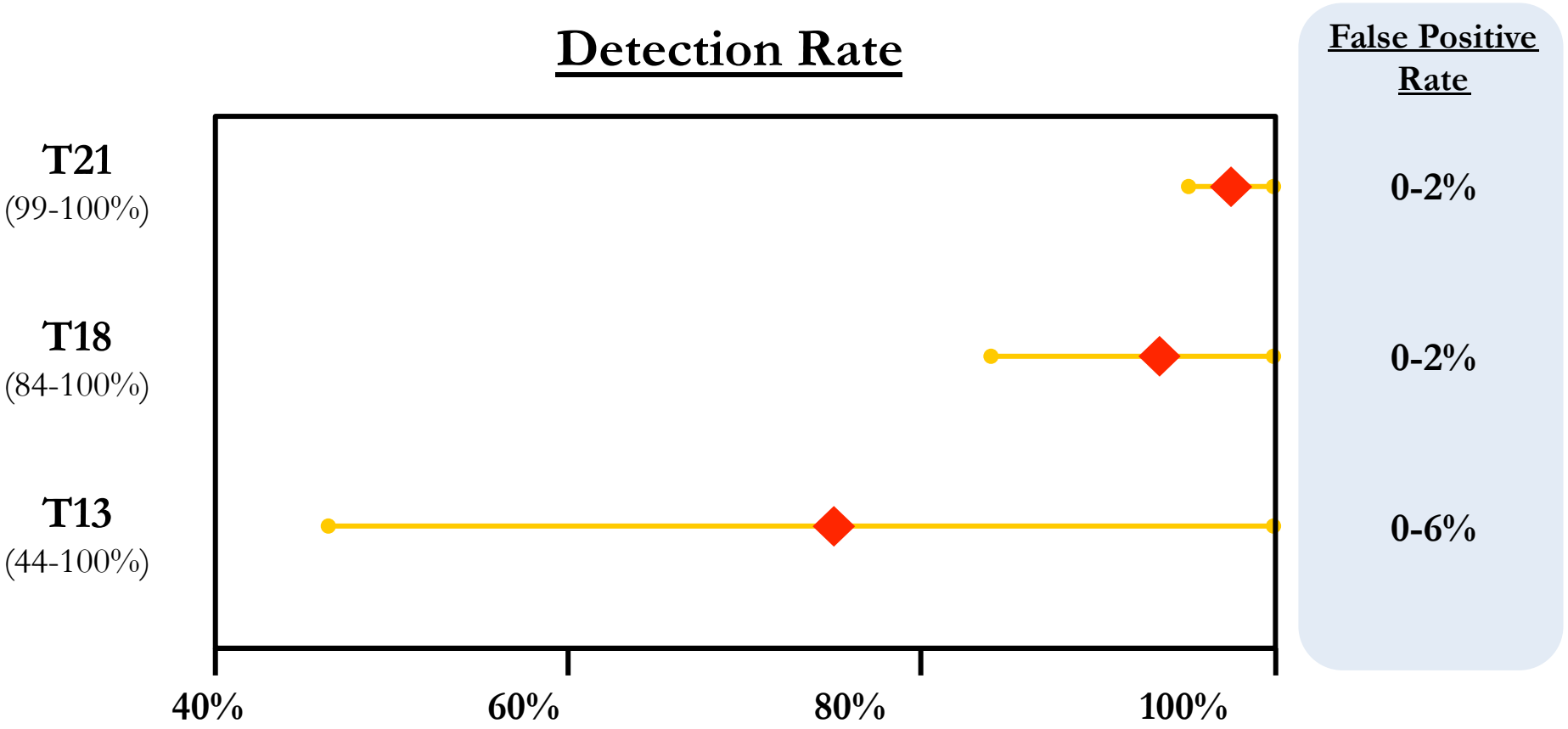


- "Unclassified" zone for values between 2.5-4
- Disproportionate number of positives in this zone

Bianchi, DW, et al. Genome-wide fetal aneuploidy detection by maternal plasma DNA sequencing. Obstet Gynecol. 2012 May;119(5):890-901.

LABCO Quality Diagnostics

MPSS Performance

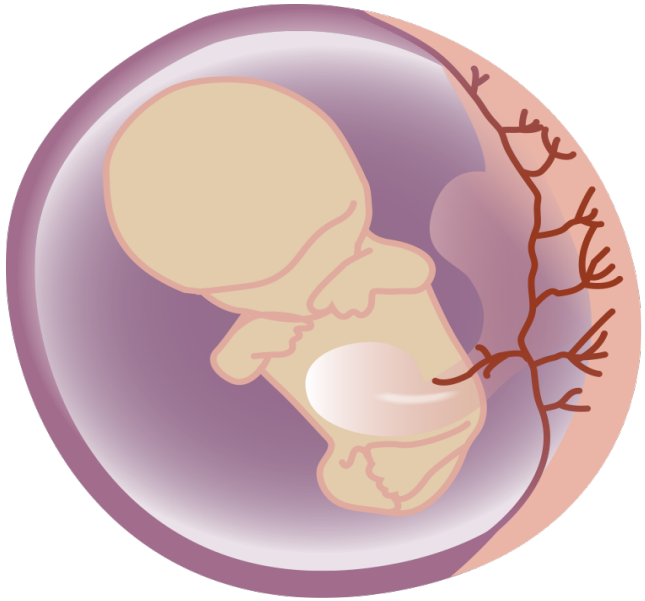


Palomaki GE, Kloza EM, Lambert-Messerlian GM, et al. DNA sequencing of maternal plasma to detect Down syndrome: an international clinical validation study. *Genet Med* 2011 Nov;13(11):913–20.; Bianchi DW, Platt LD, Goldberg JD, Abuhamad AZ, Sehnert AJ, Rava RP, Genome-Wide Fetal Aneuploidy Detection by Maternal Plasma DNA Sequencing. *Obstet Gynecol.* [Epub ahead of print] 2012 Feb 22.; Chiu et. Al BMJ 2011;342:c7401 Chen et.al (2011) http://www.plosone.org/article/info:doi/10.1371/journal.pone.0021791

LABCO Quality Diagnostics

T18, T13 y Mosaicismos Confinados a Placenta

- **El cariotipo de la placenta no siempre refleja el fetal**
- **Más frecuente para Chr 13 y 18 que Chr 21**



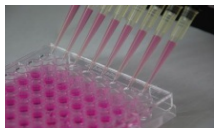
cfDNA se origina en placenta

- Probablemente el trofoblasto
- Paragonable a un "cariotipo semidirecto"
- CPM podrian generar falsos negativos y falsos positivos, en particular para T13 y T18

LABCO
Quality Diagnostics

TARGETED NIPT 21, 18, 13

DANSR™



(Digital ANalysis of Selected Regions)

- **Directed assay for cfDNA isolation and analysis.**
- **Targeted method allows for high throughput DNA sequencing**

FORTE™

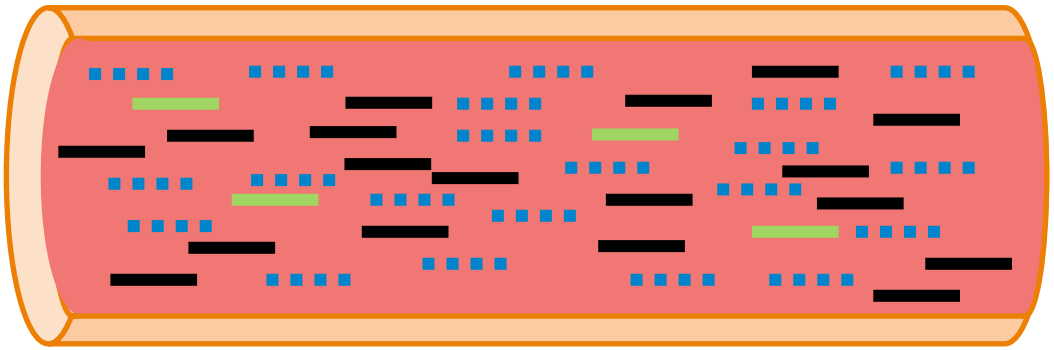
(Fetal-fraction Optimized Risk of Trisomy Evaluation)

- **New analysis that provides a trisomy risk score**
- **Incorporates DANSR assay results (chromosome counts, fetal fraction), maternal and gestational age**

High throughput and scalable test
Clinically interpretable results to patients

Harmony™ PRENATAL TEST

Assay Comparison – Targeted vs MPSS



cfDNA in blood

- Chr 21, 18, 13 cfDNA
- Other Chr cfDNA
- Unmapped cfDNA

DANSR™ (Directed)

Directed analysis

More efficient

MPSS (Shotgun)

Random analysis of cfDNA

LABCO Quality Diagnostics

Validacion/Aplicación Clínica

Study	Status	Description
NICE (Non-Invasive Chromosomal Evaluation)	**Published – Editor's choice in The Gray Journal (August 2012)**	**Multi-center (50 sites) clinical validation study, combined high risk and low risk women. Largest NIPT cohort study.**
Average Risk (Nicolaides)	**Published – The Gray Journal (2012, avail online)**	**Exclusive average-risk study of Harmony test in 1st trimester pregnancy**
Ariosa Blinded	**Published – Editor's choice in The Gray Journal (April 2012)**	**Blinded study with risk score reporting**
Nicolaides Blinded	**Published – Editor's choice in The Gray Journal (April 2012)**	**1st trimester blinded study**
Proof of Concept	**Published – cover article Prenatal Diagnosis (Jan 2012)**	**Initial description of directed cfDNA approach with combined average-risk and high-risk women**
Trisomy 13	**Published– The White Journal (2012, avail online)**	**Performance for T13 detection with combined average-risk and high-risk women**
Fetal Fraction – NICE substudy	**Published – J Mat Fet Med (2012, avail online)**	**Fetal fraction same in high-risk and low-risk women**
Fetal Fraction	**Published – Fetal Diagnosis and Therapy (2012)**	**Fetal fraction correlated to placental mass**
NITE (Non-Invasive Trisomy Evaluation)	Enrolled	Multi-center European blinded study
NEXT (Non-invasive EXamination of Trisomy)	Enrolling	Multi-center blinded study of average risk women comparing Harmony to 1st trimester combined screening

NICE Study

RESEARCH www.AJOG.org

GENETICS

Non-Invasive Chromosomal Evaluation (NICE) Study: results of a multicenter prospective cohort study for detection of fetal trisomy 21 and trisomy 18

Mary E. Norton, MD; Herb Brar, MD; Jonathan Weiss, MD; Ardeshir Karimi, MD; Louise C. Laurent, MD, PhD; Aaron B. Caughey, MD, PhD; M. Hellen Rodriguez, MD; John Williams III, MD; Michael E. Mitchell, MD; Charles D. Adair, MD; Hanmin Lee, MD; Bo Jacobsson, MD; Mark W. Tomlinson, MD; Dick Oepkes, MD, PhD; Desiree Hollemon, MSN, MPH; Andrew B. Sparks, PhD; Arnold Oliphant, PhD; Ken Song, MD

OBJECTIVE: We sought to evaluate performance of a noninvasive prenatal test for fetal trisomy 21 (T21) and trisomy 18 (T18).

STUDY DESIGN: A multicenter cohort study was performed whereby cell-free DNA from maternal plasma was analyzed. Chromosome-selective sequencing on chromosomes 21 and 18 was performed with reporting of an aneuploidy risk (High Risk or Low Risk) for each subject.

RESULTS: Of the 81 T21 cases, all were classified as High Risk for T21 and there was 1 false-positive result among the 2888 normal cases, for a sensitivity of 100% (95% confidence interval [CI], 95.5–100%) and a false-positive rate of 0.03% (95% CI, 0.002–0.20%). Of the 38 T18 cases, 37 were classified as High Risk and there were 2 false-positive results among the 2888 normal cases, for a sensitivity of 97.4% (95% CI, 86.5–99.9%) and a false-positive rate of 0.07% (95% CI, 0.02–0.25%).

CONCLUSION: Chromosome-selective sequencing of cell-free DNA and application of an individualized risk algorithm is effective in the detection of fetal T21 and T18.

Key words: aneuploidy detection, cell-free fetal DNA, Down syndrome, noninvasive prenatal diagnosis, trisomy

Cite this article as: Norton ME, Brar H, Weiss J, et al. Non-Invasive Chromosomal Evaluation (NICE) Study: results of a multicenter prospective cohort study for detection of fetal trisomy 21 and trisomy 18. Am J Obstet Gynecol 2012;207:x.ex-x.ex.

Currently, the most effective and commonly used prenatal screening

★ EDITORS' CHOICE ★

ing tests have false-positive rates of 2-3% and false-negative rates of ≥5%.[1-4] Pos

NICE Study

- 50 participating clinical sites in U.S. and Europe
- Largest cohort study to date – All eligible subjects evaluated
- Study population was women undergoing invasive testing for any indication and thus included low risk women

	Sensitivity	Specificity	False Positive Rate
Trisomy 21	**100%** (81/81)	**99.97%** (2887/2888)	**0.03%** (1/2888)
Trisomy 18	**97%** (37/38)	**99.93%** (2886/2888)	**0.07%** (2/2888)

Norton ME et al. (2012) American Journal of Obstetrics and Gynecology

Average Risk Study

REPORTS OF MAJOR IMPACT www.AJOG.org

AQ: 3

Noninvasive prenatal testing for fetal trisomies in a routinely screened first-trimester population

AQ: 1,au Kypros H. Nicolaides, MD; Argyro Syngelaki, RM; Ghalia Ashoor, MD; Cahit Birdir, MD; Gisele Touzet, MD

AQ: 2 **OBJECTIVE:** We sought to assess performance of noninvasive prenatal testing for fetal trisomy in a routinely screened first-trimester pregnancy population.

STUDY DESIGN: This was a cohort study of 2049 pregnant women undergoing routine screening for aneuploidies at 11-13 weeks' gestation. Plasma cell-free DNA analysis using chromosome-selective sequencing was used. Laboratory testing on a single plasma sample of 2 mL was carried out blindly and results were provided as risk score (%) for trisomies 21 and 18.

RESULTS: Trisomy risk scores were given for 95.1% (1949 of 2049) of cases including all 8 with trisomy 21 and 2 of the 3 with trisomy 18. The trisomy risk score was >99% in the 8 cases of trisomy 21 and 2 of trisomy 18 and <1% in 1937 (99.9%) of the 1939 euploid cases.

CONCLUSION: Noninvasive prenatal testing using chromosome-selective sequencing in a routinely screened population identified trisomies 21 and 18 with a false-positive rate of 0.1%.

Key words: first trimester, noninvasive prenatal diagnostics, prenatal screening, trisomy 18, trisomy 21

Cite this article as: Nicolaides KH, Syngelaki A, Ashoor G, et al. Noninvasive prenatal testing for fetal trisomies in a routinely screened first-trimester population. Am J Obstet Gynecol 2012;207:x.ex-x.ex.

In the last 40 years, screening and diagnosis of fetal aneuploidies has

★ EDITORS' CHOICE ★

women with singleton pregnancies attending for their routine first hospital

Average Risk Study

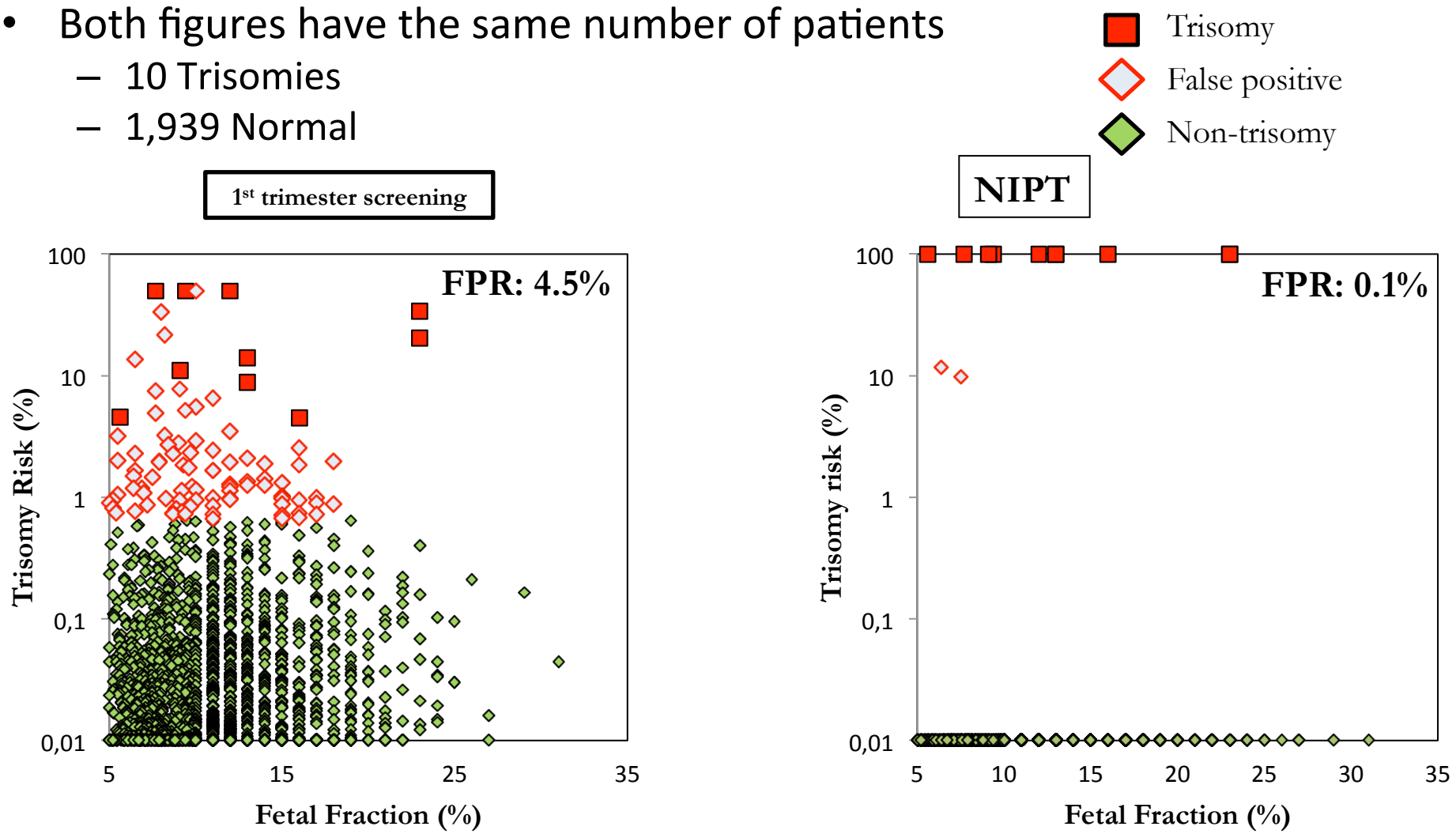
- Independent blinded study
- Patient population:
  - 1st trimester pregnancy (11-13 weeks gestation)
  - General screening population of 2,049 women
- Results
  - NIPT test detected all trisomy cases
    - Trisomy 21: 8 of 8; Trisomy 18: 2 of 2
    - Risk score of >99% given for each trisomy
  - False positive rate
    - NIPT: 2 of 1,939 (0.1%)
      - No false positives for trisomy 21
      - 0.1% false positives for trisomy 18
    - Conventional screening (serum + NT ultrasound): 87 of 1,939 (4.5%)

Nicolaides KH et al. (2012) American Journal of Obstetrics and Gynecology

LABCO Quality Diagnostics

Average Risk Study – Risk Score Comparison

- Both figures have the same number of patients
  - 10 Trisomies
  - 1,939 Normal



Low False Positives

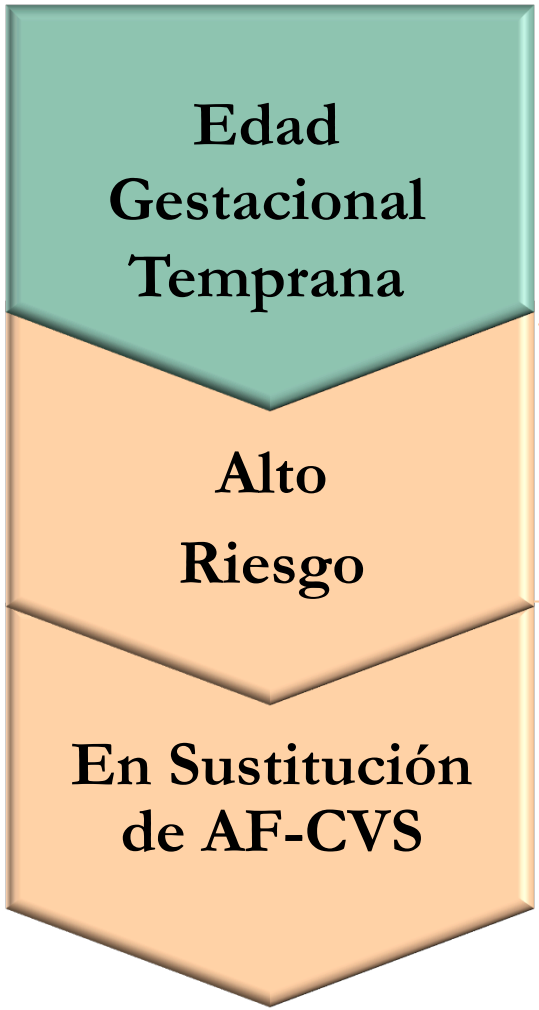
	False positive rate					List price
	T21	T18	T13	Y	Total	
Harmony™ PRENATAL TEST[1-3]	<0.1%	<0.1%	<0.1%	N/A	**<0.3%**	**$795**
MaterniT21™ PLUS	0.2%	0.28%	0.97%	0.6%	**2.0%**	**~$2,700**

Targeted NIPT shows false positive rates 5-7x lower than MPS

LABCO Quality Diagnostics

1. Norton et al, *Am J of Obstet and Gyn*, 2012; 2. Nicolaides KH et al, *Am J Obstet Gynecol* 2012; 3. Ashoor G et al., *Ultrasound Obstet Gynecol* 2012 (online); 4. Palomaki GE et al, *Genet Med* 2011; 5. Palomaki et al, *Genet Med* 2012; 6. MaterniT21 report example accessed Aug 2012

Potencial Utilidad Clínica del NIPT



- NIPT + Ecografía: Probable mejora de la eficiencia del cribado actual
- NIPT detecta las trisomías comunes con precisión
- Ecografía centrada en anomalías no relacionadas a las trisomías

- Descartar trisomías mas frecuentes con elevada especificidad
- NIPT solo detecta 3 de las posibles anomalías cromosómicas
- Técnica invasiva necesaria de todas formas para confirmar eventuales resultados positivos

- No es un test diagnostico, resultados de riesgos van confirmados con técnicas invasivas
- Solo útil para descartar trisomías mas frecuentes
- La utilización de los arrays ha ampliado enormemente el poder diagnóstico de las técnicas invasivas

LABCO
Quality Diagnostics

SEQUENCES OF PRENATAL TESTS

COUNSELLING (MATERNAL AGE/ HISTORY)

Non Invasive Screening
1st Trimester

mid/low

High/Ultrasound

NIPD

NIPD?

CVS / AF Confirmations

CVS / AF

QF-PCR

QF-PCR

aCGH

LABCO
Quality Diagnostics

SEQUENCES OF PRENATAL TESTS

COUNSELLING (MATERNAL AGE/ HISTORY)

Low Risk Screening (Anxiety)

NIPD

Common Trisomies
Residual risk 1:1200

CVS / AF

QF-PCR

aCGH

Whole Genome

LABCO
Quality Diagnostics

Position Statement MPS

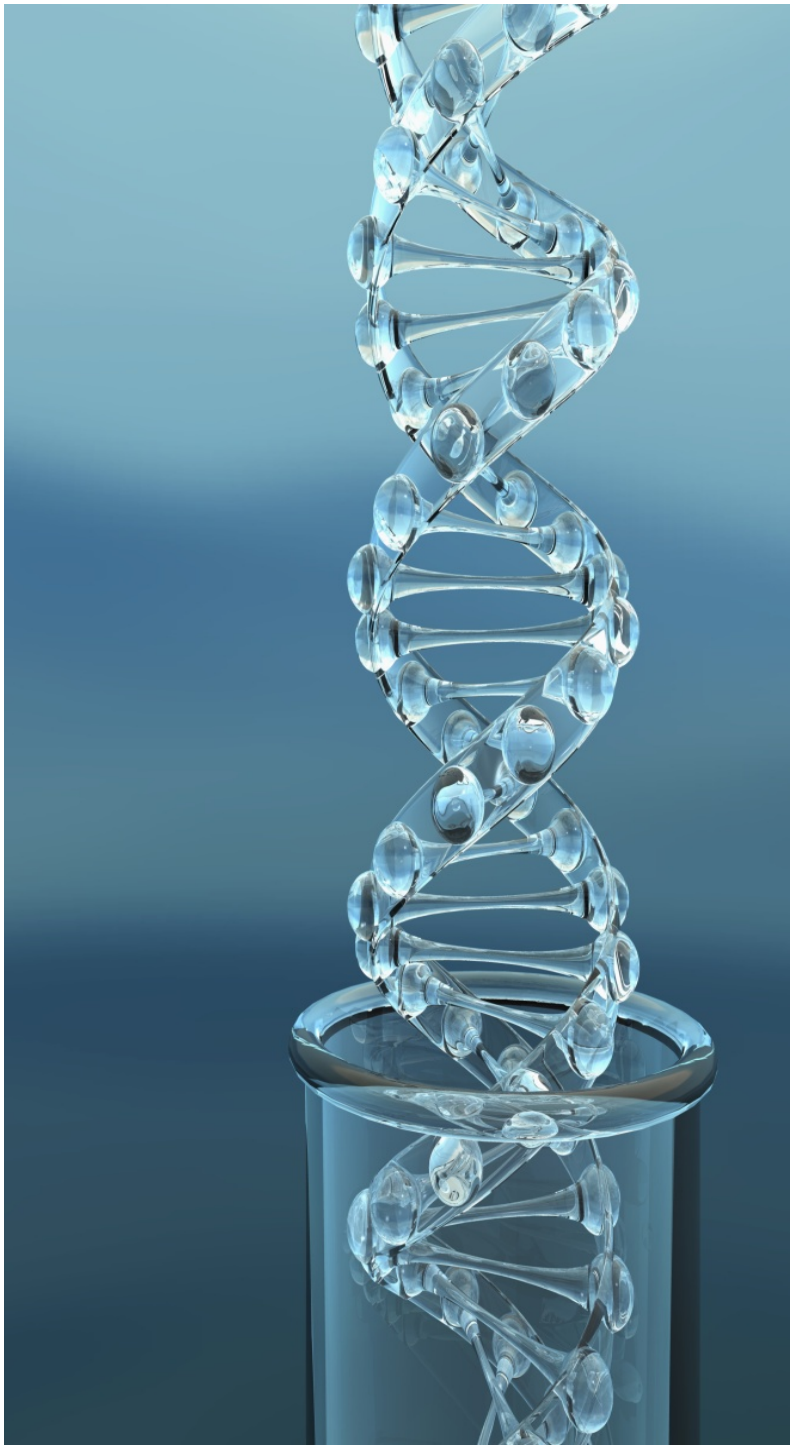
- The test is Advanced Screening not Diagnostic

- Only detects about half Chromosome Abnormalities detected by AF/CVS in women with positive screening

- More data are needed before its application in population screening

- Suitable for recognized high risk pregnancies but only after Genetic Counseling

LABCO
Quality Diagnostics



LABCO
Quality Diagnostics

GRÀCIES!

Vincenzo Cirigliano

Genética Molecular
Labco, Barcelona
vincenzo.cirigliano@labco.eu