FETALDNA
IL TEST PRENATALE DI ULTIMA GENERAZIONE

AFFIX LABEL

APPLICATION FORM AND INFORMED CONSENT

PLEASE COMPLETE ALL RELEVANT DATA IN CAPITAL LETTERS

REQUIRED TEST:

☐ BASIC ☐ BASIC PLUS ☐ KARYOTYPE ☐ KARYOTYPE PLUS ☐ TOTAL SCREEN

PATIENT

Name

Surname

Date of birth
DD / MM / YYYY ☐☐ / ☐☐ / ☐☐☐☐

Address

Post Code City

Country

Phone Number

Email

By signing below, I hereby acknowledge that I have completely read and fully understand the present informed consent. I declare that I have had the opportunity to ask my doctor about the objectives and possible risks of the test, and I get satisfactory answers. I am aware that it would be advisable to request professional genetic counseling before and after the test. I am also aware of the possibility of visiting the website www.fetaldna.it to obtain further information regarding the latest regulatory updates and the technical or medical information concerning FetalDNA. I am aware that the information contained on the website www.fetaldna.it does not replace medical advice, diagnosis or treatment. I authorize the processing of personal data in compliance with the law on privacy (Lgs 196/2003 and s.m.) for the purposes of carrying out the required test.

Date
DD / YY / YYYY ☐☐ / ☐☐ / ☐☐☐☐

Patient's signature

DOCTOR / LABORATORY

Name of the doctor

Surname of the doctor

Doctor's phone number

Laboratory / Clinical Diagnostic Center of Belonging

Address

Post Code City

Email

Date
YY / MM / YYYY ☐☐ / ☐☐ / ☐☐☐☐

Doctor's signature
that has collected the informed consent

PREGNANCY

Parity

Pregnancy ☐ Single ☐ Monochorional twin ☐ Bicorial twin

Date Last Period
DD / MM / YYYY ☐☐ / ☐☐ / ☐☐☐☐

Actual gestational age at the date of collection WEEKS ☐☐ DAYS ☐

Spontaneous pregnancy ☐ ART homologous ☐ ART heterologous (A) ☐☐

Weight Height

Clinical History

INFORMED CONSENT
GENERAL INFORMATIONS

In the preamble, at the end of this informed consent, you declare that you have well understood the following general information valid for all commercial screening tests called NON-invasive PRENATAL test (NIPT), that is CfDNA test.

- **NIPT is a non-diagnostic screening test**, analyzing free DNA fragments circulating in maternal blood, called fetal FreeDNA or CffDNA resulting from trophoblast (the cell structure forming the placenta). These DNA fragments trace, in the vast majority of cases, the composition of fetal DNA. NIPT is a screening test that evaluates the risk of the fetus being a carrier of chromosomal anomalies.
  NIPT presents a very low number of false negatives and false positives that in the international literature is reported between 0.1-0.3% of cases.
- It is absolutely recognized by medical science and guidelines that the diagnosis of certainty is provided exclusively by prenatal invasive diagnosis tests (amniocentesis and CVS Test). We have therefore been well informed that the results of this test do not guarantee correct diagnostic accuracy.
- NIPT is a molecular analysis based test using both Next Generation Sequencing and Digital PCR. The existing international literature on such methods of NIPT is so vast that it cannot be reported in the present consent. An essay of this will be available to us on request or can be viewed on the major international MEDLINE (eg. https://www.ncbi.nlm.nih.gov/pubmed).
- Although NIPT is performed through the use of the most innovative molecular technologies, it is possible that the survey does not give a result and should be repeated. This happens in about 1% of the cases in the literature.
- According to the international guidelines, NIPT should not be performed when there is an increase in the nuchal translucency (above 3 mm) or rather appears hydrops or hygroma (they are CVS test or Amniocentesis). The use of NIPT in case of suspected or confirmed fetal pathology should be performed only on the explicit request of the attending physician also on the basis of the gestational age achieved.
- When the screening test provides a pathological result, this must be confirmed by prenatal invasive diagnosis (amniocentesis/ chorionic villus test). These procedures will be scheduled at our Centre in Rome for free, both for the sampling technique and for the genetic examination.
- The reporting times vary depending on the type of examination required and may be subject to slippages based on technical problems or the need for further analytical feedback

Signature / Signatures
to confirm

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FETALDNA
IL TEST PRENATALE DI ULTIMA GENERAZIONE

INFORMED CONSENT
CHOICE TEST TO EXECUTE

I hereby declare that I have received exhaustive information regarding the level of Non Invasive Prenatal screening Test (NIPT screening) test that I/we have chosen and requested, having marked it in the box indicating it (see Barred Box) and subscribed at the bottom.

☐ **Basic FetalDna** investigates exclusively on the most common forms of chromosomal anomaly, that is **Down syndrome** (trisomy of chromosome 21), 18 or **Edwards syndrome** and 13 or **Patau syndrome**, as foreseen by the current guidelines. Although this test is performed by adding to the Next Generation Sequencing technology, a control in Digital PCR with a very high sensitivity, it's (as for all NIPT'S), always a screening test, not diagnostic. This test you must add, as indicated by the guidelines, to the ultrasound study of the fetal nuchal translucency test and represents an overcoming of the traditional biochemical tests included in the screening of the first pregnancy's trimester (example Bi/test, etc.).
On request it can be supplied also the fetal sex but as said, not the chromosomal anomalies of sex.
This seeks to further that the diagnostic certainty is supplied only by invasive tests (Amniocentesis and CVS Test).
Reporting times are included within five working days but may be increased when of technical difficulties encountered on the analysis of DNA or if the test should be repeated.
I am aware that the present NIPT, although it is performed through the use of the most innovative molecular technologies may not provide a result and should be repeated (in approximately 1% of literature reports).
This occurs even when there is a low percentage of fetal DNA (generally less than 4%). In this case it is advisable to perform a diagnostic invasive since the low amount of fetal DNA in the maternal blood may indicate an increased risk of chromosomal aberration.
Do I want to be informed of fetal sex? YES ◯ NO ◯

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☐ **Basic Plus FetalDNA** investigates the 3 main fetal chromosomal aneuploidies related to chromosomes 21, 18, 13 and the X, Y sexual chromosomes, also determining fetal sex which, at our request, may be kept silent. Each person has 2 copies of each chromosome and the term aneuploidy refers to numerical anomalies of the chromosomes. The term TRISOMY means that, for that particular chromosome, 3 copies, instead of 2, of that chromosome are observed. The term MONOSOMY means that, for that specific chromosome, 1 copy, instead of 2, of that chromosome is observed. The aneuploidies studied by FetalDNA are the most important, and common, that can affect the fetus.
TRISOMY OF CHROMOSOME 21 is the most common aneuploidy and refers to the presence of an over-copy of chromosome 21. This syndrome is known as Down syndrome and represents, with an incidence of about 1/650 births, the most common form of mental retardation.
TRISOMY OF CHROMOSOME 18 is the second most common aneuploidy and refers to the presence of an older copy of chromosome 18. This syndrome is known as Edwards syndrome and is associated with a high risk of abortion. Its incidence is estimated to be present in about 1/5000 births.
TRISOMY OF CHROMOSOME 13 is caused by an extra copy of chromosome 13 and is also known as Patau syndrome. It is associated with a high abortion; newborns have different pathological conditions that often cause deaths in childhood. It is estimated to have an incidence of about 1/16000 births.
SEX CHROMOSOME ANEUPLOIDEIS are anomalies affecting the XY sex chromosomes and which can cause difficulties of language, motor and/or learning in the affected newborns. The most common of this class of aneuploidies is TURNER SYNDROME or MONOSOMY X that affects women with only one copy of the X chromosome and has an incidence of about 1/2700 births. Other aneuploidies found with FetalDNA are TRIPLE X SYNDROME, KLINEFELTER SYNDROME, and JACOB'S SYNDROME.
Reporting times are included within 5 working days but may increase in case of technical difficulties on DNA analysis or if the test should be repeated.
I am aware that, this NIPT, though it is performed through the use of the most innovative molecular technologies, may not provide a result and should be repeated (about 1% of cases in the literature). This also occurs when a low percentage of fetal DNA (generally less than 4%) is found. In this case it is advisable to perform an invasive diagnosis since the low amount of fetal DNA in maternal blood can indicate an increased risk of chromosomal abnormality.
Do I want to be informed of fetal sex? YES ◯ NO ◯

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☐ **Karyotype FetalDna** **which in other commercial tests is represented as "Kario" or "E-Kario" or "Cario"**, represents a recently introduced NIPT, which investigates, always as screening, as well as on the chromosomal alterations shown above on the **Basic FetalDNA text, that I/we have seen** (numerical alterations of chromosomes 13, 18 and 21) and changes of the sex chromosomes X and Y, also on all the other numerical alterations, called aneuploidies, (alterations of the numerical changes) of all the other chromosomes.
In other words search the existence of an abnormal number of all and 23 chromosomes related to fetal karyotype.
It will be also included the determination of fetal sex.
Reporting times are included within five working days but may be increased when of technical difficulties encountered on the analysis of DNA or if the test should be repeated.
I am aware that the present NIPT, although it is performed through the use of the most innovative molecular technologies may not provide a result and should be repeated (in approximately 1% of literature reports).
This occurs even when there is a low percentage of fetal DNA (generally less than 4%). In this case it is advisable to perform a diagnostic invasive since the low amount of fetal DNA in the maternal blood may indicate an increased risk of chromosomal aberration.
In the even that the mother has the rhesus negative and the father RH positive (both documented and visible at the time of the FetalDNA request) **it will be possible to request a free analysis of the fetal RH by crossing the box below**: YES ◯ NO ◯
if I don't know my baby's gender, I can do it.
Do I want to be informed of fetal sex? YES ◯ NO ◯

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FETALDNA
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☐ **FetalDNA Karyotype Plus** is a highly elaborate, complete, non-invasive test of circulating free fetal DNA (NIPT).
First includes all the surveys that you perform on the **FetalDNA Karyotype test**. It therefore seeks the major alterations of chromosomes (13, 18, 21) and also of the sexual chromosomes (X and Y and their numerical alterations).
Moreover, like the FetalDNA Karyotype, it also make a screening on the other numerical alterations, called aneuploidies, (alterations of the number only) of all the other chromosomes.
In other words, it seeks the existence of an altered number of all 23 pairs of chromosomes related to fetal karyotype.
The determination of fetal sex will also be included.
In this extraordinary test is also included the screening of a large number of small chromosomal alterations determined by structural rearrangements (which are called microduplications/microdeletions) at a resolution of about 10 Mb (we inform however that all NIPT surveys on the market are not able to give any certainty. **The Italian and international guidelines provide that these problems can only be detected by prenatal, invasive diagnosis, Amniocentesis or chorionic villus test, by performing a specific study with microarrays. FetalDNA Karyotype Plus** is able to expand the number of pathologies with a **screening** that allows to obtain information on the presence of the most important microdeletion syndromes in the fetus. The term microdeletion/microduplication refers to anomalies characterized by the absence of a small chromosomal tract with consequent loss of gene information (microdeletions) or by the addition of supernumerary genomic material (microduplications). Both conditions cause pathologies with complex and variable clinical and phenotypic conditions depending on the chromosome involved, the chromosomal region involved and the size of the microdeletion itself. **It is reiterated** that the NIPT does not allow any diagnostic certainty.

The following are the main microdeletion syndromes investigated in the screening:
DiGeorge syndrome, Cri-du-chat syndrome, Prader-Willi syndrome, Wolf-Hirschhorn syndrome, Jacobsen syndrome, 1p36 deletion syndrome, Angelman syndrome, Langer-Giedion syndrome, Koolen-de Vries syndrome, Hereditary Neuropathy with Liability to Pressure Palsy (HNPP), 18q deletion syndrome, Alagille syndrome (AGS), Rubinstein-Taybi syndrome, WAGR WAGR syndrome, Potocki-Shaffer syndrome, Miller-Dieker syndrome, 1q 21.1 deletion syndrome, Kleefstra (KS), Phelan-Mcdermid syndrome, Smith-Magenis syndrome.

NB: It is reiterated that the above mentioned microdelections will only be screened without any diagnostic certainty. In fact, THESE DIAGNOSES ARE NOT OBJECTIVELY POSSIBLE WITH ANY EXISTING NIPT. Their implementation is not yet approved and recognized by national and international scientific societies and from LLGG, must be considered only for scientific research and DOESN'T HAVE a clinical value.
However, in our tests, this research was scientifically reliable. It reiterates once again that, for confirmation or exclusion, you must refer only to invasive testing using Microarrays on fetal material collected through amniocentesis or CVS test.

I am aware that the present NIPT, although it is performed through the use of the most innovative molecular technologies may not provide a result and should be repeated (in approximately 1% of literature reports).
This occurs even when there is a low percentage of fetal DNA (generally less than 4%). In this case it is advisable to perform a diagnostic invasive since the low amount of fetal DNA in the maternal blood may indicate an increased risk of chromosomal aberration.

The FetalDNA Karyotype Plus also includes, free of charge, the search for the most frequent mutations in maternal cystic fibrosis. In this way, if one of these mutations is present in the mother, it will be necessary to investigate whether the fetus was healthy or even a simple carrier or (when the father was also the carrier) ran the risk of being affected by cystic fibrosis.
This in fact occurs in 25% of cases when both parents are healthy carriers.
The following is a list of the researched mutations that are the most frequent and important in maternal screening. No other mutation responsible for the same disease will be researched.
Cystic fibrosis (FC) is an hereditary disease with autosomal recessive transmission, which means it is inherited from both parents carrying an altered gene. For this genetic error an alteration of the mucus of the various organs is determined. The organs frequently affected are the liver, intestine, reproductive system and lungs where the particularly dense mucus leads to severe respiratory problems and consequent infections. With the FetalDNA Plus and FetalDNA Karyotype Plus the maternal gene analysis is performed through a screening called 1° level, which allows to analyze the most common and frequent mutations, managing to identify about 83% of the carriers. The estimated frequency, in the Italian population, of the healthy carriers (often unaware of it) is 1 in 25 – 30, that of the affected ones is 1 on 2500 – 3000.
NB: The mutations analyzed are exclusively the following: 711+1G-T, 621+1G-T, 1717-1G-A, 3849+10kbC-T, 2789+5G-A, G542X, G85E, G551D, R553X, N1303K, R117H, R1162X, L1077P, L1065P, W1282X, R347P, I507del, T338I, F508del, 1677delTA, 2183AA-G, S549R, Q552X, 852del22, R1066H, G1244E, 1259insA, D1152H, 711+5G-A, R1158X, 4382delA, 4016insT, A455E, 1706del17, I502T, 3199del6, S912X.

In the even that the mother has the rhesus negative and the father RH positive (both documented and visible at the time of the FetalDNA request) **it will be possible to request a free analysis of the fetal RH by crossing the box below**: YES ◯ NO ◯

If I don't know my baby's gender, I can do it.
Do I want to be informed of fetal sex? YES ◯ NO ◯

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☐ **FetalDNA Total Screen** is the most elaborate and complete non-invasive test of circulating free fetal DNA (NIPT) available today.

First of all it **includes the screening tests reported in the test called FetalDNA Kariotipo Plus** (for the exact acknowledgment of which is referred to in the previous paragraph, **where its limits in the screening are clearly specified**) and **also includes** the search for genetic mutations that the greatest international studies today hold to be responsible for maternal predisposition to pre-term delivery.
The following is a list of the researched mutations that are the most frequent and important in prenatal fetal screening. No other mutation responsible for the same disease will be researched.

SCONGENITAL HEARING LOSS (gene **GJB2**) with mutations: Leu90Pro / c.35del
BETA TALASSEMIA (gene **HBB**) with mutations: IVS1, G-C, +5 / IVS1, T-C, +6 / IVS2, C-A, -3 / IVS1, T-G, -3 / IVS1, G-A, +110 / IVS2, T-G, +705 / IVS2, C-G, +745 / GGT24GGA / -101C-T / -92C-T / -88C-T / -87C-G / -86C-G / -31A-G / -30T-A / -29A-G / -28A-C / 3-UNT, A-G, +4 / C-A, -32 / 3-NT, 5-BP DEL, AATAAA-A / C-T, -90 / VAL60GLU / 1-BP INS, A, CODON 47 / 2-BP DEL, CC, CODONS 38-39 / LYS17TER / GLN39TER / TRP15TER / TRP37TER / GLU43TER / LYS61TER / TYR35TER / LYS8FS / GLY16FS / SER44FS / GLU6FS / LEU106FS / PRO5FS / VAL11FS / TYR35FS / LEU14FS / TRP37FS / ASP94FS / GLY64FS / VAL109FS / PRO36FS / ALA27FS / MET1ARG / IVS1, G-A, +1 / IVS2, G-A, +1 / IVS1, T-G, +2 / IVS1, 25-BP DEL / IVS2, A-G, -2 / IVS1, G-A, -1 / IVS2, C-T, +654 / 1-BP DEL, GTG-TG / IVS2, G-C, -1 / MET1ILE / 1-BP INS, T, CODON 26 / ASP114FS
CONGENITAL ADRENAL HYPERPLASIA (gene **CYP21A2**) with mutations: ILE172ASN7 / VAL281LEU / TRP406TER / VAL281LEU, PHE306+1, GLN318TER, AND ARG356TRP / HIS62LEU / LYS121GLN
HEMOCHROMATOSIS (gene **HFE**) with mutations: HIS63ASP / SER65CYS / 5569G-A / VAL53MET / VAL59MET / GLN127HIS / ARG330MET / ILE105THR / GLN283PRO
ACHONDROPLASIA (gene **FGFR3**) with mutations: GLY380ARG AND LEU377ARG / GLY380ARG, 1138G-A / SER279CYS

HYPOCHONDROPLASIA (gene **FGFR3**) with mutations: ASN540LYS, 1620C-A / ASN540THR / ILE538VAL / LYS650ASN, 1950G-T / TYR278CYS / LYS650GLN
APERT SYNDROME (gene **FGFR2**) with mutations: PRO253ARG / SER252PHE / SER252TRP
CROUZON SYNDROME (gene **FGFR2**) with mutations: TYR340HIS / SER354CYS / TYR328CYS / SER347CYS / CYS342TRP / LYS292GLU / TRP290ARG / CYS342TYR / CYS342ARG / ALA344ALA / GLN289PRO / LYS526GLU
PFEIFFER SYNDROME (gene **FGFR2**) with mutations: THR341PRO / TRP290CYS / GLU565ALA / SER252PHE AND PRO253SER / SER267PRO / SER351CYS
LEOPARD SYNDROME (gene **PTPN11**) with mutations: TYR279CYS / THR468MET / ALA461THR / GLY464ALA / GLN510PRO
NOONAN SYNDROME (gene **PTPN11**) with mutations: GLN79ARG / THR411MET / ALA72SER / ALA72GLY / ASN308ASP / ASN308SER/ SER502THR / TYR63CYS / TYR62ASP / ASP61GLY / THR73ILE / PHE285SER
NOONAN SYNDROME (gene **SOS1**) with mutations: THR266LYS / MET269ARG / ARG552GLY / ARG552SER / TRP432ARG
NOONAN SYNDROME (gene **RAF1**) with mutations: SER257LEU / PRO261SER / THR491ARG / LEU613VAL
PHENYLKETONURIA (gene **PAH**) with mutations: IVS12DS, G-A, +1 / ARG408TRP / LEU311PRO / GLU280LYS / ARG261GLN / ARG252TRP / MET1VAL /ARG158GLN / ARG243TER / PRO281LEU / TYR204CYS / ARG243GLN / TRP326TER / ARG413PRO / TYR414CYS / TYR356TER / 3-BP DEL, CTT / IVS7DS, G-A, +1 / LEU255SER / ALA259VAL / TYR277ASP / 3-BP DEL, ATC / PHE39LEU / IVS10AS, G-A, -11 / LEU48SER / GLU221GLY / ARG261TER / 1-BP DEL, CODON 55 / ARG408GLN / PHE299CYS / IVS7DS, T-A, +2 / SER349PRO / ALA322GLY / ASP415ASN / ILE306VAL / 15-BP DEL, EX11 / PRO244LEU / MET1ILE / IVS10AS, C-T, -3 / LEU333PHE / SER359TER / LEU98SER / THR380MET / GLY46SER / ALA47VAL / SER87ARG / ARG176LEU / VAL245ALA / IVS10DS, A-G, +3 / 1-BP DEL, 1129T / PRO407LEU / ILE65THR / GLU76GLY
RETT SYNDROME (gene **MECP2**) with mutations: PHE155SER / ARG106TRP / 2-BP DEL, 211CC / ARG306CYS / ARG168TER / GLU455TER / LEU100VAL / 1-BP DEL, 710G / THR158MET / ARG294TER
AUTOSOMAL RECESSIVE POLYCYSTIC KIDNEY DISEASE (gene **PKHD1**) with mutations: SER1664PHE / SER3018PHE / VAL1741MET / ARG2671TER / ILE3553THR / ARG496TER / VAL3471GLY

We inform and confirm that any other and different mutations from those specifically researched in the test and given in the report, will not be studied and therefore the test has no possibility, in such cases, to detect the existence.
The signature of this consensus **reiterates** that it has been well understood that the search for the aforementioned list of such anomalies (as is the case for all NIPT surveys) is exclusively a screening.
However accurate and thorough is the analysis of DNA on maternal blood, **the existence or non-existence of the pathologies specified in the above lists can never be certain.**
The certainty belongs to the diagnosis (and not to the screening) and is only possible exclusively through amniocentesis and CVS test.
This case has been widely represented and parents are aware of the possibility that this examination (like all the NIPTs on the market) can provide erroneous diagnoses. For this purpose, it is specified that even **the Italian and international guidelines do not provide for the implementation of these insights through the NIPT and confirm that these problems can be identified exclusively by the invasive prenatal diagnosis, Amniocentesis or CVS test, performing a specific study using methods such as microarrays, PCR Real Time, NGS.**

NB: It reiterates once again that with regard to microdeletions these will only be researched with any diagnostic certainty (screening test). These diagnoses, in fact, ARE NOT OBJECTUVELY POSSIBLE WITH ANY EXISTING NIPT. Their execution is not yet approved and recognized by scientific societies and national and international LLGG, it must be considered only for scientific research and HAS NO clinical value. However, in our tests, this research was scientifically reliable. It reiterates once again that, for confirmation or exclusion, reference should only be made to the invasive tests using microarrays technique on fetal material taken through Amniocentesis or CVS Test.

I am aware that, this NIPT, though it is performed through the use of the most innovative molecular technologies, may not provide a result and should be repeated (about 1% of cases in the literature). This also occurs when a low percentage of fetal DNA (generally less than 4%) is found. In this case it is advisable to perform an invasive diagnosis since the low amount of fetal DNA in maternal blood can indicate an increased risk of chromosomal abnormality.

In the even that the mother has the rhesus negative and the father RH positive (both documented and visible at the time of the FetalDNA request) **it will be possible to request a free analysis of the fetal RH by crossing the box below**: YES ◯ NO ◯

If I don't know my baby's gender, I can do it.
Do I want to be informed of fetal sex? YES ◯ NO ◯

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Doctor's signature
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As already mentioned, it is not possible to include the huge bibliographic corpus, only the main guidelines which are more often referred to in this agreement are listed:

- LLGG joint position statment SIGU and SIEOG 2004 (Appropriate use of CMA Technique (Chromosomal Microarray Analysis) in prenatal diagnosis).
- LLGG joint position statment SIGU and SIEOG 2017 (Appropriate use of CMA Technique (Chromosomal Microarray Analysis) in prenatal diagnosis).
- LLGG Canadian Society "Prenatal genomic microarray and sequencing in canadian medical practice: towards consensus" (Aprile 2015).
- LLGG documento congiunto del "Royal College of Pathology", della "British Society for Genetic Medicine" [Gardiner et al., 2015].
  Position Paper American Society of Ultrasound in Ob/Gyn: Cut-off value of nuchal translucency as indication for chromosomal microarray analysis, and coll Maya, Ultrasound in Ob Gyn 26 July 2017.
- ISUOG updated consensus statement on the impact of cfDNA aneuploidy testing on screening policies and prenatal ultrasound practice First published: 1 June 2017
- Cell-free DNA Screening for Fetal Aneuploidy. ACOG. Commitee Opinion. Reaffirmed 2017.