Societat Catalana d'Obstetrícia i Ginecologia

Cribratge prenatal d' aneuploïdies comunes mitjançant *cffDNA*

Carmina Comas
3 febrer 2017

Germans Trias i Pujol Hospital
Institut Català de la Salut

Societat Catalana d'Obstetrícia i Ginecologia
Curs de Genètica Aplicada a Medicina Fetal
Divendres 3 de febrer de 2017|9:00h| Sala 9 Acadèmia Can Caralleu

NIPS: non invasive prenatal screening (cffDNA)

Objectius

- Situació actual del cribratge prenatal d' aneuploïdies
- Tècniques i tests comercials disponibles
- Fets
- Conceptes erronis
- Assessorament
- Questions no resoltes
- Recomanacions societats científiques
- Futur

Background (I)

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DS screening test

Unnecessary Worry:

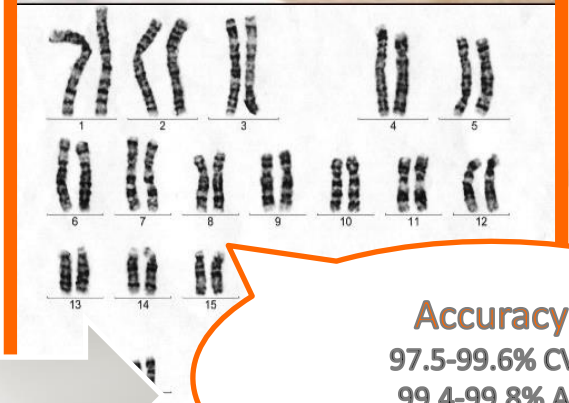
For every 20 women who test positive for Down Syndrome, only one will be carrying a baby with Down Syndrome.

False Confidence:

Of every 20 women carrying a baby with Down Syndrome, 3-4 will test negative by biochemical screening.

FPR 1.9-5.2%
FNR 12-23%

karyotyping



Accuracy
97.5-99.6% CVS
99.4-99.8% AF
Miscarriage rate 0.5-1%

NIPT

Non Invasive Prenatal Testing

Background (II)

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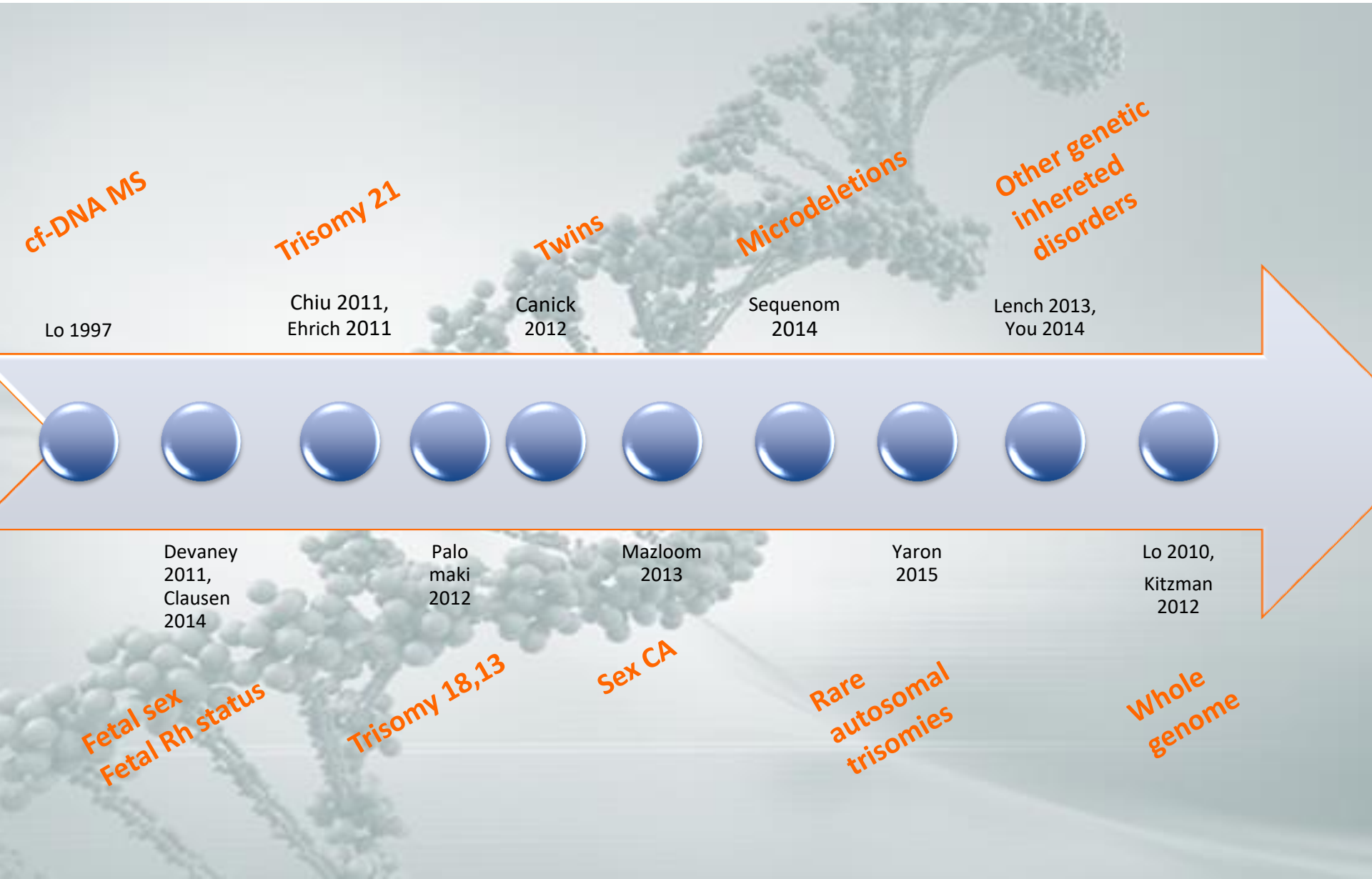
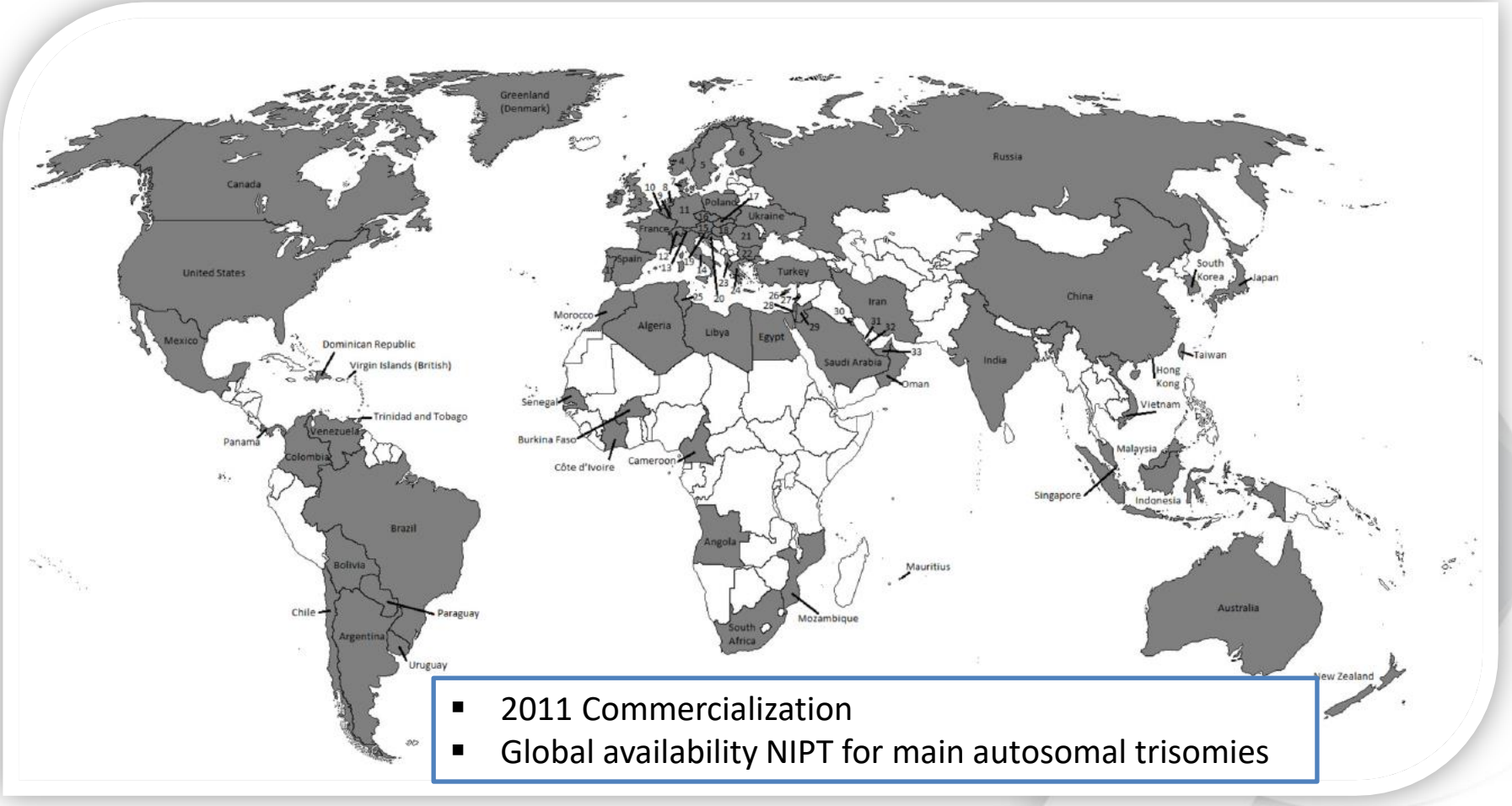
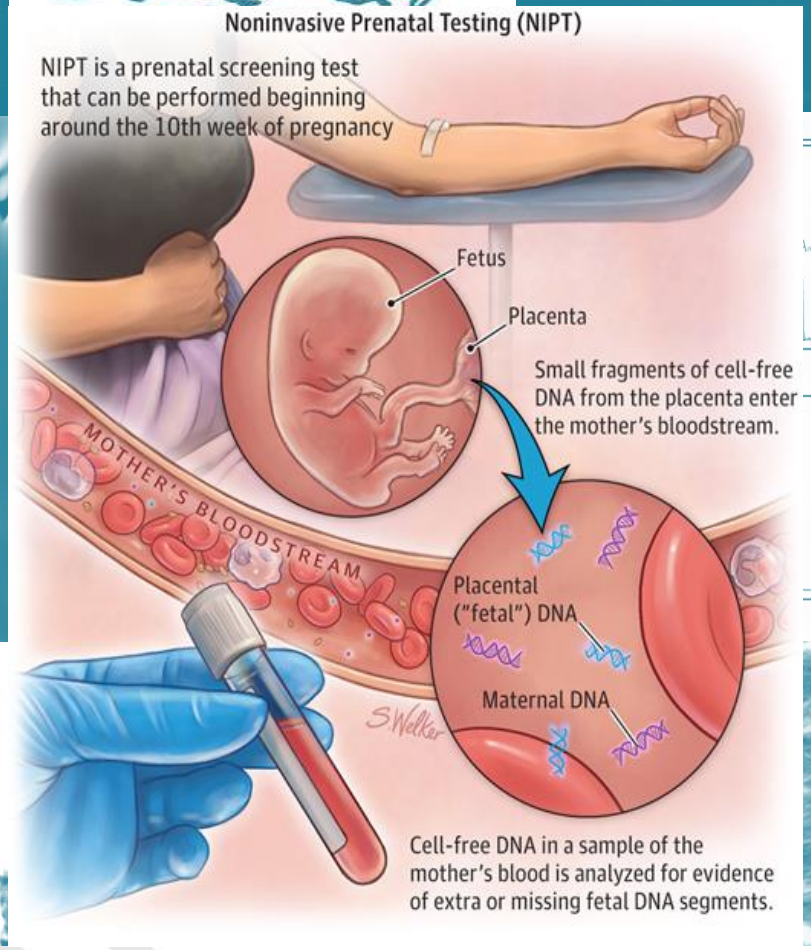


Background (III)



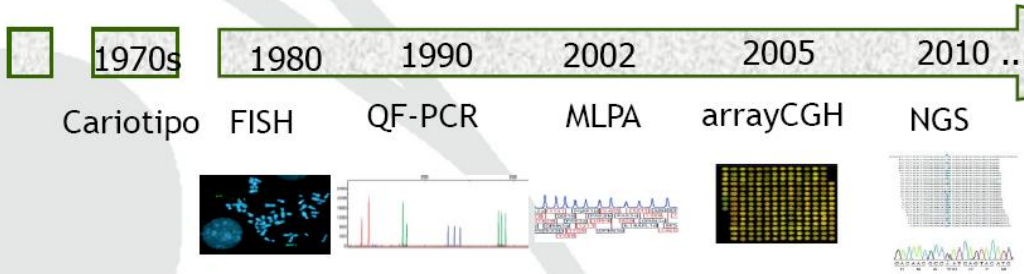
- 2011 Commercialization
- Global availability NIPT for main autosomal trisomies

Allyse M et al. NIPT: a review of international implementation and challenges. International Journal Women's Health 2015:7;113-126



NIPT: CLUE CONCEPTS

- ❑ cfDNA
- ❑ NGS
- ❑ Trophoblastic origin
- ❑ High clearance after delivery
- ❑ FF: fetal DNA/fetal+ maternal DNA (10-15%)
- ❑ Correlation GA
- ❑ Inversed correlation BMI



Counting method (I)

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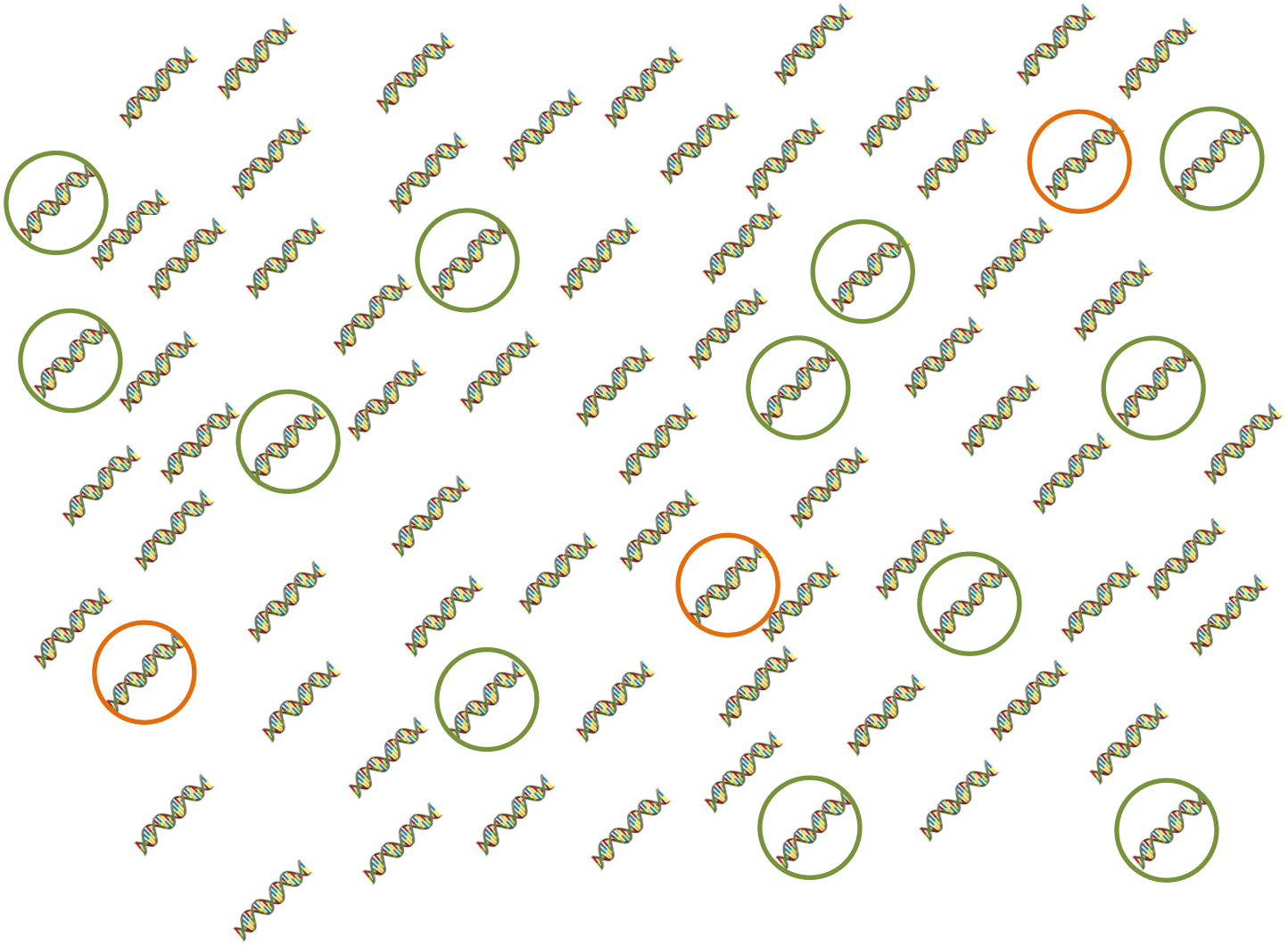
Hospital
Institut Català de la Salut



Counting method (II)

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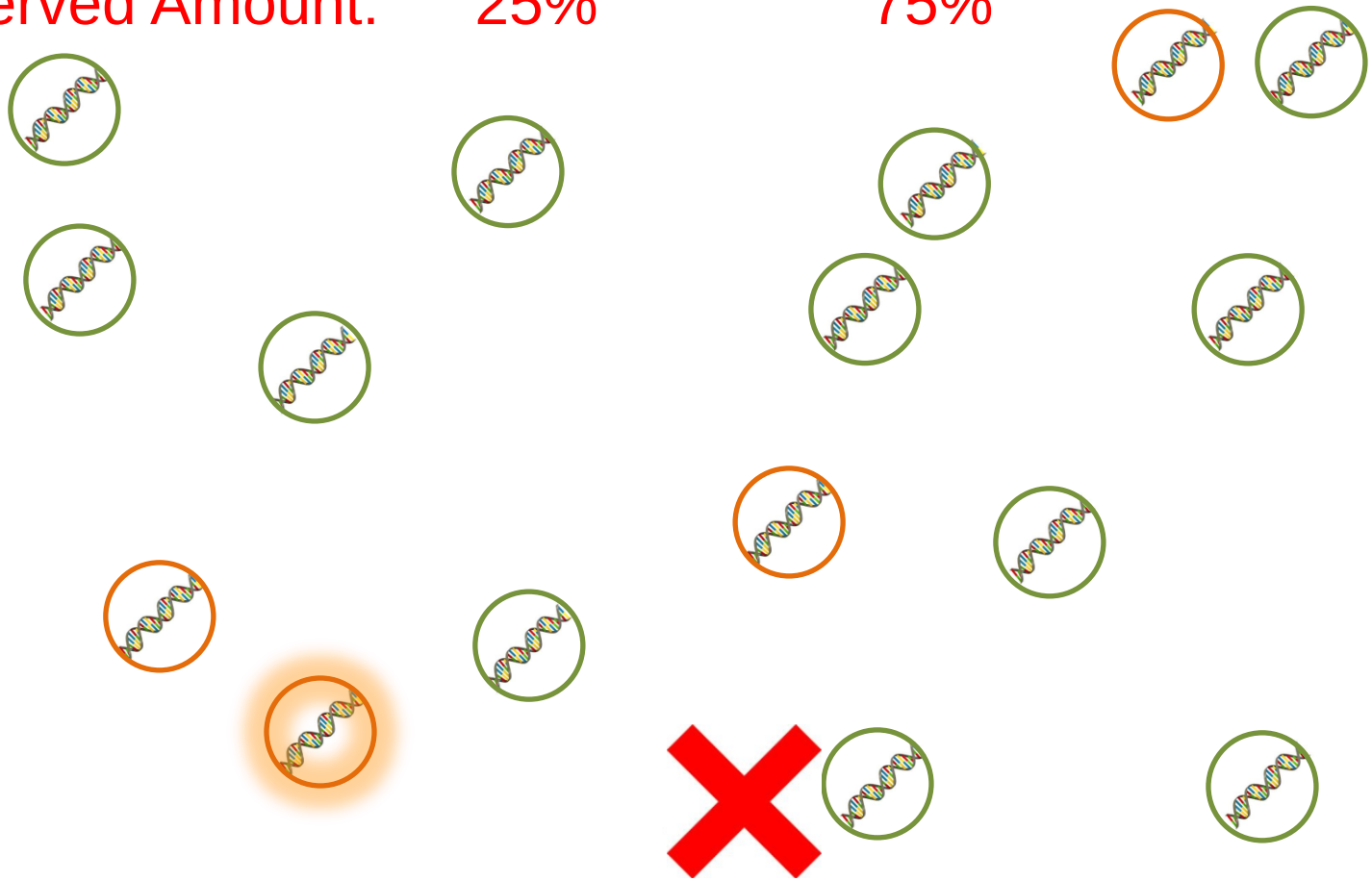
	Chrom 21 1.5% of genome	Chrom 3 6.2% of genome
Expected Amount:	20%	80%
Observed Amount:	25%	75%



Counting method (III)

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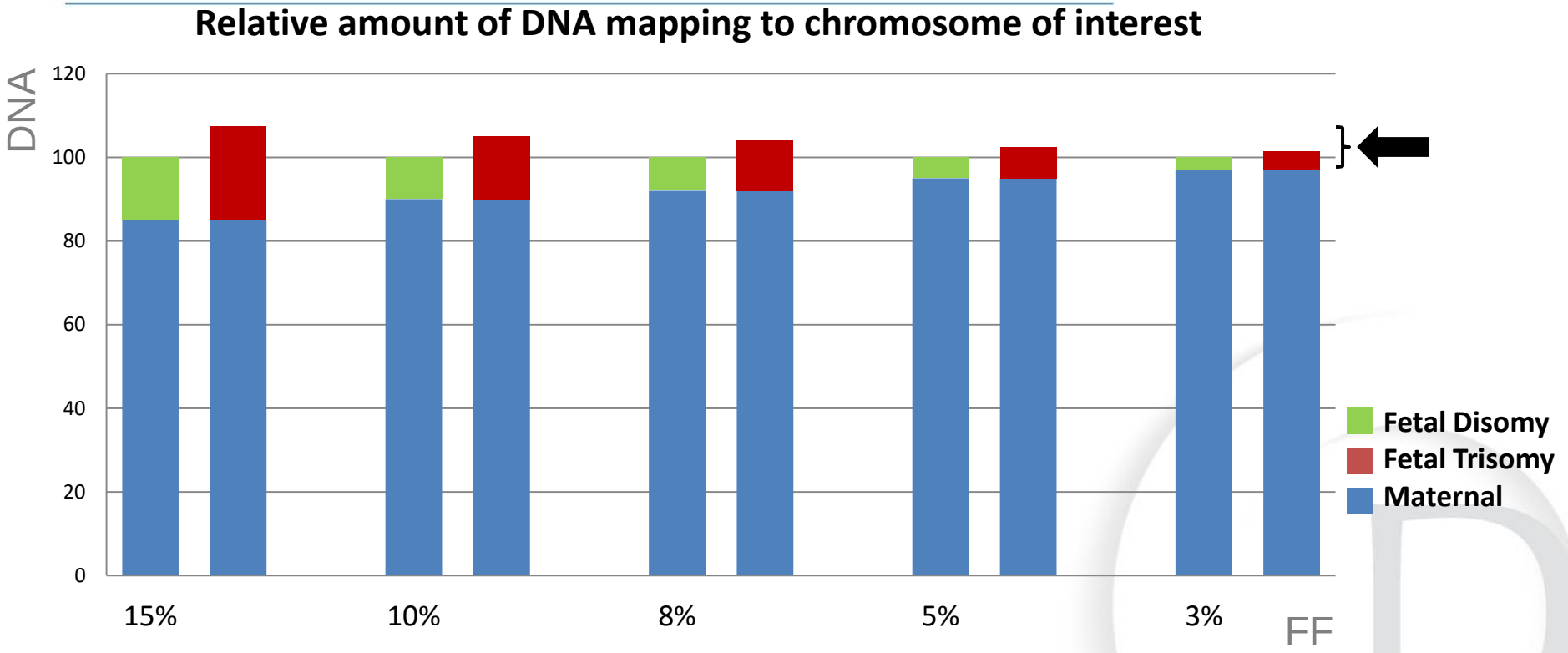

% Fetal Fraction	Counting Sensitivity[1]
>8%	>99%
4-8%	75%

1. Palomaki GE et al. DNA sequencing of maternal plasma to detect Down syndrome: an international clinical validation study. *Genet Med*. 2011;13::913-20.

Counting method (IV)

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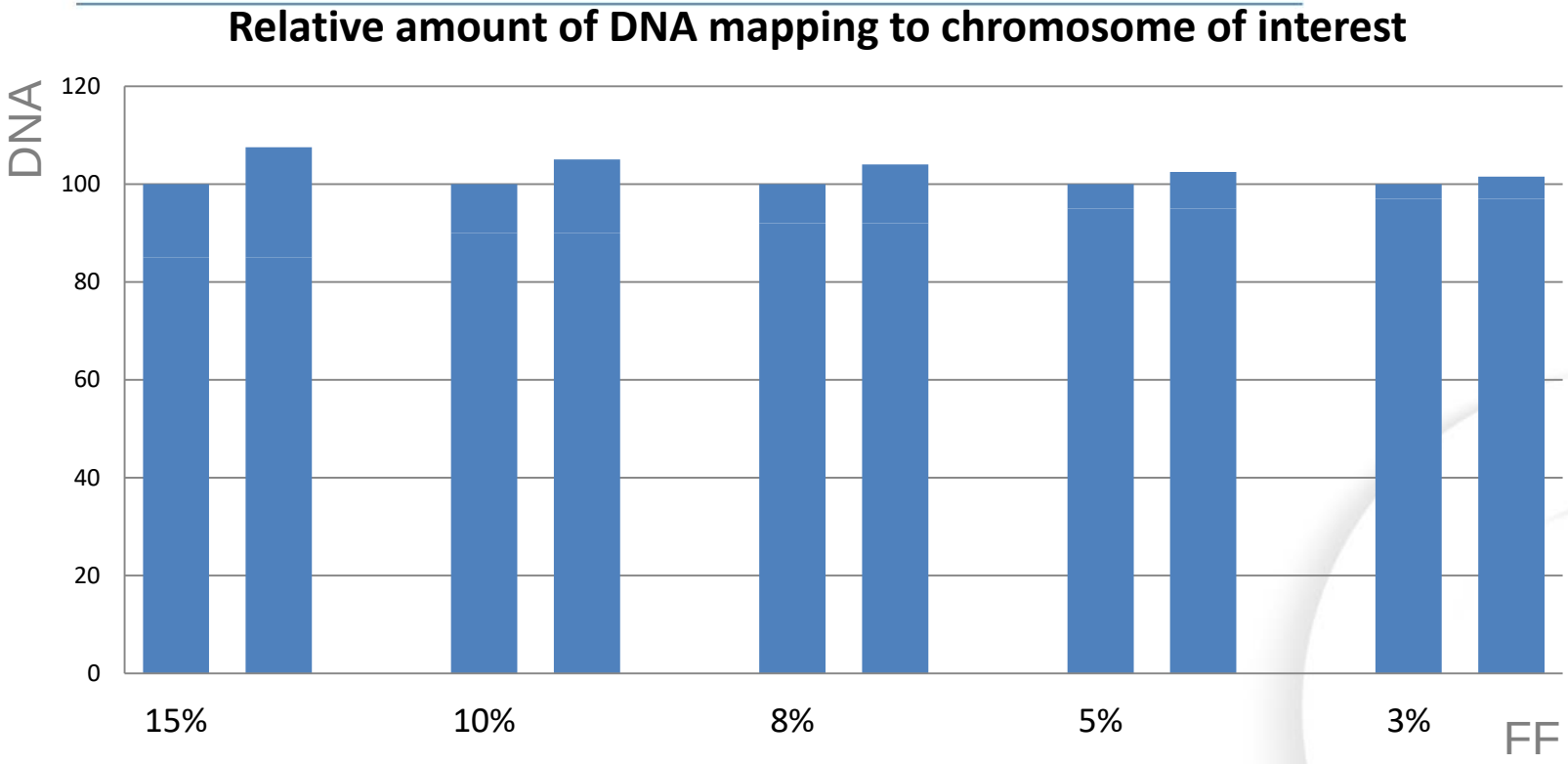

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4-8%	75%

1. Palomaki GE et al. DNA sequencing of maternal plasma to detect Down syndrome: an international clinical validation study. *Genet Med*. 2011;13::913-20.

Non-counting method: SNP-based approach

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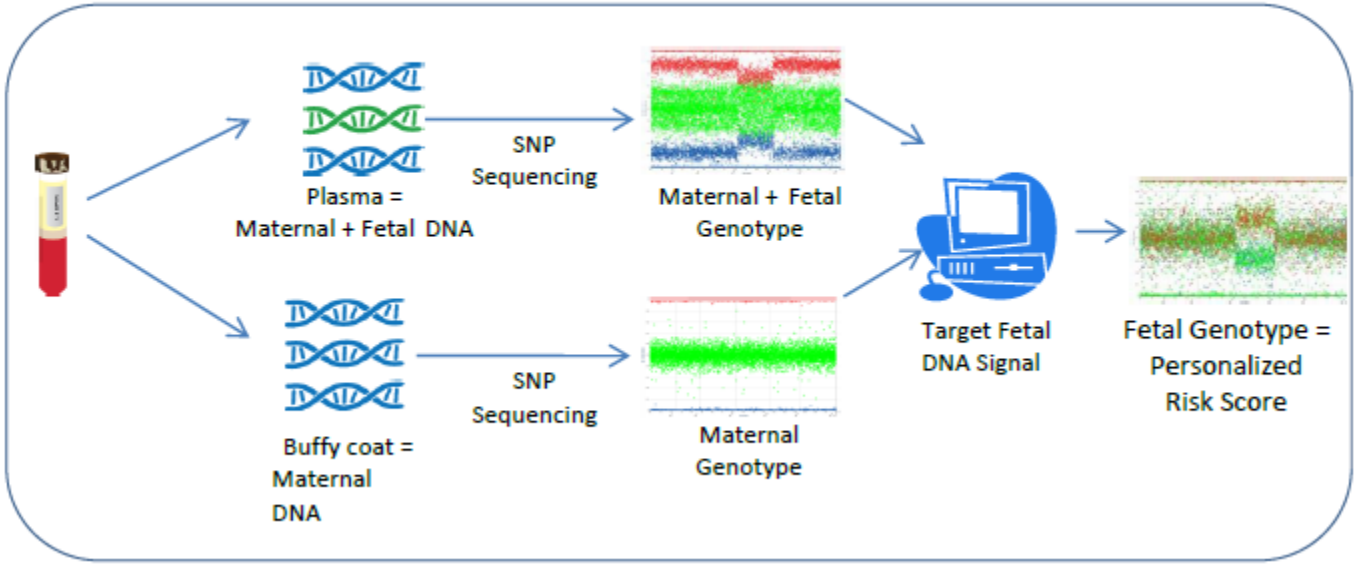
Single Nucleotide Polymorphism base pair (SNP)

A simplification of Natera's non-invasive prenatal aneuploidy test



NIPS for fetal aneuploidies

Commercial tests

data are not yet available comparing the performance and cost of these tests directly to each other

Counting method		Non-counting method
MPSS	Targeted sequencing	Targeted sequencing
Sequenom MaterniT21Plus™	Ariosa Test Harmony™	Natera Test Panorama™
Verinata/ Illumina Verifi™		
BGI NIFTY™		

RECOMMENDATIONS: <u>WHICH TEST TO CHOOSE</u>?

1. **FF included in the report**
2. **Risk-based approach adjusted FF, MA, GA and other screening tests**

Methodologies: Advantages and limitations

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Whole genome random sequencing	• High performance in T21 and 18, lower in T13 and 45,X0 • Resolution: funtion of coverage, bioinformatics • Genome-wide assessment possible
Targeted sequencing	• Improved efficiency
SNPs-based sequencing	• Reference chromosome not required • Triploidy and vanishing twin detectable • Parent origin analysis • Unsuitable for twins, consanguinity, egg donor pregnancies

Facts

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1

- **NIPT is here to stay and its scope will broaden**
  - How should it be used and implemented in routine clinical care?

2

- **The best "advanced" screening method**

Low risk	DR (%)	FPR (%)
FTS	90	4
NIPT	99	0,08

3

- **Clinical validity**
  - Singletons
  - High risk population
  - Trisomies 21,18 and 13

4

- **FF as a crucial factor affecting accuracy**
  - FF should be included in the report

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Clinical validity: updated meta-analysis

meta-analysis 2011-2015

	Chromosomal abnormality	n (cases)	DR (%)	FPR (%)
singletons	Trisomy 21	1051	99.2	0.09
	Trisomy 18	389	96.3	0.13
	Trisomy 13	139	91.0	0.13
	45,X0	177	90.3	0.23
	Other sex CA	56	93.0	0.14
twins	Trisomy 21	31	93.7	0.23

- Screening for T21 by cfDNA in singletons is significantly superior to all other traditional strategies
- The performance for T18, 13 and sex CA is lower
- Expansion to include all increases cumulative FPR eight-fold (0.09% to 0.72%)
- No-result rate: 6.9% for trisomies and 17% for sex CA
- Twins: the performance may be worse than in singletons (further data needed)

Gil MM et al. Analysis cfDNA in Maternal Blood in Screening for Aneuploidies: Updated meta-analysis. Ultrasound Obstet Gynecol 2015;45:249-266

Clinical validity: updated meta-analysis

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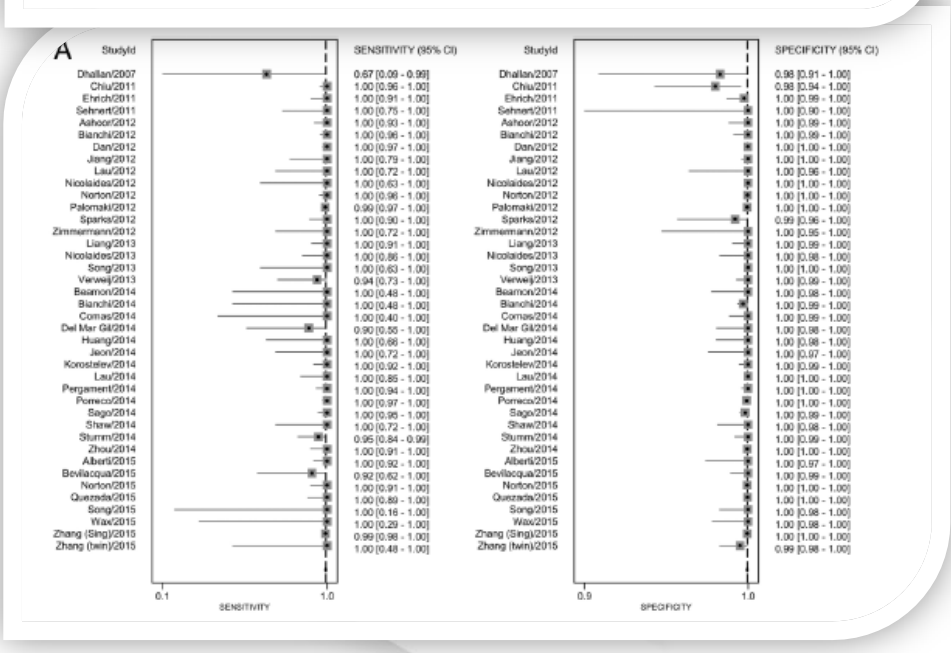
meta-analysis 2007-2015
41 articles (2012 overall)

Chromosomal abnormality	DR (%)	Sp(%)
Trisomy 21	99.3	>99.9%
Trisomy 18	97.4	
Trisomy 13	97.4	
Lower DR	First trimester, general population, twins	
No differences	Technique	

- In line with previous studies
- S/PPV: lower when
  - first trimester
  - general population
  - consecutive samples
- Test failure: 0-12.7%
  - 14% failed at repeated sample
  - Higher: earlier GA, trisomies

BMJ Open Accuracy of non-invasive prenatal testing using cell-free DNA for detection of Down, Edwards and Patau syndromes: a systematic review and meta-analysis

Sian Taylor-Phillips,[1] Karoline Freeman,[1] Julia Geppert,[1] Adeola Agbebiyi,[1] Olalekan A Uthman,[1] Jason Madan,[1] Angus Clarke,[2] Siobhan Quenby,[1] Aileen Clarke[1]



Taylor-Philips S et al. Accuracy of NIPT using cfDNA for T21,18 and 13: a systematic review and meta-analysis. BMJ Open 2016;6:e010002.doi:10.1136

Importance of FF

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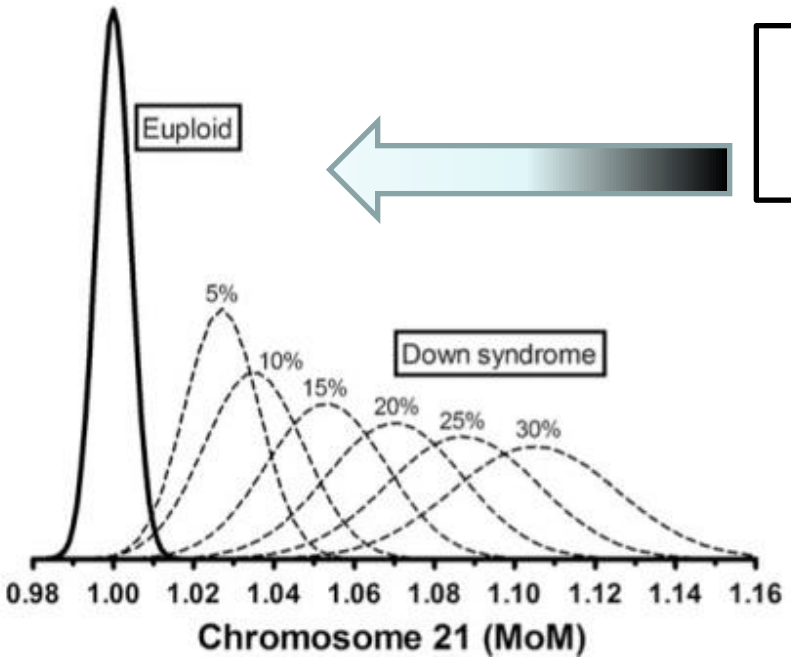

As FF decreases, there is less distinction between the euploid and aneuploid distributions

Canick, et al. Prenat Diagn 2013;33:1–8

	Sequenom MaterniT21 Plus	Verinata Verifi*	Ariosa Harmony	Natera Panorama
FF% Assessed?	✓		✓	✓
Minimum FF	4%	N/A	4%	3.8-4%
FF% Reported?	✗	✗	✓	✓

0-4% → 4-8% → >8%

FF too low to report

Low FF decreases sensitivity

FF adequate to achieve best performance

Misconceptions

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1

- *"Identification of DS with neurocognitive impairment"*
  - Women are more worried about neurocognitive impairment in general, rather than DS itself

2

- *Misnomer:*
  - *The "only Non-Invasive Prenatal Testing" :* All screening methods in prenatal diagnosis are non-invasive
  - cff -DNA: "cfp -DNA"

3

- NIPT as a "*diagnostic method replacing invasive procedures*"
  - Confusion between screening and diagnostic methods

4

- Counseling: What does really matter?
  - DR versus PPV


Genética

Un test dice adiós a la amniocentesis

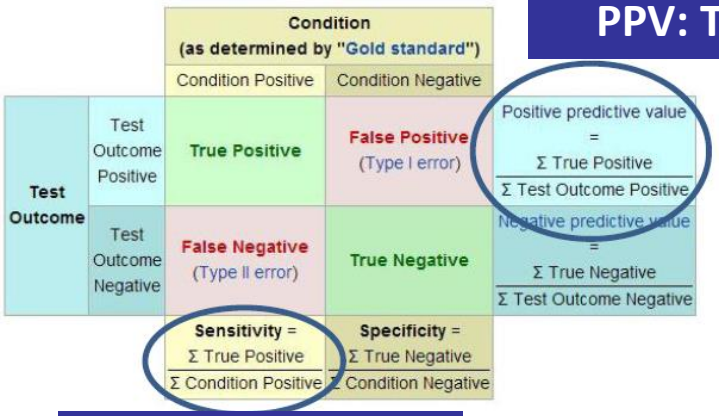
Con una muestra de sangre es suficiente para que una embarazada descarte la presencia de anomalías genéticas en el bebé a partir de la décima semana de gestación. Con una fiabilidad del 99,9%, el test NACE Plus detecta las tres más comunes, esto es, las trisomías 21, 18 y 13, que corresponden a los síndromes de Down, Edwards y Patau, respectivamente.

La incorporación de una prueba no invasiva supone una alternativa a la amniocentesis que se practicaba hasta ahora cuando había signos o sospecha de alteraciones en el feto. Sin embargo, esta entrañaba un riesgo, ya que conlleva la pér-

La amniocentesis entraña un riesgo de pérdida del feto que se evita con el nuevo test de sangre.

Borrell A. Cell-free DNA testing: inadequate implementation of an outstanding technique. UOG 2015;45:508-511

Counseling: What does really matter?



PPV: TP/T + FP

DR: TP /T

DR:
"What percentage of fetuses affected are detected"

PPV:
"What percentage of fetuses is truly affected when the test is positive?"

FTS	PPV					22q11del
	cfDNA	Combined*	T21	T18	T13	45X	
	n	222	154			18	
1/20	TP	184	140	2/3		9	1/5
	FP	38	14	2	13	9	50
5%	PPV	**83%**	91%	93%	38%	50%	18%

Dar P, et al.
Am J Obstet Gynecol 2014;211:527.e1-17.

Gross SJ et al.
Ultrasound Obstet Gynecol 2016;47:177-83

Be aware of

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1. • Need to confirm through IT
2. • Test of choice after high-risk NIPT

AC: test of choice
T13 (22% mosaicism risk)
Sex CA (60% mosaicism risk)
Normal US

CVS only first line in
T21(2% mosaicism risk)
T18 (4% mosaicism risk)
Microdeletions
Abnormal US

Unsolved questions

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1. **Analytic validity/technical accuracy**
   - Different methodologies, no comparing studies
   - None requires FDA approval (only CLIA lisence)
2. **Significance of "failed results"**
   - More concerning (high-risk; OR 9 if FF<1,5 centile)
3. **What should the test offer beyond autosomal trisomies?**
4. **Is now the time to offer NIPT to all pregnancies?**
   - Potential problems: twins, vanishing twin, insufficient FF, maternal mosaicism, maternal tumors…
   - Discordant results: biological mechanisms
5. **Lack of quality control guidelines**

Twins

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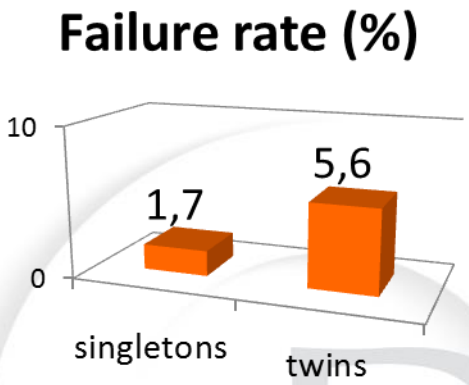
	Singletons	Twins
n	1847	515
GA (weeks)	13	13.6
Mean FF	11.7%	8.7%
Failure rate	**1.7%**	**5.6%**
Final result available	99.3%	96.9%



Lower FF*
Higher failure rate**
Limited experience

Cumulative data (758 twins)

n: 39 T21, 7 T18, 2 T13

TD: 95%, 86%, 100%
FPR: 0%

*Lowest FF
** IVF, high BMI

Bevilacqua at el. Performance of screening for aneuploidies by cfDNA in twin pregnancies. Ultrasound Obstet Gynecol 2015;45:61-66

Urge to set a quality control guidelines

Non-invasive prenatal testing performance when fetal cell-free DNA is absent

Takoudes. UOG 2015;45:112-116

	Non-pregnant patient #1		Non-pregnant patient #2	
	Test Result	Details	Test Result	Details
Harmony (Ariosa)	No	No result due to insufficient fetal DNA	No	No result due to insufficient fetal DNA
informaSeq (LabCorp)	Yes	No aneuploidy detected, female fetus	Yes	No aneuploidy detected, female fetus
MaterniT21 (Sequenom)	Yes	Negative, female fetus (fetal fraction 4.3% reported on request)	Yes	Negative, female fetus (fetal fraction 3.9% reported on request)
Panorama (Natera)	No	Unable to report due to low fetal fraction. Fetal fraction reported to be 0.6%	No	Unable to report due to low fetal fraction. Fetal fraction reported to be 0.6%
verifi (Verinata/Illumina)	Yes	No aneuploidy detected, female fetus	Yes	No aneuploidy detected, female fetus

- **This example raises concerns about the need for quality standards for NIPT**
- **Measurement of FF is a basic quality metric required to ensure the reliability of interpretation of results**

NIPS for fetal aneuploidies

Professional recommendations (I)

December 2012

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

The Society for Maternal-Fetal Medicine

COMMITTEE OPINION

Number 545 • December 2012

The American College of Obstetricians and Gynecologists Committee on Genetics
The Society for Maternal-Fetal Medicine Publications Committee

This document reflects emerging clinical and scientific advances as of the date issued and is subject to change. The information should not be construed as dictating an exclusive course of treatment or procedure to be followed.

Noninvasive Prenatal Testing for Fetal Aneuploidy

April 2013

ispd

Position Statement from the Aneuploidy Screening Committee on Behalf of the Board of the International Society for Prenatal Diagnosis, April 2013

Peter Benn (Chair), Antoni Borell, Rossa Chiu, Howard Cuckle, Lorraine Dugoff, Brigitte Faas, Susan Gross, Joann Johnson, Ron Maymon, Mary Norton, Anthony Odibo, Peter Schielen, Kevin Spencer, Tianhua Huang, Dave Wright, Yuval Yaron.

ACMG POLICY STATEMENT | Genetics inMedicine

April 2013

ACMG statement o ACMG ive prenatal screening for loidy

isuog.org

Ultrasound Obstet Gynecol 2014; 44: 122–123

CONSENSUS STATEMENT

ISUOG consensus statement on the impact of non-invasive prenatal testing (NIPT) on prenatal ultrasound practice

ispd

International Society for Prenatal Diagnosis

Building Global Partnerships in Genetics and Fetal Care

June 2015

154 Hansen Road, Suite
Charlottesville, VA 22911 U
Telephone +1.434.979.4
Facsimile +1.434.977.1
E-mail: info@ispdhome
Website: www.ispdhome

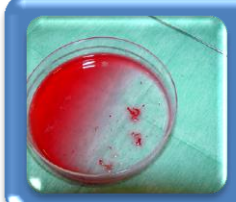
Position Statement from the Chromosome Abnormality Screening Committee on Behalf of the Board of the International Society for Prenatal Diagnosis

NIPS for fetal aneuploidies

Professional recommendations (II)

Consensus

NIPT may be considered:



"Advanced screening": invasive procedure required to confirm

medically necessary in singleton high-risk

not medically necessary in average risk

investigational in twins

NIPS for fetal aneuploidies

Update statment

Consensus 2016

Gregg AR et al. NIPS for fetal aneuploidy, 2016 update: a position statement of the ACMGG. Genet Med 2016

ACOG/SMFM's Practice Bulletin 163 May 2016

ACOG/SMFM's Committee Opinion Sept 2015

ISPD's Position Satement April 2015

ASHG's Policy Statement March 2015

"Informing all pregnant women that NIPS is the most sensitive screening option for traditionally screened aneuploidies"

	Condition	n	DR (%)	FPR (%)
singletons	Trisomy 21	1051	99.2	0.09
	Trisomy 18	389	96.3	0.13
	Trisomy 13	139	91.0	0.13

Gil MM et al. Analysis cfDNA in Maternal Blood in Screening for Aneuploidies: Updated meta-analysis. Ultrasound Obstet Gynecol 2015;45:249-266

What else?

¿Are expanded panels currently supported by clinical evidence as screening tests for routine care?

Implications of testing for additional conditions

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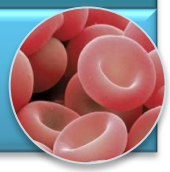
- Fetal sex
- Fetal Rh status
- Paternity (SNPs)

First applications

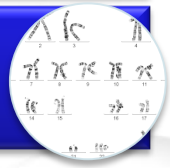


- Mild phenotype
- Higher failure rate
- Lower DR and higher FPR
- 50% sex CA are mosaics
- High incidence of maternal mosaicisms
- Other than 45,XO: indication for testing??

Sex CA

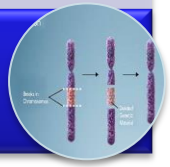
- Trisomies 9, 16, 22
- Additional FPR 0.1% each
- High IU lethality, IUGR
- Screening for lethal conditions suspected by US non justified
- No currently interventions
- Low clinical utility > 10w
- Not recommended (ACMG 2016)

Rare autosomal trisomies



- Few conditions
- Lower prevalence
- Lower PPV
- High FPR
- Few experience
- Difficult counselling

Microdeletions



- Total and cffDNA higher
- Trophoblastic apoptosis
- Confliciting results
- Future algorithms to estimates risks
- Currently no clinical utility

Prediction of APO

- Technically faisible
- Clinically unpractical

Genome wide sequencing

Sex CA: Arguments PROS and CONS

Pros

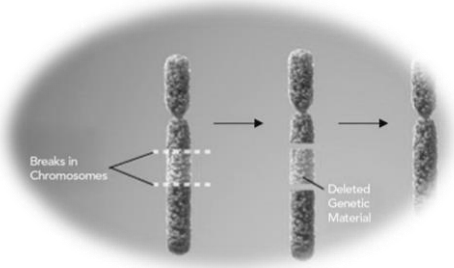
- NIPS technically available
- Individual demand
- Already diagnosed by IT
- Potential benefits
  - Early management (fertility preservation)
  - Prenatal diagnosis of genitalia abnormalities (sex discordance)
- Scientific recommendations
  - AIUM, ACOG 2013: only if medicaly indicated
  - ISMG 2014: recommended in high-risk
  - ACMG 2016: inform the availability to all

Cons

- Limited data of performance
  - Lower DR
  - High cumulative FP
  - Low PPV
  - Error 0.7%
- Maternal conditions revelead
  - Mosaics 45,X
  - Discordance TNI/US/TI
  - Maternal cancer
- Ethical concerns
  - Psychosocial harm
  - Loss of equity for access (cost)
  - Sex selection
- Scientific recommendations
  - ESHG/ASHG 2015: not recommended
  - ACOG 2016: if requested

NIPS beyond fetal aneuploidies

Microdeletions: Arguments PROS and CONS



Pros

- Significant high prevalence
- Severity
- No risk factors
- Benefit from early intervention
  - Hypocalcemia
  - Critical CHD
  - Need for delivery at 3 center
  - Preventing “postnatal diagnostic odyssey”
  - Opportunity for TOP
- Proof-of – principle
- PPV similar to FTS
- No previous screening test
- Personal utility
- Familiar utility

Cons

- Prevalence
  - Limited conditions tested (6-11%)
  - Only 70% of causal conditions
  - Similar risk pre and post test (1.7% and 1.6%)
- Technical accuracy
  - More challenging
- Clinical validity: not demonstrated
  - Variable DR 60-99%
  - High FPR (cumulative) (0.8-3%)
- Clinical utility: not demonstrated
  - Low PPV
  - Low penetrance/expressivity
- Disadvantages from early diagnosis
  - Benefit not demonstrated prospectivelly
  - Excess of medicalization ?
  - Negative postnatal effects
  - Complex and unreallistic prenatal counseling
- Doesn’t feet criteria for screening

Practical aspects and reports

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- ✓ GA (>9-10w), MA
- ✓ Previous scan
- ✓ Indications for TNI
- ✓ Contraindications for TNI
- ✓ Indications and contraindications for counting/non-counting methods
- ✓ Previous screening tests
- ✓ Always requisition form

FINAL RESULTS SUMMAR

Result

LOW RISK

Notes by the clinical reviewer, if

RESULTS DETAILS

Condition tested[1]

- Trisomy 21
- Trisomy 18
- Trisomy 13
- Monosomy X
- Triploidy/Vanishing twin
- 22q11.2 deletion syndrome

RESULTS SUMMARY

Result	*Fetal Sex*	*Fetal Fraction*
HIGH RISK for Trisomy 13	**Female**	**8.3%**

This is a screening test only. Genetic counseling and diagnostic testing should be offered to further evaluate these findings.

The Panorama risk score reflects analysis of DNA from the placenta. The placental DNA may not accurately reflect the status of the fetus, therefore no irreversible decisions should be made based upon results of this screening test alone.

RESULTS DETAILS

Condition Tested[1]	*Result*	*Risk Before Test*[2]	*Panorama Risk Score*[3]
Trisomy 21	Low Risk	1/152	<1/10,000
Trisomy 18	Low Risk	1/111	<1/10,000
Trisomy 13	**High Risk**	1/357	**>99/100**
Monosomy X	Low Risk	1/256	<1/10,000
Triploidy/Vanishing twin	Low Risk		
22q11.2 deletion syndrome	Low Risk	1/2,000	1/13,300
1p36 deletion syndrome	Low Risk	1/5,000	1/12,400
Angelman syndrome	Low Risk	1/12,000	1/16,600
Cri-du-chat syndrome	Low Risk	1/20,000	1/57,100
Prader-Willi syndrome	Low Risk	1/10,000	1/13,800

Positive Predictive Values[4]

T21: 91%
T18: 93%
T13: 33%
MX : 50%

Positive Predictive Value (PPV) is the likelihood that diagnostic testing will confirm a High Risk result. PPV provided is NOT personalized for this patient, but calculated from a published study of 17, 885 women. PPV for an individual specimen will vary based on prior risk.

NIPS for fetal aneuploidies

What should the clinician know?

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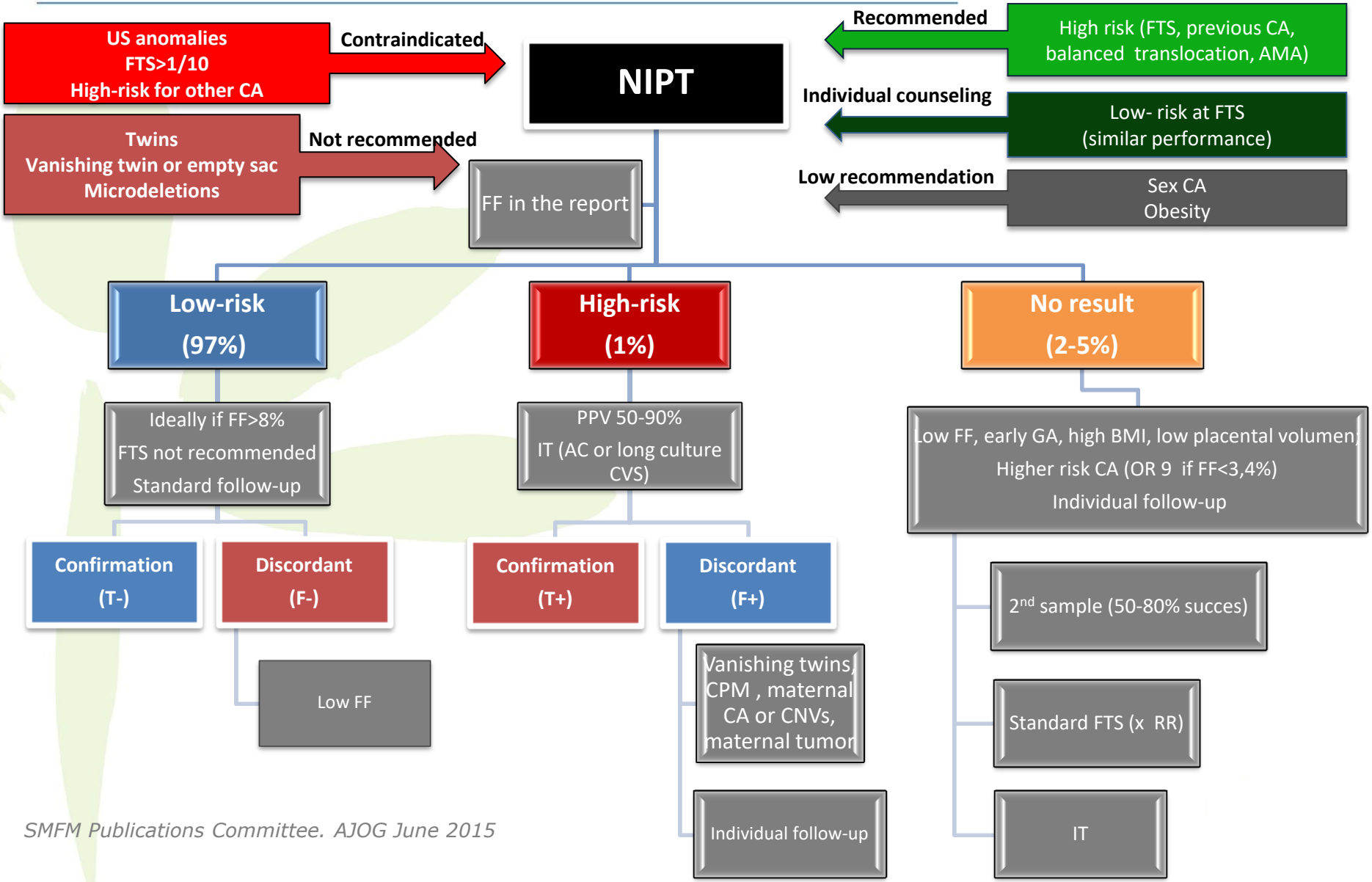

SMFM Publications Committee. AJOG June 2015

The future: next steps

Germans Trias i Pujol Hospital
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1. • Need for increased genetic counseling resources

2. • Overcoming barriers to accessibility
   - Cost
   - Labs decentralization/non-commercial labs

3. • Models of implementation
   - To whom and what to test for?
   - Approaches: Primary/Combined/Contingent strategy (risk
   - New role of US

4. • New technologies
   - NIPT using microarrays
   - Genome-wide deletions or du