Informed Consent for SABSCAN Test

1. Purpose of the Genetic Laboratory Examination

The SABSCAN test is an examination of cell-free DNA (cfDNA) from the mother's blood, performed after a spontaneous miscarriage (abortion) in its early stages. Its main goal is to detect chromosomal abnormalities of the products of conception (POC), which are the cause of miscarriages in up to 70% of cases.

SABSCAN represents an alternative to standard direct cytogenetic and molecular genetic analysis of conceptus tissue obtained through surgical intervention. It's especially suitable in situations where conceptus tissue can't be obtained or if the conceptus tissue is contaminated with maternal tissue (a common issue detected in up to 25% of samples using diagnostic molecular methods). Therefore, we recommend sending a sample for SABSCAN testing as a backup option even when the physician primarily submits POC tissue for diagnostic examination.

For the SABSCAN test, 7–10 ml of blood must be drawn from the woman's peripheral vein into a special Streck CELL-FREE DNA BCT (CE) tube under the following conditions:

- In the diagnosis of missed miscarriage.
- Before anesthesia for dilation and curettage.
- Within 24 hours after a complete miscarriage.

The success rate of the SABSCAN test depends on the FF value, which should be at least 2%. For this reason, the highest chance of obtaining a valid test result is when examining a miscarriage from the 7th gestational week. For samples taken earlier in pregnancy, a higher proportion of tests that can't be finalized must be expected.

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

2.1. Description of the Method Used

The SABSCAN screening examination uses the automated VeriSeq NIPT v2 Solution method from the American manufacturer Illumina. This test operates on the same principle as our proven and accredited NIPT (non-invasive prenatal testing) VeriSeq NIPT V2 technology, commonly used for screening chromosomal abnormalities in ongoing pregnancies.

SABSCAN analyzes cell-free DNA (cfDNA) circulating in the mother's blood plasma, which is a mixture of DNA from the placenta and maternal cells. The portion of cell-free DNA originating from the placenta (cffDNA) typically ranges from 2–20% and is referred to as Fetal Fraction (FF). A sufficient FF value is crucial for test evaluation.

The test utilizes the different lengths of fetal and maternal DNA fragments and their assignment to a specific chromosome. Deviations from the expected distribution of these DNA fragments signal an increased risk of changes in chromosome number (aneuploidies) or their parts (structural aberrations).

The standardized protocol of this method proceeds in several phases:

- Plasma and cfDNA Isolation: The input material is the patient's blood; its collection and delivery to the laboratory follow precisely defined procedures.

- Library Preparation: Isolated cfDNA is converted into a "library" by an automated protocol. During this process, cfDNA samples are labeled with index tags and adapters, which are essential for subsequent sequencing.
- Sequencing: The prepared libraries are sequenced on a NextSeq 550Dx instrument (Illumina).
- Data Analysis: The obtained sequencing data are then analyzed using VeriSeq v2 software tools (outside of the validated CE-IVD mode), Wisecondor, and BinDel.

The entire solution is designed with maximum regard for preventing sample mix-ups, minimizing the risk of errors (automation), reliability, and patient data security. Both the sample and sequencing data are processed directly in our GNTlabs by GENNET genetic laboratories.

The test focuses on:

- Aneuploidies (disorders of the number of individual chromosomes), which the test analyzes for all chromosomes, including sex chromosomes, namely with karyotypes X0, XXY, XYY, XXX.
- Structural changes of all chromosomes - deletions (losses) or duplications (doubling) of chromosome parts larger than 7 million DNA bases (7 Mb). In specific regions associated with severe clinical presentation, such as 22q11 (Di George Syndrome), SABSCAN also detects changes of a smaller scale.

Certain genomic regions are excluded from SABSCAN analysis. A detailed list is available on the manufacturer's website: https://emea.support.illumina.com/downloads/veriseq-nipt-solution-v2-excluded-regions.html.

- SABSCAN doesn't detect fetal polyploidy (i.e., multiplication of entire sets of chromosomes), which accounts for approximately 10% of early-pregnancy loss.

In the case of sample analysis for a twin pregnancy, sex chromosome analysis is limited to determining the presence of the Y chromosome. For twins, it's not possible to analyze sex chromosome aneuploidies (e.g., Turner syndrome with karyotype X0).

2.2. Options and Method of Reporting Results

The SABSCAN test analyzes all chromosomes (sex and non-sex). All suspicions of sex and non-sex chromosome aneuploidies, including structural changes of 7 Mb and smaller in selected regions, are reported. The sex of the conceptus isn't reported unless an abnormality of the sex chromosomes is the presumed cause of the miscarriage. In this case, the sex of the conceptus can be inferred from the detected and reported deviation of chromosomes X and Y (according to the result delivery settings, see section 2.3 Results).

2.3. Results

Test results will be sent to the referring physician, or by prior arrangement directly to the patient, usually within 14 working days. However, in the case of a repeat examination (see below), there may be a slight delay in issuing the results.

Possible SABSCAN test results are as follows:

- **LOW RISK:** Means there's a very low probability that the miscarriage was caused by a chromosomal abnormality.
- **HIGH RISK:** Means the test detected an increased probability of a chromosomal abnormality in the miscarriage.

- **REPEAT EXAMINATION:** Means that it wasn't possible to analyze the conceptus DNA in the first iteration in sufficient detail to provide a result. In such cases, a laboratory technician will assess the potential benefit of a repeat examination from the supplied blood sample based on the measured results from the first iteration of the SABSCAN test and decide whether to perform a repeat examination. In case of a repeat examination, there may be a delay in result delivery (the laboratory will inform the requester about the need to repeat the examination).
- **NO RESULT:** Means that it wasn't possible to finalize the SABSCAN result. The most common cause of an unsuccessful non-invasive prenatal examination is a lack of cell-free conceptus DNA (low FF) or its different properties during laboratory processing. In such a case, the laboratory claims a partial refund for the service.

For both successful and unsuccessful examinations, a genetic consultation for evaluation and proposal of further steps is recommended for the patient.

In extremely rare, unexpected situations (e.g., earthquake, floods, severe machine malfunction, etc.), the analysis won't be able to be performed. In these cases, the referring physician (or, by prior arrangement, also the patient) will be informed, and payment for the SABSCAN test won't be charged.

3. Risks and Limitations of the Procedure

3.1. Risks during Sample Collection

During blood collection from a vein, injury may occur, resulting in a hematoma at the puncture site. A local reaction to disinfectant may also appear, or, very rarely, an infection may occur.

3.2. Risks of Performing Laboratory Genetic Examination

The SABSCAN test is a screening genetic test with its limitations. Its sensitivity depends on the fetal fraction (FF) value and the mother's genetic background. A low fetal fraction is common in early stages of pregnancy and in the plasma of mothers with a higher weight (BMI above 35). Low FF is the reason for failure in approximately 5% of SABSCAN tests performed before the 7th gestational week. The SABSCAN test result may be skewed by focal changes in the placental chromosomal makeup (placental mosaicism) or a maternal chromosomal abnormality (e.g., sex chromosome mosaicism or chromosomal translocation). For this reason, this examination isn't recommended for patients with a confirmed chromosomal translocation or a clinically significant sex chromosome mosaicism.

Similar to other cfDNA-based tests, the examination may be influenced by a number of other maternal factors, such as: blood transfusions, organ transplants, immunotherapy or stem cell therapy, chronic inflammatory or autoimmune diseases, and neoplastic diseases. SABSCAN is contraindicated in bone marrow transplantation or stem cell therapy. In the case of cellular immunotherapy where exogenous DNA is introduced, or therapy with human serum albumin, at least 4 weeks must pass after the last administration of treatment before blood sampling. At least one year should pass after a transfusion.

Tests based on cfDNA analysis may generally be further skewed by, for example, the use of anticoagulant medications (e.g., Fraxiparine, Clexane). These substances can generally increase the risk of an uninformative test result.

The detection of abnormalities in the representation of cell-free DNA is probably less accurate in the examination of multiple conceptuses.

Both the referring physician and the patient confirm by their signature that they acknowledge this risk and that the blood collection was performed according to these recommendations.

The test hasn't been validated for:

- Use in triplet and higher-order conceptuses.
- Detection of polyploidy (multiplication of complete sets of chromosomes, not just one chromosome).
- Detection of balanced chromosomal rearrangements.
- Detection of sex chromosome aneuploidies other than those listed above.
- Detection of uniparental disomy (fetus inherited both chromosomes from one parent).
- Fetal monogenic/polygenic disease.

NIPT tests generally have a certain false positive rate, meaning that a result indicating an increased risk of fetal aneuploidy or structural aberration isn't confirmed by subsequent examination using an invasive diagnostic method (CVS, amniocentesis). This procedure isn't typically possible in the case of examination using the SABSCAN test. Both the physician and the patient acknowledge this and understand this limitation of the test.

A detailed overview of the limitations or specifics of tests (sensitivity and specificity) for non-invasive prenatal examination of pregnant patients is available online on the manufacturer's website VeriSeq NIPT Solution v2 Package Insert here: https://emea.support.illumina.com/.

In the case of a repeat examination (reasons see section 2.3), both the referring physician and the patient will be informed about the delay in issuing the result.

The conclusions of the genetic examination should be consulted with a clinical geneticist. The SABSCAN test result must always be interpreted individually, considering the results of previous or accompanying examinations.

GENNET, s.r.o. accepts no legal responsibility for testing that has been provided contrary to local laws regulating the provision of prenatal testing and/or prenatal health care.

4. Limitations in Usual Way of Life and Work Capacity, and Treatment Regimen

None known.

5. Alternatives to the Proposed Genetic Examination (their Suitability, Benefits, and Risks)

An alternative is the examination of aborted fetal tissue using appropriate diagnostic methods (QF-PCR, Array, etc.), possibly in combination with the results of biochemical and ultrasound examinations in the first and second trimesters of pregnancy (combined test in the first trimester, integrated test or triple test in the second trimester, and ultrasound screening in the 20th - 22nd week of pregnancy).

6. Instruction on the Examined Person's Right to Freely Decide on the Procedure for Providing Health Services

In accordance with § 28, par. 1 of Act No. 372/2011 Coll., on Health Services, the patient has the right to freely decide on the procedure for providing health services, unless other legal regulations exclude this right.

In accordance with § 31 to 33 of the Act on Health Services, the patient has the right to waive the provision of information about the result of a genetic examination and has the right to designate persons to be informed (instead of or along with her) and has the right to prohibit the provision of information about her health status to anyone.

The right to select persons who are to be or, conversely, must not be informed, is exercised by the patient by listing these persons in a separate document.

7. Statement and Consent of the Examined Person

Before the collection of my blood sample and its genetic examination, I was instructed on the purpose, nature, anticipated benefit, complications, and limitations, including the impact on health, including the health of future generations, and on the risks of unexpected findings for me and genetically related persons, by a healthcare professional on the date stated below.

I declare that the healthcare professional named below provided me with this instruction, personally explained everything clearly and understandably to me, including the risks associated with performing the examination. I had the opportunity to ask anything I didn't understand, and all supplementary questions were clearly and understandably answered.

I was also instructed about my right to waive the provision of information about my health status and also about my right to designate persons authorized to be informed about my health status and to inspect my medical documentation (contained in a separate informed consent).

I have fully understood all points of the above instruction and the answers to supplementary questions. I simultaneously declare that I have informed the physician of all facts significant for assessing my health status. I accept the warning that in case of falsity of this statement, GENNET, s.r.o. nor the attending physician are responsible for the consequences caused thereby. I undertake that if any change occurs, I will immediately inform GENNET, s.r.o. in writing.

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

7.1. Consent to Examination and Use of Samples

For the purpose stated in section 1 of this document, I consent to the collection of my peripheral venous blood sample and to the performance of this examination:

☐SABSCAN

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

☐YES ☐NO

I agree to the use of the results of the genetic laboratory examination and relevant information about my health status for scientific and educational purposes, provided that these data will be presented and published only in an anonymous form.

☐YES ☐NO

Based on this instruction, I declare that I consent to the collection of the relevant sample from my body and to the performance of the above-described genetic laboratory examination under the conditions stated above.

I am aware that I can revoke my consent at any time in writing.

Name and surname of patient: ……..

Date of birth:……..

ID: ……..

Address: ……

Email: ……

Phone: ……..

Date: ……

Signature: ……

Consent of the Legal Representative of the Examined Person (fill in only for minors or persons with limited legal capacity):

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

Date of birth: ……..Relationship to the examined: ……..

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

8. Physician's Statement

I declare that I have clearly and understandably explained the content of this instruction to the patient, especially I have familiarized her with the planned examination, its limitations, and risks.

Date: ……

Name and surname of physician: ……

Signature of physician: ……..