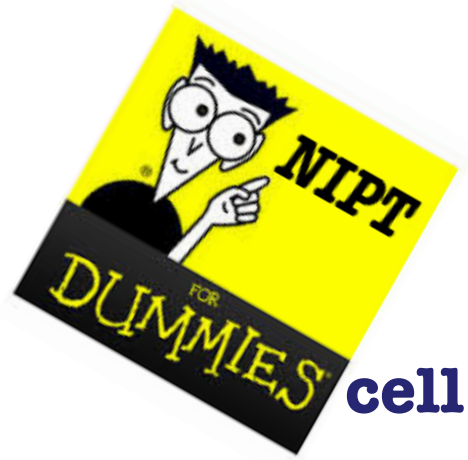
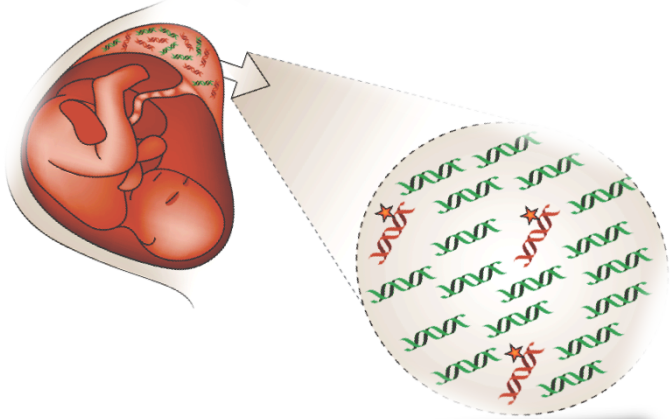


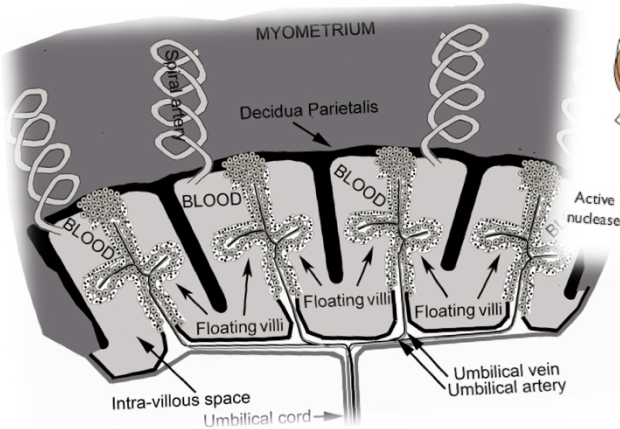
NIPT

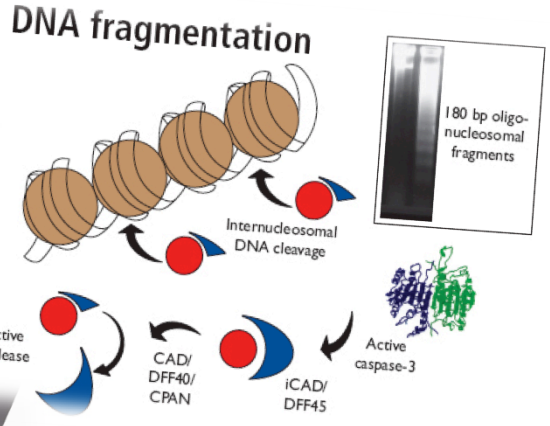
Non Invasive Prenatal Test

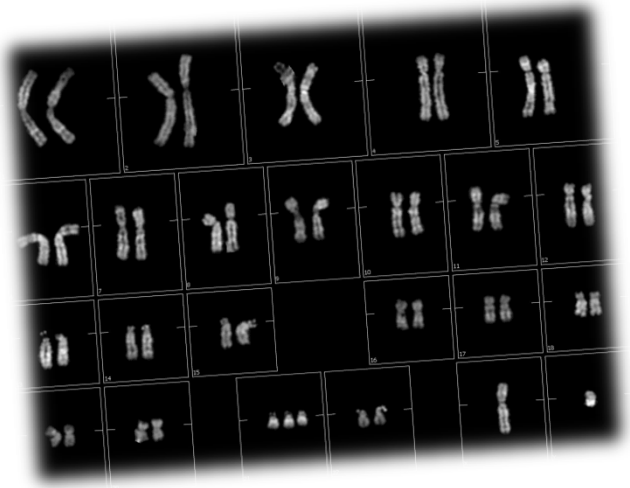
cell free fetal DNA in maternal blood

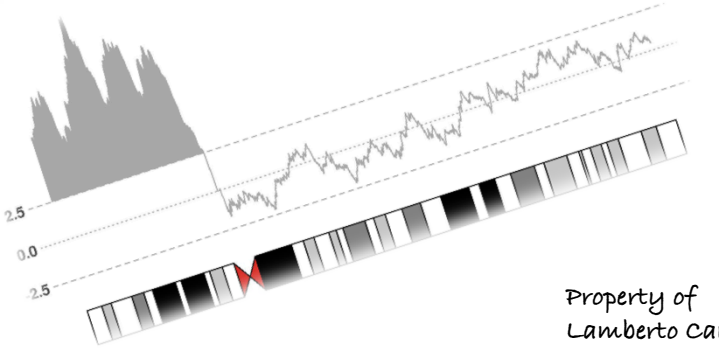
2020



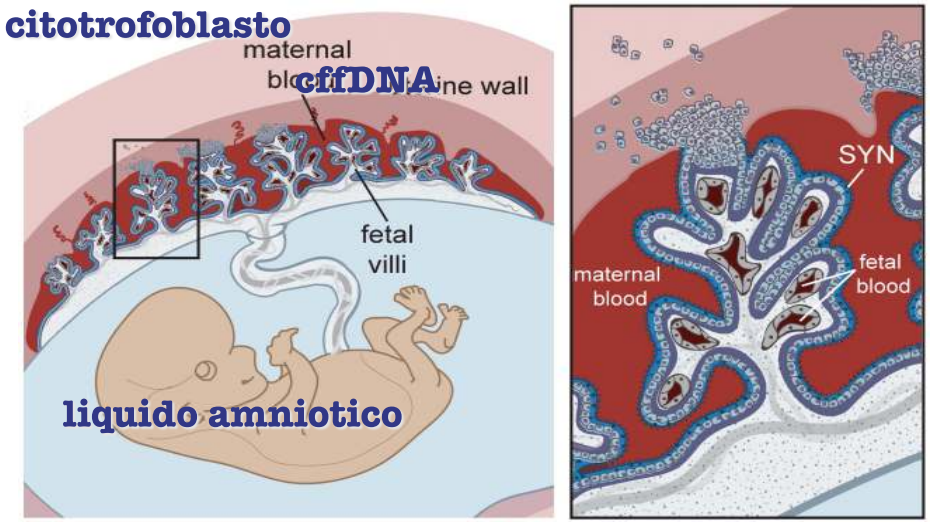






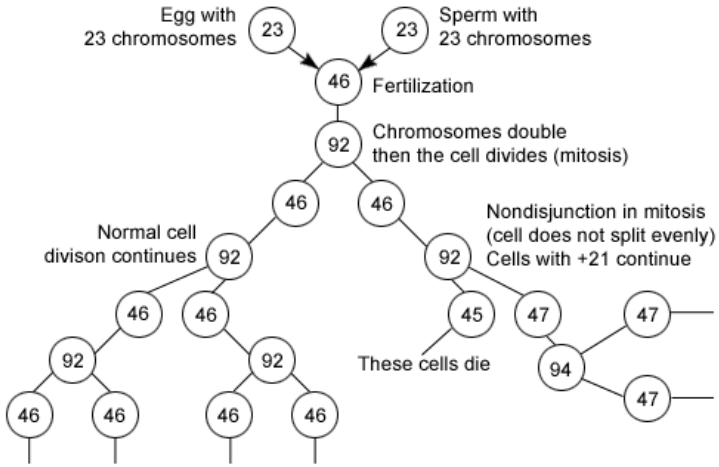


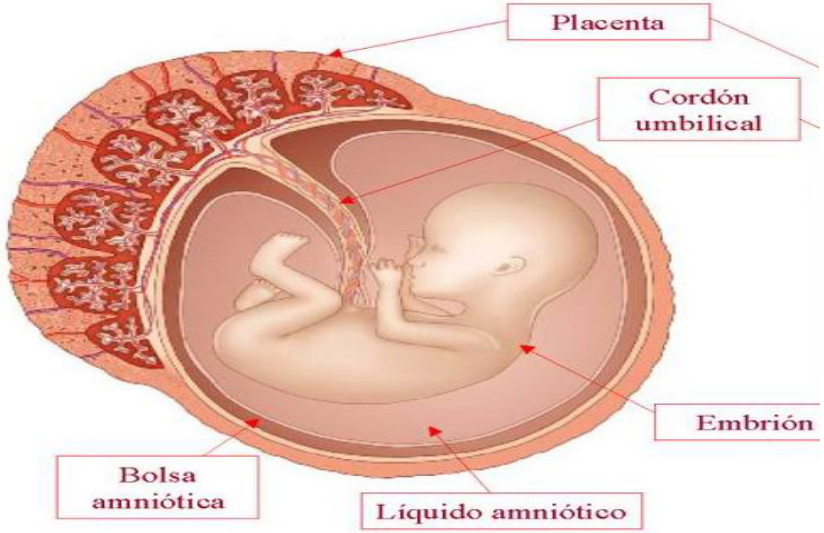
I test genetici feto-placentari usano il DNA del prodotto della fecondazione: placenta se villi coriali o DNA libero nel sangue materno (citotrofoblasto), feto se liquido amniotico (cute, epitelio renale, amnios). Il loro scopo è di intecettare il maggior numero possibile delle cause delle malformazioni congenite fetali

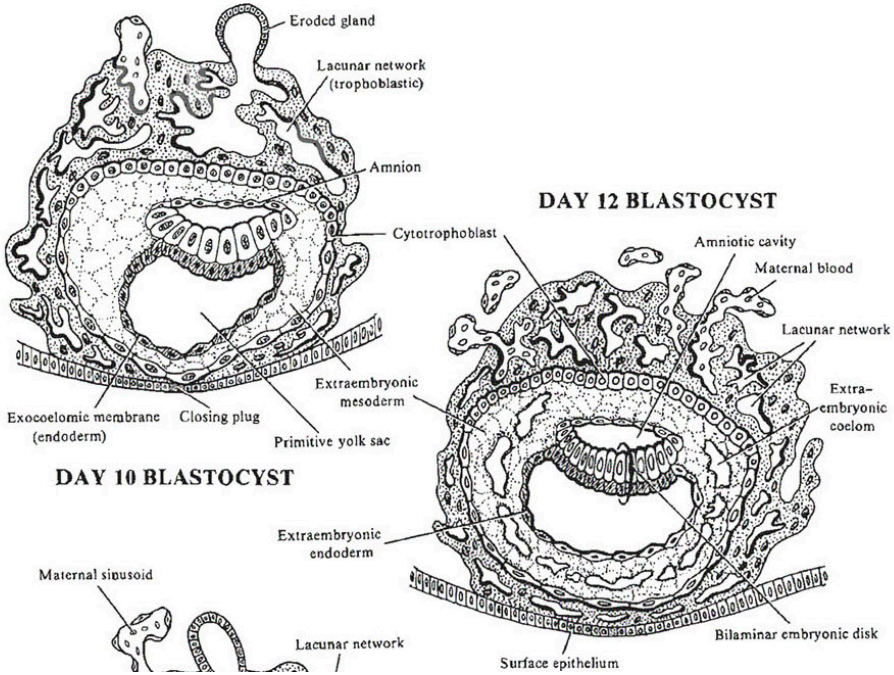


Numero casi (nati + IVG)	6849333	207225
Prevalenza anomalie congenite	2,37 % (1:42)	2,27 % (1:44)
Prevalenza anomalie cromosomiche	0,35 % (1:270)	0,32 % (1:312)

Le anomalie cromosomiche che insorgono alla fecondazione investono sia la placenta (villi coriali, DNA libero nel sangue materno) che il feto (liquido amniotico) e hanno la stessa sorte. **Le anomalie cromosomiche che insorgono oltre i 10 giorni dalla fecondazione quando la placenta il feto si separano** investono uno solo dei tessuti e danno origine alla discordanza feto placentare (<0.1%)



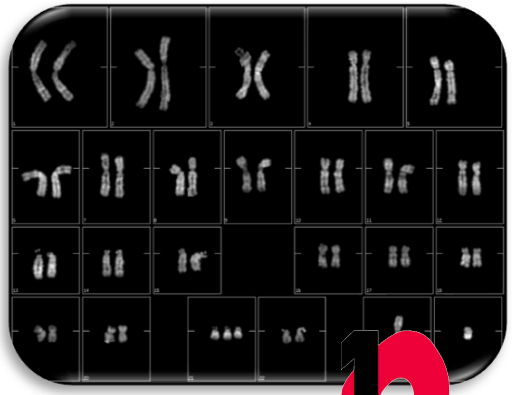


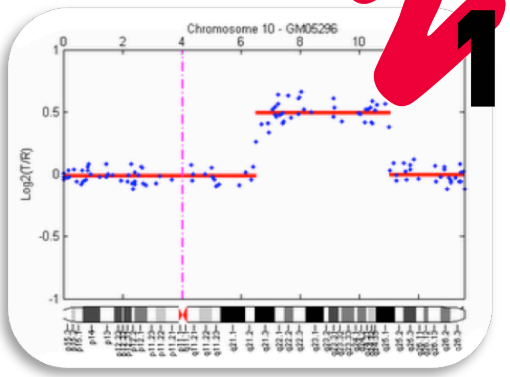


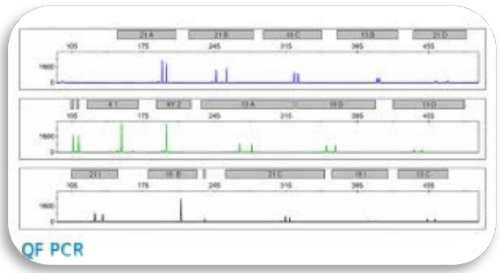
Anomalie Cromosomiche	‰ prevalence EU	CVS Cyto trophoblast direct method	ccfDNA
Totale	4,4	accuracy	accuracy
T21 T18 T13	3,1	99.91 99.93 99.84	99.96 99.97 99.82

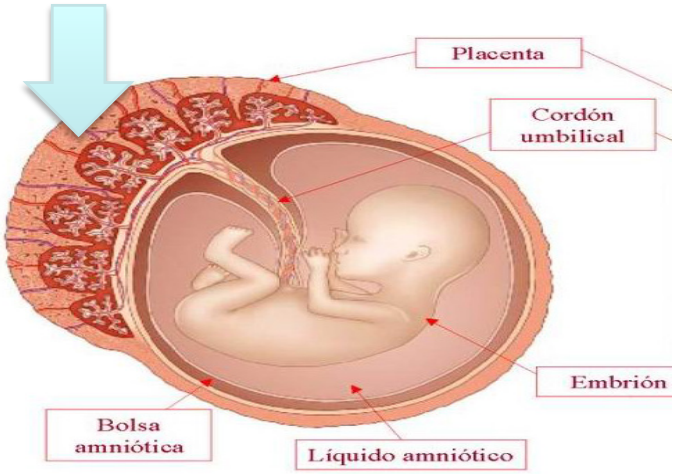
La **accuracy** di un test è: (veri positivi + veri negativi) / tutto il campione

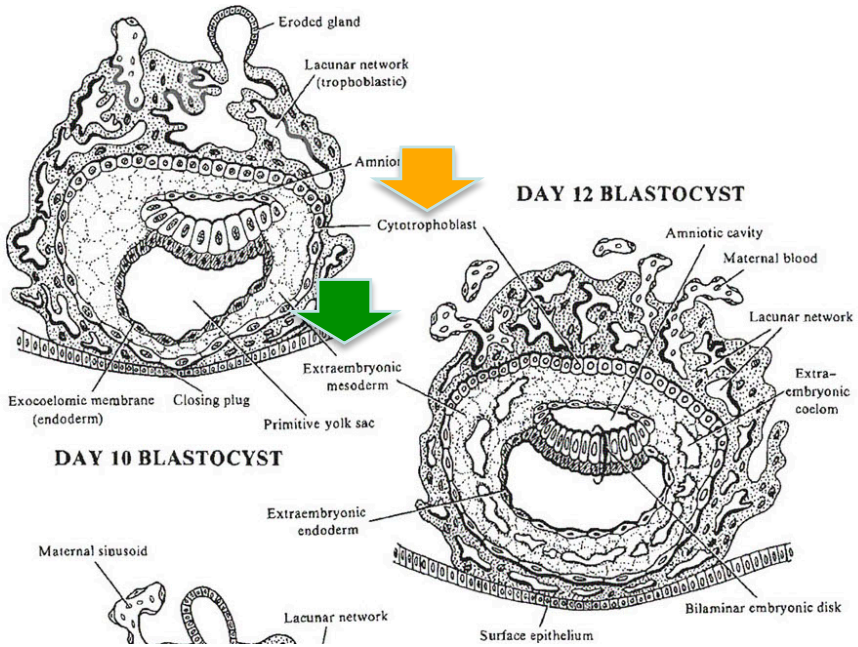
Test genetici diagnostici feto-placentari del primo trimestre di gravidanza: tecniche citogenetiche e molecolari con prelievo di villi coriali

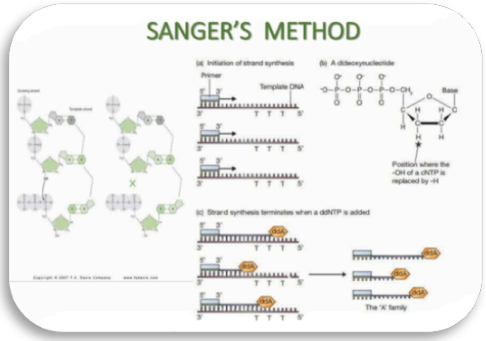


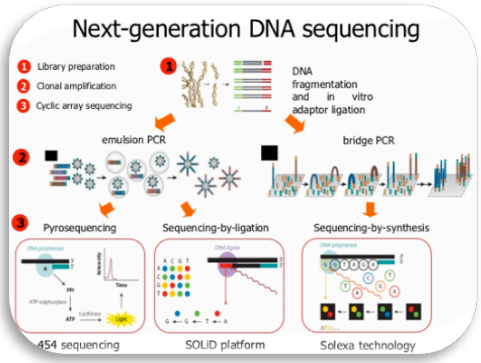


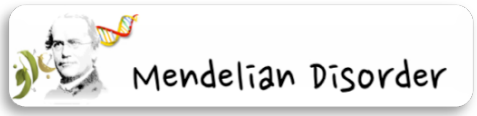




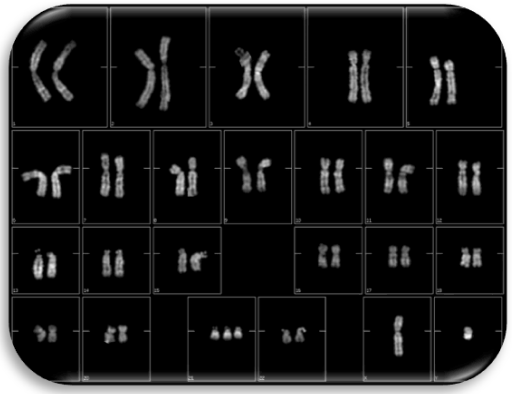


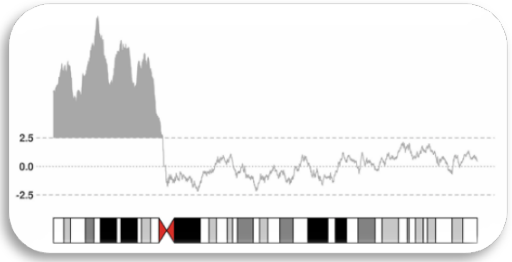


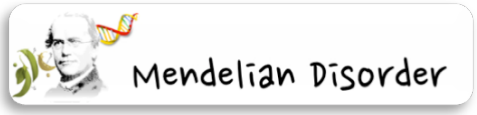


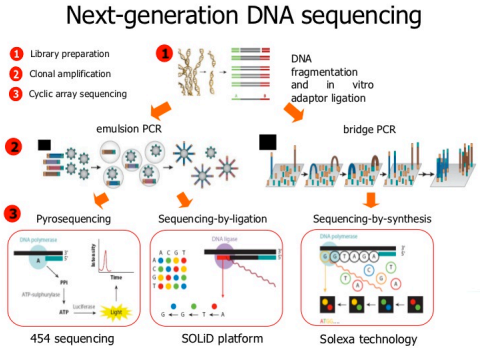


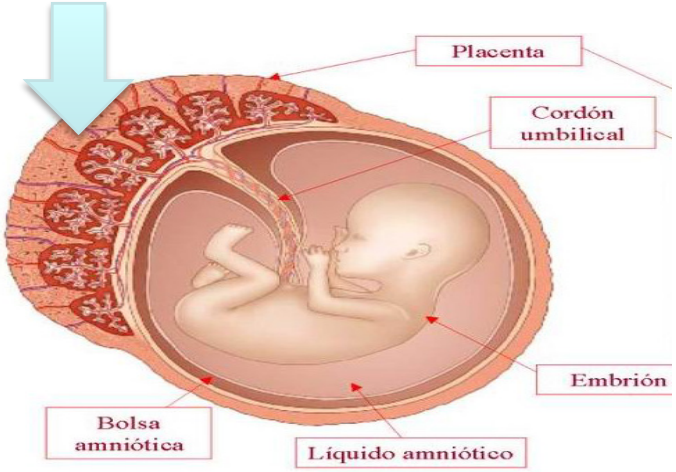
Test genetici non diagnostici feto-placentari del primo trimestre di gravidanza. DNA fetale libero nel plasma materno con prelievo di sangue materno.

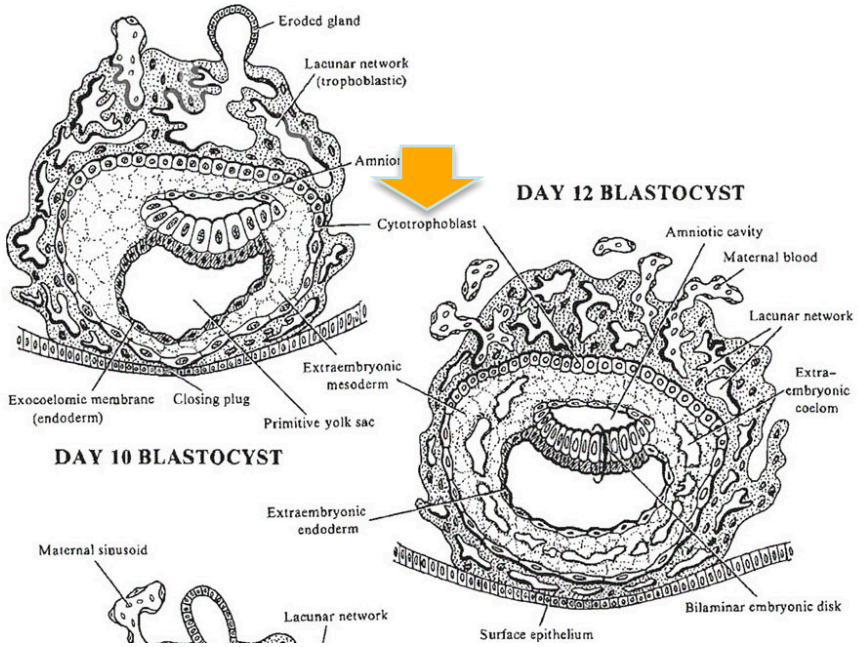










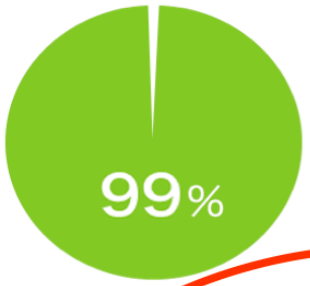


Perché non diagnostici?

1. La apoptosi delle cellule del trofoblasto nel plasma frammenta il DNA che necessita un rimodellamento con NGS.

2. La analisi è condotta su un mix di DNA materno e fetale con possibili effetti di confondimento

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

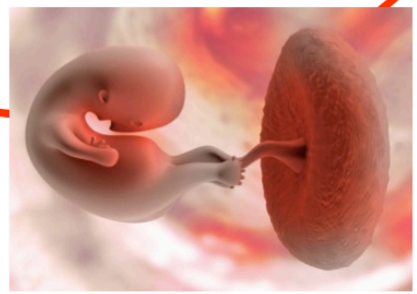


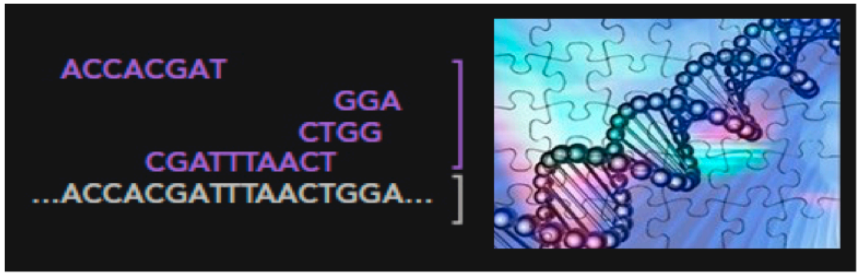
Why?

Fecondazione e errori Cromosomici:

Falsi positivi > anomalia che non c'è
Falsi negativi > tutto bene ma non è vero

Anomalie Cromosomiche	‰ prevalence EU	Liquido Amniotico	Placenta DNA Fetale plasma
Totale	4,4	accuracy	accuracy
T21 T18 T13	3,1	100 100 100	99.96 99.97 99.82





ccfDNA NIPTest. L'origine. Il DNA fetale presente nel plasma materno proviene dalla placenta

Nel sangue materno in gravidanza sono presenti cellule fetali nucleate e DNA libero non cellulare in sospensione nel plasma.
Il DNA libero non cellulare (**cffDNA**) proviene da cellule della placenta.

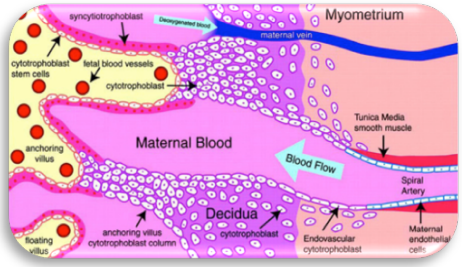
Il citotrofoblasto placentare si ancora alla decidua parietale.

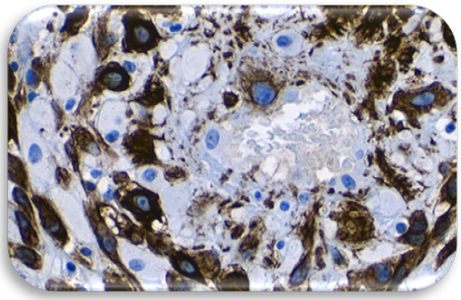
Le arterie spirali della decidua irrorano le lacune fra decidua e placenta.
Il citotrofoblasto invade e tappezza le pareti delle arterie spirali e ne causa il rimodellamento.

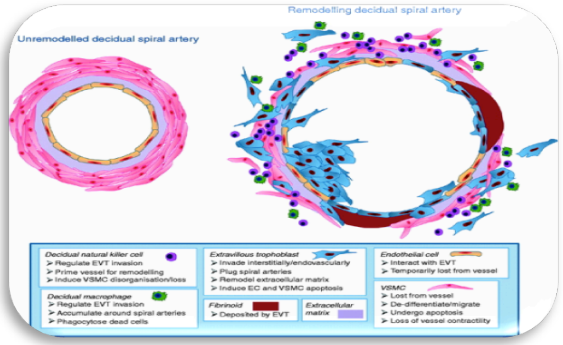
Il ricambio delle cellule del trofoblasto che tappezzano le pareti delle arterie spirali per morte cellulare o apoptosi (citochine - mediata) determina la frammentazione del DNA in degenerazione.

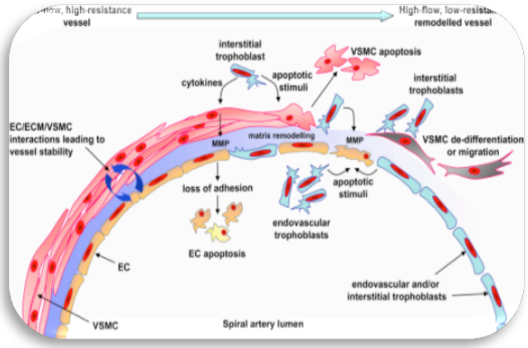
I frammenti di DNA hanno dimensioni di circa **180 bp** (paia di basi) e si sospendono nel plasma arterioso.
La presenza di DNA del trofoblasto (feto) libero nel plasma (**cffDNA**) si riscontra a partire dalla 5^ settimana di gravidanza, ma la quantità è sufficiente per i test a partire dalla 10^ settimana.

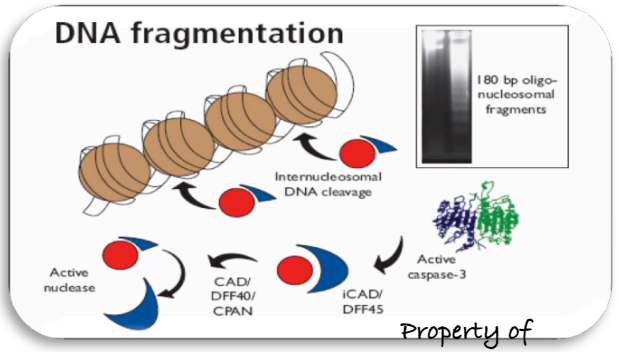
Anche la degenerazione degli epiteli materni libera frammenti di DNA sospesi nel plasma a generare un miscuglio di DNA di madre e placenta-feto.











La cattura del DNA fetale: sequenziare il genoma

MPSS, massive parallel shotgun sequencing (*Verinata Verifi, Sequenom MaterniT21, BGI Nifty*)
Analizza tutti i cromosomi.

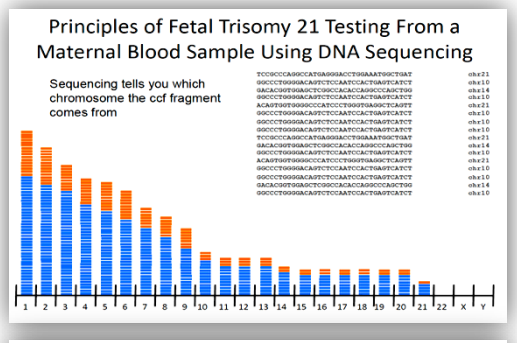
L'analisi massiccia di tutti i cromosomi evidenzia le differenze di **densità di basi GC** per cui è necessario introdurre un **Fattore di Normalizzazione (CNV)** che consente di rendere confrontabili i blocchi di lettura delle sequenze.

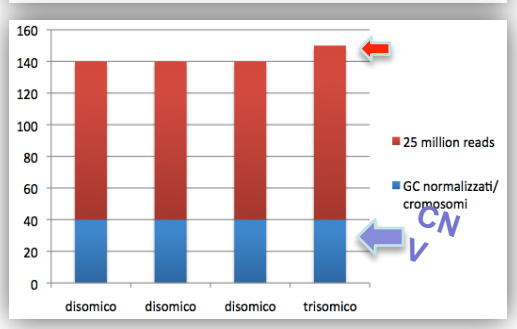
Tecniche di seconda generazione

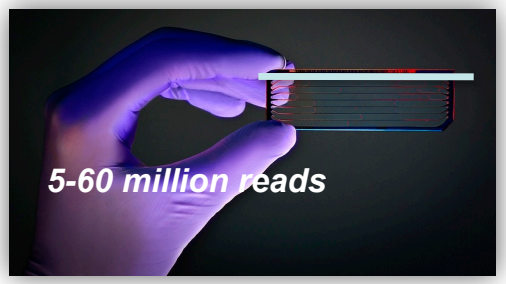
No PCR

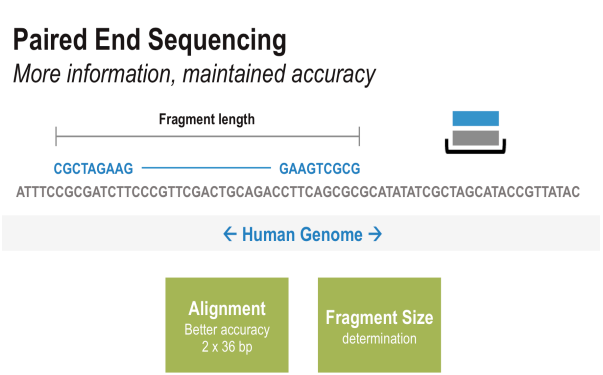
La tecnica di sequenziamento massivo consente l'analisi di tutto il genoma. Le nuove tecniche sequenziano i frammenti di DNA dai due lati con più informazioni e accuratezza. Le tecniche possono essere di diversa precisione a seconda della copertura del genoma con le letture.

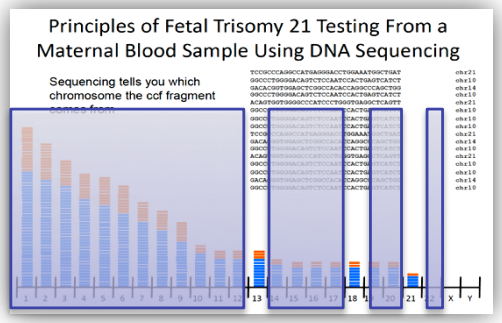
5 milioni di letture per le teniche standard, fino a 60 milioni di letture per il "whole genome".

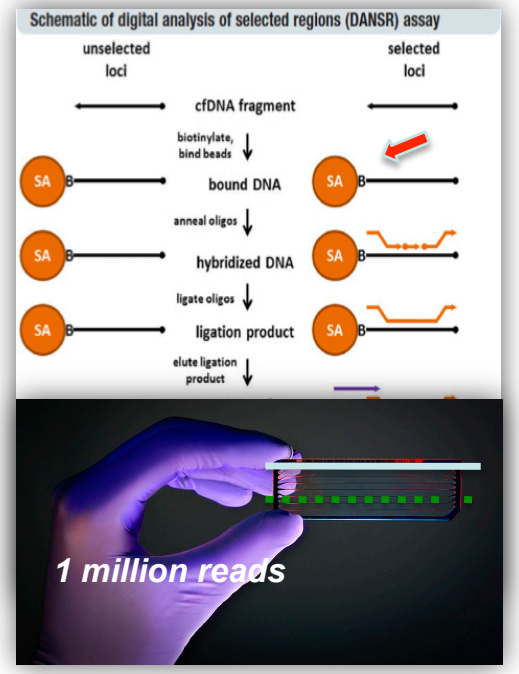


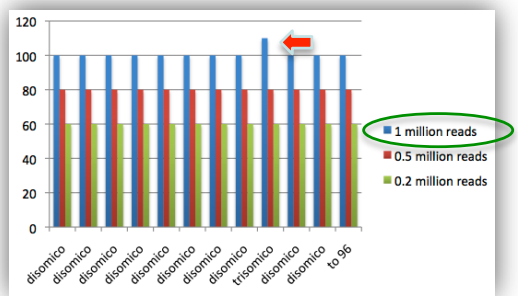












La cattura del DNA fetale: sequenziare solo i cromosomi 21, 18, 13

Per identificare una trisomia 21 (o 13 o 18) è possibile selezionare i frammenti di DNA dei cromosomi, eliminando il resto del genoma.

DANSR (*Ariosa Harmony*)

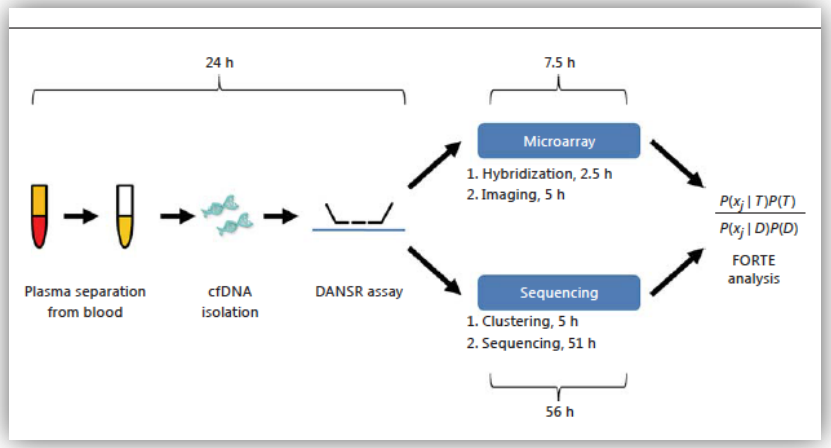
Il campione viene amplificato con PCR

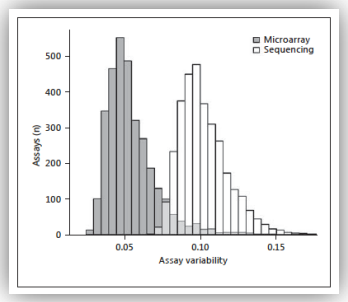
1. Sequencing: Esegue il sequenziamento (*high multiplexed*) selettivo dei frammenti di DNA solo dei cromosomi 21, 18, 13 *(clustering, sequencing).*
2. Microarrays: Esague la analisi su piattaforma array *(hybridization, imaging).* Riduce la variabilità fra campioni.

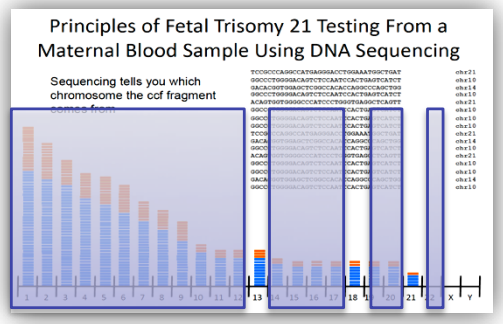
La selezione dei frammenti avviene ibridando sonde fluorescenti a:
576 marcatori STR non polimorfi dei cromosomi (21, 18, 13) per la ricerca delle trisomie
192 marcatori STR polimorfi di cromosomi fra 1 e 12 per definire la frazione fetale di ciascun campione.
Solo i frammenti agganciati alle sonde fluorescenti verranno sequenziati per il dosaggio e la elaborazione.
La tecnica esegue circa 1 milione di letture.

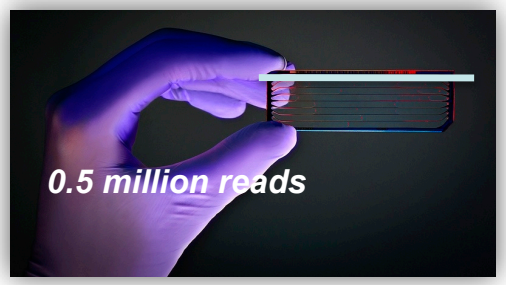
Tecniche di seconda generazione

Microarrays (*Ariosa Harmony*)









La cattura del DNA fetale: intercettare solo i cromosomi 21, 18, 13

Tecnica enzimatica e chemiluminescente

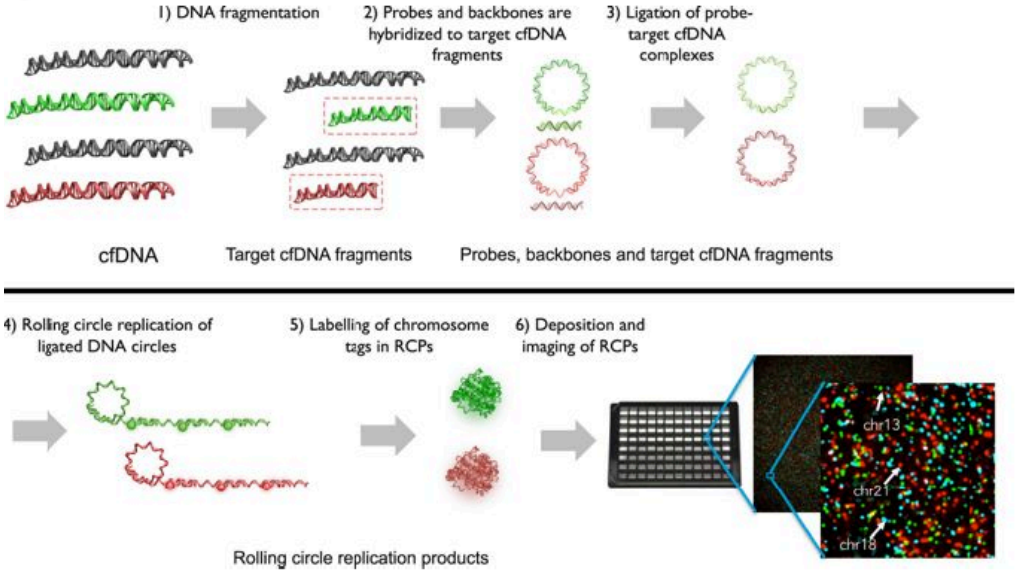
Per identificare una trisomia 21 (o 13 o 18) è possibile selezionare i frammenti di DNA dei cromosomi, eliminando il resto del genoma.

La selezione dei frammenti avviene senza sequenziamento
No PCR iniziale
Digerendo i frammenti con enzimi di restrizione, ibridando sonde fluorescenti a:
3500 loci non polimorfi dei cromosomi 21 per la ricerca delle trisomie.
Solo i frammenti agganciati alle sonde fluorescenti verranno amplificati con PCR e utilizzati
La tecnica esegue circa 0,5 milione di letture.

A sequencing and PCR-free method was developed to enable a cost efficient and high precision measurement of chromosomal aneuploidies. Thereby, we were able to eliminate expensive sequencing, complicated sample preparation protocols, PCR bias and bioinformatics. The maximum precision of digitally-enabled assays is dictated by the number of molecules analysed. To enable quantification based on high molecular count numbers without PCR, two strategies were used. Firstly, probes were designed to capture and generate labelled rolling circle replication products (RCPs) from ~3500 loci on chromosome 21, thereby increasing the number of counts per chromosome equivalent in the sample. Secondly, an optically transparent nanofilter 96-well plate was developed to capture RCPs with high yield by mechanical filtering prior to imaging, thereby increasing the number of molecules analysed from the sample.

The Vanadis NIPT assay (Fig. 1) is based on four consecutive enzymatic steps that specifically generate labelled RCPs from chromosomal DNA targets. The specificity of the DNA labelling approach eliminates the need for DNA sequencing and advanced bioinformatics data analysis. First, target chromosomes are digested into defined target cfDNA fragments using a restriction enzyme. Secondly, the digested target cfDNA fragments are mixed with a probe set where each probe carries a complementary sequence motif to the target cfDNA fragments of interest. The mixture also contains backbone oligonucleotides carrying a chromosome-specific sequence motif ("chromosomal tag") used for subsequent labelling and identification. The probes are designed to specifically guide hybridization of target cfDNA fragments, thereby allowing subsequent DNA ligation of the target cfDNA fragments to the backbone oligo, such that a single stranded DNA circle can be formed. For this to occur, the selected cfDNA fragments need to hybridize perfectly to the probe and ligate both the 3´and 5´ends to the backbone. The chromosomal tags within the backbones

Figure 1



La cattura del DNA fetale: aziende e metodiche

PIATTAFORMA ILLUMINA, (Thermofisher). Sequenziamento del genoma, Analisi del genoma, target > 7-10 Mb
PIATTAFORMA AFFYMETRIX. Ibridazione Mycroarrays. Analisi Trisomie.
System VANADIS. Mycroplate Chemiluminescenza. Analisi Trisomie.

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Table 3: Features of the intervention

Company	Brand name	Platform provider/ technology	Mechanism of action	Chromosomal anomalies detected	Sample and reporting time (days)	Indication for use/ target population
Illumina Inc. (San Diego, CA, USA)	Verifi™ prenatal test (https://www.illumina.com/)	Illumina Verifi™	NGS (WGS)	T21, T18, T13 and sex chromosome aneuploidies in singleton pregnancies T21, T18, T13 and presence of Y for women with twins through natural or reproductive methods Additional indications: T9, T16 and microdeletions (Di George, Prader-Willi/ Angelman, Cri-du-Chat, Wolf-Hirschhorn and 1p36 deletion)	3–5 days	All pregnant women ≥ 10 weeks gestation who have chosen to have T13, T18 and T13 prenatal screening Not intended to be used in isolation from other clinical findings and tests results Single, twin or egg donor pregnancies
Illumina Inc. (San Diego, CA, USA)	VeriSeq NIPT Solution Includes: the VeriSeq NIPT Workflow Manager for the VeriSeq NIPT Microlab STAR, the VeriSeq NIPT Sample Prep Kits, and the VeriSeq Onsite Server with the VeriSeq NIPT Assay Software.	Illumina VeriSeq NIPT Solution	NGS paired end (WGS)	T21, T18, T13 and sex chromosome aneuploidies	1 day (26 hours)	Intended for use in pregnant women of at least 10 weeks gestation The product must not be used as the sole basis for diagnosis or other pregnancy management decisions
Genesis Genetics (London, UK)	Genesis Serenity prenatal test (http://genesisgenetics.co.uk/genesis-serenity/)	Illumina Verifi™ and VeriSeq NIPT Solution	NGS paired end (WGS)	T21, T18, T13 and sex chromosome aneuploidies	—	Single or twin pregnancies

La cattura del DNA fetale: aziende e metodiche

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Company	Brand name	Platform provider/ technology	Mechanism of action	Chromosomal anomalies detected	Sample and reporting time (days)	Indication for use/ target population
Labco Quality Diagnostics/ Synlab International GmBH (München, Germany)	NeoBona® NeoBona® Advanced NeoBona® Advanced+ (http://www.neobona.es/)	Illumina VeriSeq NIPT Solution	NGS paired end (WGS)	**NeoBona:** T21, T18, T13 **NeoBona Advanced:** T21, T18, T13 and sex chromosome aneuploidies (singleton pregnancy only) **NeoBona Advanced+:** T21, T18, T13, T16, T9, sex chromosome aneuploidies and microdeletions (singleton pregnancy only) **Prenatal Test Extended Panel:** T21, T18, T13, sex chromosome aneuplodies and microdeletions (DiGeorge, Angelman/Prader - Willi, 1p36 deletion, Wolf -Hirschhorn y Cri-du-chat) (singleton pregnancy only) **Prenatal Test Extended Panel + All** chromosomes: T21, T18, T13, sex chromosome aneuplodies, microdeletions (DiGeorge, Angelman/Prader -Willi, 1p36 deletion, Wolf -Hirschhorn y Cri-du-chat) and all chromosome aneuploidies. (singleton pregnancy only)	10 days	Can be used in pregnancies ≥ 10 weeks of gestation Single, twin, IVF and egg donor pregnancies
Sequenom Laboratories (San Diego, CA, USA)	VisibiliT™ MaterniT 21® PLUS test (previously MaterniT 21) MaterniT® GENOME (https://www.sequenom.com/)	Illumina	NGS (WGS)	**VisibiliT™:** T21 and T18 **MaterniT® 21 PLUS:**T21, T18, T13, sex chromosome aneuploidies and 7 microdeletions (T21, T18, T13, sex chromosome aneuplodies, T16, T22 and microdeletions (Di George, Prader-Willi/ Angelman, Cri-du-Chat, Wolf-Hirschhorn, Jacobsen, Langer-Giedion and 1p36 deletion) **MaterniT® GENOME:** All chromosomes and deletions or duplications of chromosome material 7 Mb or larger, as well as seven clinically micro-deletion regions less than 7 Mb in size (Di George, Prader-Willi, Cri-du-Chat, Wolf-Hirschhorn, Jacobsen, Langer-Giedion and 1p36 deletion)	5 days	Can be utilized in pregnant women ≥ 10 weeks gestation MaterniT21 Plus is relevant for pregnancies at increased risk of fetal anomalies

La cattura del DNA fetale: aziende e metodiche

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Company	Brand name	Platform provider/ technology	Mechanism of action	Chromosomal anomalies detected	Sample and reporting time (days)	Indication for use/ target population
Natera® (San Carlos, CA, USA)	Panorama® (http://www.panoramatest.com/)	Illumina	NGS (SNP)	T21, T18, T13, sex chromosome aneuploidies and most common microdeletions, including 22q11.2 deletion syndrome, 1p36 deletion syndrome, cri du chat, Prader-Willi and Angelman	9 days	Panorama could be useful for the general pregnant population ≥ 9 weeks gestation Single pregnancies
Premaitha Health PLC (London, UK)	Iona® test (http://www.premaitha.com/the-iona-test)	Thermo Fisher Scientific	NGS (WGS)	T21, T18 and T13	3–5 days	Suitable for all pregnant women ≥ 10 weeks of gestation Intended to be used by a clinician in combination with other risk factors to estimate the risk of affected pregnancies Single, twin, surrogate or in-vitro fertilization pregnancies
Ariosa Diagnostics Inc./Roche Sequencing Solutions Inc. (San Jose, California, USA)	Harmony® prenatal test	Affymetrix® F. Hoffman-La Roche, Ltd)	Chromosomal micro-arrays	T21, T18, T13, Monosomy X*, sex chromosome aneuploidies* and 22q11.2 deletion syndrome *singleton pregnancy only	≤7 days	Intended for use in pregnant women ≥ 18 years of age, of ≥ 10 weeks´ gestation, and with ≤ 2 foetuses
LifeCodexx AG (Germany)	PrenaTest® (https://lifecodexx.com/)	Illumina	NGS (WGS)	T21, T18, T13, sex chromosome aneuploidies and 22q11.2 deletion syndrome	A few days	Available for pregnant women ≥ 9 weeks Primary diagnostic procedures in combination with ultrasound in pregnant women who are at high risk of fetal aneuploidies (≥ 35 years old, increased risk based on screening methods, ultrasound anomalies, prior pregnancies with aneuploidy, family risk, other medical reasons) Single or twin pregnancies

La cattura del DNA fetale: aziende e metodiche

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Company	Brand name	Platform provider/ technology	Mechanism of action	Chromosomal anomalies detected	Sample and reporting time (days)	Indication for use/ target population
Berry Genomics Co. Ltd. (Beijing, China)	BambniTest	Illumina	NGS (WGS)	—	—	—
BGI Diagnostics Technology Co. Ltd. (Shenzhen, China)	NIFTY™ test (http://www.niftytest.com/)	Illumina and Thermo Fisher Scientific	NGS (WGS)	T21, T18, T13, sex chromosome aneuploidies and most common microdeletions, including 2q33.1, 1p36, cri du chat, Prader-Willi, Angelman, Jacobsen, DiGeorge and van der Wonde	10 days	Available for any pregnant women ≥ 10 weeks gestation but particularly suitable for ≥ 35 years, fetal ultrasonographic findings indicative of increased risk of aneuploidy, reassurance following screening results, contraindication for invasive testing, prior pregnancies with trisomy, received In-Vitro Fertilization (IVF) treatment or suffered habitual abortion Single, twin, egg-donor and IVF pregnancies
Igenomix SL (Valencia, Spain)	NACE® test NACE® amplified test (http://nace.igenomix.es/)	Illumina	NGS (WGS)	**NACE®**:T21, T18, T13 and sex chromosome aneuploidies **NACE® amplified:** additionally T9 and T6 and six common microdeletions: 1p36, cri du chat, Prader-Willi, Angelman, Wolf-Hirschhorn and DiGeorge (singleton pregnancy only)	NACE®: 3 days NACE® amplified: 15 days	Available for pregnant women ≥ 10 weeks of gestation Specially indicated for women with abnormal 1st trimester test results, previous T21 pregnancies or suspicious ultrasonographic findings Single, twin, egg-donor and IVF pregnancies Any age, with independence of BMI
NIM Genetics (Madrid, Spain)	TrisoNIM® Advance TrisoNIM® Premium (https://www.nimgenetics.com/trisonim/)	Illumina and Thermo Fisher Scientific (NIFTY™ technology)	NGS (WGS)	**Trisomy Advance:** T21, T18, T13, sex chromosome aneuploidies and 3 microdeletions (1p36, 2q 33.1 and cri du chat) **TrisoNIM Premium:** T21, T18, T13, sex chromosome aneuploidies, T9, T16, T22 and 7 microdeletions (1p36, 1q32-q41 (van der Woude), 2q33.1, 5p (cri du chat), 10p14-p13 (DiGeorge 2), 11q (Jacobsen) and 16p12.2-p11.2	5–7 days	Can be used in pregnancies ≤ 10 weeks Single, twin, egg-donor pregnancies and IVF pregnancies

La cattura del DNA fetale: aziende e metodiche

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Company	Brand name	Platform provider/ technology	Mechanism of action	Chromosomal anomalies detected	Sample and reporting time (days)	Indication for use/ target population
Imegen Instituto de Medicina Genómica (Valencia, Spain)	Genatal 1 Genatal + Genatal 2 (Genatal Twin (https://www.imegen.es/test-prenatal-no-invasivo/)	Illumina Panorama® technology	NGS (SNP)	**Genatal 1:** T21, T18, T13 and sex chromosome aneuploidies **Genatal +:** T21, T18, T13, triploidy, sex chromosome aneuploidies and 5 microdeletions **Genatal 2** (exclusive for twin or multiple pregnancies: T21, T18 and T13)	7 days	Can be used in pregnancies ≥ 9 weeks of gestation Single, twin, egg donor pregnancies and multiple pregnancies (depending on the tests)
Genoma Laboratories (Rome and Milan, Italy)	PrenatalSafe® PrenatalSafe® Kario PrenatalSafe® Kario Plus PrenatalSafe® 5 PrenatalSafe® 3 PrenatalSafe® Plus (http://www.prenatalsafe.it/)	Illumina	NGS (WGS)	**PrenatalSafe® 3:**T21, T18 and T13 **PrenatalSafe® 5:** T21, T18, T13 and sex chromosome aneuploidies **PrenatalSafe® Plus:** T21, T18, T13, T9, T16 and 6 microdeletions (1p36, cri du chat, Prader-Willi, Angelman, Wolf-Hirschhorn and DiGeorge) **PrenatalSafe® Kario:** All chromosomes of fetal karyotype **PrenatalSafe® Kario Plus:** All chromosomes of fetal karyotype and 9 microdeletions (1p36, cri du chat, Prader-Willi, Angelman, Wolf-Hirschhorn, DiGeorge, Jacobsen, Langer-Giedion and Smith-Magenis)	3 days	Can be performed in all pregnant women ≥ 10 weeks of gestation Single, twin, egg-donor pregnancies and IVF pregnancies
Sorgente Genetica S.r.l. (Milan, Italy)	Aurora (http://www.testprenataleaurora.it/)	Illumina Verifi™	NGS (WGS)	T21, T18, T13, sex chromosome aneuploidies, T9, T16 and 5 microdeletions (1p36, cri du chat, Prader-Willi/Angelman, Wolf-Hirschhorn and DiGeorge)	10 days	Can be performed in all pregnant women but particularly recommendable for maternal age > 35 years, positive screening test for the first/second quarter, suggestive fetal US findings, contraindication for invasive testing, personal/family history of chromosomal anomalies Single, twin, egg-donor pregnancies and IVF pregnancies
Ebios Futura S.r.l. (Cuneo, Italy)	Prenataltest®	Illumina	NGS (WGS)	T21, T18, T13 and sex chromosome aneuploidies	<10 days	Can be performed in pregnant women ≥ 10 weeks gestation Single or twin pregnancies

La cattura del DNA fetale: aziende e metodiche

Screening of fetal trisomies 21, 18 and 13 by noninvasive prenatal testing

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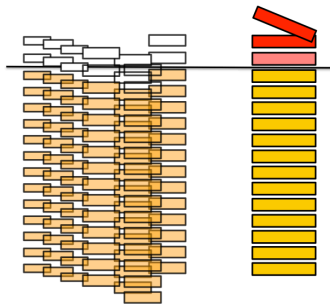
Company	Brand name	Platform provider/ technology	Mechanism of action	Chromosomal anomalies detected	Sample and reporting time (days)	Indication for use/ target population
Multiplicom NV, Belgium (Agilent Technologies, CA, USA)	Clarigo™ (http://www.multiplicom.com/)	Illumina	NGS (targeted technology)	T21, T18 and T13	6–10 days	Can be used in pregnant women ≥ 8 weeks gestation Single pregnancies
Genesupport, FASTERIS, Swiss Institute for Bioinformatics (Switzerland)	Prendia START Prendia EXTEND (http://www.prendia.ch/fr/)	—	NGS (WGS)	**Prendia START:** T21, T18 and T13 **Prendia EXTEND:** sex chromosome aneuploidies, rare chromosomal anomalies (6, 7, 14, 15, 16) and structured chromosomal anomalies of other autosomes	7–14 days	Can be performed in pregnant women ≥ 10 weeks gestation Single, twin, egg-donor pregnancies and IVF pregnancies
LabCorp Inc. (North Carolina, USA)	InformaSeq℠ (https://www.integratedgenetics.com/)	Illumina	NGS (WGS)	T21, T18, T13 and sex chromosome aneuploidies (optional)	5–7 days	Can be performed in pregnant women ≥ 10 weeks gestation Single and twin pregnancies
Vanadis Diagnostics, Perker Elmer Inc. (Sweden)	Vanadis™ NIPT system http://www.vanadisdx.com/	—	Microplate-based technology	T21, T18 and T13	2–3 days	Vanadis™ NIPT is under development. The system does not conform to 98/79 EC In Vitro Diagnostic Medical device directive and cannot beplaced on the market or put into service in EU until they have been made to comply.
NIPD Genetics (Nicosia, Cyprus)	VERACITY™ test (https://www.nipd.com/)	Illumina	NGS (targeted enrichment technology)	T21, T18 and T13 and sex chromosome aneuploidies	A few days	Can be performed in pregnant women ≥ 10 weeks gestation Single and twin pregnancies

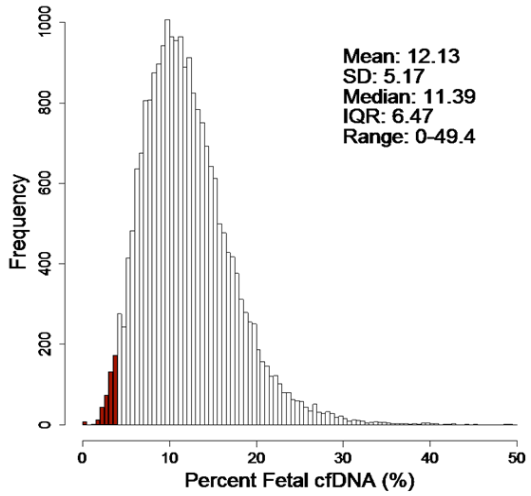
Abbreviations: IVF=in vitro fertilisation; NGS=next-generation sequencing; SNP=single nucleotide polymorphism; T6=trisomy 6; T9=trisomy 9; T13=trisomy 13; T16=trisomy 16; T18=trisomy 18; T21=trisomy 21; US=ultrasound; WGS=whole genome sequencing.

* Illumina WGS technology before 2014.



La quantità del DNA fetale: il test deve indicare la frazione fetale del campione

La frazione fetale di ciascun cromosoma per poter identificare una trisomia deve consentire un *expected ratio* >1. Harmony test si basa su polimorfismi SNP e indica Il 4%. Altri metodi metodi si basano su dimensione dei frammenti di DNA,CNV, cromosoma Y, e indicano come limite operativo il 2%.



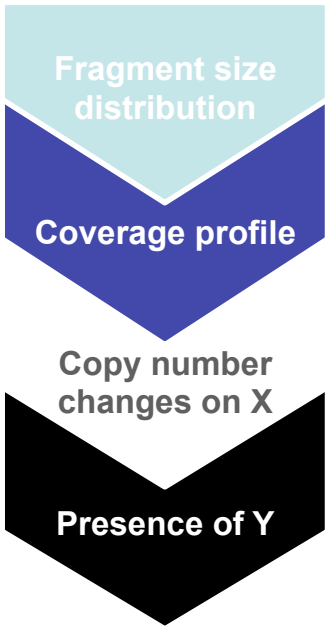
Fetal Fraction	Expected ratio for Trisomy
2%	1.01
4%	1.02
10%	1.05
20%	1.10

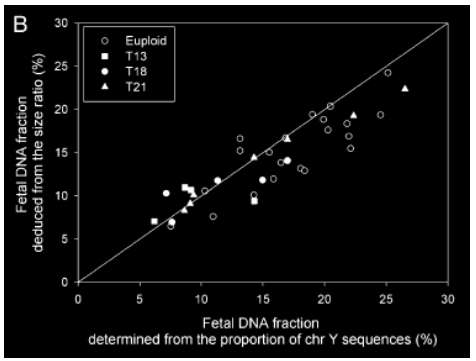
SNP
(DANSR)

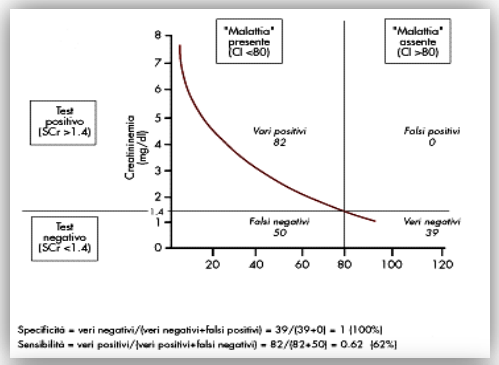
ABRSJA5517	**Maternal (buffy coat)**	A/C	G/G	C/T	A/T	A/A	A/G	C/T	C/C	A/C	A/G
	Fetal (cfDNA)					**A/G**			C/C		

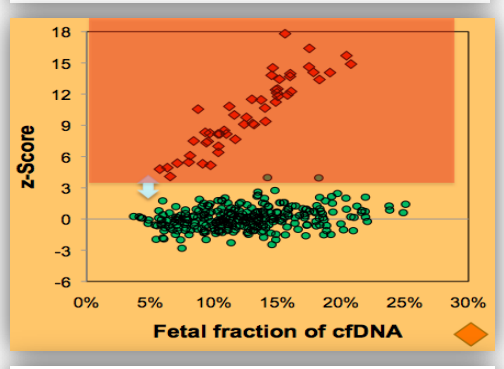
Fragment size, #X CNV & #Y (Tscore e altri)

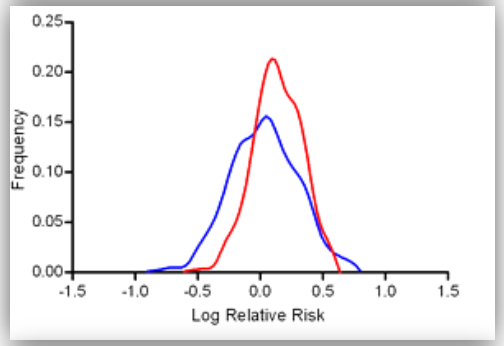
Normal
Pregnancies
.4% fail

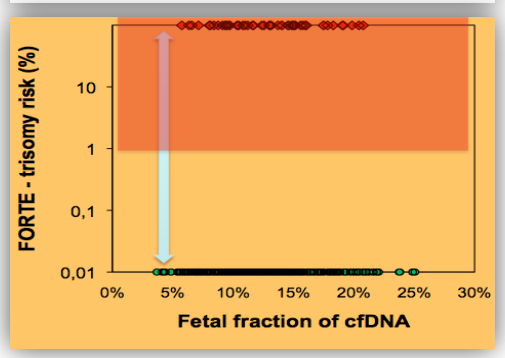












Analisi dei dati

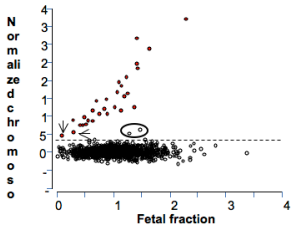
Il test calcola il rapporto di verosimiglianza fra le probabilità che i campioni contenuti in una linea di sequenza siano euploidi

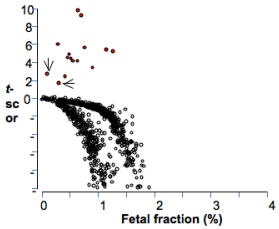
MPSS (*Verinata Verifi* **SAFER**, *Sequenom MaterniT21, BGI Nifty*) – *2012*
Algoritmo per definire il valore soglia di rischio trisomia basato su: **One sample set**
Il calcolo su singolo campione è reso necessario dal ridotto numero di test per linea di sequenza.
1) ipotesi binaria positivo-negativo con t-Student (z-score) e Likelyhood Odds Ratio (rapporto di verosimiglianza).
2) fattore di normalizzazione di sequenza CNV.
3) Variazioni di corsa fra le varie linee di sequenziamento corrette con un algoritmo z-score.
4) Definizione di un valore soglia per la trisomia (valore di z-score fra 3 e 4)
L'algoritmo non considera la frazione fetale. Quindi con **frazione fetale bassa i valori di score +/- sono molto vicini,** aumentando la possibilità di falso positivo e negativo.

DANSR – **FORTE** (*Ariosa Harmony*) – *2012*
Algoritmo per definire il valore soglia di rischio trisomia basato su: **Multiple sample set**
Il calcolo su campioni multipli è reso possibile dalle piccole dimensioni del blocchi di lettura di sequanza (1 milione di reads) che consente l'analisi in una linea di sequenza di 96 campioni che vengono paragonati fra lori.
1) Ipotesi percentuale con Odds ratio (rapporto di verosimiglianza) fra modelli disomico/trisomico, (curve normali di distribuzione)
2) Calcolo della frazione fetale
3) Montecarlo Simulation che inserisce anche età materna e epoca gestazionale nel calcolo dell'algoritmo FORTE.
L'algoritmo considera la frazione fetale. Quindi i valori dello score con **frazione fetale bassa sono normalizzati** consentendo una valutazione del rischio indipendente dalla quantità di DNA fetale.

T-SCORE *(Labco Neobona/Illumina) – 2015*
Algoritmo composto *z-score like*
1) Calcolo frazione fetale
2) Distribuzione frammenti per dimensione
3) Confronto batch-campioni / profondità di sequenza

LIFE CIRCLE *(Perkin Elmer)-2018*





Identificazione delle Trisomie Fetali con cffDNA: la validazione

Company	Sequenom	Verinata	Ariosa	Natera	BGI
NASDAQ	SQNM	ILMN			
Prodotto	MaterniT21plus	Verifi	Harmony	Panorama	Nifty
sequenziamento	MPSS	MPSS	TPS	TPS-SNPbased	MPSS
tecnologia	SEQureDx		DANSR	NATUS	
algoritmo		SAFeR	FORTE		
statistic	z-score binary	z-score binary	OR multiple	z-score binary	z-score GC correct
weeks	10	10	10	10	12
condizioni	13,18,21,gender,XX	13,18,21,gender,XX	13,18,21,gender,XX	13,18,21,gender,XX	13,18,21,gender,XX
accreditamento	CAP CLIA	CAP CLIA	CAP CLIA	CAP CLIA	
statements	ACMG ACOG ISPD	ACMG ACOG ISPD	ACMG ACOG ISPD	ACMG ACOG ISPD	
clinic-patients	2500	650	6000	300	3500/11100
failure			2%	5%	<1%
specificity 21	99,4-99,9	99,1-100	99,8-99,9	98,2-100	99,60-99,97
sensibility 21	95,9-99,7	95,9-100	95,5-100	86,3-100	100-100

Table 1 Results from four published clinical trials that measured NIPT's sensitivity and specificity in detecting common aneuploidies

	Trisomy 21		Trisomy 18		Trisomy 13	
	Sensitivity (95 % CI)	Specificity (95 % CI)	Sensitivity (95 % CI)	Specificity (95 % CI)	Sensitivity (95 % CI)	Specificity (95 % CI)
Palomaki et al. 2011	98.6 % (95.9 - 99.7)	99.8 % (99.4 - 99.9)	———	———	———	———
Palomaki et al. 2012	———	———	100 % (93.9 -100)	99.7 % (99.3 - 99.9)	91.7 % (61-99)	99.1 % (98.5 - 99.5)
Bianchi et al. 2012	100 % (95.9 – 100)	100 % (99.1 – 100)	97.2 % (85.5 – 99.9)	100 % (99.2 – 100)	78.6 % (49.2 – 99.9)	100 % (99.2 – 100)
Norton et al. 2012	100 % (95.5-100)	99.97 % (99.8 -99.99)	97.4 % (86.5-99.9)	99.93 % (99.75 - 99.98)	———	———
Ashoor et al. 2012	———	———	———	———	80 % (49-94.3)	99.95 % (99.71-99.99)

2012. Gli agreements internazionali sono configurati sui tests delle companies indicate e sulla produzione scientifica collegata ai trials clinici che hanno dimostrato i dati di sensibilità e specificità dei NIPT.

2015. La ditta Illumina, produttrice di sequenziatori NGS, e proprietaria di Verinata, si accorda con Sequenom per un "sharing revenues". Roche acquista Ariosa e Illumina lancia il test NGS di seconda generazione (Paired end sequencing) con LabcoEU (NeoBona)

2015. Su questi dati vengono stilate le linee guida di validazione dei test.

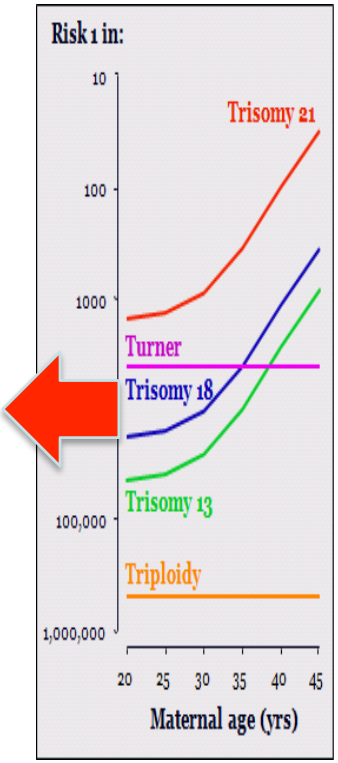

Ministero della Salute
Consiglio Superiore di Sanità
Sezione I

Linee-Guida

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

Identificazione delle Trisomie Fetali con cffDNA: European population prevalence

Casi di Anomalie Cromosomiche		Popolazione EU ‰	% Anomalie Cromosomiche
Totale	10323	4,4	100
Trisomie T21 T18 T13	7335	3,1	70 (48<77)
X0,XXX,XXY,XYY	1251	0,6	13



European Journal of Human Genetics (2012) 20, 521–526

www.nature.com/ejhg

ARTICLE

Rare chromosome abnormalities, prevalence and prenatal diagnosis rates from population-based congenital anomaly registers in Europe

Identificazione delle Trisomie Fetali con cffDNA: Casistica pubblicata e performances

		Trisomy 21	Trisomy 18	Trisomy 13

	Falsi +	Veri +	Falsi -	Prev-coorte /10000	Prev-popolaz /10000	Casi coorte
T21	**19**	**827**	**4**	120	15	67859
T18	**11**	**220**	**5**	30	5	67103
T13	**12**	**80**	**2**	13	0,6	58947

HARMONY study (Stokowski)	***False positive rate/specificity***	***0.04%***	***0.02%***	***0.02%***
23000 cases 2015	***False negative*** *False negative Population rate*	***0.7%*** *0.03%*	***2.6%*** *0.02%*	***6.2%*** *0.01*
NEOBONA /Illumina	***False positive rate/specificity***	***0.03%***	***0***	***0.02%***
6000 cases 2015/16	***False negative/detection rt.***	***0***	***0***	***0***
PRENATALSAFE Genoma	***False positive rate/specificity***	***0.02%***	***0.02%***	***0.02%***
31800 cases 2016	***False negative/detection rt*** *False negative population rate*	***0.39%*** *0.03%*	***2.08%*** *0.03%*	***0***

Identificazione delle Trisomie Fetali con cffDNA: Casistica pubblicata e performances: confronto col citotrofoblasto da villi coriali. I metodi non differiscono in specificità e sensibilità cliniche, determinate dalla biologia della placenta e del suo trofoblasto.

Casi di Anomalie Cromosomiche	‰ prevalence EU	% Anomalie Cromosomiche	CVS Cytotrophoblast (direct method)	CVS Cytotrophoblast (direct method)	cffDNA	cffDNA
Totale	4,4		Specificità%	Sensibilità%	Specificità%	Sensibilità%
T21 T18 T13	3,1	70 (48<77)	99,9 99,9 99,8	99,5 98,4 98,4	99,9 99,9 99,7	99,5 98,4 98,4
X/Y Trisomies	0,2	5	99,9	99,0	99,9	100
45,X	0,33	8	99,7	99,1	99,8	100

Totale	4,4		PPV%	NPV%	PPV%	NPV%
T21 T18 T13	3,1	70 (48<77)	96 92 62	99,98 99,99 99,99	97,7-92,2 88,7-76,6 82,0-32,8	99,99 99,99 100
X/Y Trisomies	0,2	5	85	99,00	73,40	100
45,X	0,33	8	43	99,10	61,60	100

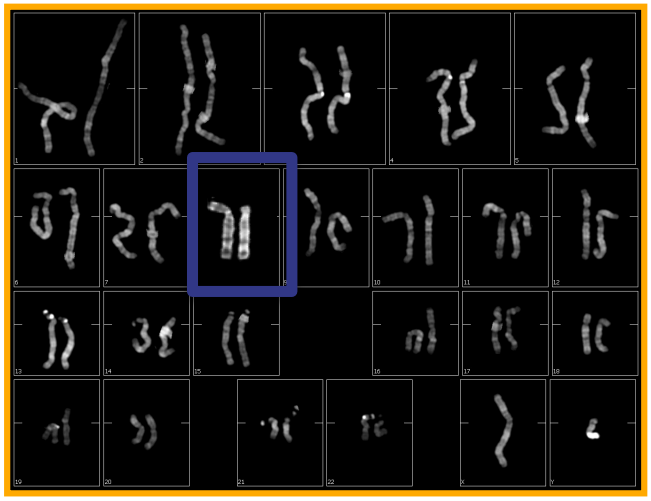
La **specificità** di un test è: N° FP/N° TN (falsi positivi/veri negativi)

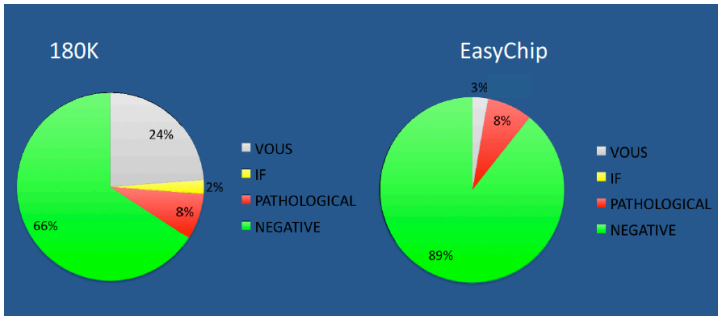
La **sensibilità** (detection) di un test è: N° FN/N° TP (falsi negativi/veri positivi)

Identificazione delle Anomalie Cromosomiche Fetali con cffDNA: European population prevalence. Analisi del genoma:

Il cariotipo convenzionale identifica anomalie di struttura più grandi di 10-15 Mb.
Una analisi molecolare aCGH o NGS calibrata a >10Mb identifica le anomalie genomiche di struttura limitando al minimo i findings di dubbio significato.
Una analisi genomica molecolare a >10Mb è in grado di identificare anomalie cromosomiche (CNV, delezioni, duplicazioni) pari al cariotipo convenzionale

Casi di Anomalie Cromosomiche	Popolazione EU	‰ prevalence	% Anomalie Cromosomiche
Totale	10323	4,4	
Anomalie cromosomiche strutt.	1737	0,7	17 (40<10)
Anomalie > 10Mb		tutto genoma	10





Identificazione delle Anomalie Cromosomiche Fetali con cffDNA: European population prevalence. Analisi del genoma: Illumina valida e commercializza la metodologia Veriseq2, 2019

NIPT DNA WHOLE GENOME ACCURACY

2017 WHOLE GENOME APPROACH 12000 CASES

PrenatalSafe®

Prenatal Diagnosis 2017

The clinical utility of genomewide cfDNA screening

Table 3 Performance of the genome-wide cfDNA screening approach

	Trisomy 21 (*n* = 12 114)	Trisomy 18 (*n* = 12 114)	Trisomy 13 (*n* = 12 114)	Sex chromosome aneuploidies (*n* = 12 114)	Rare aneuploidies (*n* = 12 114)	Segmental imbalances (*n* = 12 114)
False positive—no.	1	1	1	12	7	5
False negative—no.	0	0	0	0	0	0
True positive—no.	88	15	12	36	10	8
True negative—no.	12.025	12.098	12.101	12.066	12.097	12.101
Sensitivity (95% CI)—%	100.00% (95.89%–100.00%)	100.00% (78.20%–100.00%)	100.00% (73.54%–100.00%)	100.00% (90.26%–100.00%)	100.00% (69.15%–100.00%)	100.00% (63.06%–100.00%)
Specificity (95% CI)—%	99.99% (99.95%–100.00%)	99.99% (99.95%–100.00%)	99.99% (99.95%–100.00%)	99.90% (99.83%–99.95%)	99.94% (99.88%–99.98%)	99.96% (99.90%–99.99%)
Positive predictive value (95% CI)—%	98.88% (92.54% to 99.84%)	93.75% (67.88%–99.07%)	92.31% (62.83%–98.84%)	75.00% (63.02%–84.08%)	58.82% (40.52%–74.97%)	61.54% (39.98%–79.35%)
Negative predictive value (95% CI)—%	100.00% (99.95%–100.00%)	100.00% (99.95%–100.00%)	100.00% (99.95%–100.00%)	100,00% (99.95%–100.00%)	100.00% (99.95%–100.00%)	100.00% (99.95%–100.00%)

2019 WHOLE GENOME APPROACH 20000 CASES

Noninvasive prenatal testing for fetal subchromosomal copy number variations and chromosomal aneuploidy by low-pass whole-genome sequencing

Dongyi Yu, Kai Zhang, [...], and Yang Du

TABLE 4 Evaluation of the NIPSCCD method in detecting CNVs

CNV size	TP	FP	FN	Sensitivity (%)	PPV (%)	FNR (%)
>10 Mb	11	2	1	91.67	84.62	9.33
5 Mb–10 Mb	5	2	0	100.00	71.43	NA
<5 Mb	13	3	6	68.42	81.25	31.58
Total CNVs	29	7	7	80.56	80.56	19.44

Note. TP, true positive NIPSCCD-detected CNVs that were confirmed by amniocytes testing; FP, inconsistent CNVs that were detected by NIPSCCD while not detected by amniocytes testing were classified as false positive; FN, amniocytes testing-characterized CNVs that were not detected by the NIPSCCD method; PPV, positive predictive value; FNR, false negative rate; NA, not applicable.

PPV	Sen
61	100
71	100
83	74

2019 WHOLE GENOME VALIDATION 2000 CASES

illumina®

	Trisomy 21	Trisomy 18	Trisomy 13	RAA[d]	CNV ≥ 7 Mb	Any anomaly[e]
Sensitivity[b]	> 99.9% (130/130)	> 99.9% (41/41)	> 99.9% (26/26)	96.4% (27/28)	74.1% (20/27)	95.5% (318/333)
2-sided 95% CI[c]	97.1%, 100%	91.4%, 100%	87.1%, 100%	82.3%, 99.4%	55.3%, 86.8%	92.7%, 97.3%
Specificity	99.90% (1982/1984)	99.90% (1995/1997)	99.90 (2000/2002)	99.80% (2001/2005)	99.80% (2000/2004)	99.34% (1954/1967)
2-sided 95% CI[c]	99.63%, 99.97%	99.64%, 99.97%	99.64%, 99.97%	99.49%, 99.92%	99.49%, 99.92%	98.87%, 99.61%

a. Seven twin pregnancies reported correctly as T21 not shown in table

b. Basic screen performance is reported for T21, T18, and T13 and excludes 16 samples with known mosaics and an additional 49 samples affected with anomalies for the genome-wide screen only; genome-wide screen performance is reported for RAAs and CNVs

c. CI based on Wilson's score method

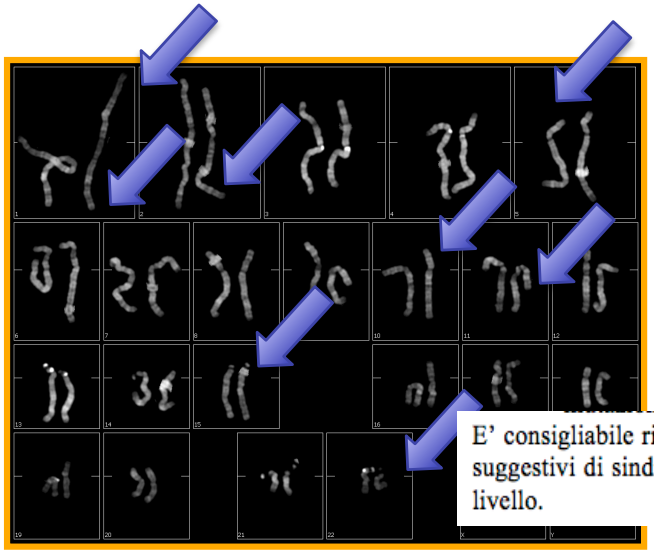
d. RAA excludes chromosomes 21, 18, and 13

e. Any anomaly includes samples from SCA basic and genome-wide screens

Identificazione delle Anomalie Cromosomiche Fetali con cffDNA: European population prevalence. Analisi delle microdelezioni:

La analisi cffDNA delle microdelezioni (<10Mb) ricerca una serie di sindromi rare con una sensibilità clinica fra il 50 e il 95%.
L'analisi è consigliata come indagine di secondo livello in concomitanza con findings ecografici e consulenza genetica.

Casi di Anomalie Cromosomiche	Popolazione EU	‰ prevalence	% Anomalie Cromosomiche
Totale	10323	4,4	
Anomalie cromosomiche strutt.	1737	0,7	17 (40<10)
Anomalie < 10Mb			3

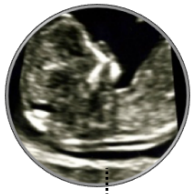


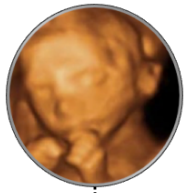
Sindrome da microdelezione	Regione cromosomica	Prevalenza (alla nascita)
Sindrome di DiGeorge	delezione 22q11.2	1/2.000 - 1/4.000
Sindrome Cri-du-chat	delezione 5p15.3	1/15.000 - 1/50.000
Sindrome di Prader-Willi	delezione 15q11.2	1/25.000
Sindrome Angelman	delezione 15q11.2	1/10.000 - 1/20.000
Sindrome da delezione 1p36	delezione 1p36	1/5.000 – 1/10.000
Sindrome di Wolf-Hirschhorn	delezione 4p16.3	1/20.000 -1/50.000
Sindrome di Jacobsen	delezione 11q23-q24.3	1/100.000
Sindrome di Langer-Giedion	delezione 8q24.11-q24.13	1/200.000
Sindrome di Smith-Magenis	delezione 17p11.2	1/15.000 - 1/25.000

E' consigliabile ricorrere all'utilizzo del **PrenatalSafe® Karyo Plus solo in determinati contesti clinici** (esempio dubbi ecografici suggestivi di sindrome da microdelezione cromosomica) per i quali risulta giustificato un approfondimento diagnostico di secondo livello.

Identificazione delle Anomalie Cromosomiche Fetali con cffDNA: Flow screen

flow





NIPT

U/S with NT

- low risk NIPT
- normal US

LOW RISK

ANATOMY U/S

- low risk NIPT
- increased NT
- abnormal US

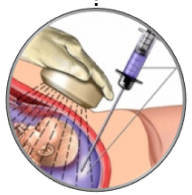
HIGH RISK

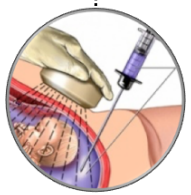
- high risk NIPT
- maternal choice

10 11 12 13 14 15 16 17 18 19 20

CVS

AMNIO





Identificazione delle Anomalie Cromosomiche Fetali con cffDNA: Flow screen

flow

- high risk NIPT
- increased NT
- abnormal US

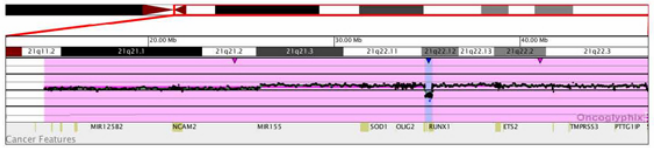
- high risk NIPT
- normal US

- abnormal US

10 11 12 13 14 15 16 17 18 19 20

CVS

AMNIO



- Deletion/duplication
- 500 kbases DNA

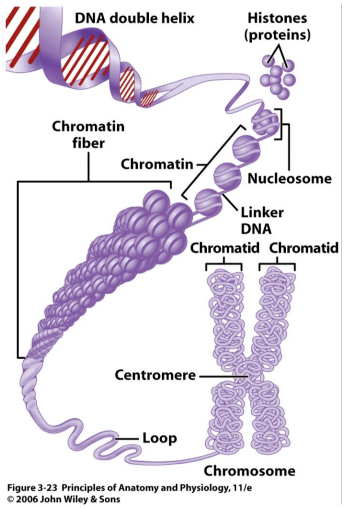
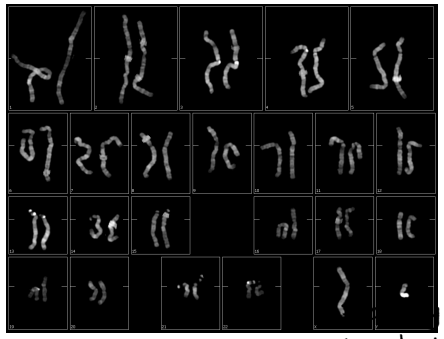


Figure 3-23 Principles of Anatomy and Physiology, 11/e
© 2006 John Wiley & Sons

- Deletion/duplication
- 15 Mbases DNA



of
Lamberto Camurri Ph.D.