CENTRO ANALISI MONZA

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Certitude for you

LABCO
Quality Diagnostics

NIPT onfield

DNA fetale circolante e
Test Prenatali non invasivi

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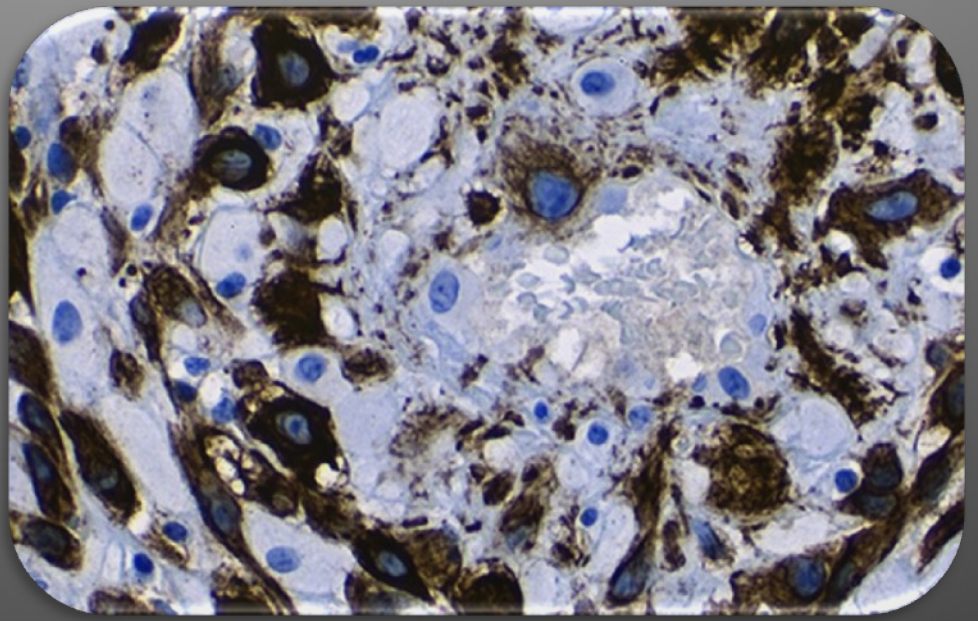
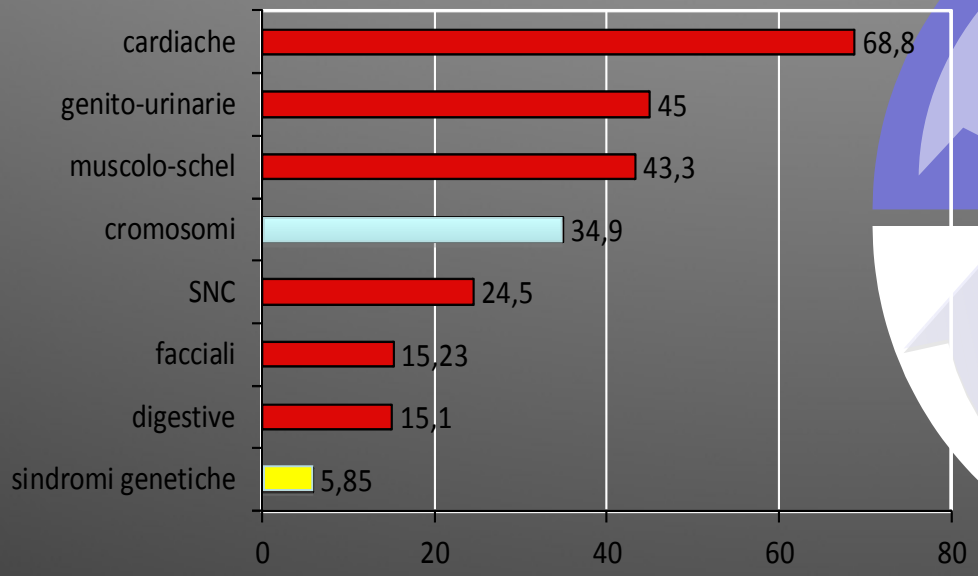
Università Tor Vergata Roma

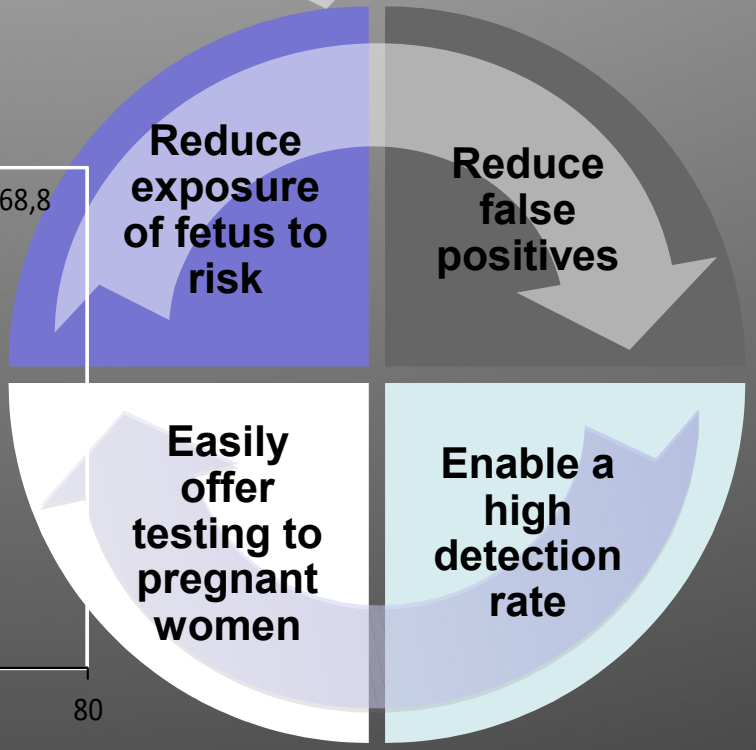


NIPT

NON INVASIVE PRENATAL TEST

ANOMALIE FETALI





NIPT

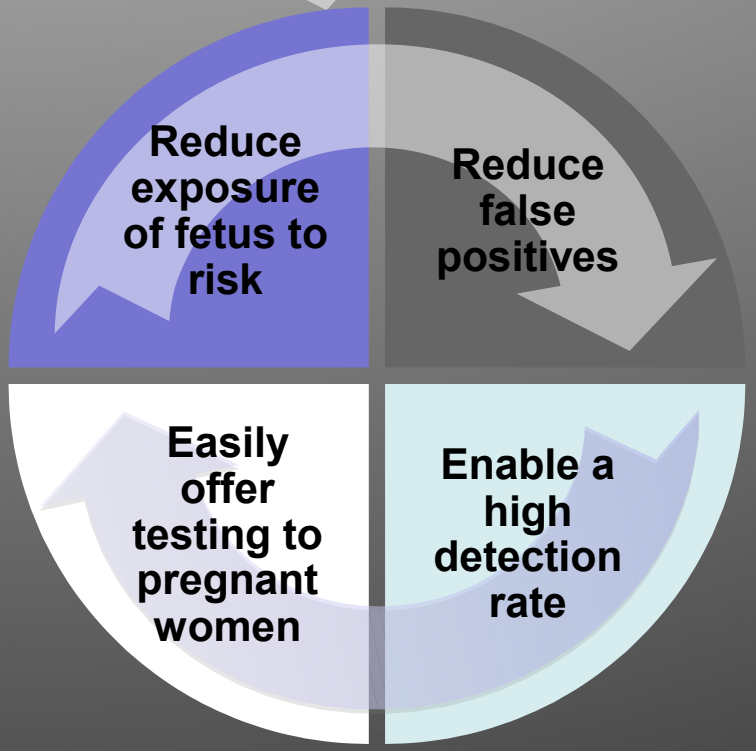
NON INVASIVE PRENATAL TEST

CONSULENZA PRE TEST

La disponibilità di varie tecniche che utilizzano il DNA fetale per la ricerca di anomalie genetiche nel corso della gravidanza rende tassativa la consulenza pre-test, che rappresenta lo strumento di elezione per informare la gestante/coppia sulle diverse opzioni disponibili.

Infatti, è stato dimostrato che la comprensione delle potenzialità e dei limiti dei test su DNA e cffDNA è fortemente compromessa, in assenza della consulenza.

La consulenza pre-test deve essere effettuata da uno specialista esperto di medicina fetale.



NIPT

NON INVASIVE PRENATAL TEST

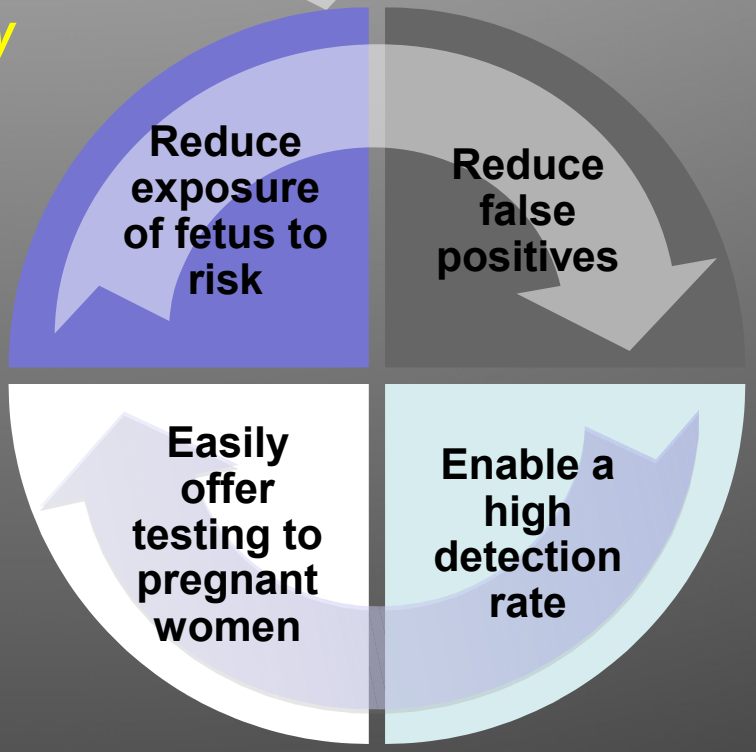
Non Invasive Tests General Sensitivity

NIPTcffDNA *Cromosomi*
60-90% *>maternal age*
2.5 / 1000 nati + IVG

US secondo livello Malformazioni Maggiori
90%
23 / 1000 nati

Sindromi genetiche mendeliane

CF	*75-80%*	*0.4 / 1000 nati*
SMA	*93%*	*0.1 / 1000*
FRAXA	*99%*	*0.8m-0.5f / 1000*
SNS	*90%*	*0.3 / 1000*



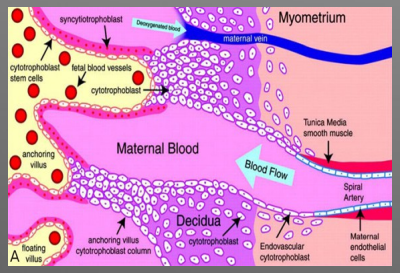
NIPT

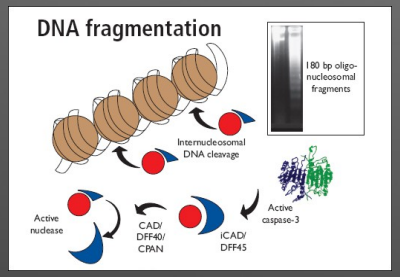
NON INVASIVE PRENATAL TEST

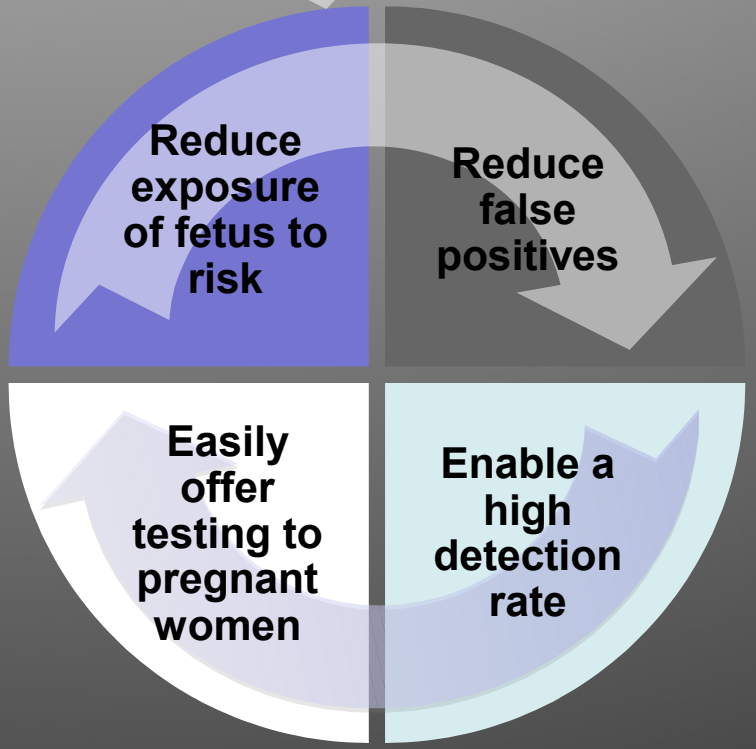
NIPTcffDNA *Cromosomi*

60-90% *>maternal age*

2.5 / 1000 nati + IVG

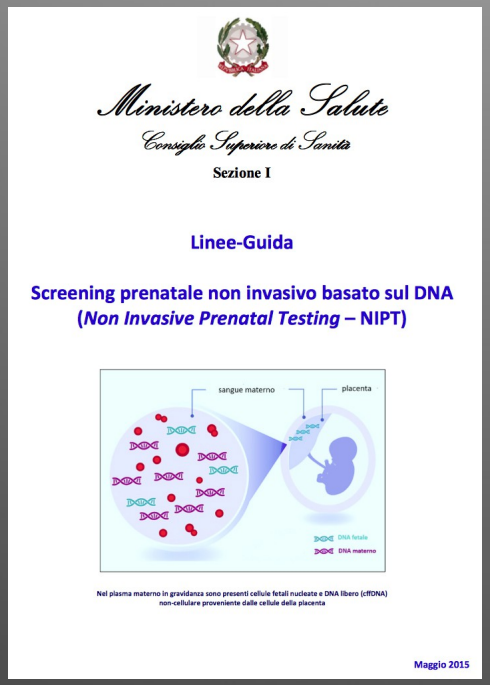






NIPT

NON INVASIVE PRENATAL TEST



Ministero della Salute

Consiglio Superiore di Sanità

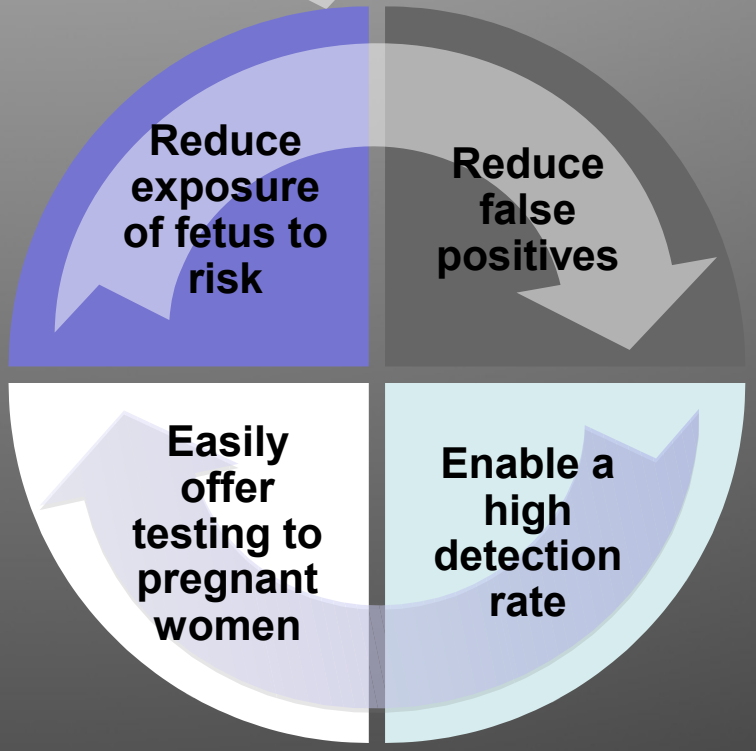
Sezione I

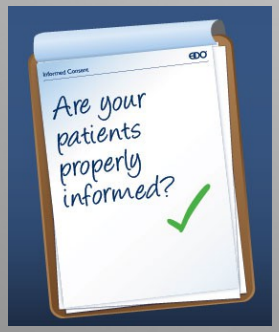
Linee-Guida

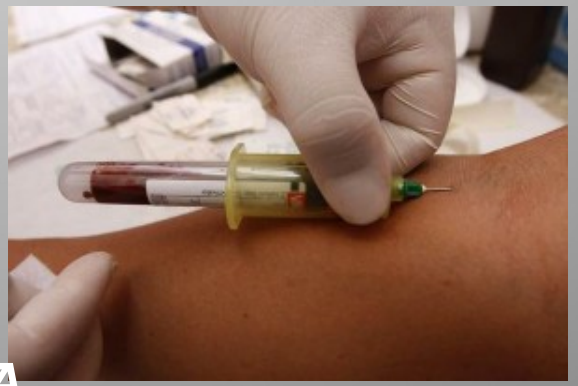
Screening prenatale non invasivo basato sul DNA
(*Non Invasive Prenatal Testing* – NIPT)

Nel plasma materno in gravidanza sono presenti cellule fetali nucleate e DNA libero (cffDNA) non-cellulare proveniente dalle cellule della placenta

Maggio 2015





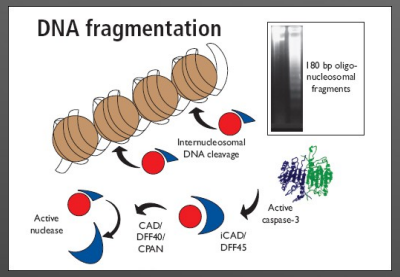


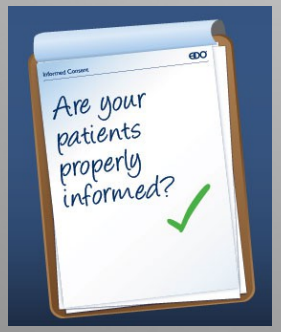
Screening non invasivi cffDNA

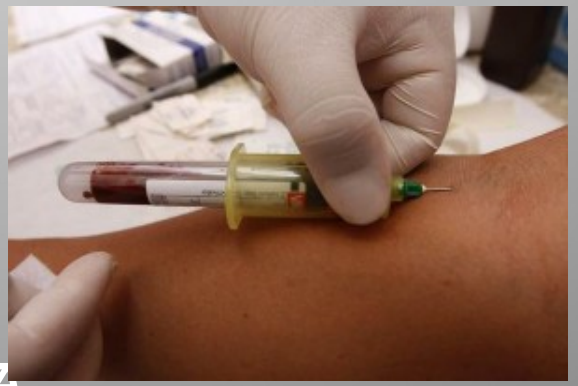
Anomalie Cromosomiche

T21 1/700 nati
T18 1/2
T13 1/5
X/Yaneupl.
RCA del/dup

CA cases	n.	‰ prevalence	% CA
Total	10323	4,4	
T21 T18 T13	7335	3,1	71 53 - 13 - 5
X-Y trisomies	473	0,2	5
X0	778	0,33	8
RCA	1737	0,7	17





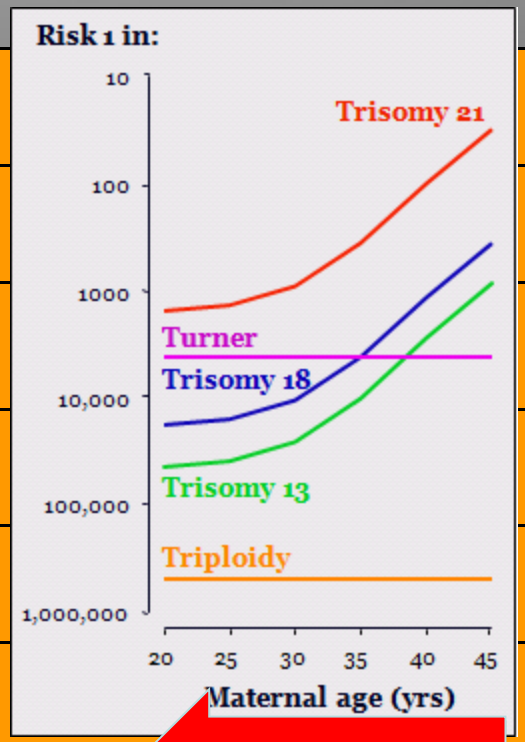


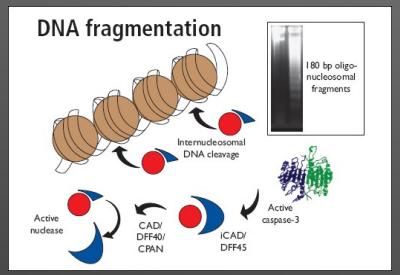
Screening non invasivi cffDNA

Anomalie Cromosomiche. SENSIBILITA' GENERALE 60>90%

T21 1/700 nati
T18 1/2
T13 1/5
X/Yaneupl.
RCA del/dup

CA cases		% CA
Total		
T21 T18 T13		77>48
X-Y trisomies		5
X0		8
RCA		10>40





Screening non invasivi cffDNA

Anomalie Cromosomiche. SENSIBILITA'

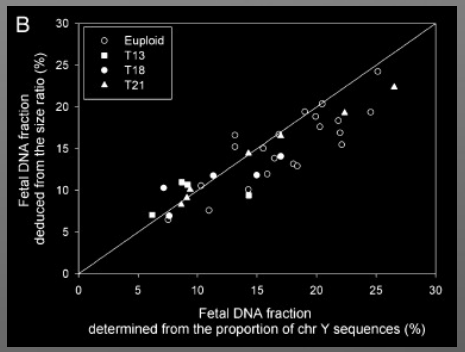
Fetal fraction and Expected ratio for trisomy

Fetal Fraction	Expected ratio for Trisomy
4%	1.02
10%	1.05
20%	1.10
40%	1.20

SNP (DANSR)

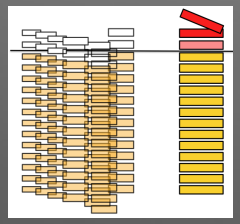
ABRSJA5517	Maternal (buffy coat)	A/C	G/G	C/T	A/T	A/A	A/G	C/T	C/C	A/C	A/G
	Fetal (cfDNA)					**A/G**			C/C		

Fragment size, #X CNV & #Y (Tscore)



Fetale

Materno



Fetal fraction and Expected ratio for trisomy

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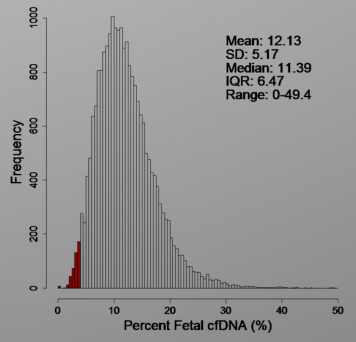


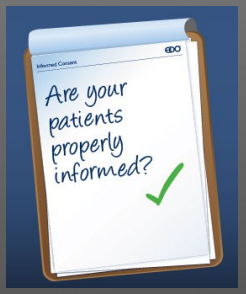
NIPTonfield 1000

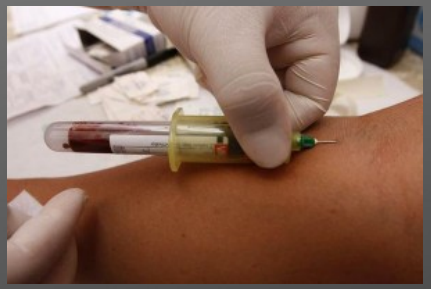
Fetal fraction and test failure

Cases	1200
Successful 1st tier	1187
Successful 2nd tier	9 (0,7%)
Low DNA	7
High variance/1 obese	2
Double fail	4 (0,3%)
Low DNA-HV / FIV ovod.	2
High Variance / obese	2

Fetal fraction and gestation weeks

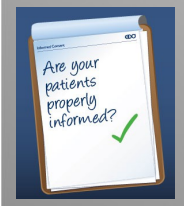
Mean weeks	12,34
Mean fetal fraction	11,5%



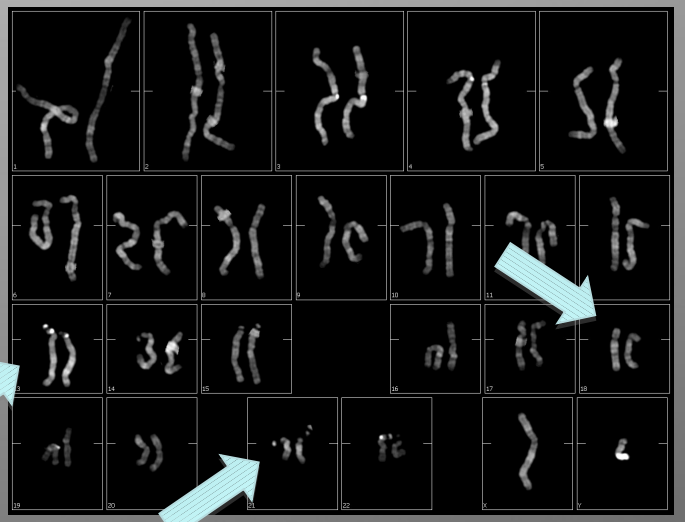


Screening non invasivi cffDNA

Anomalie Cromosomiche. SENSIBILITA' SPECIFICITA'



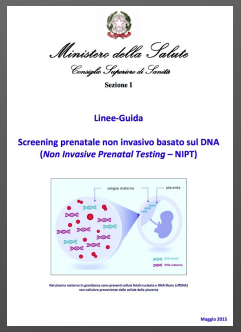
Validation and aneuploidy risk prediction: **high**



Frequenza Anomalie Cromosomiche Autosomi

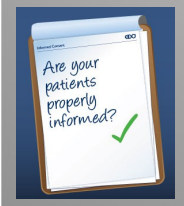
T21 **1/700**
nati+ivg
T18
1/2000
T13

		Trisomy 21	Trisomy 18	Trisomy 13
CVS trophoblast	False positive rate/specificity	0.08%	0.06%	0.2%
62000 cases*	False negative Population rate False negative/sensitivity	0.02% 0.74%	0.01% 1.59%	NS 0.74%
NIPT total 2013-2015	False positive rate/specificity	0.09%	0.13%	0.13%
	False negative Population rate False neg. /detection rate	0.08% 0.8%	0.06-0.12% 3.7%	0.18-0.36% 9%

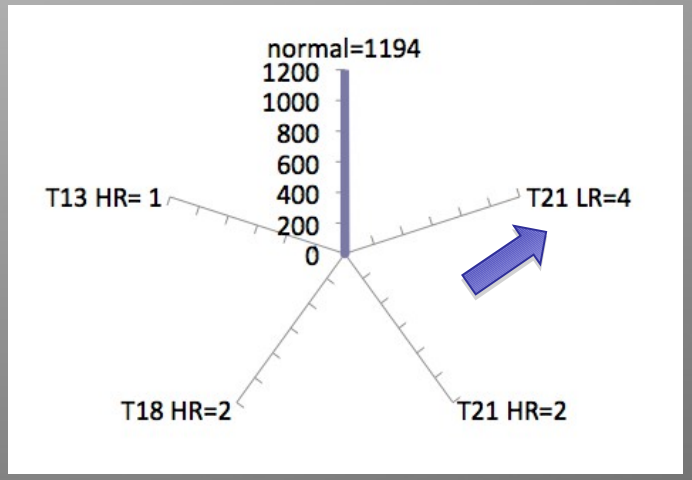


Screening non invasivi cffDNA

Anomalie Cromosomiche. SENSIBILITA' SPECIFICITA'



Validation and aneuploidy risk prediction: **high**



Frequenza Anomalie Cromosomiche Autosomi

T21 **1/700**
nati+ivg
T18
1/2000 nati
T13

		Trisomy 21	Trisomy 18	Trisomy 13
CVS trophoblast	False positive rate/specificity	0.08%	0.06%	0.2%
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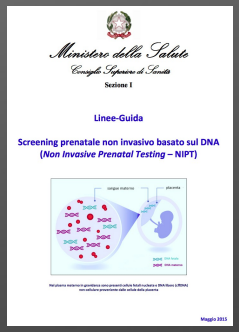


Screening non invasivi cffDNA

Anomalie Cromosomiche. SENSIBILITA' SPECIFICITA'

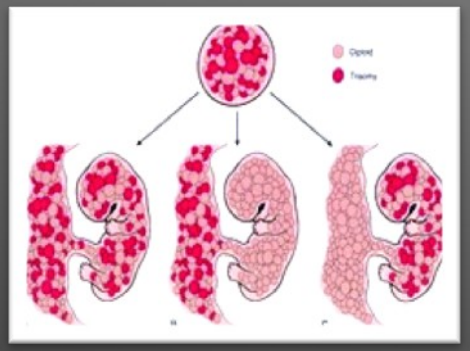
Validation and Aneuploidy risk prediction: **medium**

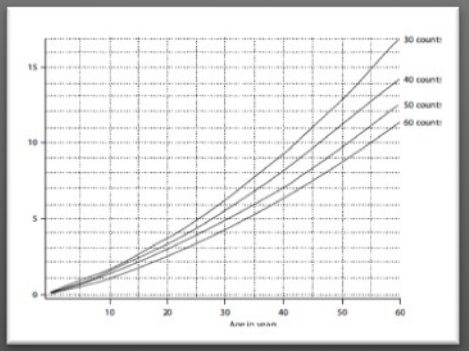
Frequenza Anomalie Cromosomiche SEX

45X

47XXX,47XXY,47XYY

65-90%





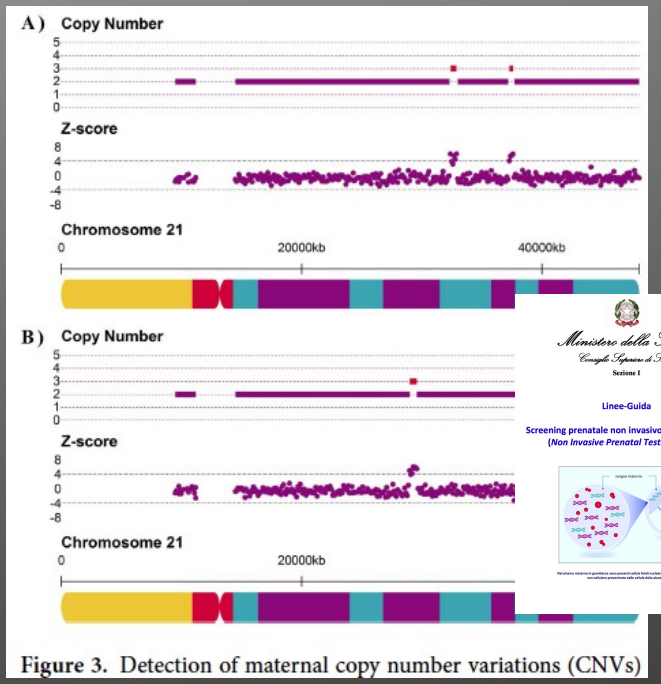
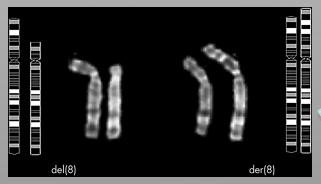


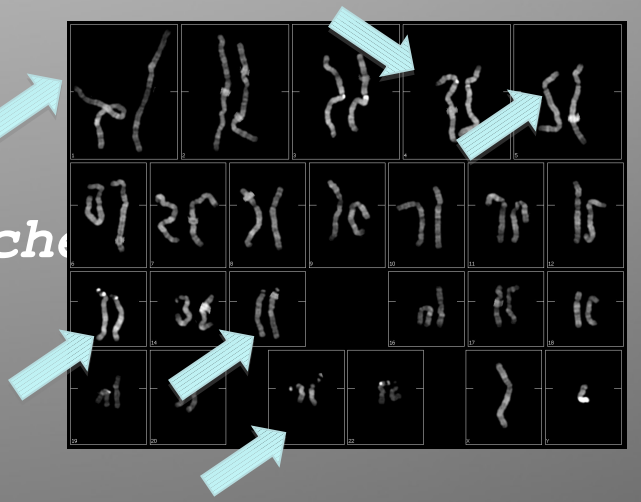
Figure 3. Detection of maternal copy number variations (CNVs)

Screening non invasivi cffDNA

Anomalie Cromosomiche. SENSIBILITA' SPECIFICITA'

Validation and Aneuploidy risk prediction: **low/not yet validated….**



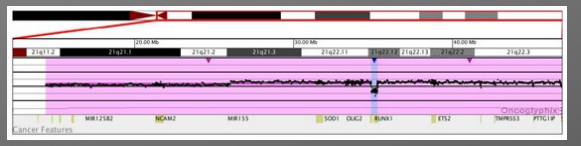


Frequenza Anomalie Cromosomiche

RCA (10-40%) >1/1800

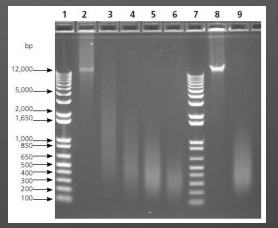
MICRODELEZIONI SELEZIONATE

CNV > DELEZIONI DUPLIC. > 10 Mb



1. Because tested with simulated fragmentation by…. DNA sonication …… than NGS and sequencing…High false positive rates

2. **Few clinical retrospective studies**



Screening non invasivi cffDNA

Anomalie Cromosomiche. SENSIBILITA' SPECIFICITA'

1. Clinical validation of a noninvasive prenatal test for genomewide detection of fetal copy number variants (1166 cases)

TABLE 3
Clinical performance for indicated abnormalities and fetal sex

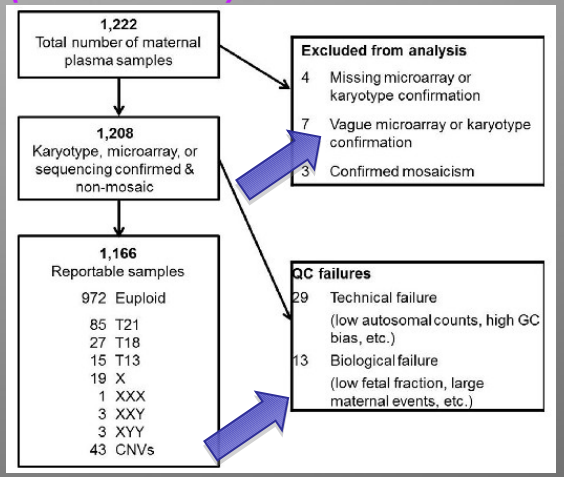
Abnormality	Concordant positive	Discordant positive	Concordant negative	Discordant negative	Sensitivity (95% CI)	Specificity (95% CI)
T21	85	0	1081	0	100% (94.6–100%)	100% (99.6–100%)
T18	27	0	1139	0	100% (84.4–100%)	100% (99.6–100%)
T13	15	0	1151	0	100% (74.7–100%)	100% (99.6–100%)
SCA	26	1	1117	0	100% (84.0–100%)	99.9% (99.4–100%)
CNVs[a]	42	1	1122	1	97.7% (86.2–99.9%)	99.9% (99.4–100%)

Analyte	Concordant male	Discordant male	Concordant female	Discordant female	Accuracy (95% CI)
Fetal sex	583	4	578	1	99.6% (98.9–99.8%)

CI, confidence interval; *CNVs*, copy number variants; *SCA*, sex chromosome aneuploidy; *T13*, trisomy 13; *T18*, trisomy 18; *T21*, trisomy 21.

[a] Includes 8 samples with detected whole chromosome trisomies, and 35 samples with subchromosomal CNVs.

Lefkowitz et al. Clinical validation of genomewide cell-free DNA testing. Am J Obstet Gynecol 2016.



2. Copy number variants using low-coverage whole-genome sequencing of plasma DNA

Table 3. Performance of detecting CNVs events in 919 pregnant women who

Sensitivity	Specificity
88.89%	99.32%
72.73%	99.09%
84.21%	98.42%

Screening non invasivi cffDNA

Anomalie Cromosomiche. SENSIBILITA' SPECIFICITA'

1. Clinical validation of a noninvasive prenatal test for genomewide detection of fetal copy number variants (1166 cases)
2. Copy number variants using low-coverage whole-genome sequencing of plasma DNA

but no PPV because

sequencing depth, GC contribution

low incidence (low PPV)

fetal fraction

CNV size / CNV confined

maternal CNV

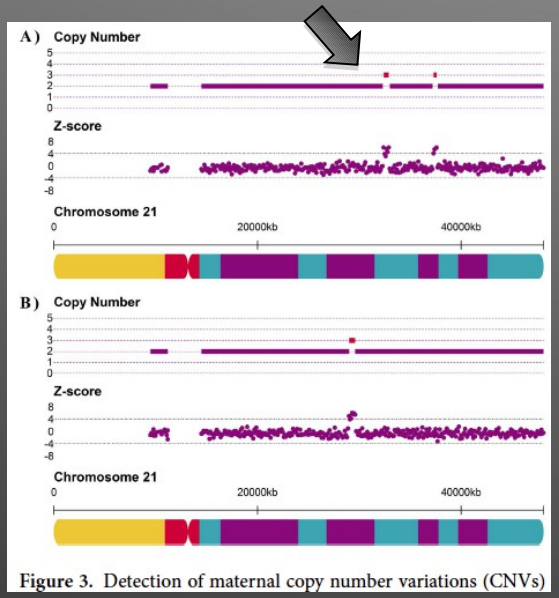
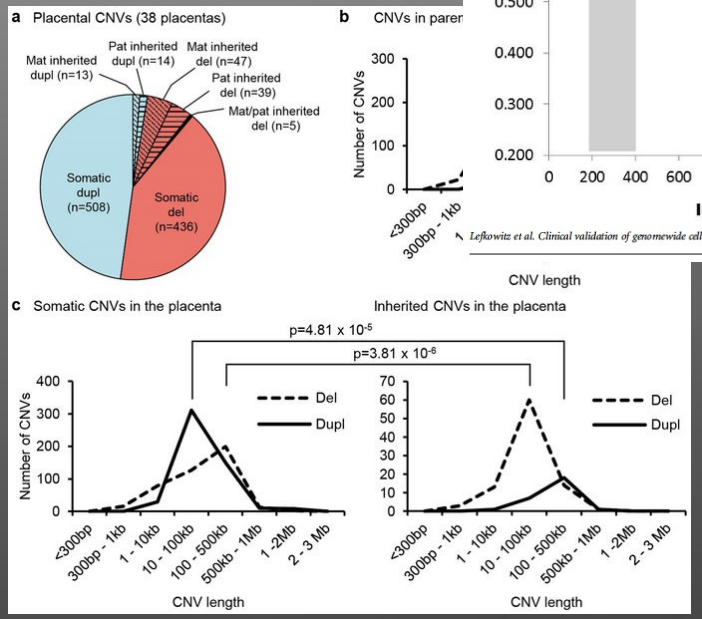
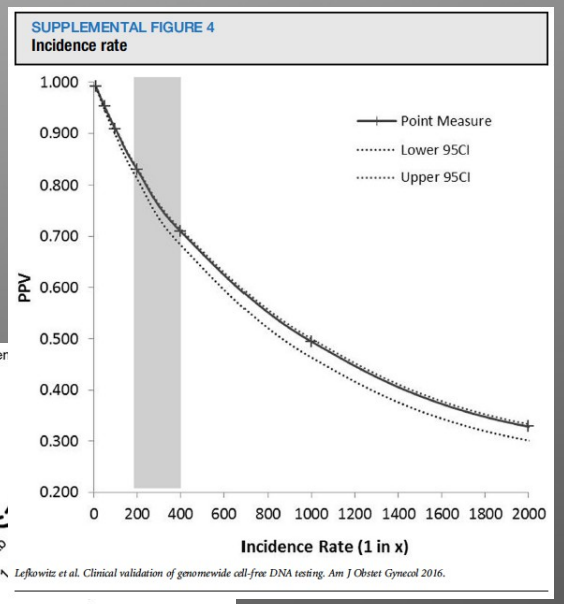


Figure 3. Detection of maternal copy number variations (CNVs)





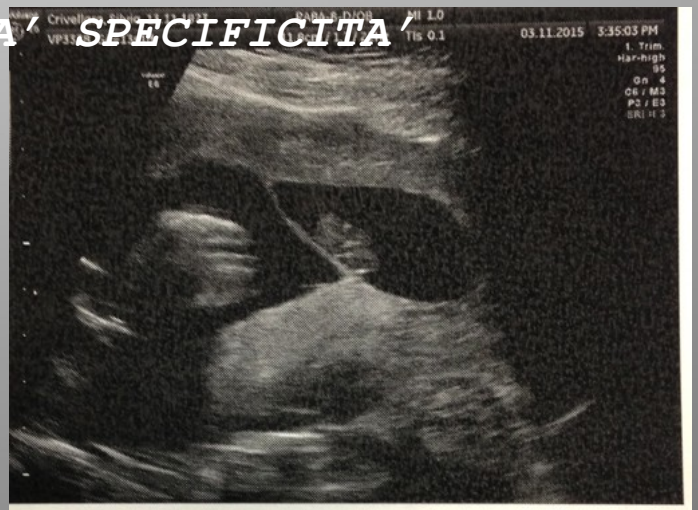
Screening non invasivi cffDNA

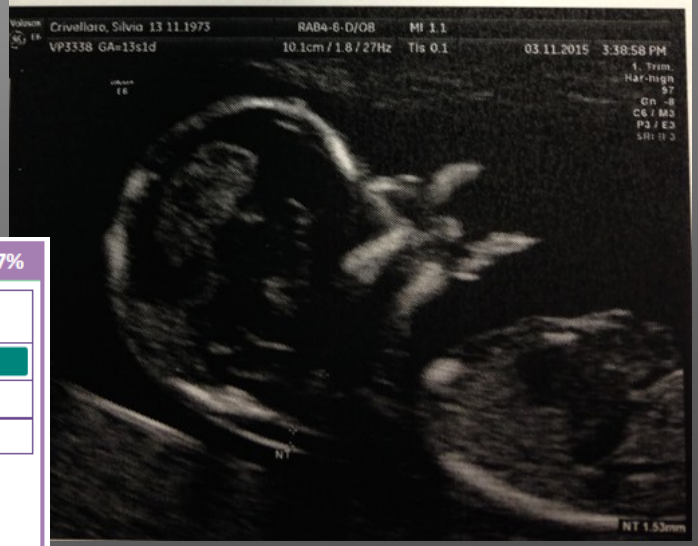
Anomalie Cromosomiche. SENSIBILITA' SPECIFICITA'

Twin pregnancies

The presence of a vanishing fetus is a common cause of trophoblast mosaicism. cfDNA NIPT is not validated to investigate it, but the high sensitivity of the test often reveals the presence of a vanishing fetus .

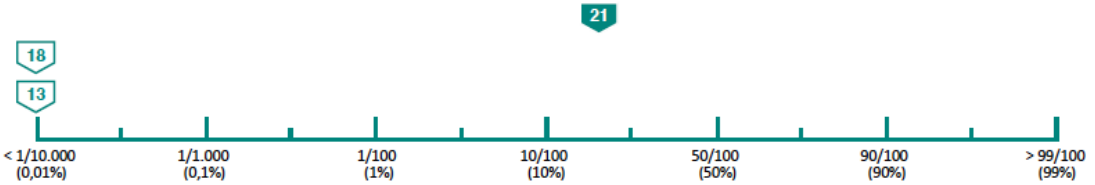
The risk of trisomy 21 with chance 23% is caused by a trisomic no more living fetus and a normal ongoing fetus.





Risultati del test — **Percentuale fetale di cfDNA: 9,7%**

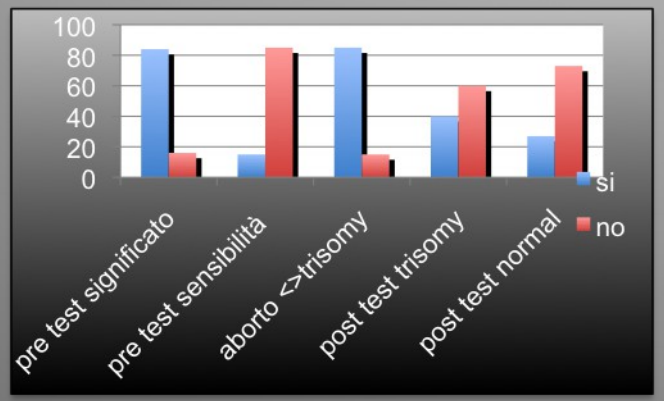
CROMOSOMA	RISULTATO	PROBABILITÀ	RACCOMANDAZIONI
Trisomia 21 (T21)	**Rischio Alto**	**23/100 (23%)**	**Consulenza genetica e test supplementari**
Trisomia 18 (T18)	Rischio Basso	Inferiore a 1/10.000 (0,01%)	Rivedere i risultati con la paziente
Trisomia 13 (T13)	Rischio Basso	Inferiore a 1/10.000 (0,01%)	Rivedere i risultati con la paziente

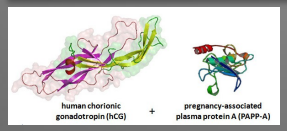


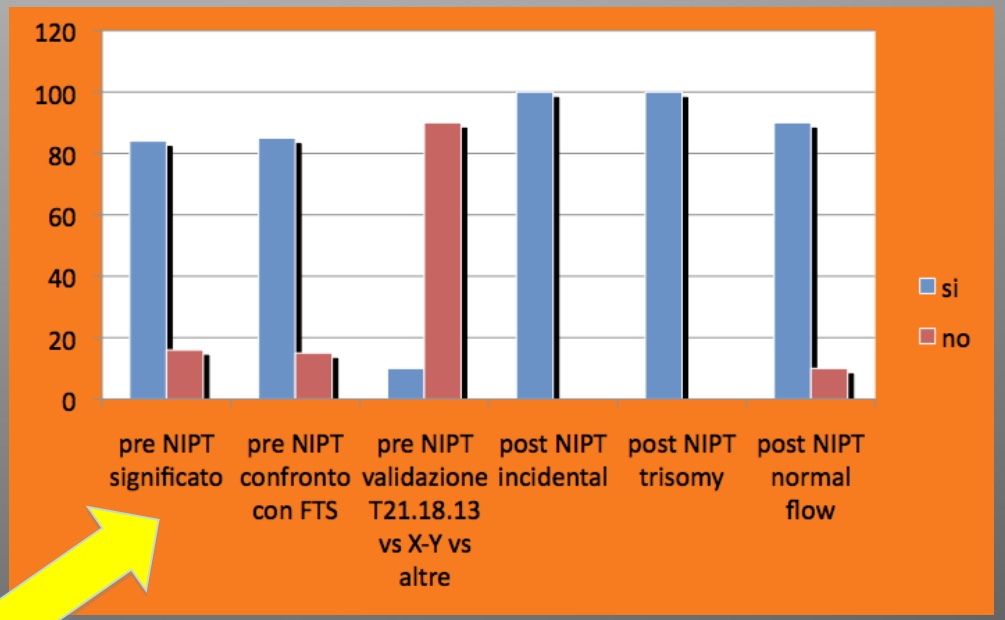
Screening non invasivi cffDNA

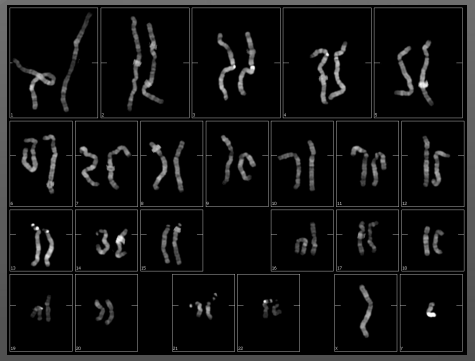
Anomalie Cromosomiche. SENSIBILITA' SPECIFICITA'

Patient information and understanding **NIPTonfield 1000**









Screening non invasivi cffDNA

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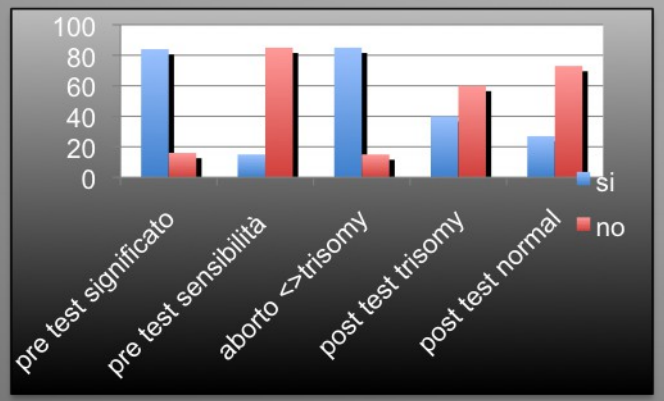
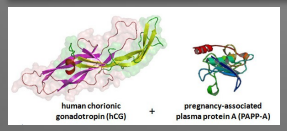
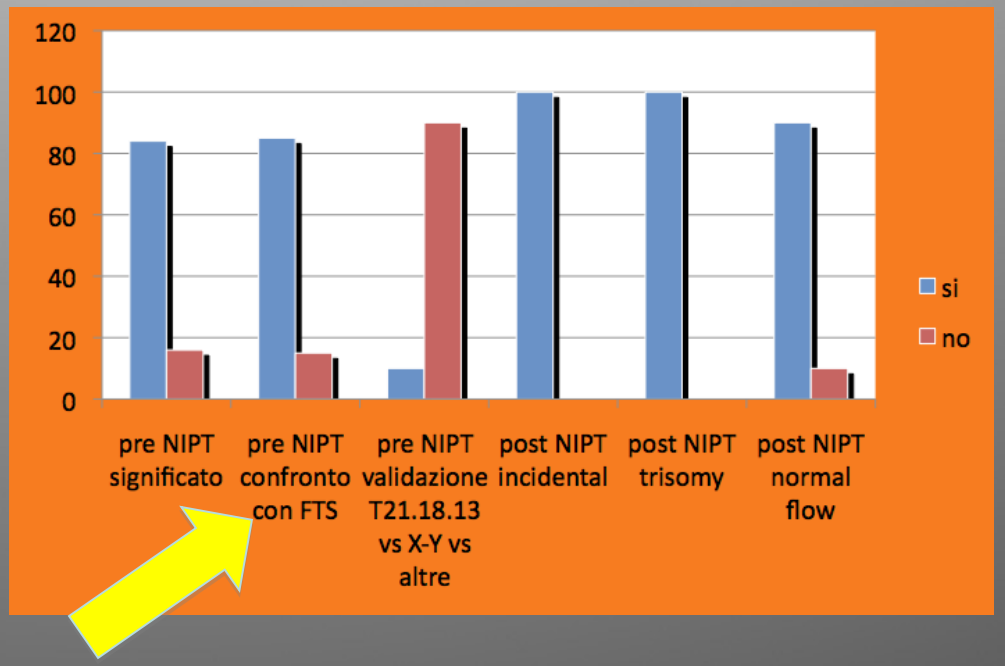






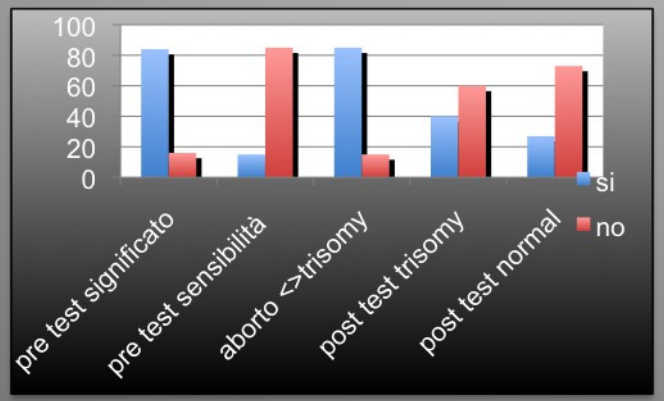
2015 NEXT STUDY 16000 parallel cases: data trisomy 21

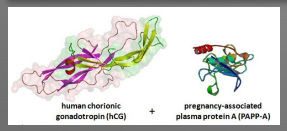
	cffDNA	vs	FTS
DETECTION RATE	36/36 (100%)		28/36 (77.8%)
FALSE POSITIVE RATE	9/15050 (0.06%)		818/15050 (5.4%)
PPV	80%		3,4%

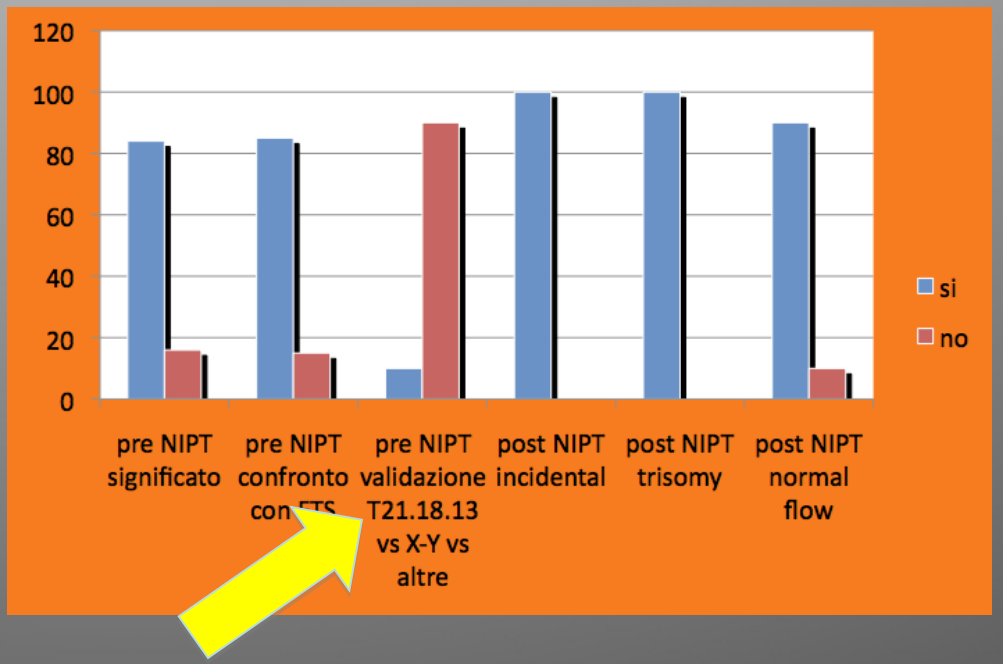
Screening non invasivi cffDNA

Anomalie Cromosomiche. SENSIBILITA' SPECIFICITA'

Patient information and understanding **NIPTonfield 1000**







Anomalie Cromosomiche NIPT validazione

T21 T18 T13 *XXX XXY XYY X0* *Rare Chromosome Anomalies*

>99 *60-90*

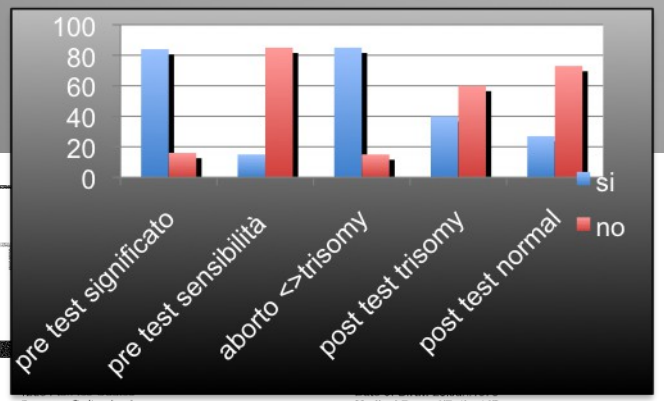
no on-field clinical data

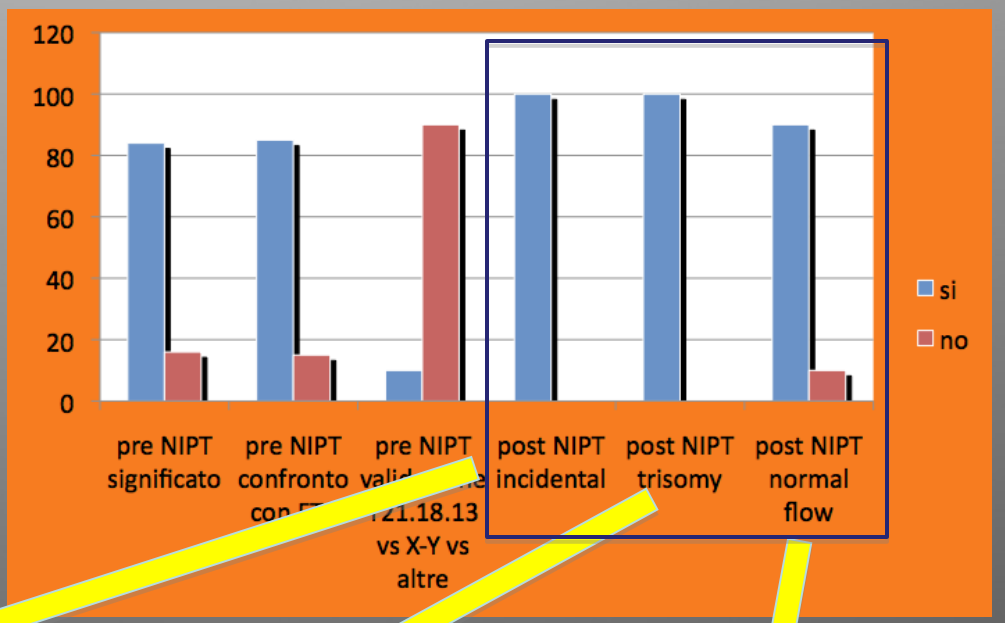
Validated *Validated*

Screening non invasivi cffDNA

Anomalie Cromosomiche. SENSIBILITA' SPECIFICITA'

Patient information and understanding **NIPTonfield 1000**





Geneva - Switzerland

Medical Record/Patient ID:
Gestational Age at Draw (weeks): 12
Indication:
Counselor:
Client Sample ID:

PRENATAL ANEUPLOIDY TEST RESULTS -- Singleton Pregnancy

	RESULT	INTERPRETATION
	No aneuploidy detected	Results consistent with two copies of chromosome 21
	No aneuploidy detected	Results consistent with two copies of chromosome 18
	No aneuploidy detected	Results consistent with two copies of chromosome 13
s	No aneuploidy detected	Results consistent with two sex chromosomes (XX)

Comments: A detection of 5MB on the long arm of chromosome 13 was established with no direct diagnostic implications. Further chromosomal analysis is recommended.
Clinical correlation with ultrasound findings and other screening tests is indicated.

SAMPLE ID

Order ID:	Sample ID:	Draw Date: 02.Mar.2015	Receipt Date:06.Mar.2015

Test Method:
Non Invasive Prenatal Test: Nucleic Acid extraction, DNA sequencing using NGS technology, and analysis of sequencing results to determine fetal aneuploidy.

Limitations of Test:

Disclaimer:

No Call Rate ≤ 0.1%

Genoma SWISS BIOTECHNOLOGY
a company of esperite

This prenatal test is sold by Genoma SA, 12 chemin des Aulx, 1228 Plan-les-Ouates, Switzerland, tel: +41 58 1000 168, a wholly owned subsidiary of Esperite NV group. Esperite N.V., Piet Heinstraat 11, 7204 JN Zutphen, The Netherlands, tel. +31 (0) 575 599 100.
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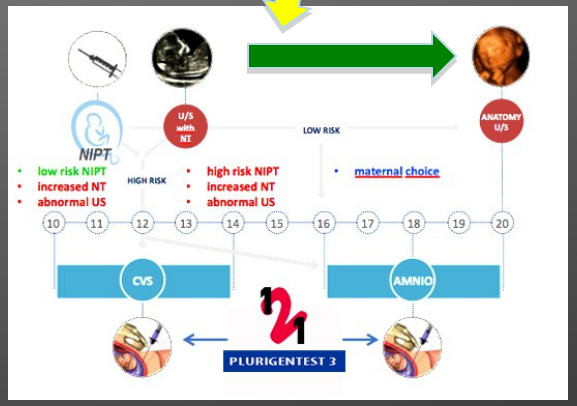
TRD02.0-1114-ENXX

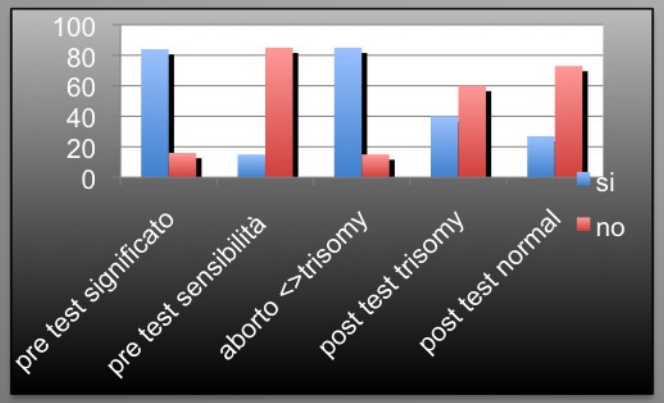
False Positive Population rate Case to case (PPV)	T21 0.09% 7/100	T18 0.13% 23/100	T13 0.13% 66/100	45X 0.23% 62/100

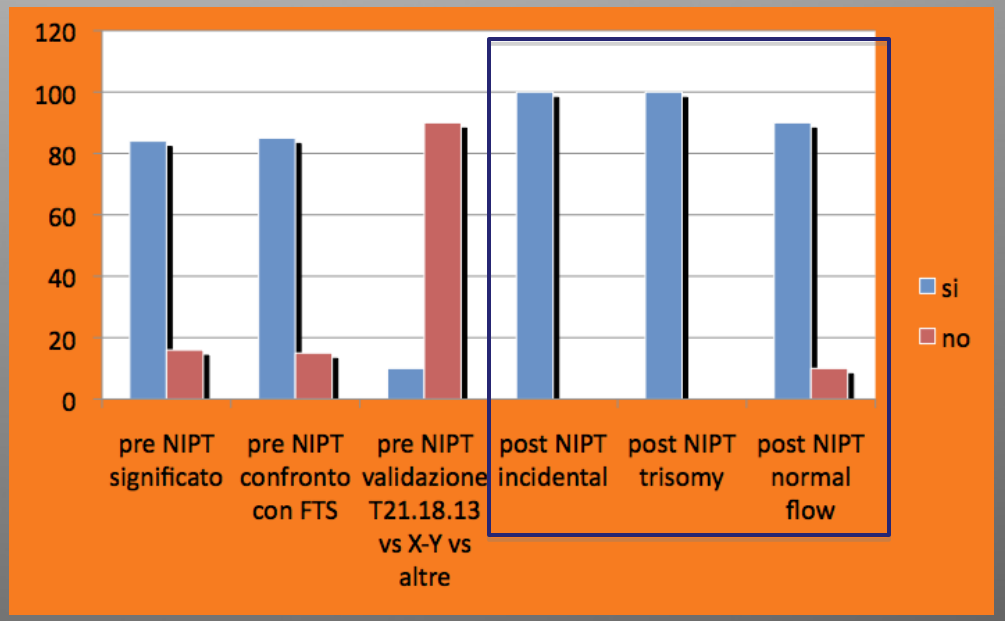


Screening non invasivi cffDNA

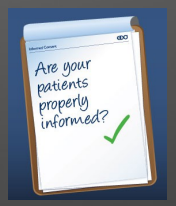
Anomalie Cromosomiche. SENSIBILITA' SPECIFICITA'

Patient information and understanding **NIPTonfield 1000**





Indice di comprensione dopo consulenza genetica pre test passa dal 25-40% al 85-100%

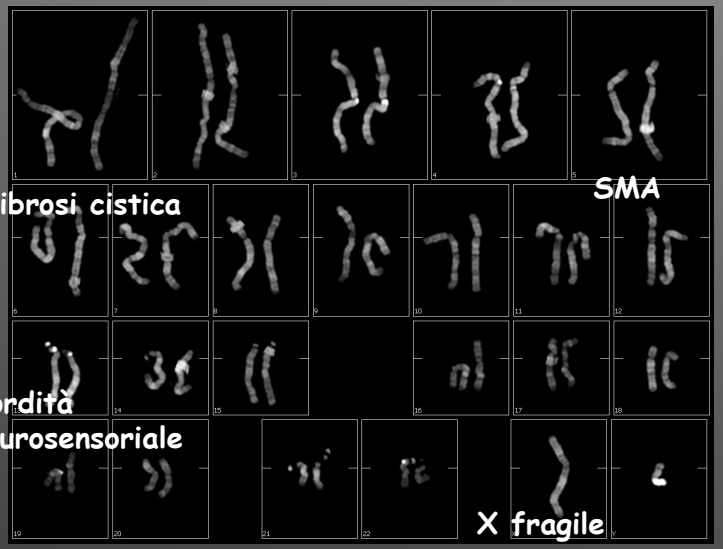


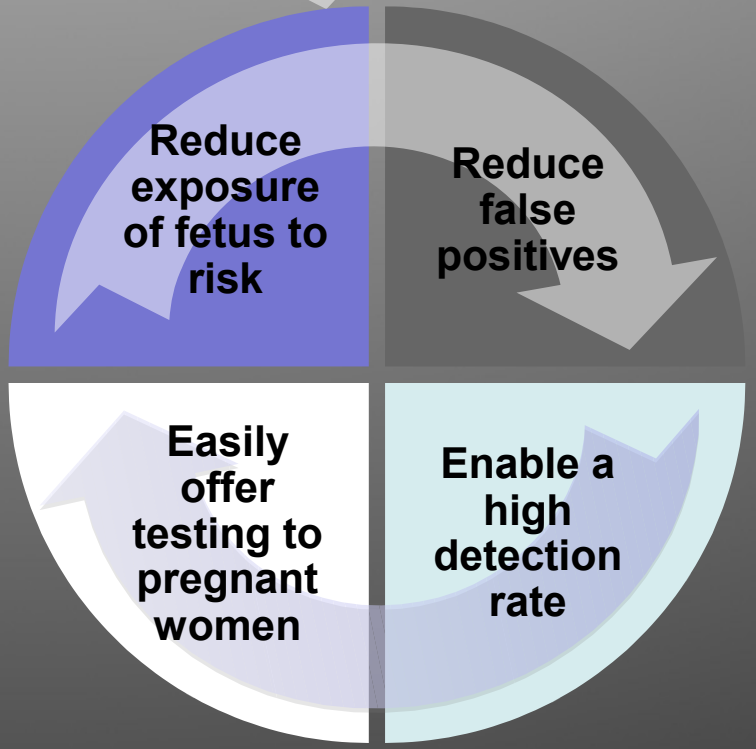
NIPT

NON INVASIVE PRENATAL TEST

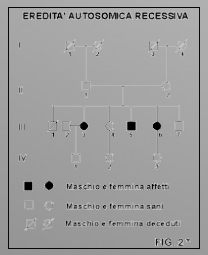
Sindromi genetiche mendeliane

CF	*75-80%*	*0.4 / 1000 nati*
SMA	*93%*	*0.1 / 1000*
FRAXA	*99%*	*0.8m-0.5f / 1000*
SNS	*90%*	*0.3 / 1000*



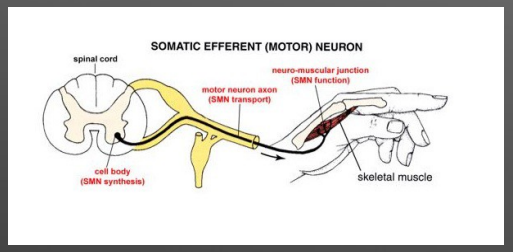


PluriGenTEST™



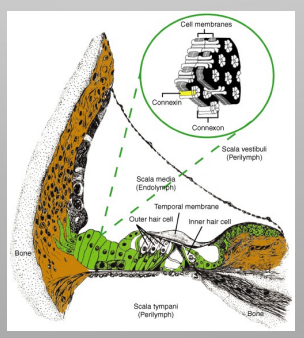
Fibrosi cistica

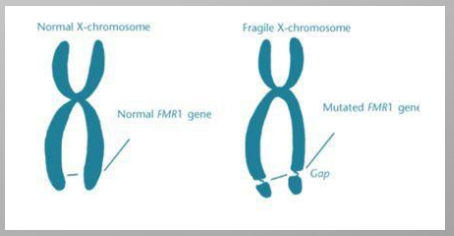
	SINTOMI FC	ETA' DI COMPARSA	MALATTIA CON CUI VENGONO CONFUSI
RESPIRATORI	○ Tosse frequente ○ Catarro ○ Stanchezza ○ Perdita di peso e appetito	Dalla nascita all'età adulta	○ Asma ○ TBC ○ Bronchite cronica senza cause
INTESTINALI	○ Feci frequenti e abbondanti ○ Pancia gonfia ○ Dolori addominali ○ Arresto di crescita o perdita di peso	Dalla nascita all'età adulta	○ " Colite" ○ Celiachia ○ "Intolleranza alimentare"
	○ Occlusioni intestinali	Più frequenti nell'infanzia ma possibili anche in altra età	○ Appendicite ○ Malformazione intestinale



SMA

Sordità neurosensoriale



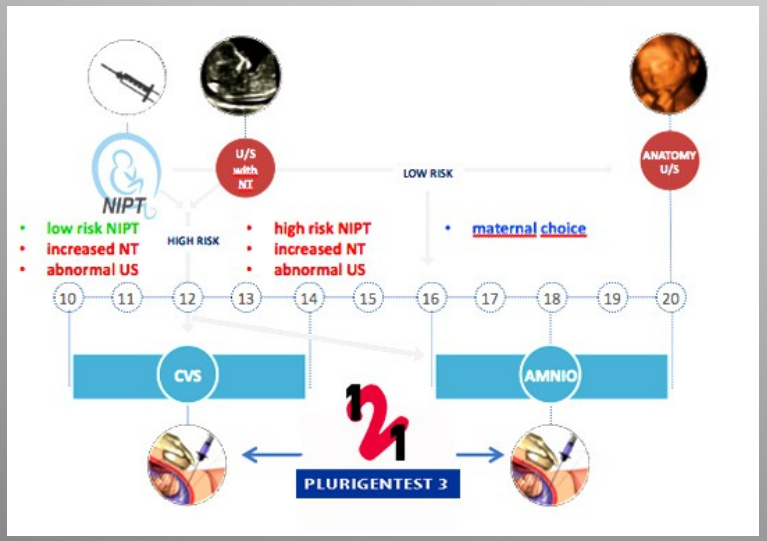


X fragile

Ricerca portatori malattie mendeliane

Fibrosi Cistica	sensibilità tecnica/malattia 75-80%
Atrofia muscolare spinale	sensibilità tecnica/malattia 92%
Sordità NS	sensibilità malattia 90% (rischio residuo 1/350)
Ritardo mentale FRAXA	sensibilità metodo 99%

	casi	freq/attese	freq/ottenute	Diagnosi fetale
Totale	543			
PGT1	275			
FC +-/--	8			
FC +-/+-	2	1>25	1>34	2 (--)
SMA	4	1>50	1>70	1 (--)
Sordità Cx	3	1>35	1>90	
FRAXA	1	1>260	1>275	1 (-)



Finally…

How and when…

			PLURIGEN						
					CVS				AF
				NIPT					
Private					FTS/NT				
		CONSULENZA							
	8	9	10	11	12	13	14	15	16
				CONSUL					
HCS facility					FTS/NT				
						NIPT			
					CVS				AF

Who and where…

LLGG.NIPT Raccomandazioni generali

I Centri che offrono il test devono avere competenze nella diagnosi ecografica, nella diagnosi prenatale; devono avere competenze qualificate per la consulenza pre-test e post-test; devono garantire la tracciabilità del campione ed essere collegati con il laboratorio che esegue il test. Nel caso il laboratorio sia all'estero o a distanza è necessario un efficiente contatto a sostegno della interpretazione dei risultati.

NIPTonfield1000

Poliambulatori con competenze integrate.
Visita ostetrica/ecografia. Consulenza genetica pre test. NIPT e/o altre indagini. Consulenza e/o Supporto post test. Decorso ecografico.

NIPT non è per "il venditore di medicine"…