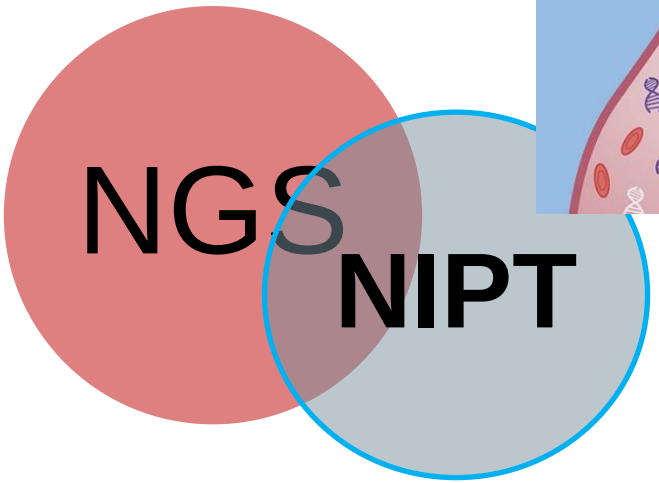
eurofins | Genoma

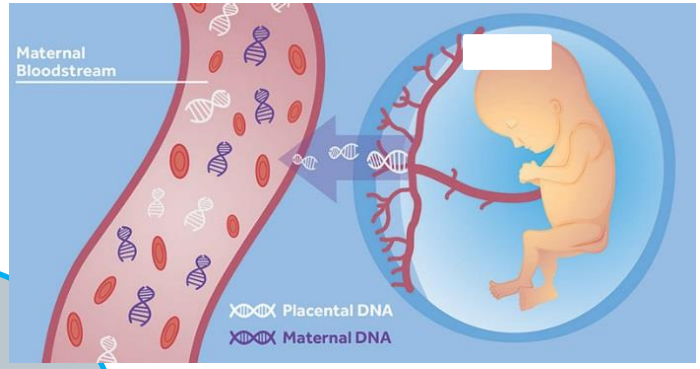
Francesca Spinella, PhD
Medical Scientific Liaisons

PAST | PRESENT | FUTURE

Non Invasive Prenatal Testing

for detection of fetal aneuploidies

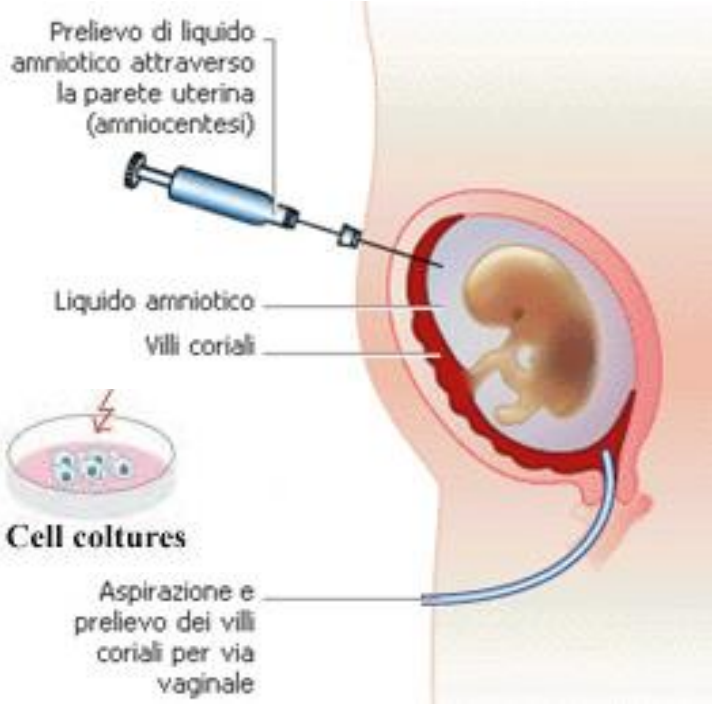


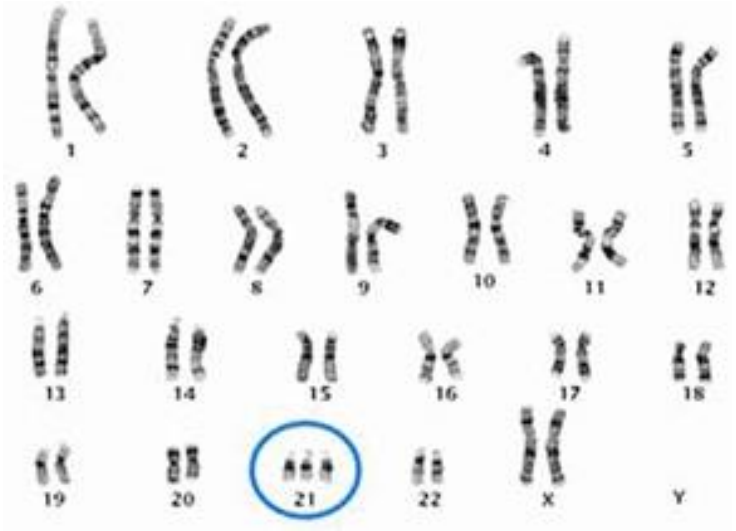


Placenta-derived cfDNA

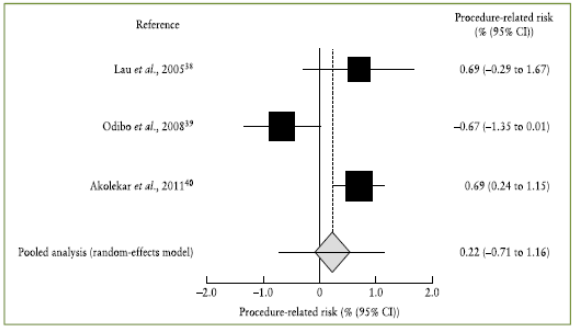
DIAGNOSI PRENATALE

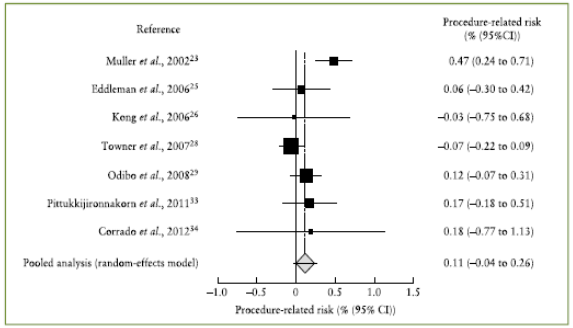
Test invasivo su campioni di villi coriali o amniciti





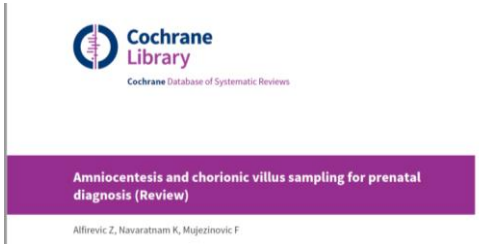
Complicanze della villocentesi e dell'amniocentesi





CVS 0.2%

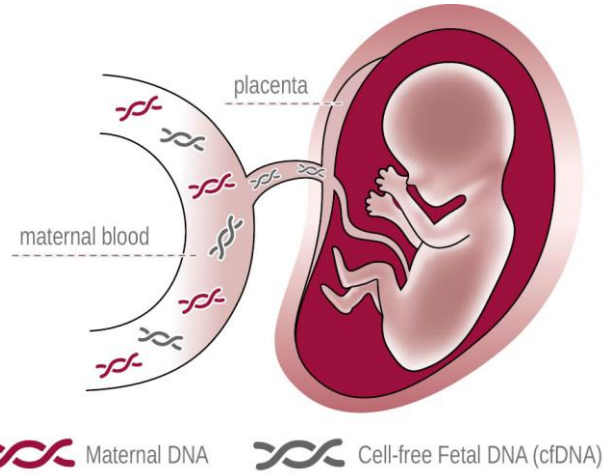
AC 0.1%



Non Invasive Prenatal Testing

for detection of fetal aneuploidies

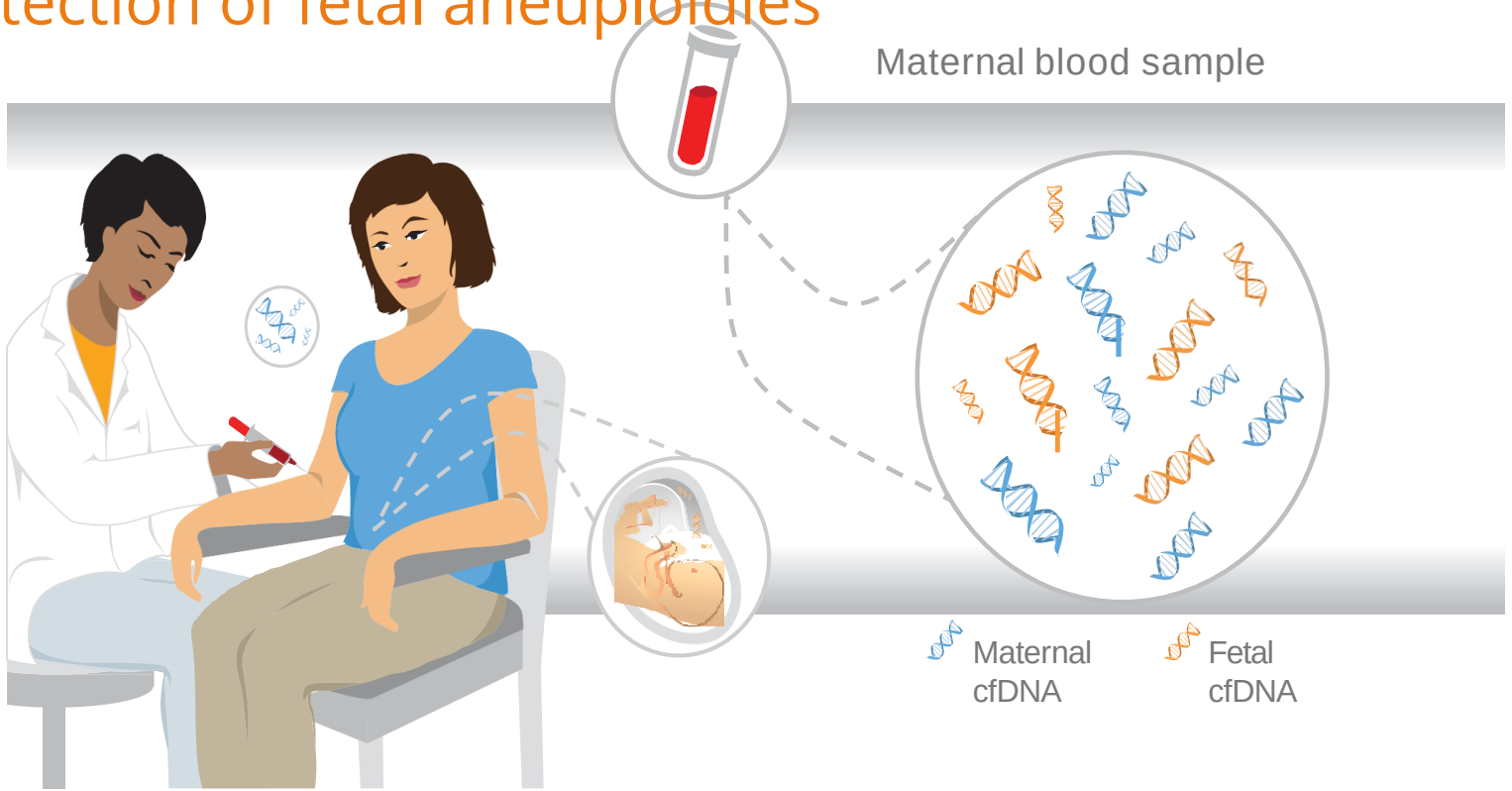
- Evidenzia aneuploidie comuni
- Rare
- Aberrazioni strutturali (delezioni e duplicazioni)
- **Vantaggi**:
  - Non è invasiva
  - Non necessita coltura cellulare
  - Si esegue precocemente



- **LIMITI**
- E’ un test di SCREENING
- In caso di positività è necessario eseguire un test diagnostico invasivo

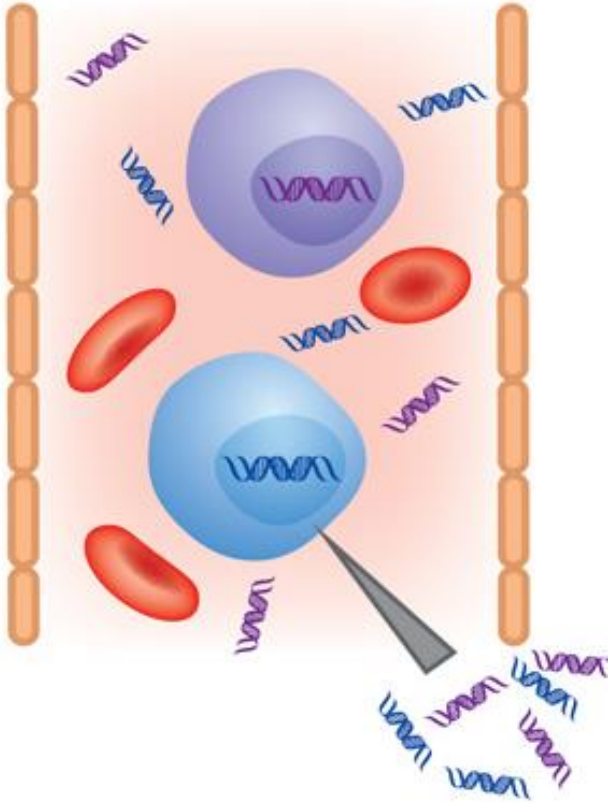
Non Invasive Prenatal Testing

for detection of fetal aneuploidies

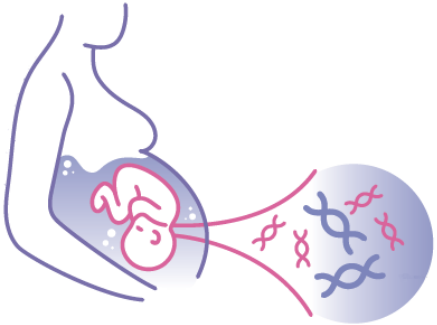


Il cell free DNA

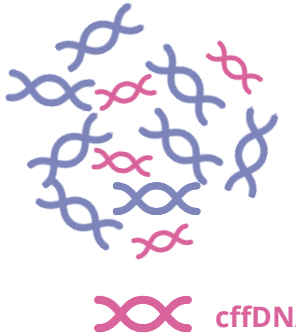
- Cf-DNA deriva prevalentemente da apoptosi delle cellule del sinciziotrofoblasto;
- Il cfDNA consiste in corti frammenti di DNA (154-200pb)
- Il cfDNA fetale (cffDNA) rappresenta ≈10% (3-20%) del cfDNA totale nel plasma materno
- Il cfDNA è rivelabile a partire dalla 5°settimana di gestazione. La sua concentrazione aumenta nelle settimane successive e scompare subito dopo il parto
- La quantità di DNA fetale circolante dalla 10° settimana è sufficiente per garantire l'elevate specificità del test



NIPT
WORKFLOW



Maternal blood sample



~145-200 bp

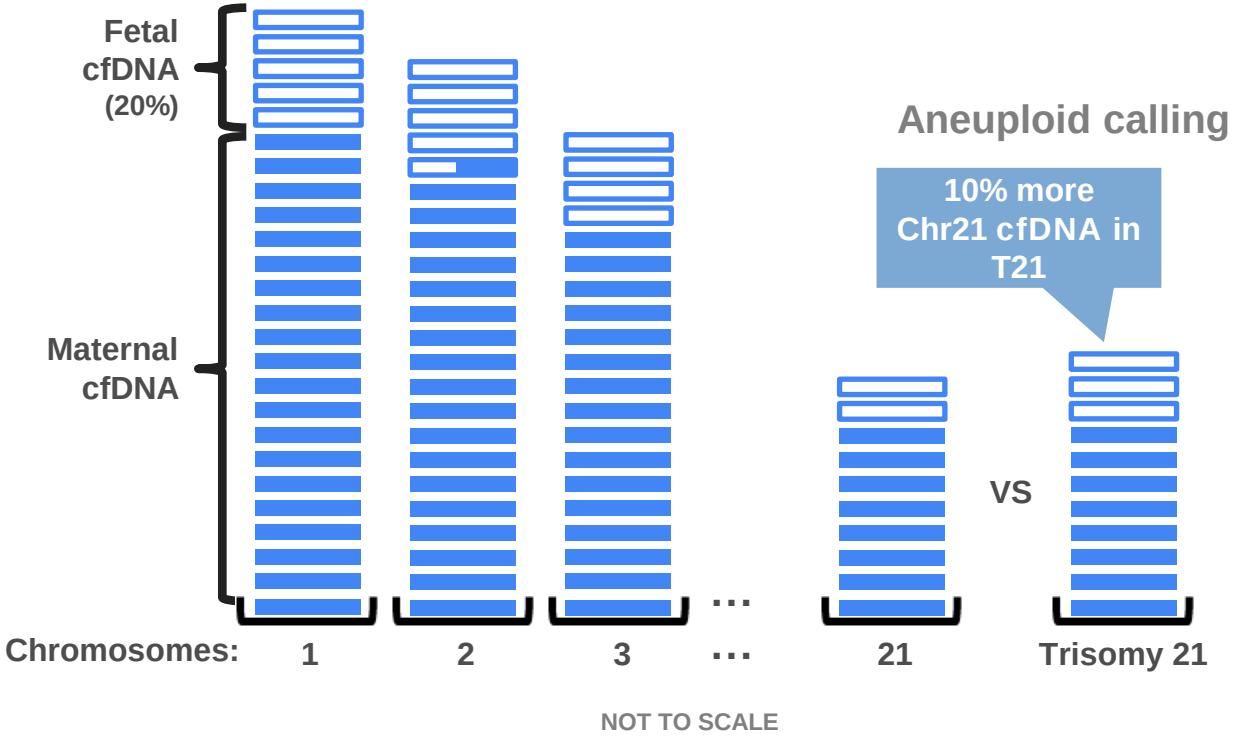
Whole genome sequencing

Sequence alignment

Read count

- Automated workflow and traceability
- CE-IVD reagents and workflow
- Reduced TAT (3-5 Days)

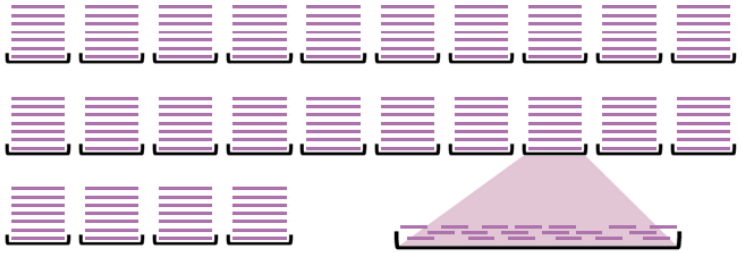
Analysis of MPS Identifies fetal aneuploidies through count



Massively Parallel Sequencing (MPS) vs Targeted approaches

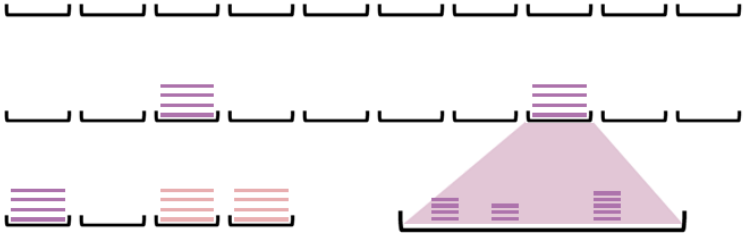
MPS provides across-the-genome coverage

- Benefits
  - Higher resolution (~28 Million tags)
  - Lower assay failure rates
- Drawbacks
  - More expensive

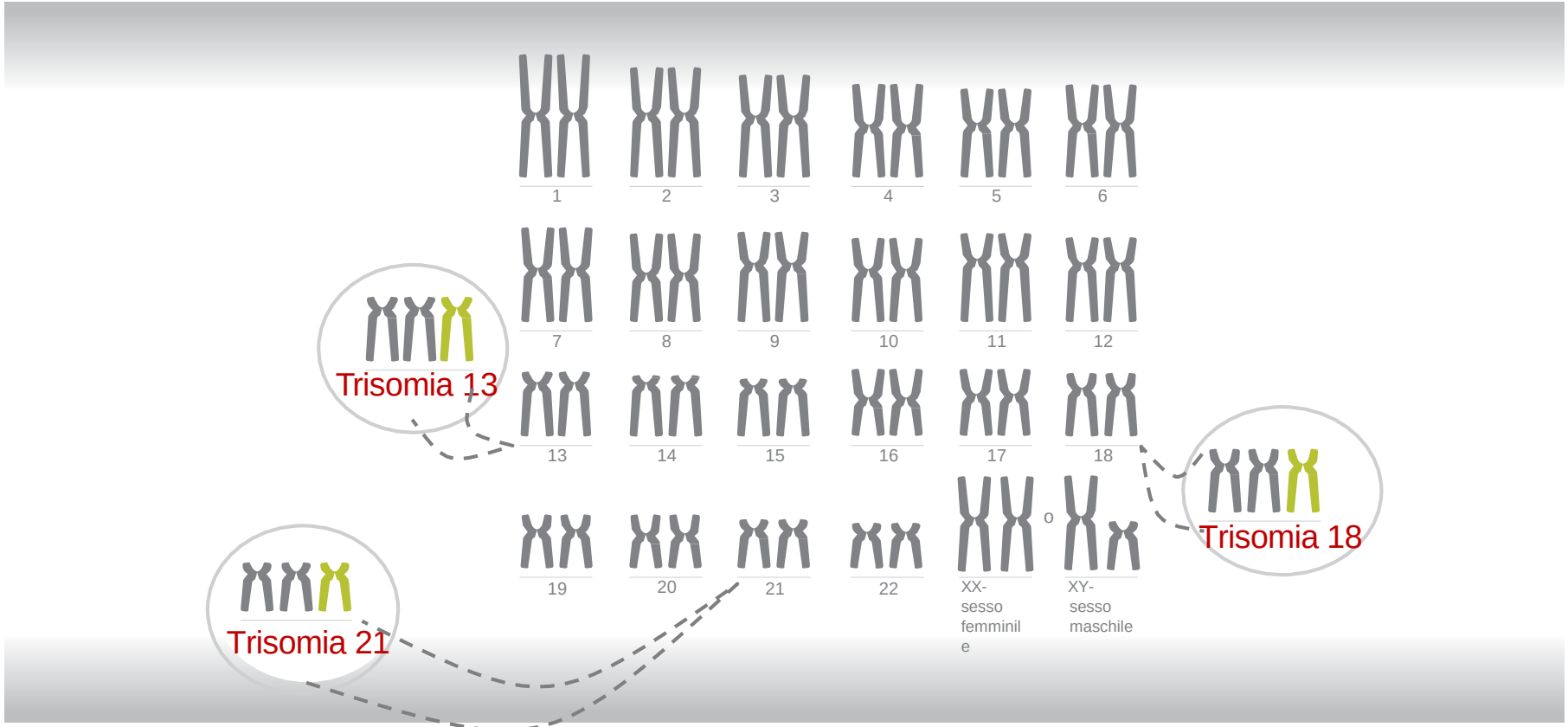


Targeted sequencing is limited to few chromosomes, loci

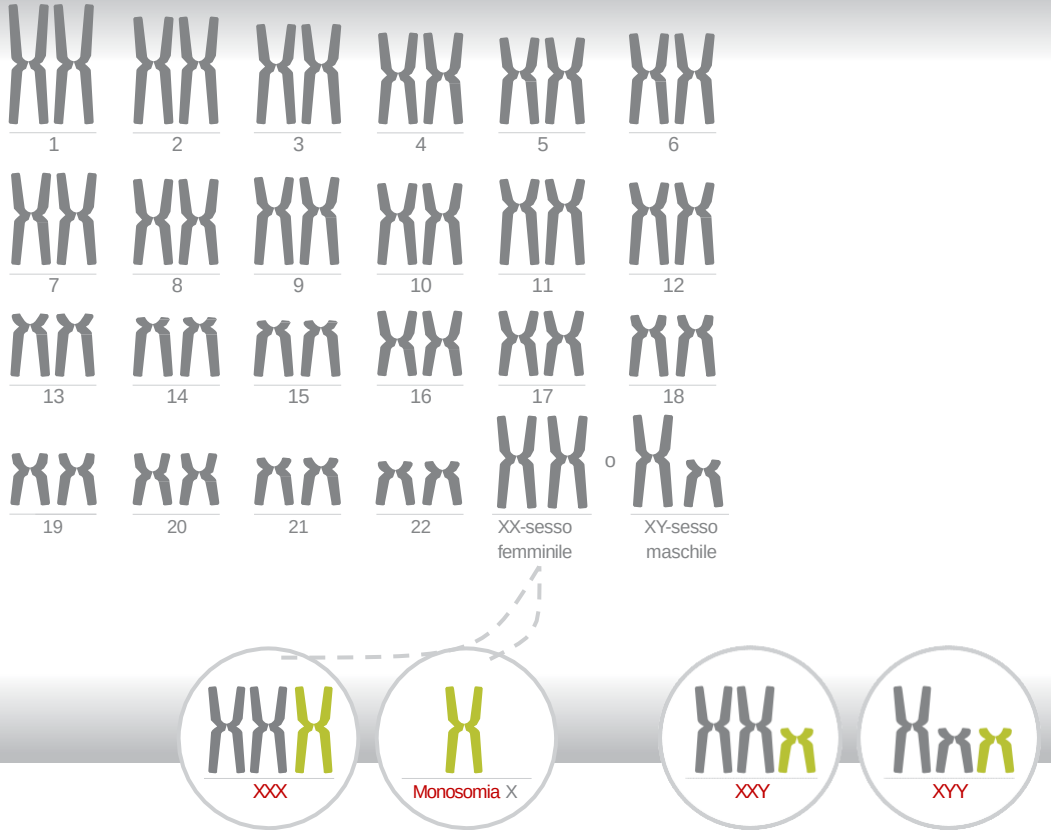
- Benefits
  - Cheaper
- Drawbacks
  - Higher assay failure rates
  - Lower resolution (1.15M to 6.5M tags)



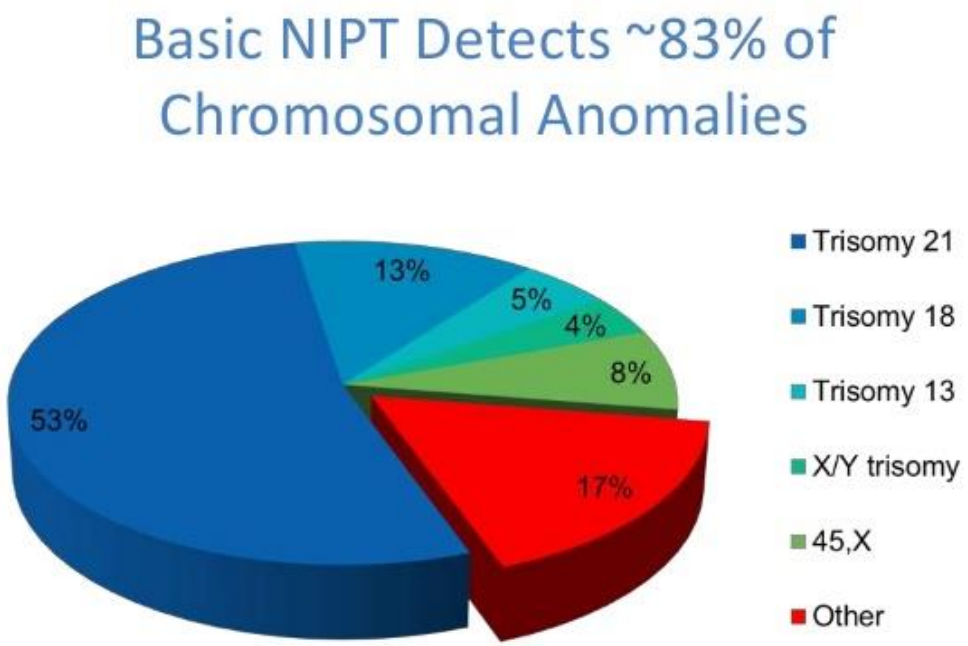
NIPT for COMMON prenatal aneuploidies



NIPT for COMMON prenatal aneuploidies



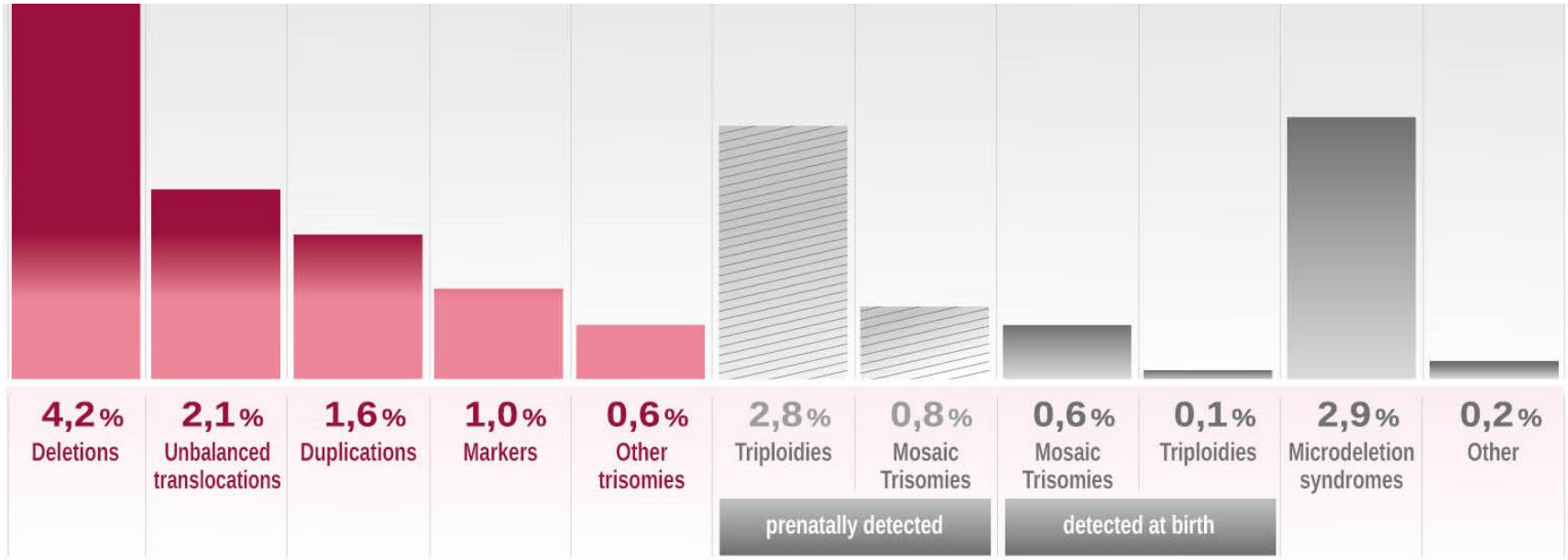
Prevalence of chromosomal abnormalities detected by Conventional cfDNA screening



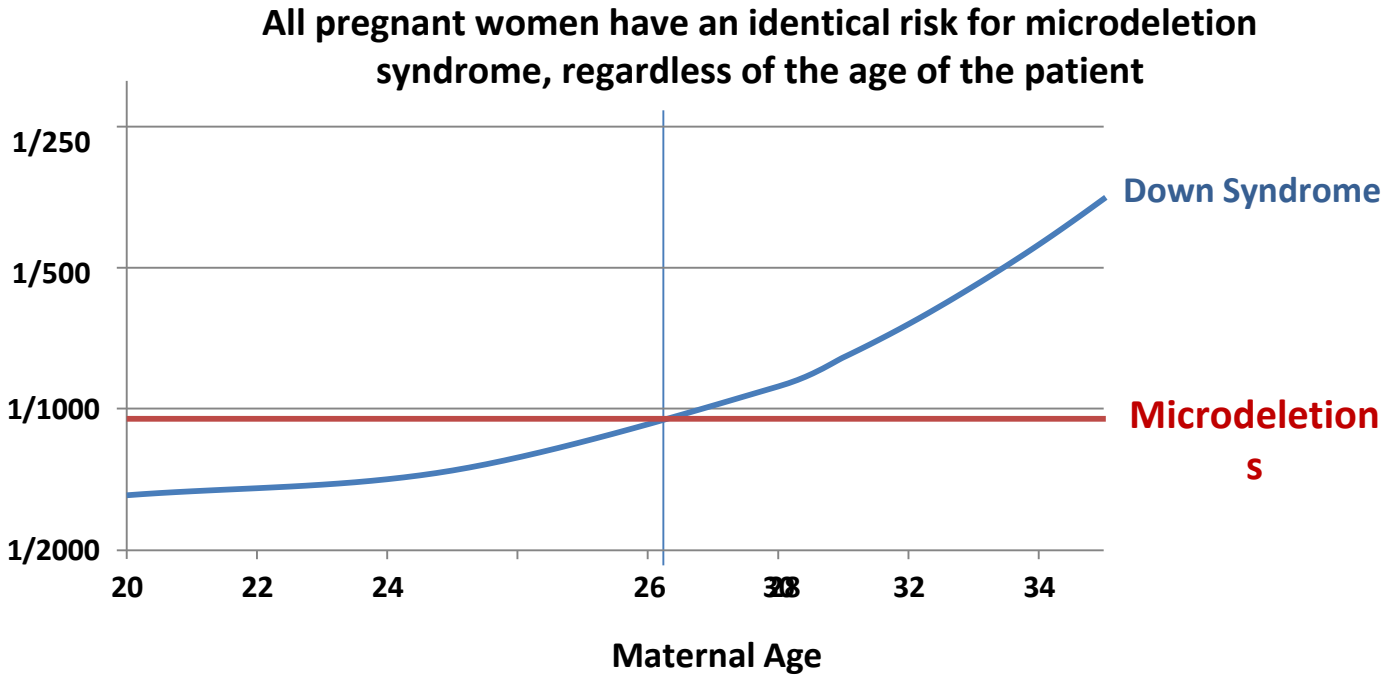
Wellesley et al. (2012)
Eur J Hum Genet; 20:521–6

Modified from Wellesley et al. European Journal of Human Genetics 2012;**20**:521–526

Types of chromosome anomalies not detected by conventional NIPT



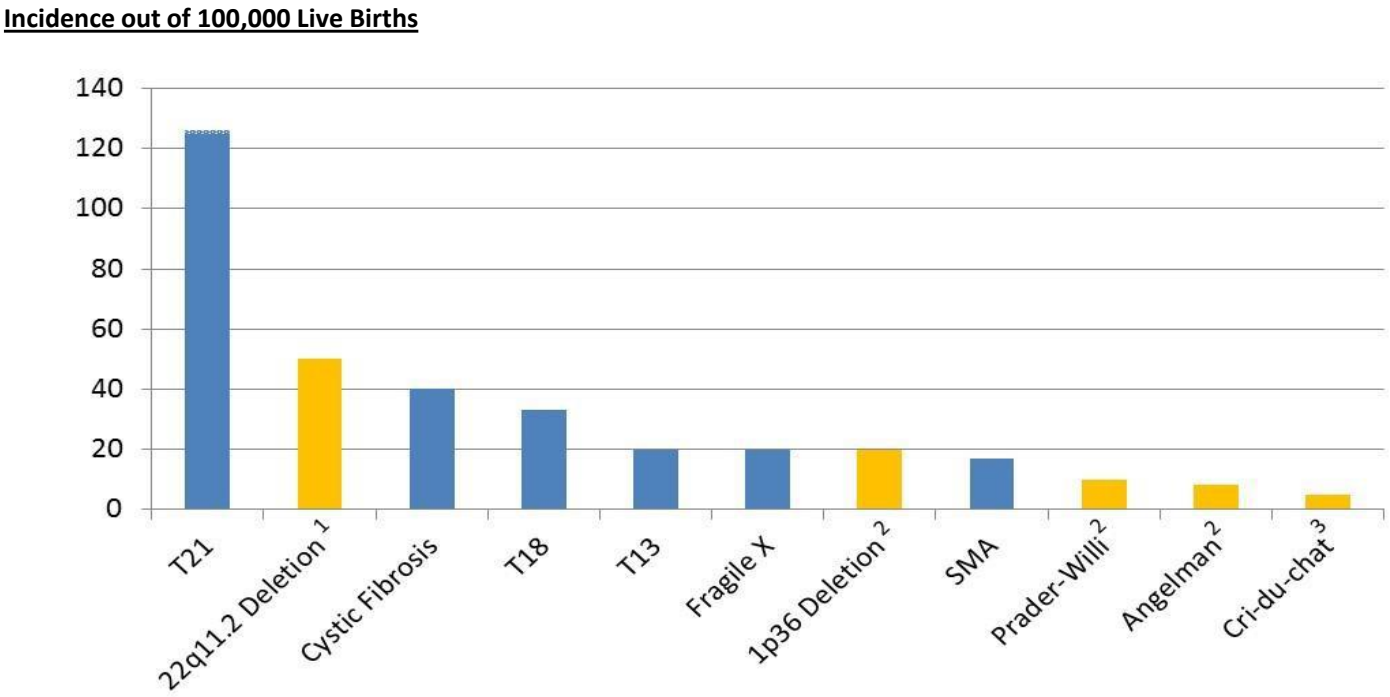
Prevalence of microdeletion compared to Down syndrome



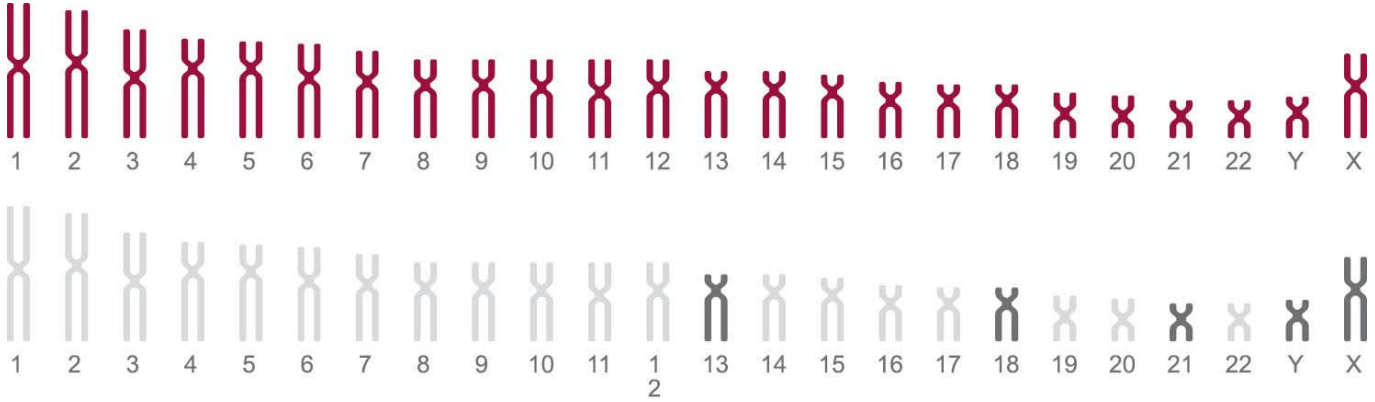
Down Syndrome prevalence from Snijders, et al. *Ultrasound Obstet Gynecol 1999;13:167–170.*
Total prevalence shown for 5 microdeletions using higher end of published ranges from Gross et. al., *Prenatal Diagnosis 2011; 39, 259-266;* and www.genetests.org. Total prevalence may range from 1/1,071 - 1/2,206.

Frequency of microdeletion and genetic or chromosomal diseases evaluated during pregnancy

- The 22q microdeletion syndrome (DiGeorge) is more common than cystic fibrosis

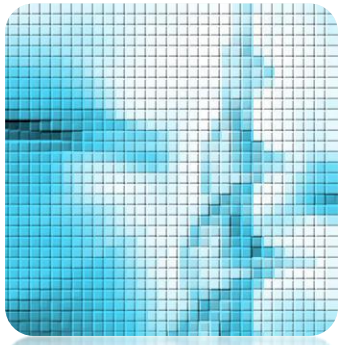


Genome-wide NIPT



- Genome-wide NIPT analyzes every chromosome in the genome.
  - **Aneuploidies**
  - **structural chromosomal aberrations** (deletions or duplications) across the fetal genome
- providing **karyotype-level** insight (like amniocentesis).

More Sequence Counts Equals Greater Precision



Low Resolution
(1–5M sequence reads/sample)

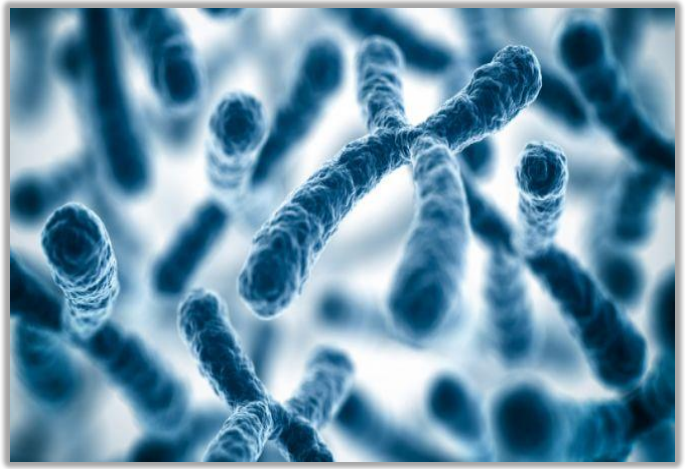
VS.

High resolution
(>10M sequence reads/sample)

- More sequence reads/sample provide greater resolution and confidence in the output.

Genome-wide NIPT

- Standard **(common anuploidies)**
  - **Trisomy 21 (Down syndrome)**
  - **Trisomy 18 (Edwards syndrome)**
  - **Trisomy 13 (Patau Syndrome)**
  - **Monosomy X (Turner syndrome)**
  - **XXX (Trisomy X)**
  - **XXY (Klinefelter syndrome)**
  - **XYY (Jacobs syndrome)**



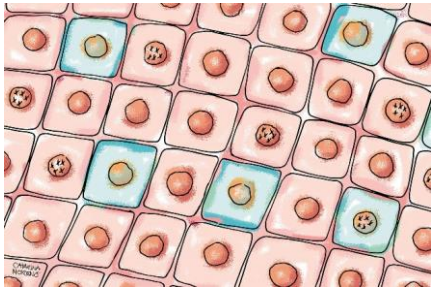
- **common aneuploidis+ microdeletion +rare trisomy**
  - **Deletion syndrome 22q11.2 deletion syndrome** (**DiGeorge**)
  - **1p36 deletion syndrome**
  - **Angelman syndrome** (15q11.2 deletion syndrome);
  - **Prader-Willi syndrome** (15q11.2 deletion syndrome);
  - **Cri du Chat syndrome** (5p- syndrome);
  - **Wolf-Hirschhorn syndrome** (4p- syndrome)

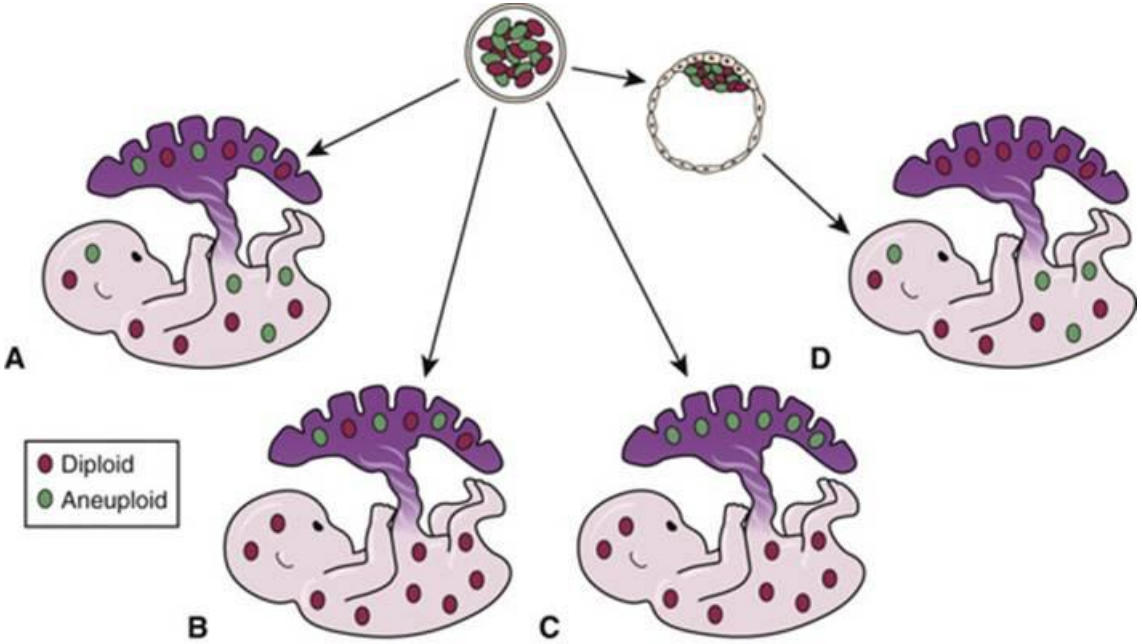
- **Rare trisomy**
  - **Trisomia 9**
  - **Trisomia 16**
  - **ect**

Limits

Biological & Technical

Fetal mosaicismll Test result may not reflect your baby's chromosomes.
Instead, they may reflect chromosomal changes in the placenta (confined placental mosaicism) or fetal mosaicism.



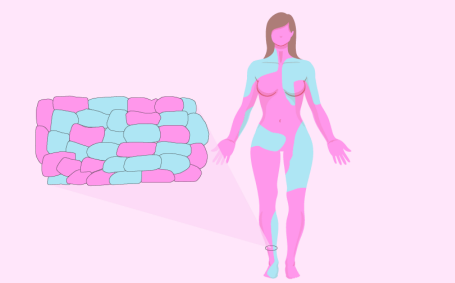


Limits

Biological & Technical

Maternal aneuploidies

Maternal aneuploidies (complete or mosaic), a neoplasm or a transplant could lead to alterations in the outcome.

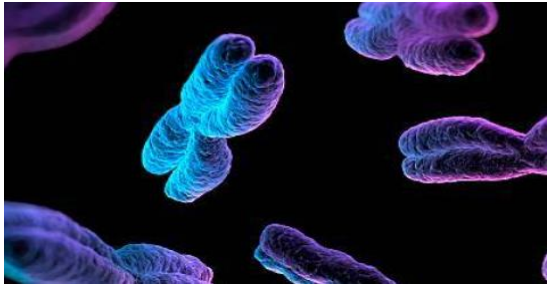


Low fetal fraction:

The amount could be affected by maternal BMI or weight.

Detection limits:

The analysis does not identify polyploidies, balanced rearrangements, deletions and duplications below the resolution limit, mosaicisms; These abnormalities can be detected with invasive diagnostic analysis.



La valutazione di un risultato positivo o di un test non conclusivo può comportare sia un test prenatale invasivo che ulteriori studi sulla madre.

Limits

Biological & Technical

Bichoral twin pregnancies:

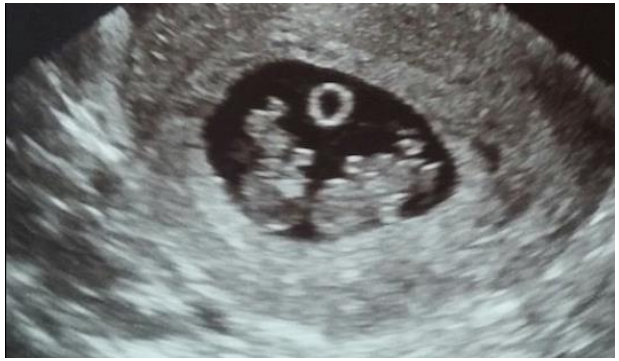
Microdeletions and aneuploidies of **sex chromosomes will not be screened**.

We will not report the sex of the babies, **only the presence or absence of the Y chromosome.**

Only PrenatalSafe 3, Karyo, and Complete will be allowed.

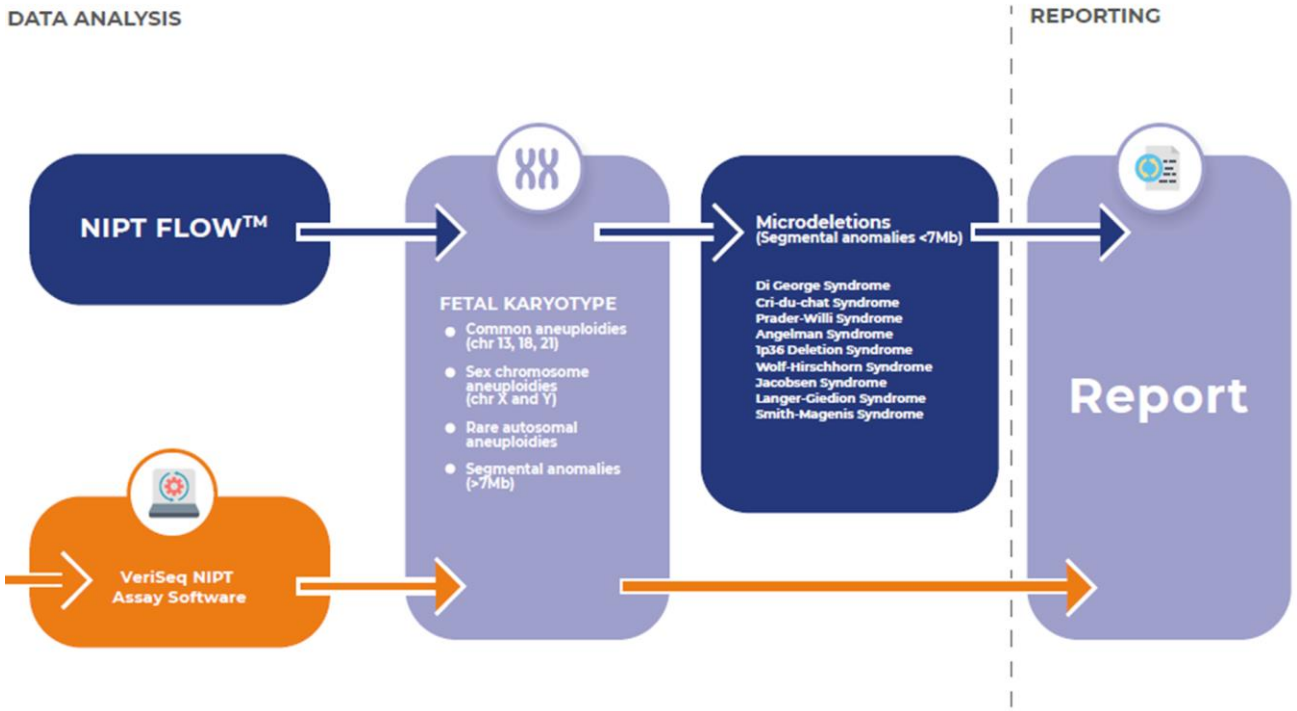
Vanishing twin:

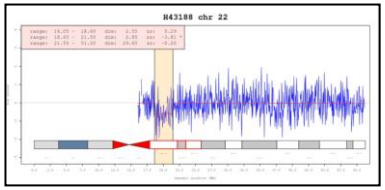
There is no data in the scientific literature on how many weeks after abortion fetal DNA will no longer be detectable. This data must be reported on the acceptance form, supplemented with as much information as possible.



NIPT FLOW ™

BIOINFORMATIC DATA ANALYSIS





2.5Mb Del22q11,2

- Analysis of high depth sequencing data
- Microdeletions of 3-7 Mb
- Partial Del/Dup of chromosome X

Prenatalsafe®

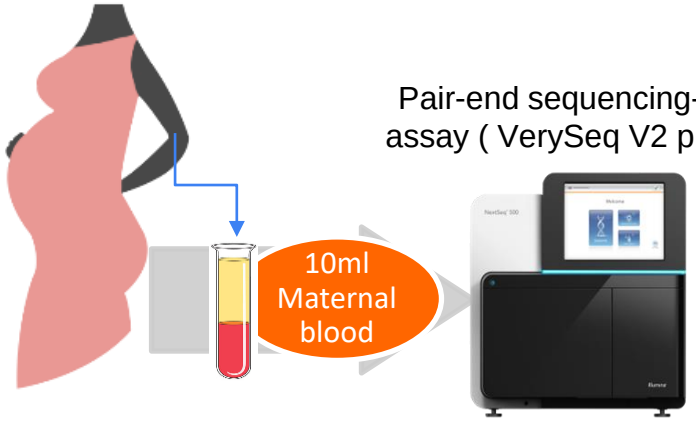
Validation Study

Aim of the study

To evaluate the performance of VeriSeqv2+Eurofins Genoma algorithm in the detection of genome-wide fetal anomalies including trisomies, SCAs, RAAs, and partial deletion/duplication >7Mb and <7Mb

Study population and Demographics

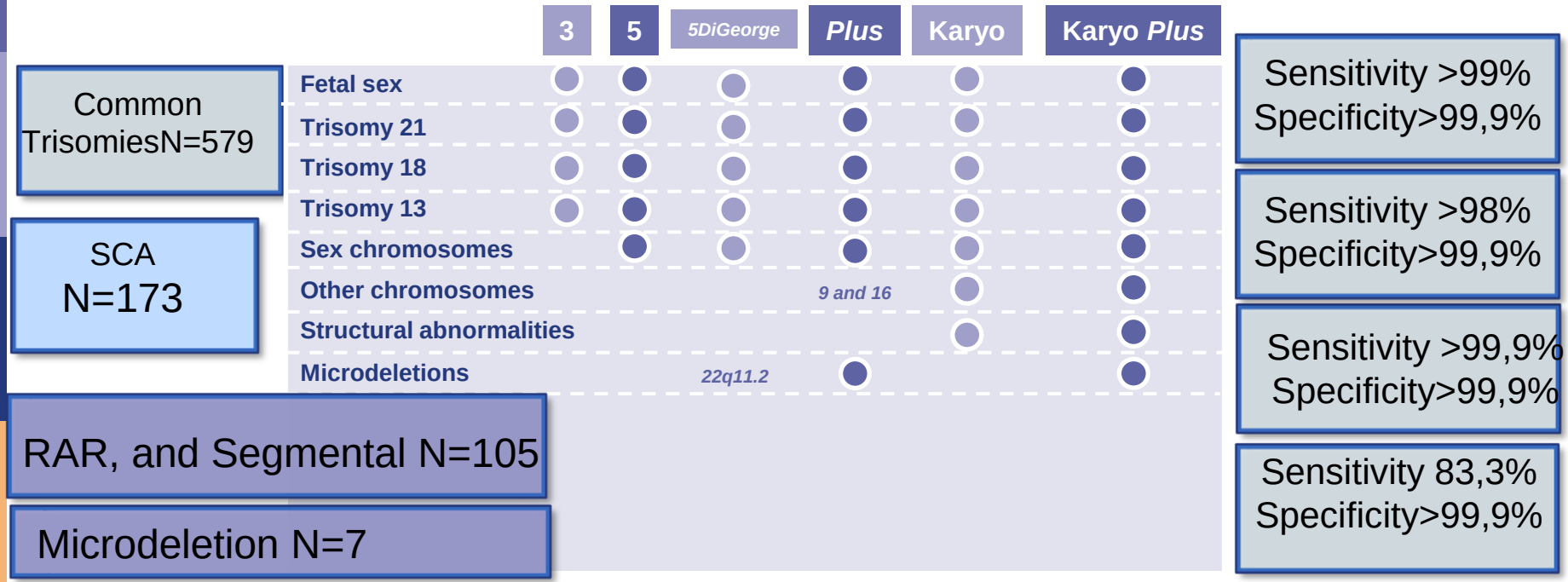
Sample pool collected from November 2019 to Dicember 2021
N=71883



Pair-end sequencing-based assay (VerySeq V2 protocol)

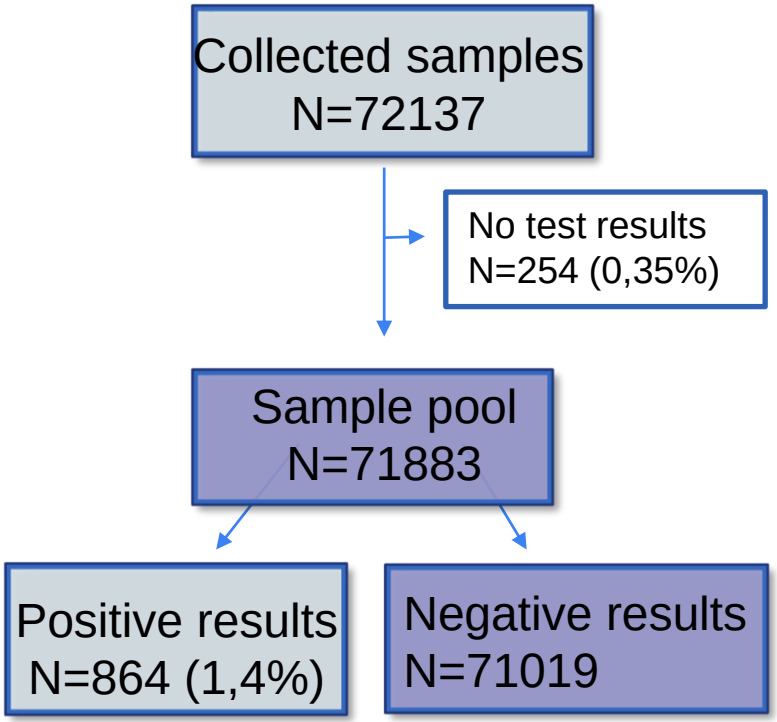
Patient demographics for study cohort	
Maternal Age (years)	
Median	38
Range	20-50
Gestational Age (week)	
Median	12
Range	9.4-28.2
Trimester (week)	
First (9-13,9 weeks)	92%
Second (14-27,9 weeks)	8%
Third (over 28 week)	0.20%
Fetal Fraction (%)	
Median	9%
Range	2%-26%

Performance



Prenatalsafe®

Validation Study Results



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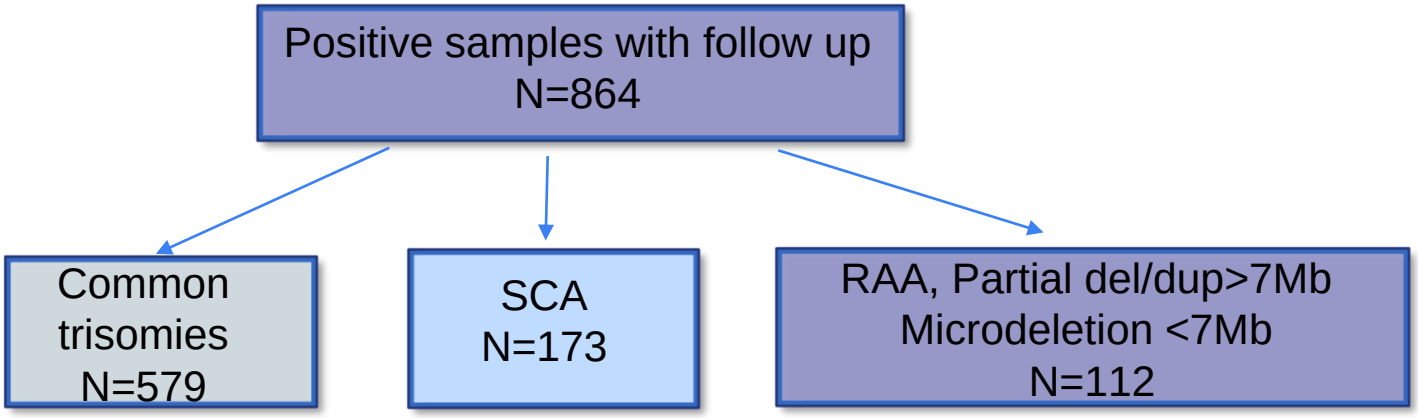
Follow-up



Confirmed by either invasive prenatal diagnosis or by any abnormality detected on ultrasound

Performance

Detection of trisomies 21, 18, 13

Common trisomies N=579

	Trisomy 21	Trisomy 18	Trisomy 13
True positive	437	93	37
False positive	3	1	8
True negative	71392	71775	71828
False negative	2	0	0
Sensitivity (95% CI)	99.54% (98.36%- 99.94%)	99.9% (96.11% -100.00%)	99.9% (90.51%- 100.00%)
Specificity (95% CI)	100% (99.99% - 100.00%)	100% (99.99% - 100.00%)	99.99% (99.98% -100.00%)
PPV (95% CI)	99.32% (97.92% - 99.78%)	98.94% (92.91% -99.85%)	82.22% (69.82% -90.24%)
NPV (95% CI)	100% (99.99%-100.00%)	100% (99.99%-100.00%)	100% (99.99%-100.00%)

Sensitivity >99%
Specificity>99,9%

Performance

Detection of Sex Chromosomal Aneuploidies

SCA
N=173

	X0	XXX	XXY	XYY
True positive	52	27	51	26
False positive	13	0	3	1
True negative	65724	65775	65747	65776
False negative	1	0	0	0
Sensitivity (95% CI)	98.11% (89.93% -99.95%)	100% (87.23%-100.00%	100% (93.02%-100.00%)	100% (86.77%-100.00%)
Specificity (95% CI)	99.98% (99.97% -99.99%)	100% (99.99%- 100.00%)	99.99% (99.99% -100.00%)	99.99% (99.99% -100.00%)
PPV (95% CI)	80% (69.88% -87.34%)	100% (99.99%-100.00%)	94.44% (84.57%- 98.14%)	96.3% (78.55% -99.46%)
NPV (95% CI)	100% (99.99%-100.00%)	100% (99.99%-100.00%)	100% (99.99%-100.00%)	100% (99.99%-100.00%)

Sensitivity >98%
Specificity>99,9%

Performance

Rare autosomal aneuploidies, segmental anomalies

RAR, and Segmental N=105

Tot. Genoma-wide N=46724	RAA	Segmental abnormalities (>7 Mb)
True positive	33	20
False positive	36	16
True negative	46630	46681
False negative	0	0
Sensitivity (95%CI)	100% (89.42%-100.00%)	100% (83.16%-100.00%)
Specificity (95%CI)	99.92% (99.89%- 99.95%)	99.97% (99.96%- 99.99%)
PPV (95%CI)	47.83% (39.81%- 55.96%)	55.56% (43.37% - 67.11%)
NPV (95%CI)	100% (99.99%- 100.00%)	100% (99.99%- 100.00%)

Sensitivity >99,9%
Specificity>99,9%

Positive cases without follow-up (n°): RAA(25); Segmental abnormalities >7Mb (7); Microdelelions(2)

Performance

Microdeletion

Microdeletion N=7

Tot. Genoma-wide N=46724	Microdeleltions** (segmental abnormalities <7 Mb)
True positive	5
False positive	2
True negative	28743
False negative	1
Sensitivity (95%CI)	83.33% (35.88% -99.58%)
Specificity (95%CI)	99,99% (99.99%- 100.00%)
PPV (95%CI)	71.43% (37.40% -91.27%)
NPV (95%CI)	100% (99.99%- 100.00%)

Sensitivity 83,3%
Specificity>99,9%

**Investigated microdeletions: Di George Syndrome, Cri-du-chat Syndrome, Prader-Willi Syndrome, Angelman Syndrome, 1p36 Deletion Syndrome, Wolf-Hirschhorn Syndrome, Jacobsen Syndrome, Langer-Giedion Syndrome, and Smith-Magenis Syndrome

Selected clinical cases:
Focus on Genome Wide NIPT

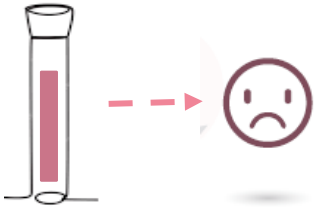
Case Study 1

eurofins | Genoma

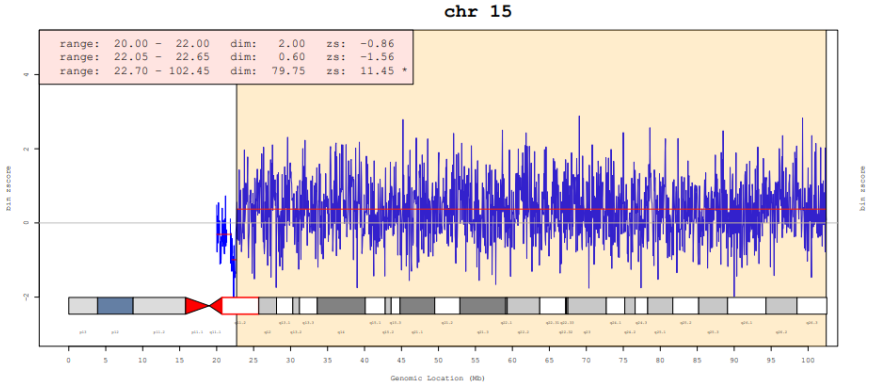
Prenatalsafe

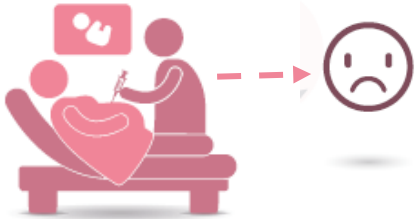
35-year-old

12+5 weeks of gestation



SUSPECTED Trisomy
15q11.2q26.3





Results confirmed

arr[GRCh37] 15q11.2q26.3
(18,697,268-100,171,678)x2~3

Karyotype 46,XX [90] / 47,XX,+15 [10]

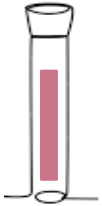
Generalized mosaicism
Presence of two or more chromosomally different cell lines in both the placenta and the fetus

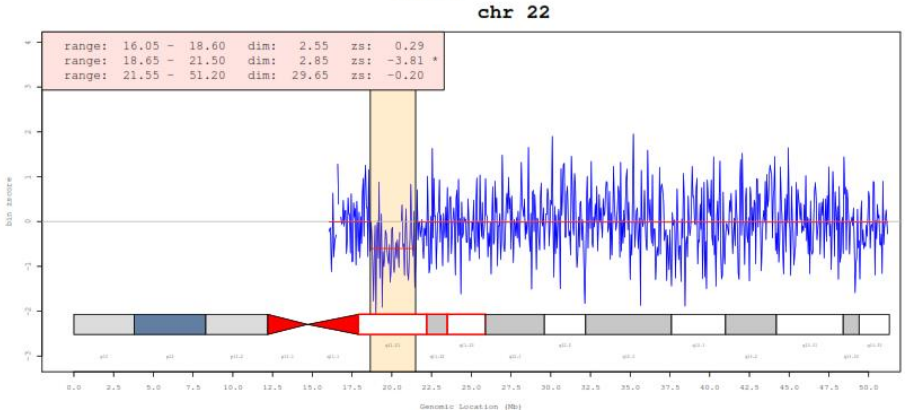
Case Study 2

eurofins | Genoma

Prenatalsafe

31 years old
20 weeks of gestation





Prenatalsafe Karyo Plus identified the presence of microdeletion of chromosome 22 of 2.85 Mb

Ultrasound
Suspected DeGeorge syndrome

Amniocentisis
Results confirmed

Karyotype study by Array-CGH confirmed the presence of a microdeletion of the chromosome 22 of 2,8 Mb attributable to DiGeorge syndrome (Del22q11.21)

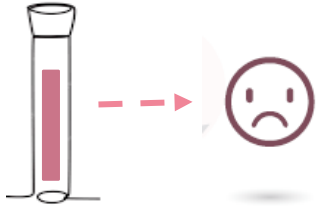
Case Study 3

eurofins | Genoma

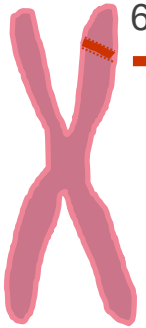
Prenatalsafe

39-year-old

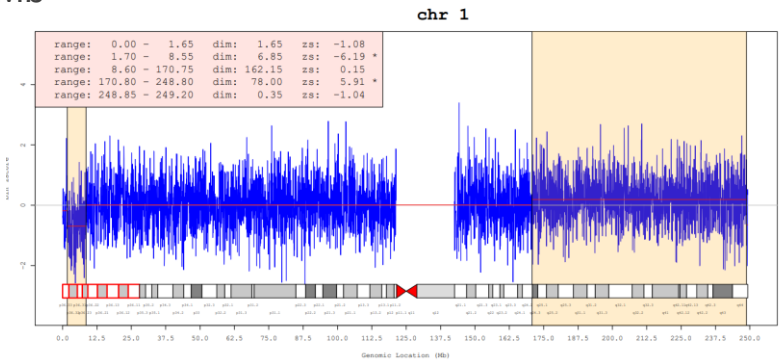
19 weeks of gestation

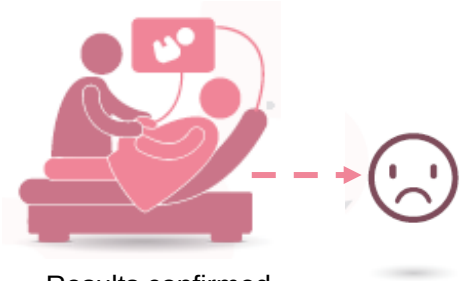


SUSPECTED del(1)(p36.33p36.23)
(6.8Mb)



6.8Mb





Results confirmed

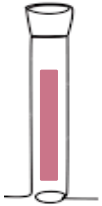
Abnormal Result
detected fetal karyotype with del(1)(p36.33p36.23)

Case Study 4

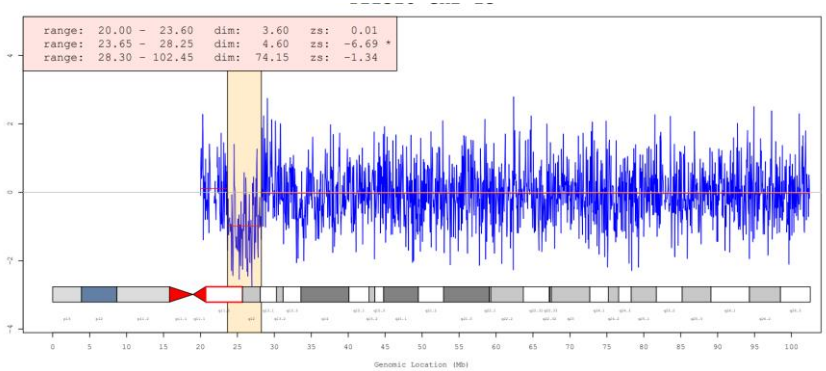
43-year-old

15+6 weeks of gestation

Prenatalsafe



SUSPECTED del15



Amniocentisis
Results confirmed

del(15)(q11.2q12) (3.7Mb)

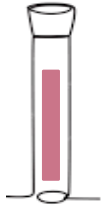
Selected clinical cases:
Focus on false positive

False positive case-Case Study 1

Prenatalsafe

37-year-old

12+3 weeks of gestation



NIPT

SUSPECTED T13

14 weeks of gestation:
Amniocentesis
46XY (76 met); 47,XY+13 (1 met)
CPM or TFM?

Ultrasound

No abnormality

19 weeks of gestation
Cordocentesis
Normal male karyotype

39 week of gestation→Labor induction

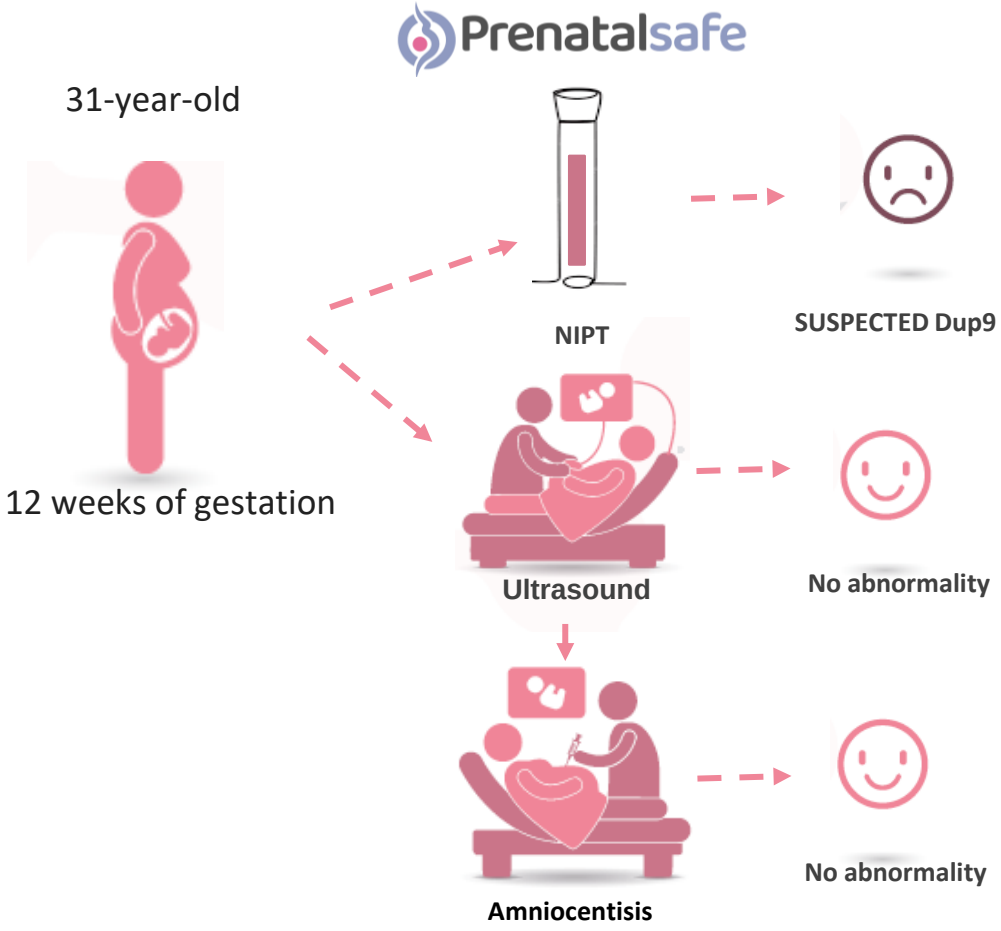
Cordocentesis

No abnormality

aCGH on Placenta at delivery
→T13 mosaicism
Confirmed CPM

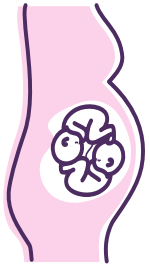
Bonanni et al., 2022

False positive case-Case Study 2

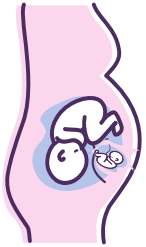


Patient with Hodgkin's lymphoma
dup(9)(p24.3p24.1)(8,6 Mb)

Twin and Vanishing Pregnancies



Twin

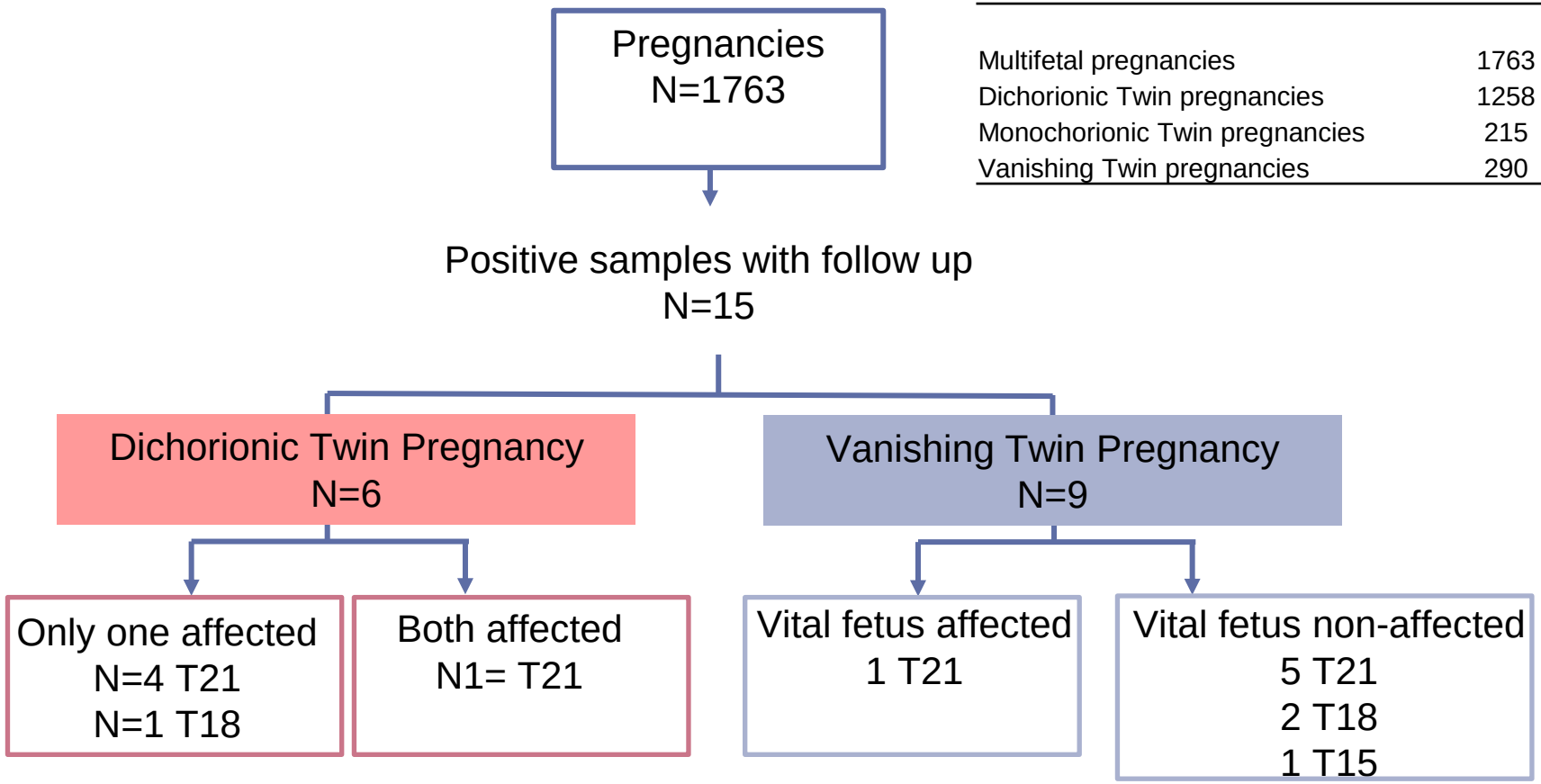


Vanishing

Results

Twin and Vanishing Pregnancies

Characteristics	N
Multifetal pregnancies	1763
Dichorionic Twin pregnancies	1258
Monochorionic Twin pregnancies	215
Vanishing Twin pregnancies	290

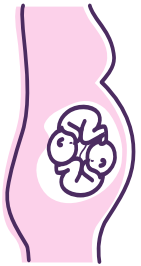


Case Study 1

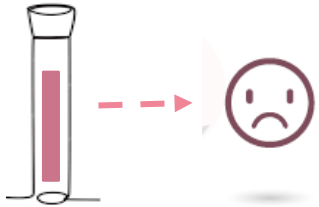
eurofins | Genoma

Prenatalsafe

31 year old,



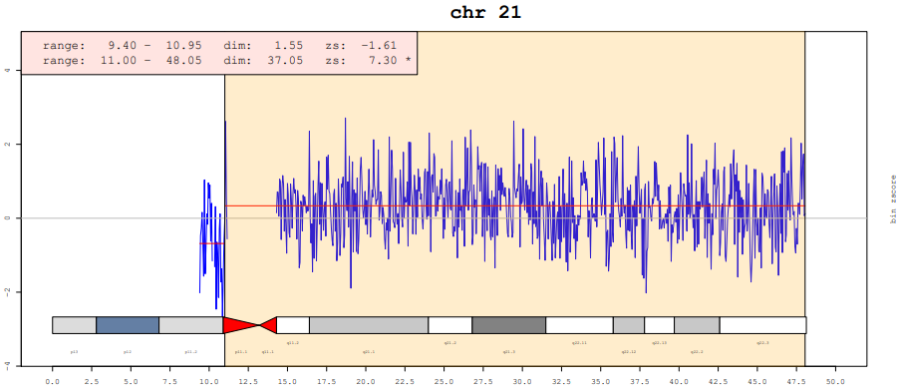
16+1 weeks of gestation

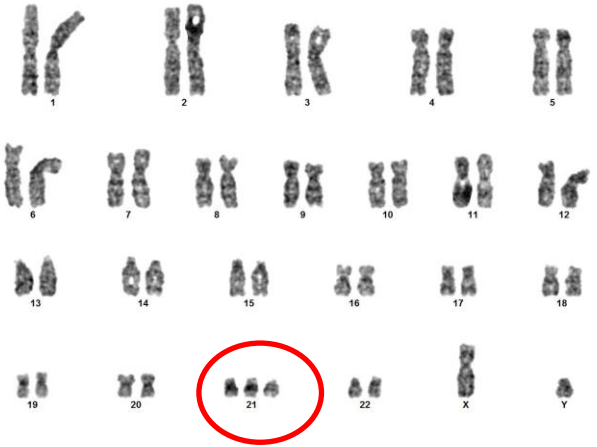


Suspected ONE FETUS AFFECTED
1)46,XY
2)47,XY+21

Amniocentesis

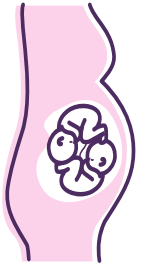
Results confirmed





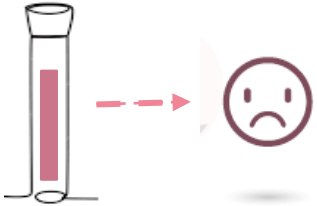
Case Study 2

32 year old,



16 weeks of gestation

Prenatalsafe



Suspected BOTH Fetuses
1)47,XY+21
2)47,XY+21

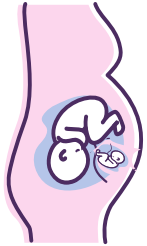
Amniocentesis

Results confirmed

Both fetuses were impacted by the aneuploidy. The array-CGH confirmed the prediction of one twin versus both was affected.

Vanishing Case Study 1

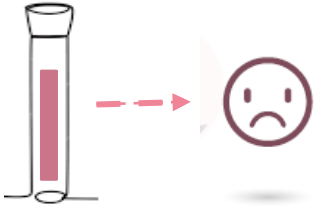
38 year old,



Vanishing

10 weeks of gestation

Prenatalsafe



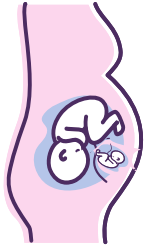
SUSPECTED vanishing with Vital fetus FETUS AFFECTED
21q11.2q22.3(14,530,938-48,020,049)x3

Amniocentesis

Results confirmed

Vanishing Case Study 2

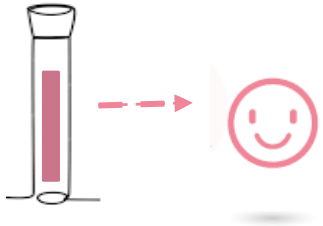
38 year old,



Vanishing

13 weeks of gestation

Prenatalsafe



SUSPECTED Vital fetus Healthy
Affected fetus in resorption
1)47,XY+21

Results confirmed

The array-CGH allowed to exclude that the abnormality detected was at the expense of the ongoing twin, confirming the prediction about the status of the vital fetus.

RESULTS

- The integrated use of Veriseq NIPT solution v2, along with an in-house-developed algorithm allowed to detect Common, SCA, RAA, Deletions/Duplications and microdeletions <7Mb;

- PPV for common chromosomal aneuploidies was 97.9%, and for T21 (PPV=99.3%) and T18 (PPV=98.9%), exceed the excellent performance of previous statistical evaluations.

- PPV for T13 (82.2%) and for X0 (80%)was lower compared to those of T21 and T18. related to the relatively high number of FP, maybe due to its propensity to be associated wit CPM but also maternal mosaicism.

- RAA, Deletion/Insertion and microdeletion were detected in 1 out of every 414 performed GW-NIPT (0,24%) and accounted for 13% (113/868) of all abnormal NIPT results.

- PPVs for RAT was 48%, for Deletion/Insertion 55% and and microdeletion <7Mb 71%, with a sensitivity for 22q11.2 deletion syndrome of 99.9%

Thank You

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