INFORMED CONSENT FOR PRENASCAN

1. Purpose of Genetic Laboratory Testing

PRENASCAN is a non-invasive prenatal screening test that detects the risk of fetal abnormalities due to variations in the number of chromosomes (called aneuploidy) or parts of the chromosomes (structural aberrations). The most common abnormality is trisomy of chromosome 21, characterised by three copies of the chromosome instead of the usual two, which leads to fetal Down syndrome.

The examination requires 7-10 ml of blood taken from the peripheral vein of the pregnant woman anytime between 10 and 26 weeks of gestation. PRENASCAN analyses the free circulating fragments of cell-free DNA (cfDNA) in the mother‘s plasma. This cfDNA enters the mother‘s blood plasma from both fetal placental cells and maternal cells. The proportion of free fetal DNA, called cfDNA (cell-free fetal DNA), is about 10% of the total amount of cfDNA in the plasma and is referred to as the fetal fraction. The amount of fetal fraction is reported in the results report and is important for the performance of the test and its evaluation.

2. Description of the Proposed Examination, its Limitations, and the Method of Reporting Results

2.1. Description of the Utilised Method

SThe screening examination of cfDNA in the plasma of pregnant women is performed using an automated, CE-IVD (compliant with EU regulations for in-vitro diagnostic devices) certified method known as the VeriSeq NIPT v2 Solution, provided by the American manufacturer Illumina. The standardised protocol of this method begins with the isolation of plasma and cfDNA, using the patient‘s blood as the input material, following a defined procedure for sample collection and delivery to the laboratory. To prepare the isolated cfDNA for PRENASCAN purposes, an automated protocol transfers it into a „library“ through a process that labels the samples of isolated cfDNA with index tags and adapters necessary for subsequent library sequencing. Sequencing is conducted on the NextSeq 550Dx machine by Illumina. The obtained sequencing data is then analyzed on the VeriSeq On-site server, equipped with VeriSeq NIPT Assay Software v2. The entire solution is designed with the utmost consideration for sample integrity, error risk reduction (automation), reliability, and patient data security. Both the sample and sequencing data are processed directly in our genetic laboratories at GNTlabs by GENNET.

PRENASCAN analyses cell-free DNA (cfDNA) in the mother‘s blood plasma. Based on the varying lengths of cfDNA fragments from both the fetus and the mother, it calculates the risk of deviations in the number of chromosomes comprising an individual‘s overall genetic makeup (genome) or their parts. Deviations from the expected distribution of DNA fragments increase the risk of changes in the number (aneuploidy) of a specific chromosome or its parts (structural aberrations). PRENASCAN is primarily focused on assessing the risk of the presence of extra chromosomes, known as trisomies, in:

- Chromosome 21 causing Down syndrome,
- Chromosome 18 causing Edwards syndrome,
- Chromosome 13 causing Patau syndrome.

PRENASCAN detects over 99% of these relatively common trisomies. PRENASCAN can also reveal significant deviations in other areas of the genome and can be used with high accuracy to determine the genetic sex of the fetus in singleton pregnancies.

In the case of analysing all chromosomes, both sex and non-sex chromosomes are analysed for aneuploidies and structural changes (deletions or duplications of chromosome segments) of at least 7Mb in size (7 million DNA base pairs). The test has been validated for the detection of the following sex chromosome aneuploidies: XO, XXX, XXY, and XYY. Certain regions of the genome are excluded from the PRENASCAN analysis. The list of excluded regions can be found on the manufacturer‘s website at https://emea.support.illumina.com/downloads/veriseq-nipt-solution-v2-excluded-regions.html.

In the case of analysing a sample from a twin pregnancy, it is possible to detect only the presence or absence of the Y chromosome (thus, we are unable to predict the gender of both fetuses – in the case of detecting the Y chromosome, it could be a sister and a brother or two brothers).

The accuracy of calculating the risk of trisomies and other genetic deviations depends on the fetal fraction.

2.2. Options and Method of Reporting Results

The patient can decide whether she wants to know the results of the analysis of all chromosomes (known as genome-wide analysis) or only of chromosomes 13, 18, and 21, and whether she wants to know the result of the genetic analysis of the fetal sex*.

The method of reporting results is set as follows based on the patient's decision:

A) Complete analysis of all chromosomes and determination of the fetal sex. In this case, all suspicions of deviations in the number of both sex and non-sex chromosomes, including structural changes of at least 7Mb in size, are reported. The result of the genetic analysis of the fetal sex is also reported.

B) Complete analysis of all chromosomes but without reporting the fetal sex. In this case, all suspicions of deviations in the number of both sex and non-sex chromosomes, including structural changes of at least 7Mb in size, are reported. The result of the genetic analysis of the fetal sex is not reported unless the test detects an aberration in sex chromosomes.

C) Analysis of only chromosomes 13, 18, and 21 and analysis of sex chromosomes to detect anomalies. The result of the genetic analysis of the fetal sex is reported.

D) Analysis of only chromosomes 13, 18, and 21 and analysis of sex chromosomes to detect anomalies. The result of the genetic analysis of the fetal sex is not reported unless the test detects an aberration in sex chromosomes.

**However, it should be noted that if the test predicts the presence of aneuploidy or other abnormalities in sex chromosomes, in such cases, professional judgment and the best interest of the patient and the fetus are considered more important, and suspicions of deviations in sex chromosomes will be reported, thereby providing the patient with information about the genetic sex of the fetus and associated implications.*

2.3. Results

The examination results will be sent to the referring physician and, if agreed upon in advance, also directly to the patient, typically within 7 working days. In a small number of cases, the sample may need to be reanalysed, leading to a slight delay in the report.

- **LOW RISK** indicated in the result means that there is a very low probability that the child will have the specified genetic anomaly.
- **HIGH RISK** means that there is an increased likelihood of the child having the specified genetic anomaly. In such cases, to confirm the results, it is necessary to perform invasive prenatal diagnostic tests, such as amniocentesis, for example.
- **REPEAT TESTING** indicates that, in a small number of cases**, it was unfortunately not possible to analyse the fetal DNA in sufficient detail to provide a result. In such instances, the test will be repeated using the provided blood sample or a new blood sample from the patient may be requested.
- **NO RESULT** means that we were unable to conclude PRENASCAN result on repeated attempts**. The most common reasons for the failure of non-invasive prenatal testing are typically the insufficient presence of fetal free DNA or variations in its characteristics during laboratory processing. In the event of repeated test failure, it is recommended that the patient seeks genetic counselling for assessment and further recommendations. Genetic counselling appointments can be scheduled at our facility, GENNET, s.r.o. In such cases, the payment for the PRENASCAN test will be refunded to you.

***Repeat testing and/or no result apply to a maximum of 1.2% of all received samples.*

In exceptionally rare, unforeseen circumstances (e.g., earthquakes, floods, severe equipment malfunctions, etc.), the analysis may not be possible or may be delayed. In such cases, the patient and her physician will be notified. If, in these situations, it is not possible to conduct the test or obtain a valid result, the payment for the PRENASCAN test will be refunded to you.

3. Risks of the Procedure, Complications, and Limitations

3.1. Risks during Sample Collection

During the blood collection from the vein, there is a risk of vein injury, which may lead to the formation of a bruise at the collection site. Local reactions to disinfection may also occur, and in extremely rare cases, an infection could occur.

3.2. Risks of Genetic Laboratory Testing

PRENASCAN is a screening genetic test with its limitations. The results of PRENASCAN depend on the amount of fetal fraction and the genetic background of the mother. In approximately 1.2% of cases, there is a low fetal fraction, and the result cannot be issued or is unclear. Low fetal fraction is more common in early stages of pregnancy, in mothers with higher body mass (BMI above 35), and it may persist throughout the pregnancy. PRENASCAN results can also be affected by differences in the genetic makeup of the placenta and the fetus (fetoplacental mosaicism) or chromosomal abnormalities in the mother (e.g., mosaic sex chromosomes or chromosomal translocations). **For this reason, this test is not recommended for patients with confirmed chromosomal translocation or clinically significant mosaic sex chromosomes.**

Similarly to other cfDNA-based tests, the examination can be influenced by various factors on the part of the pregnant patient (mother), such as: blood transfusions, organ transplants, immunotherapy or stem cell therapy, chronic inflammatory or autoimmune diseases, and cancer. **If the patient has undergone a bone marrow transplant, stem cell therapy, or received a blood transfusion within the past year before the testing, then the PRENASCAN test cannot be performed. In the case of cellular immunotherapy involving exogenous DNA or therapy with human serum albumin, at least 4 weeks must elapse from the last treatment before the blood sample collection.**

Tests based on the analysis of cfDNA **can generally be further affected, for example, using blood-thinning medications (such as Fraxiparine or Clexane). These substances can generally increase the risk of obtaining a non-informative test result, necessitating repeat blood collection. Therefore, it is advisable to perform the blood collection before administering another dose of such medication (i.e., as far as possible from the last dose).**

The detection of variations in the representation of free DNA is likely to be less accurate in the case of twins, where it is influenced by the type (monozygotic, dizygotic twins) and condition of the placenta. In up to 4% of twin pregnancies, it is not possible to conclusively determine the test. When examining the gender of twins, it is only possible to detect the presence or absence of the Y chromosome. In the case of the presence of the Y chromosome, it is not possible to determine whether it is a sister and brother or two brothers.

Furthermore, the result may be distorted in the case of the „vanishing twin syndrome“, where testing can only be performed if detected before the 8th week of pregnancy, and blood collection is carried out at least 8 weeks from its detection. We note that the result can be up to 5% falsely positive.

The referring physician and the patient, by their signatures, acknowledge that they are aware of this risk, and that the blood collection was performed according to these recommendations.

The test **has not been** validated for:
- Use in triplet or higher-order pregnancies,
- Detection of polyploidy (multiplication of complete sets of chromosomes, not just a single chromosome),
- Detection of balanced chromosomal rearrangements,
- Detection of other than the above-mentioned aneuploidies of sex chromosomes,
- Detection of uniparental disomy (the fetus inherits both chromosomes from one parent),
- Fetal diseases caused by monogenic/polygenic conditions.

The test also has a certain rate of false positives, meaning that a result indicating an increased risk of aneuploidy or structural fetal abnormalities may not be confirmed by subsequent diagnostic invasive methods (chorionic villus sampling (CVS), amniocentesis (AMC)).

A detailed overview of limitations or test-specific characteristics (sensitivity and specificity rates) is available online on the VeriSeq NIPT Solution v2 Package Insert on the manufacturer‘s website at https://emea.support.illumina.com/.

In cases of insufficient quality of the fetal free DNA or variations in its characteristics during laboratory processing, it may be necessary to repeat the examination, either from the same submitted sample or by requesting a new blood sample. In such cases, both the referring physician and the patient will be informed, as this will lead to a delay in the result issuance - see section 2.3.

The conclusions of the genetic examination should be discussed with a clinical geneticist. The result of the PRENASCAN test must always be interpreted individually, taking into consideration the results of previous examinations. We always recommend a detailed ultrasound examination in the 20th - 22nd week. In the case of a positive result, targeted (diagnostic) examination of fetal tissues obtained through invasive procedures such as chorionic villus sampling (CVS) or amniocentesis (AMC) is indicated.

For this reason, PRENASCAN is ideally conducted based on the physician‘s indication between the 12th and 22nd week of pregnancy, following the results of the first-trimester combined test or second-trimester screening. This timing allows for timely performance of invasive prenatal diagnostics with results available no later than the 24th week of pregnancy. If the patient decides for PRENASCAN later - after the 24th week of pregnancy, when the fetal defect proven by the clinical geneticist cannot be used to indicate termination of the pregnancy for genetic reasons, she bears full responsibility for this decision. GENNET, s.r.o. does not accept any legal responsibility for testing that has been provided in violation of local laws governing the provision of prenatal testing and/or prenatal healthcare.

4. Information about Limitations in Daily Life and Work Capacity, and the Treatment Regimen

Not known.

5. Alternatives to the Proposed Genetic Examination (their suitability, benefits, and risks)

The assessment of the risk of the most common fetal aneuploidies through prenatal screening tests based on the results of biochemical and ultrasound examinations in the first and second trimesters of pregnancy (first-trimester combined test, integrated test, or triple test in the second trimester, and ultrasound screening in the 20th - 22nd week of pregnancy) is available. Screening for free DNA is not the method of choice when fetal abnormalities are detected via ultrasound because, in most such cases (80%), it does not provide any new information. The definitive confirmation of fetal chromosomal abnormalities can only be achieved through targeted (diagnostic) examination of fetal tissues obtained through invasive procedures, such as chorionic villus sampling (CVS) or amniocentesis (AMC).

6. Information about the Right of the Individual Undergoing the Examination to Freely Decide on the Provision of Healthcare Services

In accordance with § 28, paragraph 1, of Act No. 372/2011 Coll., on healthcare services, the patient has the right to freely decide on the course of action in the provision of healthcare services unless other legal regulations exclude this right.

In accordance with § 31 to 33 of the Act on healthcare services, the patient has the right to waive the provision of information about the result of the genetic examination and has the right to specify the individuals who should be informed (instead of or along with her) about it, as well as the right to express a prohibition on providing information about the health status to anyone. The patient has the right to select the individuals who should or should not be informed by listing them in a separate document.

7. Statement and Consent of the Examined Person

Prior to the blood sample collection and its genetic examination on the date specified below, I was informed by a healthcare professional about its purpose, nature, anticipated benefits, complications, and limitations, including its impact on health, including that of future generations, as well as the risks associated with unexpected findings for me and genetically related individuals.

I declare that the healthcare professional specified below provided me with this information, explaining everything clearly and comprehensibly. I had the opportunity to ask questions about anything I did not understand, and all additional questions were answered clearly and comprehensibly.

I was also informed about my right to waive the provision of information about my health status and my right to determine individuals authorised to be informed about my health status and to access my medical records (as contained in a separate informed consent).

I fully understood all the points of the above-mentioned information and the answers to supplementary questions.

Simultaneously, I declare that I have disclosed all facts significant for the assessment of my health status to the physician. I accept the warning that in case of any falsification of this declaration, GENNET, s.r.o., or the attending physician is not responsible for the resulting consequences. I undertake to promptly inform GENNET, s.r.o. in writing of any changes.

Furthermore, I declare that in the event of unexpected complications requiring urgent additional procedures necessary to protect my health, I consent to the performance of these necessary and urgent procedures.

7.1. For the Purposes Outlined in Section 1 of this Document, I consent to the following:

☐ **cfDNA analysis of all chromosomes** or ☐ **analysis of chromosomes 13, 18, and 21**

I wish to receive the result of the cfDNA analysis of the fetal sex.

☐ YES ☐ NO

I agree that GENNET, s.r.o. may use my samples for quality control of DNA diagnostics (the sample will be used as a control for another patient's examination).

☐ YES ☐ NO

I consent to the use of the results of the genetic laboratory test and relevant information about my health condition for scientific and educational purposes, provided that this data will be presented and published only in anonymous form.

☐ YES ☐ NO

Based on this information, I declare my consent to the collection of the relevant sample from my body and the execution of the genetic laboratory test as described above.

With my signature, I confirm that I will not use the laboratory result for the purpose of gender selection. I am aware that I can withdraw my consent at any time in writing.

Patient's Full Name:
Date of Birth:
ID number:
Address:
Email:
Phone:

Date:.....

Signature:.....

Consent of the Legal Representative of the Examined Person (to be filled out only for minors or legally incapacitated persons):

Name and surname of the legal representative/guardian of the examined person:

……..

Date of Birth:..... Relationship to the examined person:.....

Date:..... Signature of the legal representative (guardian):…….....

8. Physician's Statement

I declare that I have clearly and comprehensibly explained the content of this information to the patient, particularly informing her about the planned examination, its limitations, and risks.

Date:.....

Name and Surname of the Physician: .....

Physician's Signature: .....