FETALDNA
IL TEST PRENATALE DI ULTIMA GENERAZIONE

APPLICATION FORM AND INFORMED CONSENT
INFORMATION ON THE POTENTIAL AND LIMITATIONS OF THE TEST

PLEASE COMPLETE ALL RELEVANT DATA IN CAPITAL LETTERS

AFFIX LABEL

PATIENT

Surname

Name

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

Place of birth

Address - Post Code - City

Country

C.F.

Phone Number

Email

Date
DD / MM / YYYY ☐☐ / ☐☐ / ☐☐☐☐

TEST REQUIRED

- ☐ BASIC
- ☐ BASIC PLUS ☐ DIGEORGE SYNDROME
- ☐ BASIC PLUS + 90 MICRODELETIONS
- ☐ KARYOTYPE
- ☐ KARYOTYPE PLUS
- ☐ KARYOTYPE PLUS + MONOGENIC DISEASES
- ☐ TOTAL SCREEN
- ☐ MONOGENIC DISEASES
- ☐ MINI CGS 1
  BASIC PLUS + 90 MICRODELETIONS + MATERNAL CARRIER SCREENING
- ☐ MINI CGS 2
  KARYOTYPE + MATERNAL CARRIER SCREENING
- ☐ MINI CGS 3
  KARYOTYPE PLUS + MONOGENIC DISEASES + MATERNAL CARRIER SCREENING

PREGNANCY (ALL FIELDS ARE REQUIRED)

I want to be informed of **fetal sex?** Yes ☐ NO ☐

PARITY

PREGNANCY

Single ☐ Monochorional twin ☐ Bicorial twin ☐

Spontaneous pregnancy ☐ Homologous IVF ☐ Heterologous IVF (Age) ☐☐

Date Last Period
DD / MM / YYYY

☐☐ / ☐☐ / ☐☐☐☐

Actual gestational age at the date of collection

WEEKS ☐☐ DAYS ☐

ETHNIC GROUP
(required)

Caucasian ☐ African ☐ North African ☐

Asian ☐ Other ☐

Weight (required) Height (required)

Smoker Yes ☐ No ☐

Clinical History

DOCTOR / LABORATORY

Surname of the doctor
(required)

Name of the doctor
(required)

Doctor's phone number

Laboratory / Clinical Diagnostic Center of Belonging
(required)

Address

Post Code City

Email

Date
DD / MM / YYYY ☐☐ / ☐☐ / ☐☐☐☐

Doctor's signature
that has collected the informed consent

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INFORMATION ON THE POTENTIAL OF THE TEST AND ACCEPTANCE OF ITS LIMITATIONS

TEST SELECTION

THE ANALYSES AVAILABLE WITHIN THIS TEST MUST BE SELECTED BY THE PREGNANT WOMAN UNDER THE GUIDANCE OF A SPECIALIST, WHO WILL ENSURE THAT THIS INFORMED CONSENT IS FULLY UNDERSTOOD IN ACCORDANCE WITH THE NEEDS AND REQUESTS OF THE PARENTAL COUPLE.

I hereby declare that I have received detailed information regarding the NIPT screening test that I have selected and requested.

I fully understand that, although the test I am undergoing demonstrates extremely high diagnostic accuracy, as stated by the current guidelines in use in our country, definitive diagnostic certainty is provided exclusively by invasive tests (Amniocentesis and Chorionic Villus Sampling).

I also understand that all fetal DNA tests (NIPT) do not provide definitive diagnostic certainty. While rare, cases of both false positives and false negatives have been reported, and I accept this rare eventuality.

Additionally, I understand that the likelihood of incorrect interpretation of fetal sex, as estimated in global literature, is 3%.

☐ **BASIC** exclusively investigates the most common forms of chromosomal anomalies, **Down syndrome** (trisomy of chromosome **21**), **Edwards syndrome** (trisomy of chromosome **18**), and **Patau** syndrome (trisomy of chromosome **13**), with a **sensitivity of 99,8%**, as outlined in the current **g**uidelines. **If requested, fetal sex determination can also be provided; however, as previously stated, it does not include the detection of sex chromosome anomalies.**

☐ **BASIC PLUS** investigates the three main fetal chromosomal **aneuploidies** associated with **chromosomes 21, 18, and 13** (as previously described), as well as the X and Y sex chromosomes, with a **sensitivity of 99,8%**. It also determines fetal sex, which, upon our request, can remain undisclosed.

If requested, the test can be extended to include screening for **DiGeorge Syndrome**, a sporadic genetic condition caused by the deletion of a portion of chromosome 22, with an incidence of approximately 1 in 3,500 live births.

I confirm my request for the screening of **DiGeorge Syndrome**? YES ☐ NO ☐

☐ **BASIC PLUS + 90 MICRODELETIONS** investigates the three main **fetal chromosomal aneuploidies** associated with **chromosomes 21, 18, and 13,** as well as the **X and Y sex chromosomes (with a sensitivity of 99,8%)**. The test also includes the screening of a large number of small chromosomal alterations caused by structural rearrangements (referred to as **microdeletions/microduplications**) at an average resolution of 5Mb, which is nonetheless correlated to the fetal fraction (**sensitivity** increases with a higher fetal fraction, up to **90%**). Higher sensitivity values cannot be achieved by any NIPT test. The term microdeletions/microduplications refers to anomalies characterized by the absence of a small chromosomal segment, resulting in the loss of genetic information (microdeletions), or the addition of extra genomic material (microduplications). Other molecular variants than microdeletions and microduplications associated with the same conditions cannot be detected by this test.

The complete list of investigations is as follows:

1p31, microduplication
1p36, microdeletion
1q21q32, monosomy
1q21.1, microdeletion
1q21.1, microduplication
1q23--qter, trisomy
1q41-q42, microdeletion
1q42--qter, monosomy
2p15-p16.1, microdeletion
*2q22.3, **Mowat-Wilson***
2q33.1, microdeletion
2q33.1, microduplication
2q35, microduplication
2q37, microdeletion
3p11--p21, monosomy
*3q22, **Dandy-Walker***
3p25--pter, monosomy
3q29, microdeletion
3q29, microduplication
*4p16.3, **Wolf-Hirschhorn***
4q21q31, monosomy
4q31--qter, monosomy
*5p, **Cri-du-chat***
5q12, microdeletion
*5q35.3, **Sotos***
*6p21, **Cleidocranial Dysplasia***
6q24-q25, microdeletion
7q11.23, microduplication
*7q11.23, **Williams-Beuren***
7q21.q31, trisomy
7q32--qter, monosomy
7q32--qter, partial trisomy
8p23.1, microdeletion
8p23.1, microduplication
8q12.1-q21.2, microdeletion
*8q13.3, **Branchio-Oto-Renal Syndrome***
8q21--qter, monosomy
8q21.11, microdeletion
*8q24.11, **Langer-Giedion***
9p, microdeletion
9q22.3-q33, microdeletion
9q33.2-q34.3, microduplication
*9q34, **Kleefstra***
10q26, microdeletion
*11p, **Potocki-Shaffer***
*11p13, **WAGR***
11p15-p14, microdeletion
*11q, **Jacobsen***
11q23.3-q25, microdeletion
12q14, microdeletion
13q14, microdeletion
13q21--qter, monosomy
13q21--qter, trisomy
14q11-q22, microdeletion
14q24-qter, trisomy
*14q32.13, **Wilms tipo 1***
*15q11, **Angelman***
*15q11-q13, **Prader-Willi***
15q14, microdeletion
15q22--qter, trisomy
15q26-qter, microdeletion
15q26-qter, microduplication
*15q26.1, **Congenital Diaphragmatic Hernia Type 1***
16p11.2-p12.2, microdeletion
16p11.2-p12.2, microduplication
*16p13.3, **Rubinstein-Taybi***
17q11.2, microdeletion
17q11.2, microduplication
*17p11.2, **Potocki-Lupski***
*17p11.2, **Smith-Magenis***
*17p13.3, **Miller-Dieker***
*17q21, **Koolen-de Vries***
17q21.31, microduplication
18p, microdeletion
18pter--q12, trisomy
18q, microdeletion
18q12--qter, trisomy
19p13, microduplication
19q13.11, microdeletion
20p, trisomy
*20p12, **Alagille***
20q13.1-q13.3, microduplication
*22q11.2, **DiGeorge***
22q11.2, microduplication
*22q13, **Phelan-mcdermid***
Xp11.3, microdeletion
Xp11.23-p11.22, microduplication
*Xp21.3, **Lissencefalia X-linked***
Xq27.3-q28, microduplication
Xq28, microdeletion

☐ **KARYOTYPE** extends the investigation of numerical alterations to all chromosomes. This NIPT, In other words, detects the presence of an **altered number in all 23 pairs of chromosomes related to the fetal karyotype**, including the sex chromosomes X and Y (with a **sensitivity of 99,8%**).

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☐ **KARYOTYPE PLUS** is a highly elaborate, complete, non-invasive test of circulating free fetal DNA (NIPT). It includes all the investigations mentioned so far: **numerical alterations**, known as aneuploidies (alterations in chromosome number only), for **all chromosomes**, including **13**, **18**, **21**, and the **sex chromosomes** (**X** and **Y**, along with their numerical alterations) with a **sensitivity of 99,8%**. Additionally, it includes the analysis of **90 syndromes caused by microdeletions/microduplications**, at an average resolution of 5Mb, which depends on the fetal fraction (**sensitivity increases with a higher fetal fraction, up to 90%**). Higher sensitivity values cannot be achieved by any test. The list of the 90 main syndromes investigated in this screening will be included in the results and is clearly outlined on the FetalDNA.it website and on the previous page under the section 'Base Plus+90 Microdeletions.'

The FetalDNA Karyotype Plus also includes, at no additional cost, the screening for the most common maternal Cystic Fibrosis mutations.

I confirm that I request the screening for **the most common maternal Cystic Fibrosis mutations?** YES ☐ NO ☐

☐ **MONOGENIC FETAL DISEASES (can be requested individually or in combination with the levels described above)**

This test is a screening test and not a diagnostic one. Although highly accurate, the results do not have diagnostic value and must be assessed within the pregnant woman's clinical condition and genetic family history. It is not a substitute for invasive prenatal diagnostic procedures (Chorionic Villus Sampling or Amniocentesis).

Hereditary Monogenic Diseases

*Autosomal Recessive Polycystic Kidney Disease (**PKHD1** gene)*
*Beta-Thalassemia (**HBB** gene)*
*Congenital Adrenal Hyperplasia (**CYP21A2** gene)*
*Congenital Deafness (**GJB2** gene)*
*Fetal Cystic Fibrosis (**CFTR** gene)*
*Hemochromatosis (**HFE** gene)*
*Phenylketonuria (**PAH** gene)*
*Rett Syndrome (**MECP2** gene)*

Monogenic Diseases due to de novo mutations

*Achondroplasia (**FGFR3** gene)*
*Apert Syndrome (**FGFR2** gene)*
*Crouzon Syndrome (**FGFR2** gene)*
*Hypochondroplasia (**FGFR3** gene)*
*LEOPARD Syndrome (**PTPN11** gene)*
*Noonan Syndrome (**PTPN11** gene), (**RAF1** gene), (**SOS1** gene)*
*Pfeiffer Syndrome (**FGFR2** gene)*
*Thanatophoric Dysplasia (**FGFR3** gene)*

I have been fully informed that it is not possible to obtain definitive evidence of these anomalies in the fetus through maternal blood testing. The test I am undergoing has a **sensitivity of up to 90%**. Higher values are NEITHER realistic NOR documented and cannot be achieved by any NIPT test based on fetal DNA. Certainty (as reiterated by the Guidelines of our country) can only be provided by invasive tests (Amniocentesis or Chorionic Villus Sampling).

☐ **TOTAL SCREEN** represents a highly advanced and comprehensive non-invasive prenatal test (NIPT) performed on circulating free fetal DNA.

It analyzes all **23 pairs of chromosomes** related to the fetal karyotype, including the investigation of **sex chromosome aneuploidies (X, Y)** as well as the three main fetal chromosomal aneuploidies associated with **chromosomes 21, 18, and 13**: **Down syndrome** (Trisomy 21), **Edwards syndrome** (Trisomy 18), and **Patau syndrome** (Trisomy 13), with a **sensitivity of 99,8%.**

It also includes the screening of 90 small chromosomal alterations caused by structural rearrangements (referred to as **microdeletions/microduplications)** at an average resolution of 5Mb, which depends on the fetal fraction (**sensitivity increases with a higher fetal fraction, reaching up to 90%**). Additionally, it investigates genetic mutations responsible for **fetal monogenic diseases** as listed in the previous section, with a **sensitivity of up to 90%.**

The FetalDNA Total Screen also includes investigations related to the pregnant woman, specifically:

- Screening for mutations responsible for **maternal Cystic Fibrosis**.
- Screening for exon 7 and 8 deletions of the SMN1 and SMN2 genes associated with **Spinal Muscular Atrophy** (this test excludes the vast majority of molecular alterations linked to SMA; however, extremely rare mutations cannot be detected with this test).
- Screening for **infectious agents** present in the maternal blood, allowing early detection prior to the positivity of antibody tests routinely used during pregnancy (while reliable and thorough, it does not rule out fetal damage caused by infections occurring before or after the test).
- Screening for mutations associated with **predisposition to preterm birth** (this test does not exclude preterm birth caused by other clinical factors).
- Biochemical risk assessment for **preeclampsia** (provides a risk value that, although highly useful for the treating physician, does not offer diagnostic certainty).
- Screening for the most common mutations responsible for **hereditary thrombophilia** (these investigations, considered by much of the international literature to be useful for preventing maternal-fetal complications such as miscarriage, growth restriction, placental abruption, and thrombosis, must be evaluated in the clinical context and do not exclude the existence of other factors causing the same conditions).

☐ **MINI CGS 1** includes the investigations indicated in the Base Plus + 90 Microdeletions combined with Maternal Carrier Screening, which analyzes:

- Cystic Fibrosis, Congenital Deafness, and Spinal Muscular Atrophy (SMA) inherited from both parents (recessive transmission).
- Muscular Dystrophy and Fragile X Syndrome inherited from healthy carrier mothers (X-linked transmission).

If any positive results are found in the Maternal Carrier Screening, I will be contacted for genetic counseling.

☐ **MINI CGS 2** includes the investigations indicated in the Karyotype Plus combined with Maternal Carrier Screening, which analyzes:

- Cystic Fibrosis, Congenital Deafness, and Spinal Muscular Atrophy (SMA) inherited from both parents (recessive transmission).
- Muscular Dystrophy and Fragile X Syndrome inherited from healthy carrier mothers (X-linked transmission).

If any positive results are found in the Maternal Carrier Screening, I will be contacted for genetic counseling.

☐ **MINI CGS 3** includes the investigations indicated in the Karyotype Plus and monogenic diseases combined with Maternal Carrier Screening, which tests for:

- Cystic Fibrosis, Congenital Deafness, and Spinal Muscular Atrophy (SMA) inherited from both parents (recessive transmission).
- Muscular Dystrophy and Fragile X Syndrome inherited from healthy carrier mothers (X-linked transmission).

If any positive results are found in the Maternal Carrier Screening, I will be contacted for genetic counseling.

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- Regarding the detection of fetal anomalies in maternal blood (NIPT), I fully understand that the test I am undergoing, as stated by the current Guidelines in use in our country, does not provide diagnostic certainty, which is exclusively offered by invasive tests (Amniocentesis and Chorionic Villus Sampling). I also understand that all fetal DNA tests (NIPT) do not provide definitive diagnostic results. Although rare, cases of false positives and false negatives have been reported. I accept this rare eventuality. Furthermore, the possibility of incorrect fetal sex determination is 3%. While this occurrence has no clinical relevance, it must be acknowledged due to its emotional impact.
- Additionally, NIPT does not detect balanced chromosomal rearrangements. It may fail to detect fetal and/or placental chromosomal mosaicisms (two cell lines with different chromosomal configurations). It does not analyze all point mutations associated with the investigated genes, and its sensitivity does not exceed 90%. It does not detect methylation defects, triploidy, polyploidy, or any chromosomal and molecular rearrangements that cannot be identified using NIPT techniques.
- If the screening test yield a positive result, current guidelines require confirmation through invasive prenatal diagnosis Chorionic Villus Sampling or Amniocentesis). These procedures will be scheduled at our Rome center completely **free of charge**, covering both the sampling technique and genetic testing.
- Result reporting times vary depending on the type of test requested and may be delayed due to technical issues or the need for additional analytical checks.
- I undertand that this NIPT, although performed using the most advanced molecular technologies, may not provide a result and might need to be repeated (approximately 1% of cases in the literature). This can also occur when a low percentage of fetal DNA is detected (generally below 4%). In such cases, an invasive diagnostic procedure is recommended, as a low level of fetal DNA in maternal blood may indicate an increased risk of chromosomal abnormalities. FetalDNA (like all NIPT tests) is performed by quantitatively comparing the DNA of selected chromosomes in maternal blood with that of fetal origin. Most of this DNA is of maternal origin, with only a small proportion being fetal. The test determines if the amount of DNA from a specific chromosome deviates from the expected amount. For example, an excess of DNA from chromosome 21 could indicate that the fetus has three copies of this chromosome (causing Down syndrome) instead of the usual two. The minimum threshold of 4% required to obtain a sufficiently reliable diagnosis has been defined through statistical models based on the minimum number of readings of aneuploid chromosome fragments sufficient to detect fetal aneuploidy at various levels of fetal fraction (FF). According to this model, at low FF levels, differences in circulating cfDNA between pregnancies with fetal trisomies and those with euploid fetuses may not be detectable, leading to false negatives. A factor associated with low fetal cfDNA percentages and the potential failure of the test is increased maternal body weight. The increased amount of maternal cfDNA in obese women may mask the fetal fraction, complicating the screening for aneuploidies and increasing the risk of test failure due to a high body mass index (BMI >30 in obesity and between 25 and 30 in overweight cases).
- It is emphasized and reiterated that any mutations other than those specifically targeted and reported in the test results will not be investigated, and the test has no capability to verify their presence.
- When the test needs to be repeated, a new blood sample is collected at no additional cost.
- In dizygotic twin pregnancies, it is not possible to distinguish the condition of each fetus or accurately assess sex chromosome aneuploidies. However, the presence or absence of the Y chromosome can be detected. If the Y chromosome is identified, it cannot be determined whether one or both fetuses are male. In pregnancies that began as twin or multiple gestations followed by spontaneous miscarriage of one or more fetuses with reabsorption of the gestational sac (vanishing twin), the maternal blood may contain free fetal DNA from the miscarried fetus. This could interfere with the quality of results, leading to false positives if the miscarriage was caused by chromosomal aneuploidies in the lost fetus. Similarly, there may be discrepancies in sex determination (e.g., male sex identified due to the presence of the Y chromosome originating from the miscarried fetus).
- In cases of chromosomal mosaicism (with a frequency of approximately 1-2%), result discrepancies (false positives or false negatives) may occur. Specifically, the test may produce a positive result (aneuploidy detected), but the chromosomal anomaly is confined to the placenta due to chromosomal mosaicism. In such cases, the fetus may present a normal karyotype during invasive prenatal diagnosis (false positive). Conversely, the test may produce a negative result (aneuploidy not detected), but the fetal DNA without aneuploidy may be confined to the placenta due to chromosomal mosaicism, resulting in a fetus with an aneuploid karyotype during invasive prenatal diagnosis (false negative).

FIELD REQUIRED

☐ I hereby declare that I have **FULLY UNDERSTOOD** the limitations of the selected screening test.

☐ I hereby declare that I have **NOT FULLY UNDERSTOOD** the limitations of the selected screening test.

Signature / Signatures
pregnant woman _____

Doctor's signature
that has collected the informed consent _____

Your privacy is a priority for ALTAMEDICA. Artemisia SpA, whose registered office is located in Rome at Viale Liegi, 41 as the data controller, informs you that your data will be handled in compliance with the applicable laws and EU Regulation No. 2016/679. Your identity and all data related to your personal information will remain confidential, and only authorized personnel will have access to this information, along with competent authorities when required by local jurisdiction laws. We wish to inform you that your personal data will be processed solely for the following purposes: (1) To fulfill obligations arising from the provision of services you have subscribed to; (2) For research purposes, scientific publications, and presentations, provided that your data remains anonymous and cannot be identified during data analysis, and any identifiable data will be removed from any publication.

In accordance with personal data protection laws, the requesting party must have the patient's consent to perform the requested diagnostic tests and process their data, which will be stored for no longer than permitted by the current legislation.

You may exercise your rights at any time, including access, rectification, objection, deletion, withdrawal, automated decision-making, restriction, and portability, by contacting the company at Artemisia spa con sede in Viale Liegi 41 - Roma via registered letter with acknowledgment of receipt, or at the following certified email address: artemisiaspa@pec.it. Alternatively, you may contact the company's designated Data Protection Officer (DPO) at dpo@artemisia.it.

☐ **Authorize** ☐ **Do not authorize**

I authorize/do not authorize the sending of the report to my email address

Signature / Signatures
pregnant woman _____